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Victor R. Preedy
Vinood B. Patel
Editors

Handbook of Famine, Starvation, and Nutrient Deprivation

From Biology to Policy

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With 479 Figures and 226 Tables

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Preface

There are numerous definitions of the terms malnutrition, undernutrition, and starvation, many of which are used interchangeably. Strictly speaking, malnutrition also refers to an "imbalance" and includes an excess of nutrients. However, within the context of this handbook we are primarily concerned with a lack or deficiency of one or more dietary components rather than an excess. Embedded within the deficiency states is "acute restriction" whereby food is withdrawn or deliberately not consumed at all. For example, individuals may refrain from eating to provide blood samples for subsequent analysis or food may be withdrawn from patients before surgery. Further downstream is the consumption of a fraction of the diet, or none at all. This may arise when there are restrictions in the amount of food available to an individual or population. The causes of such restrictions in dietary intake are varied and include poverty, conflict, and regional famine. In the sociogeographical context, refugees and displaced persons may also be vulnerable to undernutrition.

Some diseases will impact on the total food consumed, for example, when there are physical impediments (intestinal obstruction or dysphagia) or anorexia. There may also be restrictions in the availability of single micro- or macronutrients such as vitamins, minerals, proteins, lipids, or dietary energy. There may be increased bodily demands for certain nutrients in some diseases, but these may not be met by the existing diet, thus resulting in deficiency states.

The impact of such dietary restrictions is variable. Deficiencies in micro- or macronutrients will impact on cells, organs, individuals, and even populations. Quality of life measures, for example, are impaired in anorexia and in famine. Some communities are blighted by the absence of specific micronutrients which impact on physical and mental health: endemic iodine deficiency is a good example of this.

It is important to understand the causes of nutrient deficiencies and also to be aware of the impact on the cell-to-community continuum. The knowledge gained from understanding how cells and organs respond to nutrient deficiencies may be transferable to understanding and treating deficiency diseases. At the population level, it is important to know how to diagnose and treat nutritional deficiencies. In the wider context, food waste and food insecurity are at opposite ends of the spectrum but have in common the disparities between provision and need. Policies

and procedures to address the aforementioned are required to reduce food waste and food insecurity.

There is a wide range of information that interlinks the complexities of undernutrition, disease, famine, sociology, food waste, food insecurity, poverty, provision, need, policies, and procedures. Hitherto, this has been sporadically distributed across different publications. This is resolved in the *Handbook of Famine, Starvation, and Nutrient Deprivation: From Biology to Policy.* It has wide coverage and also includes social aspects, refugees, conflict, hunger, anorexia, screening tools, medical causes of malnutrition, endocrinology, metabolism, tissue systems, life stages, micronutrients, modeling, cellular and molecular biology, international aspects, and management.

There are 12 parts as follows:

1. General Aspects of Famine and Undernutrition: Setting the Scene
2. Effects of Famine
3. Food Insecurity, Security, and Waste
4. Biosocial and Social Aspects, Inequalities, Low Income, Refugees, and Conflict
5. Hunger and Anorexia
6. Screening Tools, Classifications, and Applications
7. Medical Causes of Malnutrition, Prevalence, and Impact
8. Effects of Undernutrition, Endocrinology, Metabolism, and Tissue Systems
9. Life Stages, Pregnancy, the Young and Elderly
10. Micronutrients
11. Modeling Systems, Cellular and Molecular Effects
12. International Aspects, Policy, Management, Case Study, and Resources

The editors recognize the fact that it has been difficult to allocate specific chapters to the different parts. Some chapters may be suitably placed in different parts of the book. Nevertheless, the information in the *Handbook of Famine, Starvation, and Nutrient Deprivation: From Biology to Policy* is wide ranging. To bridge the intellectual divide and to provide guidance, each chapter has three sections as follows:

Policies and Protocols
Dictionary of Terms
Summary Points

Contributors are authors of international and national standing, leaders in the field, and trendsetters. Emerging fields of nutritional science and important discoveries are also incorporated in this book.

This book is designed for nutritionists and dietitians, public health scientists, doctors, epidemiologists, biologists, health-care professionals of various disciplines, policy makers, governmental bodies, and strategists. The *Handbook of Famine,*

Starvation, and Nutrient Deprivation: From Biology to Policy is designed for teachers and lecturers, undergraduates and graduates, researchers, and professors and as a library resource.

Victor R. Preedy
Vinood B. Patel
The Editors

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About the Editors

Victor R. Preedy (**B.Sc., Ph.D., D.Sc., FRSB, FRSPH, FRCPath, FRSC**), is a staff member of the Faculty of Life Sciences and Medicine at King's College London. He is also a member of the Division of Diabetes and Nutritional Sciences (research) and the Department of Nutrition and Dietetics (teaching). Professor Preedy is also Director of the Genomics Centre of King's College London.

Professor Preedy graduated in 1974 with an honors degree in biology and physiology with pharmacology. He gained his University of London Ph.D. in 1981. In 1992, he received his membership of the Royal College of Pathologists, and in 1993, he gained his second doctorate (D.Sc.) for his outstanding contribution to protein metabolism in health and disease. Professor Preedy was elected as Fellow to the Institute of Biology in 1995 and to the Royal College of Pathologists in 2000. In 2004, he was elected as Fellow to the Royal Society for the Promotion of Health and to the Royal Institute of Public Health. In 2009, he became Fellow of the Royal Society for Public Health and, in 2012, Fellow of the Royal Society of Chemistry. Professor Preedy has carried out research at the National Heart Hospital (part of Imperial College London), the School of Pharmacy (now part of University College London), and the MRC Centre at Northwick Park Hospital. He has collaborated with research groups in Finland, Japan, Australia, the USA, and Germany. He is a leading expert in the science of health and has a long-standing interest in nutrition and disease. He has lectured nationally and internationally. To his credit, Professor Preedy has published over 600 articles, which include peer-reviewed manuscripts based on original research, abstracts and symposium presentations, reviews, and numerous books and volumes.

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Dr. Vinood B. Patel (**B.Sc., Ph.D., FRSC**) is a Reader in Clinical Biochemistry at the University of Westminster and Honorary Fellow at King's College London. Dr. Patel graduated from the University of Portsmouth with a degree in pharmacology and completed his Ph.D. in protein metabolism from King's College London in 1997. His postdoctoral work was carried out at Wake Forest University Baptist Medical School studying structural-functional alterations to mitochondrial ribosomes, where he developed novel techniques to characterize their biophysical

properties. Dr. Patel is a nationally and internationally recognized scientist, and in 2014 he was elected as Fellow to the Royal Society of Chemistry. He presently directs studies on metabolic pathways involved in diabetes and liver disease, particularly related to mitochondrial energy regulation and cell death. He is currently conducting research to study the role of nutrients, antioxidants, phytochemicals, iron, alcohol, and fatty acids. His other areas of interest include identifying new biomarkers that can be used for the diagnosis and prognosis of liver disease and understanding mitochondrial oxidative stress in Alzheimer's disease and gastrointestinal dysfunction in autism. Dr. Patel has edited biomedical books in the areas of health preventive nutrition and biomarkers and has published over 150 articles.

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Part I

General Aspects of Famine and Undernutrition: Setting the Scene

Biafran Famine

1

Mikael Norman and Peter Ueda

Contents

Abstract

Following ethnic, economic, and religious tensions, the republic of Biafra unilaterally declared independence from the rest of Nigeria in 1967. This action triggered the Nigerian civil war in which the inflow of food and supplies to Biafra was blocked.

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The result was extensive famine, regarded as one of the great nutritional disasters of modern times. During the two-and-a-half years of armed conflict, an estimated one to three million people died, most of them from starvation.

Forty years later, adults in Enugu (the former capital of Biafra) who had been conceived or born during the famine and who survived the famine show health problems with two-to-three times higher prevalence of hypertension, glucose intolerance, and overweight than those born before or after the war. These findings support that undernutrition early in life have significant and adverse impact on human development and physiological design, eventually contributing to an increased risk for noncommunicable diseases in adulthood such as ischemic heart disease, stroke, and diabetes. The long-term effects of undernutrition during pregnancy and in infancy should be considered and receive high priority when setting goals for global health, education, and economic agendas.

Keywords

Sub-Saharan Africa · Nigeria · Biafran famine · Developmental origins of health and disease · Fetal undernutrition · Intrauterine growth restriction · Low birth weight · Metabolic syndrome · Diabetes · Hypertension · Overweight · Obesity

List of Abbreviations

BMI	Body mass index
OR	Odds ratio
SBP	Systolic blood pressure

Introduction

Epidemiological evidence supported by experimental data suggests that public health problems emerging in adult life are not only determined by genes and adult life-style, but also by perinatal factors acting before and shortly after birth (Barker 1998; Gluckman and Hanson 2004; Armitage et al. 2004; Gluckman et al. 2009). According to this concept, early living conditions – of which fetal nutrition is a key component – shape not only our ability to survive birth and infancy, but also our physiological capacity to respond to health challenges occurring later in life. In this way, developmental plasticity in response to early under- or malnutrition can be considered an important, underlying mechanism explaining why adults born small – a proxy for fetal starvation – are at increased risks for cardiovascular disease and diabetes.

In the twenty-first century, the prevalence of obesity, hypertension, and insulin resistance, i.e., the metabolic syndrome (sometimes also including a proinflammatory and prothrombotic state) has increased (Manson et al. 2004; Franks et al. 2010). The metabolic syndrome is the most important predictor of cardiovascular disease and type 2 diabetes (Rapsomaniki et al. 2014; Ekblom-Bak et al. 2009). In developed countries

around 15% of the adult population is affected and in the developing world, rates of metabolic syndrome are rapidly becoming even higher. As a result, 80% of all new cases of diabetes and 85% of all deaths in cardiovascular disease are estimated to occur in low-middle income countries by 2025–2030 (Joshi et al. 2008).

Although infections still plague many Sub-Saharan African countries, non-communicable diseases are emerging as leading causes of morbidity and death (Unwin 2001). Besides better economy and successful vaccination and educational programs, this change in epidemiology is commonly attributed to rural-to-urban shifts in adult lifestyle, typically involving obesity-promoting diet, lack of exercise, and cigarette smoking (Yach et al. 2004). However, there may also be a significant perinatal contribution to this development. In particular, people who suffered from undernutrition in utero and who later in life become exposed to nutritional affluence are thought to run the greatest risks of cardiovascular disease and diabetes (Barker et al. 1993; Barker 1998; Gluckman and Hanson 2004).

The role of undernutrition during pregnancy for future childhood and adult health has been evaluated in different settings exposed to famine, most of them outside Sub-Saharan Africa (1991; Ravelli et al. 1998, 1999; Roseboom et al. 1999, 2001; Victora et al. 2003, 2008; Bhargava et al. 2004; Richter et al. 2004; Sachdev et al. 2005; Grajeda et al. 2005). Given limited resources, previous and ongoing maternal – infant undernutrition (Black et al. 2008) and growing numbers affected by cardiovascular disease, obesity, diabetes, and hypertension (Ike 2009; Unwin 2001), the significance of poor fetal-maternal health for adult disease risk, would be of great importance to clarify in Sub-Saharan Africa as well. To do so, researchers from Sweden and Nigeria focused on long-term health among adult survivors of the Biafran famine occurring almost 50 years ago in Nigeria (Hult et al. 2010).

The Biafran Famine

In 1960, Nigeria became independent from United Kingdom. As with other new African states, the borders of the country did not reflect earlier ethnic boundaries, resulting in social unrest. As a culmination of ethnic and religious tensions, civil war broke out in Nigeria on 6 July 1967, after the Igbo people in the south-eastern part had declared independence as the Republic of Biafra. The struggle for control over the large amount of oil in the southeastern Nigeria is also likely to have contributed to armed conflict.

Disapproving of the secession, federal Nigerian forces rapidly encircled Biafran territory and inflow of food to this densely populated enclave was stopped. The resulting famine was extensive and has been regarded as one of the greatest nutritional disasters of modern time (Miller 1970). Of the estimated one to three million Biafrans that lost their lives, only a small fraction (10%) died in military actions and the rest from starvation. International relief actions were launched but they were insufficient and the majority of Igbos did not get access to food from these aid programs (Aall 1970). The war ended in January 1970, Fig. 1.

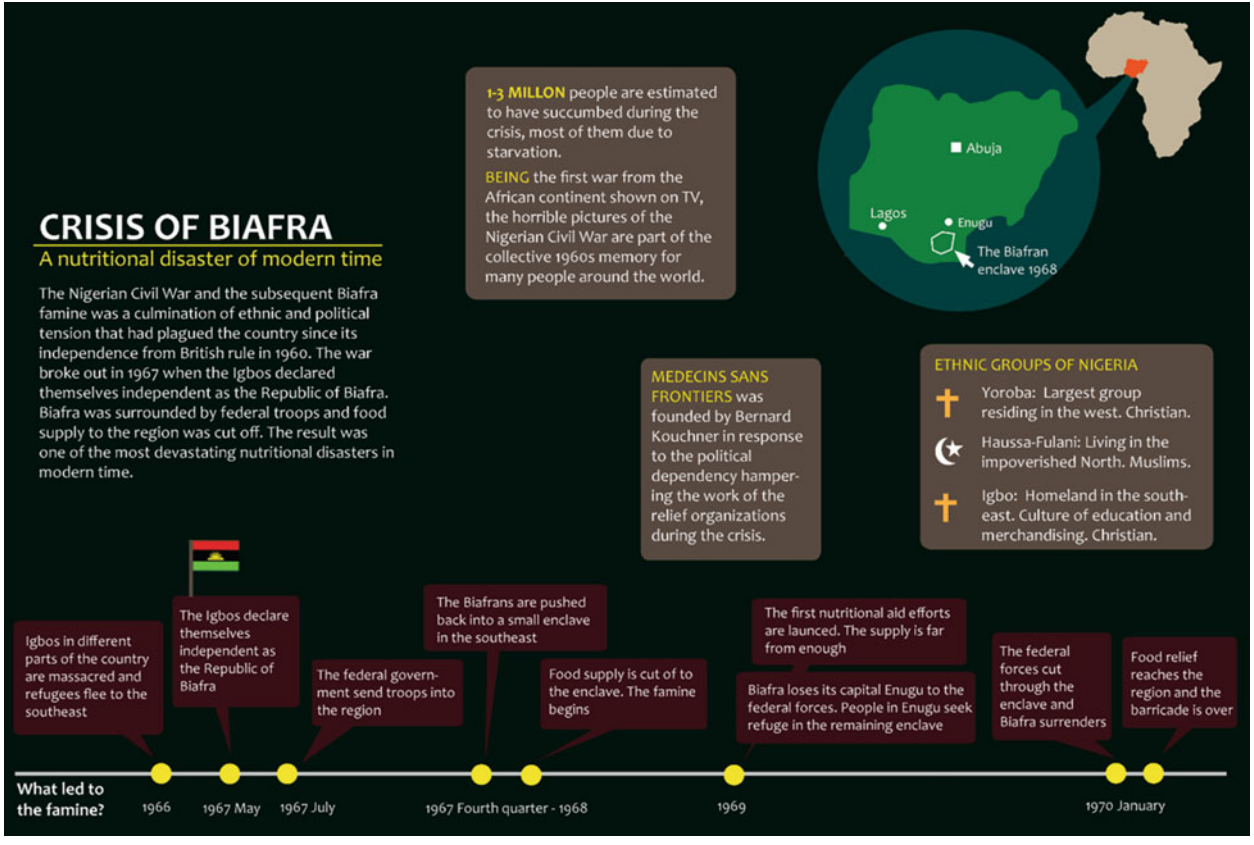

Fig. 1 Crisis of Biafra

Adult Health Forty Years Later

To examine the association between early life exposure to malnutrition and adult life health indicators, a cohort study was performed in 2009 in Enugu (the former capital of Biafra). In total, 1,339 adults aged 36–44 years and thus born before (1965–1967), during (1968–January 1970), or after (1971–1973) the years of famine were recruited at market places of the city, i.e., people representative for today's urbanization in sub-Saharan Africa and thought to be at the highest risk for noncommunicable diseases. Cardiovascular risk factors including blood pressure, plasma glucose, and body mass index were measured (Hult et al. 2010) and adjusted for sex and BMI.

The results showed that people born during the famine had higher systolic blood pressure (mean difference + 7 mmHg; p < 0.001), increased plasma glucose (+0.3 mmol/L; p < 0.05), and waist circumference (+3 cm, $p < 0.001$) than those born before or after the famine. As shown in Fig. 2, those born during the years of famine also had a higher risk of hypertension. Similarly, in this group the risks of impaired glucose tolerance and overweight were elevated. The highest risk for elevated systolic blood pressure was seen in those exposed to fetal-infant famine and ending up with overweight in adult life, Fig. 3.

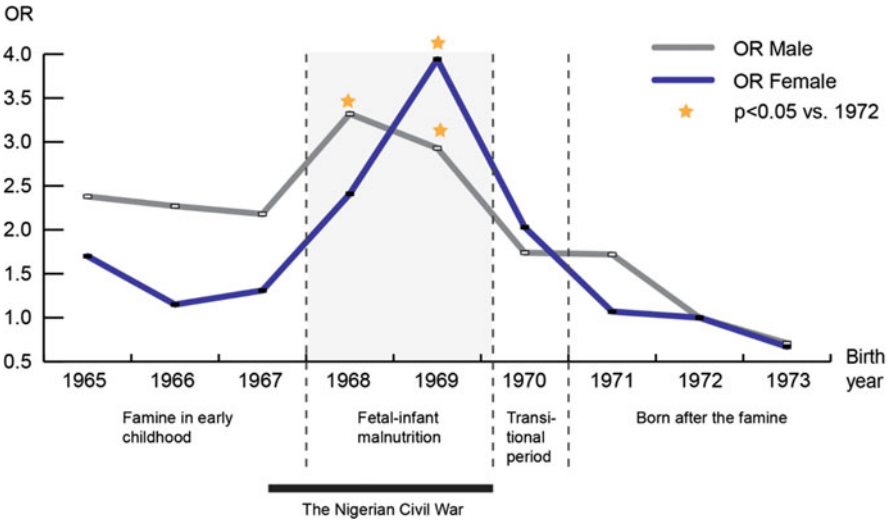

Fig. 2 Risk of hypertension in 2009 by year of birth before, during, and after the Biafran famine. Odds ratios (*OR*) for high systolic (≥140 mmHg) blood pressure in men and women in Enugu, the former capital of Biafra, at follow-up in 2009 according to year of birth (1972 reference) (With permission (this figure has a creative commons license); from Hult M, Tornhammar P, Ueda P, et al. (2010) Hypertension, Diabetes and Overweight: Looming Legacies of the Biafran Famine. PLOS ONE 5(10): e13582. ▶ https://doi.org/10.1371/journal.pone.0013582, http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0013582)

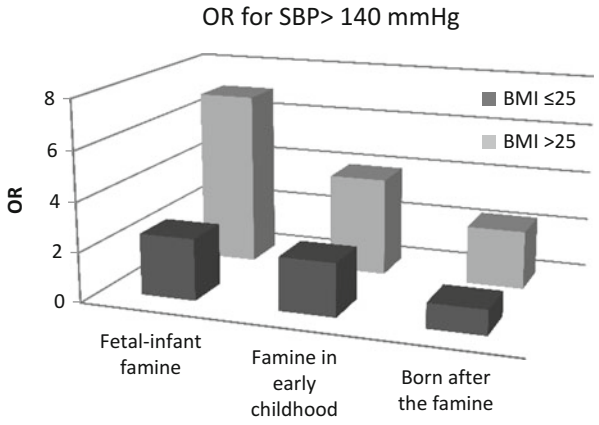

Fig. 3 Joint effects of exposure to famine in early life and adult overweight in survivors of the Biafran famine, as risk factors for a systolic blood pressure (SBP) ≥140 mmHg at 40-years-of-age. *OR* crude odds ratio, *BMI* body mass index. The *bars* represent the risk of high blood pressure for people born during the Biafra famine compared with those born after the famine, among individuals who are normal weight and overweight, respectively

What Could Be the Mechanisms?

During the Biafran famine, the fetal supply line was likely to be seriously compromised in most pregnant women. Although no birth records exist from that time, signs of almost universal fetal and infant undernutrition are obvious on photographs taken during the famine. Some misclassification may have occurred in the above mentioned study, but if anything, this could only have introduced a conservative bias, underestimating the true long-term effects of undernutrition in fetal life and in infancy.

Fetal starvation – as reflected by intrauterine growth retardation and low birth weight at term birth – can cause lasting endothelial dysfunction in small and large arteries, predisposing the affected individuals to atheroma formation and cardiovascular disease (Leeson et al. 1997; Martin et al. 2000a, b; Singhal and Lucas 2004). In addition, the vascular phenotype characterizing people born thin include smaller and stiffer arteries, premature intima-media thickening and capillary rarefaction, all of which are risk factors for later cardiovascular disease (Martyn and Greenwald 1997; Martin et al. 2000b; Singhal and Lucas 2004; Brodszki et al. 2005; Gale et al. 2006; Mitchell et al. 2008; Clough and Norman 2011). Poor fetal kidney development resulting in fewer nephrons may have contributed to the increased risk for hypertension seen in adult survivors of the Biafran famine (Brenner and Mackenzie 1997) and thinness at birth has previously been associated with lower glucose tolerance in childhood (Law et al. 1995) and adult life (Phillips et al. 1994a, b), especially after reduced protein intake during pregnancy and lactation (Smith 2006). Interestingly, maternal undernutrition may also result in behavioral changes in the offspring, such as sedentariness and increased appetite (Vickers et al. 2000, 2003) which could be in the casual pathway for development of overweight and obesity after famine in early

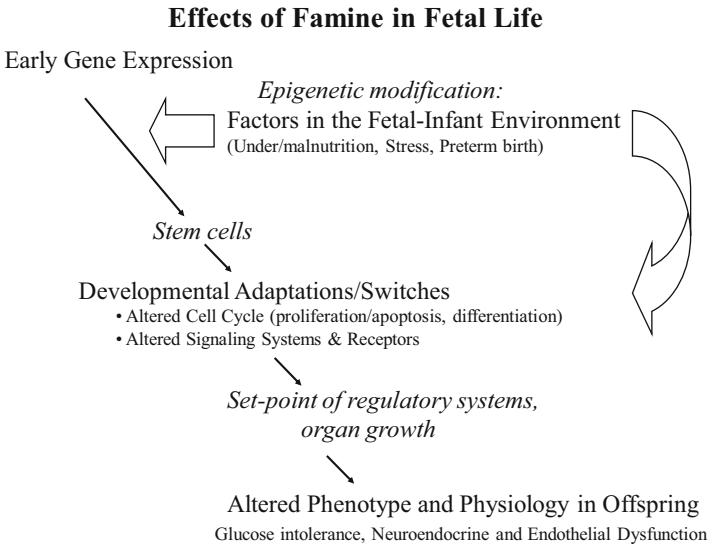

Fig. 4 Early life mechanisms shaping the phenotype

life. Accordingly, different adaptive responses to pre- and perinatal undernutrition can alter the phenotype in terms of physiology, neuroendocrine, and behavioral responses, processes sometimes referred to as "developmental programming," Fig. 4.

Protein Undernutrition in Fetal Life and Later Health

The Biafran famine was characterized by a particularly severe scarcity of proteins, manifested in a large number of infants and children suffering from kwashiorkor (Miller 1970). In experimental models, protein deficit in fetal life results in abnormal glucose homeostasis and vascular endothelial dysfunction in the adult offspring (Armitage et al. 2004).

The role of fetal protein restriction with regard to programming of high blood pressure is less clear. Besides the nutritional insult, pregnant women in former Biafra were living under conditions of war. Such stress for mothers and infants could also have contributed to higher blood pressure in later life, possibly mediated via sympathoadrenal overactivity (Johansson et al. 2007) or exaggerated responses to stress in the hypothalamic-pituitary-adrenal system (Welberg and Seckl 2001; Fish et al. 2004).

Role of Epigenetics

Besides direct effects of fetal-infant undernutrition on cell division, signaling systems, and organ size, early fetal gene expression may also be epigenetically modified in response to environmental exposures (Murphy and Jirtle 2003). One of the best studied epigenetic control mechanisms – in which gene expression is changed

without altering the genome – is DNA methylation (Jirtle and Skinner 2007). DNA-methylation status at birth has been related to both maternal pregnancy diet and to degree of adiposity in her child at age 9 years (Godfrey et al. 2011), and to aortic stiffness – a risk factor for cardiovascular disease – at school-age (Murray et al. 2016). Experimental data also suggest that diet restriction during pregnancy may increase the risk for type 2 diabetes in later life through epigenetic mechanisms (Sandovici et al. 2011).

The Importance of Timing

The importance of timing for later effects of early life famine has been demonstrated in follow-up studies of the Dutch famine, occurring in the end of the Second World War. These studies indicate that undernutrition during different parts of pregnancy may result in different adult risk profiles and outcome, with the highest adult disease risk seen in those exposed to undernutrition early in pregnancy (Painter et al. 2005; Roseboom et al. 2011; van Abeelen et al. 2012b, d). Although available information on the Biafran famine does not allow for a detailed analysis of the timing of the insult, the striking dose-response effect found between birth during years of famine and over-risk for hypertension in adult life (Fig. 2) supports the idea of a causal relationship.

Starvation and Growth in Childhood

As illustrated by the Biafran and Dutch famine follow-up studies (van Abeelen et al. 2012a, b, c), exposure to starvation not only in fetal-infant life but also in childhood is associated with increased risks (but not as pronounced as exposure in fetal-infant life) for hypertension and diabetes in adult life. Children and young adults with accelerated or exaggerated growth following famine in early life seem to be at highest risks (Eriksson et al. 2000; Adair and Cole 2003).

Implications

The famine triggered by the Nigerian civil war represents double misfortune with obvious immediate suffering and health problems for those people exposed to famine, followed in the next generation by increased risks for noncommunicable diseases for those who were conceived and born during famine and survived. Such health problems – originating from reproduction during famine and emerging later in adult life – are not restricted to those investigated herein, but also include increased prevalence of musculoskeletal problems, immune disorders, cognitive disabilities, and psychiatric diseases (Gluckman and Hanson 2006), Fig. 5. Therefore, the Biafran famine has and will have significant impact on health of the affected families, on the health care system, society, and economy.

The implications are important. On a population level, a 2–3 mmHg increase in mean blood pressure has been translated into an estimated increase in cardiovascular

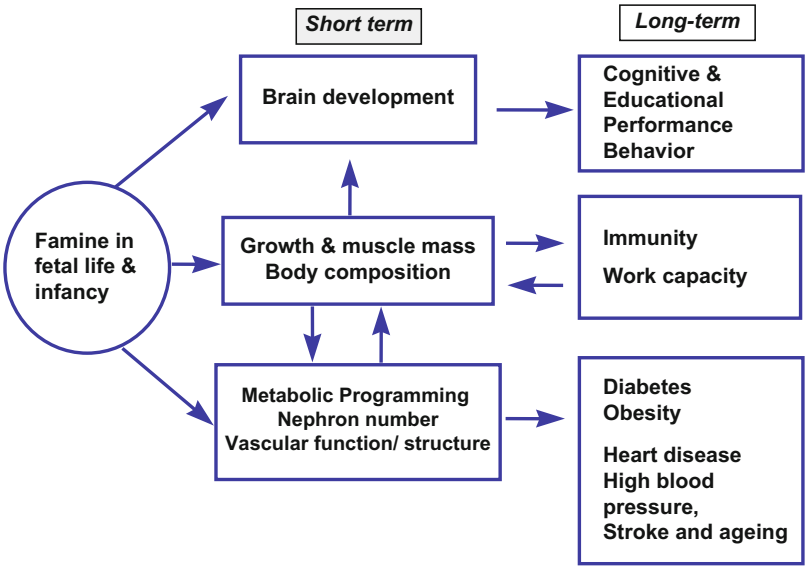

Fig. 5 Conceptual framework of mechanism behind the association between early exposure to the Biafra famine and health outcomes later in life

deaths by 25% and stroke by 32% (Yusuf et al. 2000). Given the combination of elevated blood pressure and glucose intolerance resting on a basis of prevalent obesity before middle age – characteristic for today's urban Nigeria – it is not surprising that morbidity and deaths from stroke and coronary heart disease are increasing. The increasing burden of noncommunicable diseases therefore poses a massive challenge to build-up sufficient health care systems infrastructure in sub-Saharan Africa. Although nutritional disasters with the same severity as the Biafra famine are perhaps not seen in sub-Saharan Africa today, maternal starvation, fetal growth restriction, and infant malnutrition are common and ongoing public health problems on the African continent. Prevention of fetal and infant undernutrition should be given high priority in national health, education, and economic agendas on how to limit the increase of noncommunicable diseases in many African countries.

Policies and Protocols

Measuring and Interpreting Blood Pressure, Blood Glucose, and Body Mass Index

In this chapter we have described health in 40-year-old survivors conceived and born during the Biafran famine. The health outcome variables of these people included determination of three common predictors of cardiovascular disease and diabetes: blood pressure, glucose levels in blood, and body mass index. When

measuring such outcomes, it is important to consider predefined standard operating procedures and to use validated devices (Topouchian et al. 2006). When interpreting data and results, it is also important that common and generally accepted criteria for hypertension, glucose intolerance/diabetes, and overweight/obesity (Alberti et al. 2006) are used.

Global and Local Governance over Health Care, Education, and Nutrition

In this chapter we have described that famine among pregnant women and their infants represents double misfortune: besides the immediate suffering and risk of death for both the woman and her fetus or newborn infant, famine in fetal-infant life will – even after the situation has resolved – results in a weaker construction and design of human physiology which cannot be fully repaired or compensated for later in childhood and adult life. This will result in increased risk for noncommunicable diseases in later life. Given that severe undernutrition and famine still is ongoing, in several cases in the same settings that now are facing an increasing burden of noncommunicable disease, nutrition, and health of pregnant women and their infants is the best investment for the future and should receive highest priority in global and national health, education, and economic agendas.

Dictionary of Terms

- **Endothelium** – The endothelium is the inner cell-lining of all blood vessels. The endothelium has many important function related to how the blood vessels function. The endothelium can actively open or close blood vessels, activate clotting, and open the vascular wall for white blood cells fighting infections in the tissue. Endothelium dysfunction is one of the earliest signals of increased risk for cardiovascular disease.
- **Capillary rarefaction** – The capillaries are the smallest blood vessels through which oxygen and nutrients can be released to the tissues and surrounding cells. The total surface area of the capillaries is comparable to that of a normal football court. Capillary rarefaction means loss of capillaries and surface area for exchange of oxygen and nutrients to tissues. Fewer capillaries will also contribute to build up pressure in the vascular system.
- **Intima-media thickness** – The intima-media is a part of the vascular wall. Thickening of the intima-media in arteries – for example to the brain – signals early atherosclerosis and increased risk for stroke in the future.
- **Atheroma** – An atheroma constitutes a local thickening in an artery caused by fatty accumulation and inflammation of the vessel wall. Atheroma precedes the formation of arterial plaques which means that the thickening of the blood vessel inner wall has been calcified. Once plaques are present, the vessel lumen is narrowed and eventually occluded by a clot. Depending on where the occluded

artery is located, it will result in myocardial infarction (heart attack), stroke, or peripheral ischemia, usually in a foot or leg.
- **Cardiovascular disease** – means ischemic heart disease (myocardial infarction or heart attack), stroke or more rare complications to atherosclerosis.
- **Glucose intolerance** – means elevated levels in blood or plasma of glucose but still not fulfilling criteria for diabetes. The limits are different for random blood glucose, blood glucose after an overnight fast or after ingestion of a standard amount of glucose. Glucose intolerance is associated with overweight and obesity and can precede gestational or type 2 diabetes. In turn, diabetes is closely related to accelerated vascular aging and atherosclerosis.

Summary Points

- Middle-aged survivors of the Biafran famine – regarded as one of the great human disasters of modern times – suffer today from hypertension, glucose intolerance, and overweight, predicting increased prevalence of cardiovascular disease and diabetes.
- These findings support that undernutrition early in life irreversibly affect human physiology so that noncommunicable diseases in adulthood – such as heart attacks, stroke, and diabetes – will increase.
- Perinatal contributions to adult disease are most pronounced in countries undergoing a rapid rural-to-urban transition in life-style and diet.
- Prevention of undernutrition during pregnancy and in infancy should therefore receive high priority in global health, education, and economic agendas.

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The Great Irish Famine 1845–1850: Social and Spatial Famine Vulnerabilities

2

Declan Curran

"Several persons, residents of Castlebar, have informed us that while digging potatoes in their fields they encountered an intolerable stench, which, after examination, they found to proceed from the putrid state of the esculents they were in the act of unearthing [. . .] Should this fearful malady spread among the crops of the rural population, dreadful indeed must be the consequences to the poor, whose sole dependence in this country is the potato crop."
[Excerpt from Mayo Telegraph, reprinted in Freeman's Journal, Friday 19 September 1845, p. 4]

Contents

Abstract

This review chapter of the Great Irish Famine (1845–1850) discusses the famine onslaught in terms of uneven "famine vulnerabilities": pre-existing social and spatial disparities that characterized pre-famine Ireland and exacerbated the

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famine hardship experienced by poorer classes in rural areas. More generally, this chapter advocates that episodes of famine be understood in terms of a complex interaction between the immediate catalyst of famine and the pre-existing social and spatial variations that define the local context in which the dire consequences of famine unfold.

Keywords
Pre-famine agriculture · Pre-famine textile industry · Landlords · Tenants · Excess mortality · Potato crop · Potato blight · Emigration · Eviction · Relief policies · Historiography

Introduction

Early reports of the potato blight's arrival on Irish shores in September 1845 provide visceral accounts of a harvest rotting where it had been planted and warn of imminent, inevitable suffering on a national scale. However, as alluded to in the Mayo Telegraph excerpt above, the famine maelstrom into which Ireland was about to be thrust would not afflict all localities to the same extent. Rather it would reveal entrenched famine vulnerabilities which had crystalized in the prevailing socioeconomic and political landscape of the pre-famine decades (Curran 2015). These pre-existing famine vulnerabilities reflected national fissures that were both social – an estimated three million landless laborers and smallholders dependent on the potato as a subsistence foodstuff – and spatial – an established regional geography of deprivation and poverty. This chapter provides an overview of these pre-existing social and spatial famine vulnerabilities and traces their influence on subsequent famine mortality and famine relief efforts.

The immediate devastation wrought by the Great Irish Famine (*An Gorta Mór*) of 1845–1850 has been well documented: with an estimated 1 million famine-related deaths from a population of 8.5 million in 1845 and an emigration outflow in excess of 1 million Irish inhabitants, the Famine onslaught culminated in a 20% population decline over the period 1845–1851 (Boyle and Ó Gráda 1986; Mokyr 1983; Ó Gráda 2012). The longer term imprint of the famine on Irish development has also been well established, characterized as it was by continued population decline and emigration into the early decades of the twentieth century (Vaughan and Fitzpatrick 1978). These demographic trends were accompanied by a transformation of Irish social and economic structures, as landless laborers and smallholders – those who had borne the brunt of famine-era hardship – faced further difficulties in a post-famine agricultural landscape that was shifting from tillage to pasture, undergoing a consolidation of landholdings, and becoming increasingly mechanized (Guinnane 1997; Ó Gráda 2007).

This chapter begins by situating the famine onslaught and aftermath in the context of Ireland in the first half of the nineteenth century. While the famine left

an indelible mark on subsequent Irish socioeconomic development, the famine unfolded in a particular Irish socioeconomic setting that had established itself over the preceding decades.

Pre-Famine Ireland

The late 1700s and early 1800s represent a period of rapid Irish social and economic change, which can be seen as the product of intertwined domestic and international factors. In an international context, a series of technological improvements in textiles and a major geopolitical event, the Napoleonic Wars of 1793–1815, provided a great stimulus to Irish economic development and, through their interaction with existing social and economic structures within Ireland, these developments had far reaching consequences for Irish living standards. The Napoleonic Wars, in particular, brought great benefit to Irish agricultural interests, conferring on Ireland a near monopoly status in British wartime food importation due to blockade of continental trade (Daly 1981). These economic developments were accompanied by an unprecedented population explosion, particularly in Connaught and Munster whose greater proportion of small farmers reaped the benefits of the wartime boom (Donnelly 2002). This period also saw the emergence of textiles as an important cottage industry, supplementing rural farm incomes (Daly 1986). Linen was the most successful of the Irish textile industries and by 1800 linen had developed into a major rural cottage industry, emerging in north east Ulster and spreading across the northern half of the country and into isolated parts of the west. Many landless laborers and smaller tenant farmers earned additional income, either by spinning yarn or weaving coarser fabrics, while many weavers rented a plot of land where they grew potatoes but paid the rent from weaving earnings.

The end of the war in 1815, however, along with the collapse of the domestic textile industry, ushered in an era of economic instability between 1815 and the eve of the famine. The reopening of the British market to grain imports from continental Europe and the demobilization of the British army and navy created a more challenging environment for Irish agriculture. Economic difficulties intensified in 1820, as falling agricultural prices eroded public confidence in the abilities of small private Irish banks to meet their obligations and triggered a wave of bank closures (Cullen 1972; Ó Gráda 1994). Within a two-week period in May–June 1820, seven of the fourteen banks servicing the southern counties of Ireland had folded (Barrow 1975; Collins 1988). Further economic difficulties ensued in 1825–1826, in part as a spillover from financial crisis within the British financial system but also due to deflationary pressures arising from the implementation of the currency union between Ireland and Britain envisaged by the 1800 Act of Union. The economic consequences of the 1800 Act of Union, which abolished the Irish parliament and established a new political unit known as the United Kingdom of Great Britain and Ireland, involved the following steps: abolition of intra-Union trade barriers,

establishment of a common external tariff, consolidation of the exchequers, and the merger of the two currencies. The first two steps were implemented in 1801, the exchequers were consolidated in 1817, and the currencies merged in 1826 (Cullen 1972; Geary and Stark 2002).

Irish agriculture did overcome its post-war slump, as prices of grain, livestock, and dairy began to recover in the 1830s, and export volumes expanded once again (Ó Gráda 1994). However, the domestic textiles industry, which had been the second major area of economic expansion in eighteenth-century Ireland and had been an important source of supplementary income for the rural poor, entered into a terminal decline in the decades prior to the famine. While Belfast's large population of skilled weavers and existing mills enabled the region to establish the region as an early center of mechanized fine linen spinning, the rural cottage textile industry could not compete in the face of large-scale mechanized production (O'Malley 1981).

This decline of Irish industry and fluctuations in Irish agriculture led to a deterioration of the position of the landless poor and small tenant farmers, who accounted for at least half of the population prior to the famine, and left them particularly vulnerable to the famine onslaught when it struck. Landless laborers and smaller tenant farmers were now almost exclusively dependent on farming, and in particular the potato crop, for subsistence.

Pre-Existing Famine Vulnerabilities

The socioeconomic transformations experienced in the pre-famine decades created uneven famine vulnerabilities across the country, along both social and spatial lines, which exacerbated Ireland's susceptibility to famine. These distinct famine vulnerabilities are exemplified by the disparities evident in pre-famine living standards across social groups and regions and, related to this, the uneven economic geography which prevailed in Ireland at this time.

The 1841 census provides an invaluable insight into the socioeconomic variation prevailing within pre-famine Irish society. The 1841 census categorizes respondents as: (i) property owners, and farmers of more than 50 acres; (ii) artisans, and farmers of 5–50 acres; (iii) laborers and smallholders up to 5 acres; and (iv) "means unspecified." For rural districts of the country as a whole, the first two categories accounted for 30% of families. A further 68% of rural families consisted of laborers, small holders with less than five acres, and less prosperous artisans, while 2% of families were unspecified. However, these first two categories of larger landholding combined ranged from 40% to 42% in some eastern counties to below 23% in the western counties of Donegal, Sligo, Leitrim, Roscommon, Mayo, Galway, and Clare. Regional disparities in living standards are also evident from the illiteracy data reported in the 1841 census, which put illiteracy in the 16–25-year age cohort at 27.6% and 29.5% for Ulster and Leinster, respectively, compared to 48.5% and 62.5% for Munster and Connaught, respectively. The proportion of illiteracy for older age cohorts in Munster and Connaught resided within a range of 60–80%

(Mokyr and Ó Gráda 1988). The divergence in living standards between Irish social groups in the half century prior to the famine has also been documented in Mokyr and Ó Gráda's research, which finds that while the urban and middle classes may have experienced some moderate increase in their incomes, the landless poor experienced increasing impoverishment. Though the nutritional content of the potato and widespread access to heating fuel in the form of turf may have ameliorated conditions somewhat for landless laborers and cottiers, the collapse of the cottage textile industry had a devastating impact in many rural areas and led to an increased dependence on the potato crop as a means of subsistence. The analysis of Mokyr and Ó Gráda draws on a number of sources: responses to the Poor Inquiry Commissioners, a body appointed by the British parliament in 1835 which collected responses from 1,590 Catholic and Protestant clergymen on the conditions facing the Irish poor; consumption data of sugar, tea, and tobacco; as well as indicators of human capital formation such as illiteracy and school attendance.

A unique economic geography had established itself in Ireland prior the famine. The regional dispersion of agriculture across the country reflected the fact that commercial farming was prominent on the better and drier land of east and south east, which was also nearer to the British market, while the transport of grain from the midlands was facilitated by a network of canals. The more remote areas of the west, north-west, and south-west suffered from poorer soil quality, a wetter climate, and from greater difficulties in gaining access to export markets due to high transport costs (Cullen 1972; Daly 1986; Mokyr 1983). Concurrent regional and social obstacles to pre-famine agricultural commercialization are also identified by Ó Gráda (1988): the west of the country engaged in less commercial farming due to farm sizes being smallest and dependence on the potato as a subsistence crop being greatest, while smallholders and laborers nationwide engaged in less commercial farming as they consumed the subsistence potato crop produced on their plot of land and paid their rent mostly in labor.

As noted above, the retrenchment of the textile industry from a cottage-based rural dispersion to an industrialized core of north east Ulster increased the dependence of rural areas on agriculture. Underpinning the structure of Irish pre-famine agriculture was the contentious issue of land ownership: agricultural land in pre-famine Ireland largely resided in the hands of several thousand landlords, most of whom were descendants of families granted land either by Cromwell or the British Crown in the seventeenth century. Landlords were typically of Anglo-Irish stock and Protestant religion, though some traditional Irish landlords had survived. Few landlords were actively involved in managing their estates (Daly 1981). Landlords invested little in the maintenance or improvement of their estates, leaving the introduction of more efficient equipment or methods to the stronger tenants (Ó Gráda 1994). Instead, landlords rented their land on long-term leases in order to receive a secure fixed income. These leases were often granted to large tenants, known as middlemen, who then sublet portions of land to numerous smaller tenants (Daly 1981). This role of middlemen as intermediate landlords was all but eradicated during the famine years as their small tenants fell into insurmountable rent arrears (Donnelly 2002).

The labor requirements of tillage farming contributed to the division of landholdings into smaller plots. Commercial famers made agreements with permanent laborers known as *cottiers*, whereby the cottier provided his labor to the farmer for a fixed daily rate. The cottier would also rent a portion of land (*conacre*) from the farmer on a short-term lease for a fixed annual sum payable as days worked for the farmer. On this parcel of land, the cottier could build a cabin and grow the subsistence potato crop. Casual laborers (*spalpeen*) also rented conacre plots, but their employment was less secure than that of cottiers. These casual laborers often received monetary payment, although potatoes, turf, and other provisions were also used as a means of payment (Crotty 1966; Daly 1986). Subdivision of land also took place on smaller noncommercial farms. In some cases, small- and medium-sized farmers supplemented their income by subletting small plots of land to laborers. Subdivision was also undertaken within families in order to provide for a son or act as a dowry for a daughter's marriage. This process of subdivision led to the formation of ever smaller landholdings as families were pushed on to marginal land suitable only for potato cultivation. As Daly notes, by the eve of the Famine, the potato crop sustained an entire socioeconomic system (Daly 1997, p. 39).

The role of the potato in the average Irish diet was far greater than in the rest of Western Europe, with Irish daily potato consumption per capita more than double the Prussian or Netherlands equivalent (Vanhaute et al. 2007). Despite being unsuited to storage or transportation, the potato's high nutritional content, relatively dependable yield even in poor soil during the pre-famine years, and its suitability as a foodstuff for both man and livestock led to an over-dependence on the crop, particularly among the poorer layers of Irish society. It has been estimated that, in the pre-famine years, potato consumption of the average adult male among the laborer, cottier, and smallholder classes was 12–14 lb per day (Bourke 1993). Ó Gráda estimates that on the eve of the famine, Irish laborers, 40% of the Irish population, accounted for over 60% of human annual potato consumption. Cottiers (17% of the population) and small farmers (6% of the population) accounted for 13% and 5% of annual potato consumption, respectively (Ó Gráda 2012, p. 46). By 1845, the potato's share in tilled acreage was little short of one-third and about three million people were largely dependent on it for food (Ó Gráda 1988).

The Famine Onslaught

The immediate cause of the Great Irish famine was the fungus *Phytophthora infestans*, which decimated the Irish potato crop in the harvesting seasons of 1845, 1846, and 1848 (Bourke 1993). The presence of the fungus was detected in Belgium in late June 1845, possibly introduced via potato imports from South America (Neiderhauser 1993). By mid-July, the fungus had spread from Belgium to the Netherlands, and by mid-August it had been detected in France, Germany, and Southern England. By late August, *Phytophthora infestans* had arrived in Ireland, where it was first observed in the Dublin area (Dowley 1997). When the potato blight struck Ireland in Autumn of 1845, it destroyed about one-third of that year's potato

crop and nearly the entire potato crop of 1846. While 1847 marked a respite from potato blight, the 1846 crop failure led to a severe shortage of seed which resulted in the area planted in 1847 being only about 25% of that of the previous year. The potato blight returned with a vengeance in 1848, destroying most of that year's harvest (Dowley 1997).

Notwithstanding difficulties in reconstructing famine-era excess mortality statistics, estimates place the level of excess mortality due to the Irish Famine at one million deaths, nearly one-eighth of the entire population (Mokyr 1983). Indeed, this estimate would be greater were it to include births which did not take place due to the famine, estimated be the region of 300,000, or famine-related deaths abroad (Boyle and Ó Gráda 1986). Outright starvation was not a major cause of death during the famine years (Geary 1997). The vast majority of those who perished as a result of the famine succumbed to dysentery, typhus, typhoid fever, and other hunger-induced infectious diseases. Contagious fevers, such as typhus, were particularly virulent due to a famine-induced breakdown in personal hygiene, overcrowded workhouses, and famine-era migration and vagrancy (Daly 2007).

As the famine's grip tightened on the poorer classes of Irish society, famine-related mortality was distributed very unevenly across the country. The distribution of famine-induced excess mortality across Irish province has been estimated as: Connacht, 40.4%; Munster, 30.3%; Ulster, 20.7%; and Leinster, 8.6% (Donnelly 2002, pp. 176–178). Regional mortality rates also reflect the protracted nature of the famine. The south and west of the country bore the brunt of the Poor Law Amendment in 1847 (discussed below), which placed the full burden of financing poor relief on the Irish rate payer and thereby prompted large-scale evictions by landlords as they sought to lessen their poor rate obligations (Ó Gráda 1994; Donnelly 2002).

Famine conditions also triggered a mass exodus from Irish shores, with over one million people emigrating from Ireland between 1846 and 1851 (Cousens 1960; Boyle and Ó Gráda 1986). Irish emigration was not immediately impacted by the failure of the potato crop in Autumn 1845. It was the second, more widespread, season of blight in 1846 that triggered an immediate large-scale emigration flow in which migrants undertook risky winter transatlantic crossings with little or no food provisions (MacDonagh 1966). The first wave of emigrants were mainly poor cottiers, but were soon followed by smallholders of all types. It is the 1847 transatlantic crossings in particular that have come to be associated with appalling levels of suffering and mortality, the infamous "coffin ships" on which Irish emigrants made their way to ports in America and Canada, and outbreaks of disease at the landing posts (MacDonagh 1966; Daly 1986). While the relatively less severe potato deficiency in Autumn 1847 led to a brief respite in emigration flows, the total failure of the potato crop in 1848 lead to a resumption of emigration at levels previously experienced in 1846 and early 1847. Mortality rates were particularly high on crossings to Canada in 1847. Miller (1985, p. 292; 2008, p. 67) estimates that at least 30,000 emigrants may have died on the Canadian route or in fever hospitals on their arrival. This third season of blight appears to have broken the morale of those cottiers and small farmers who had up to that point held on to their landholdings.

Famine conditions also triggered internal migration within Ireland, though the extent and consequences of this remain under-researched aspects of the famine (Ó Gráda 2007). Cousens characterizes internal migration during the famine as a short-lived influx in to larger urban centers, which was curtailed as relief efforts at a local level were restricted to local residents (Cousens 1960). Donnelly also depicts a rural to urban migration pattern, with large crowds from the countryside congregating in urban areas and at workhouses, food depots, and soup kitchens (Donnelly 2002, pp. 172–173).

Policy Measures Aimed at Famine Relief

Initial publicly funded famine relief initiatives were implemented as temporary measures, funded in part by the British exchequer, with the aim of addressing an exceptional situation. In November 1845, the Tory government of Sir Robert Peel undertook the covert purchase of £100,000 worth of Indian Corn and meal in the United States, with the supplies arriving in Ireland from February to June 1846 (Donnelly 2002). Local relief committees and a network of food depots were set up to distribute the food, though with some delay. As Gray notes, Peel's commitment to free trade and the abolition of the Corn Laws limited the extent to which he was prepared to engage in such pragmatic interventions and by April 1846 Peel had eschewed anything other than marginal interventions in the food market (Gray 2007).

In early 1846, a scheme of public works, mostly involving road improvements, was established in order to provide employment so that the destitute could purchase food. The public works schemes were overseen by either county grand juries, in which case the entire cost of the works was borne by the county, or a local Board of Works, whereby half the funds advanced were to be repaid to the British treasury and half treated as a grant chargeable to the British consolidated fund. The public works schemes quickly came to be regarded by government officials as an unproductive use of public expenditure, a system open to widespread abuse, and a diversion of productive labor away from agriculture (Gray 2007). By March 1847, the public works employed seven hundred thousand people (one-twelfth of the Irish population). However, the public works schemes did not succeed in containing the famine, as they did not target the neediest, paid too low a wage, and exposed the malnourished and poorly clothed to harsh weather conditions (Ó Gráda 2007). Under the Whig government of Sir John Russell, which came to power in July 1846, the public works scheme was replaced in Spring 1847 with a system of soup kitchens. This measure was intended to be temporary, lasting until a revised Poor Law system had been put in place (Donnelly 2002). The soup kitchens were financed by local ratepayers and private subscriptions from local landowners, and at their peak in early 1847 provided meal-based gruel for three million people daily (Ó Gráda 1988). The soup kitchen scheme was brought to an end in September 1847, at a time when mortality rates appeared to wane, food prices had fallen, and demand for seasonal

work was anticipated. However, a further season of complete potato crop failure was to follow in 1848.

The British government's relief policy after September 1847 sought to bring famine relief measures under the auspices of the existing Poor Law system. The rationale here was to shift the burden of financing relief from the British treasury to Irish rate-paying landlords and tenants, with the workhouse system becoming the main thrust of subsequent relief efforts (Daly 1986). The Poor Law Amendment of 1847 allowed for the provision of outdoor relief to anyone unable to work due to age, disability, or ill-health, together with orphans and widows with two or more children (Crossman 2006). However, the able bodied poor could only avail of relief within the workhouse, unless circumstances such as the workhouse being full or infected rendered outdoor relief necessary. One particular provision within the 1847 Poor Law Amendment, the infamous Gregory Clause, prohibited tenants with landholdings of more than one-quarter of an acre from accessing relief. The Gregory Clause triggered a wave of land clearances and evictions, as landlords sought to remove impoverished cottiers from their property (Donnelly 2002). Estimates of the number of evictions over the course of the Famine vary greatly: Vaughan (1984) estimates that over 70,000 families were evicted over the period 1846 and 1853, while analysis undertaken by O'Neill (2000) puts the number of evicted families at 144,759 for the period 1846–1854.

The geography of land clearances yet again illustrates where heaviest burden the famine-era suffering was borne, with the heaviest toll recorded in Connaught and Munster. Indeed, one county in Connaught (Mayo) was the scene of 10.5% (26,000 tenants) of all evictions in Ireland during the years 1849–1854 (Donnelly 2002, pp. 156–157). As a result of the Gregory Clause, many smallholders were forced to give up their land in order to qualify for relief, losing their homes in the process and swelling the numbers dependent on the workhouse system. The numbers seeking relief within the workhouses rose to 932,284 in 1849, with a further 1,210,482 seeking outdoor relief (Daly 1986). The overcrowded workhouse system, with its regime of hard labor and conditions that spread contagious diseases, led to very high mortality rate within workhouses. Ó Gráda (2007, pp. 47–49) estimates that about one-quarter of all famine-induced excess mortality occurred within the workhouse system.

Conclusion

Irrespective of country or time period, all famine episodes are characterized by appalling levels of death and suffering. The Great Irish Famine (1845–1850) inflicted an estimated 1 million famine-related deaths on a pre-famine population of 8.5 million, not to mention the attendant wave of averted births believed to be in the region of 300,000, and triggered an emigration exodus in excess of 1 million Irish inhabitants. Irish population decline and emigration continued into the early decades of the twentieth century. Irish social structures were dramatically altered

by the famine, as landless laborers and smallholders – who had been the major casualties of the famine – struggled to adapt as post-famine agriculture switched from labor-intensive tillage to land-intensive pasture. As outlined in this chapter, episodes of famine should be understood in terms of a complex interaction between the immediate catalyst of famine and the pre-existing social and spatial variations that define the local context in which the dire consequences of famine unfold.

While the catalyst for subsistence, as opposed to man-made, famines may often be unanticipated climatic or environmental events, the human face of famine-era suffering ensures that famines are always inherently political. As such, issues such as inadequacy of government policy initiatives to alleviate famine-era distress have raised the contentious issue of culpability in interpretations of the Great Irish famine. In documenting the historiography of the Irish Famine, Lee (1997) and Donnelly (2002) note that academic scholarship had long been dismissive of the genocidal interpretation embodied by John Mitchell's *The Last Conquest of Ireland (perhaps)*, published in 1861. This divergence in famine narratives is clearly illustrated by the contrast between the two most prominent book length studies of famine to emerge prior to the 1990s: The Great Hunger: Ireland, 1845–1849 Woodham-Smith (1962), which shared many of Mitchell's sentiments, and The Great Famine: Studies in Irish History, 1845–1852 by Edwards and Williams' (1957), a revisionist work which eschewed the traditional nationalist view of the famine.

Since the 1980s, a more nuanced characterization of the Irish famine has emerged which challenges both nationalist and revisionist narratives (Curran et al. 2015). These "post-revisionist" studies have been influenced conceptually by contemporary studies of hunger and poverty and methodologically by the emergence of new statistical and econometric techniques (see for example, the quantitative work of Mokyr (1983) as well as the Ó Gráda (1998) discussion of Amyrta Sen's food entitlement view in the Irish context). One notable contribution to the discussion of famine culpability is that of Peter Gray, who analyses the response of British government and public opinion to the famine, in the context of famine-era debates about the nature and future of Irish society (Gray 1999). Gray, drawing on archival material and the personal correspondence of contemporaries, examines the prevailing ideologies among elite British politicians and civil servants during the famine years. What emerges is a dogmatic distain among British policymakers for publicly funded relief efforts, predicated on ideas of moralism, a providentialist view of the famine, and lassaiz faire economics, which had deadly consequences for those enduring the deteriorating famine conditions. However, even the very concept of *famine culpability* is one that warrants further interrogation. As Noack et al. (2012, p. 12) contend, a distinction should be made between culpability which centers on adequacy of government relief efforts and humaneness of intentions in the face of the crisis, and culpability in which the government is the instigator of the crisis.

Dictionary of Terms

- **An Gorta Mór** – Irish language term for the Great Irish Famine, 1845–1850.
- **Act of Union** – The Act of Union (1800) abolished the Irish parliament and established a new political unit known as the United Kingdom of Great Britain and Ireland.
- **Phytophthora Infestans** – A destructive plant pathogen which caused the potato blight that decimated the Irish potato crop in 1845, 1846, and 1848.
- **Cottier** – A peasant farming a smallholding of not more than half an acre.
- **Conacre** – A system of letting land in small patches or strips, usually for tillage.
- **Spalpeen** – A poor migratory farm worker in Ireland.
- **Gregory clause** – A provision of Poor Law Amendment Act introduced by the British government in 1847 which prohibited anyone holding one-quarter of an acre or more of land from receiving any assistance under the Irish Poor Law; also known as the *Quarter Acre Clause*.

Summary Points

- Great Irish Famine (1845–1850) unfolded in the context of pre-existing social and spatial disparities.
- The end of the Napoleonic Wars (1815) ushered in a period of economic instability that particularly affected those dependent on agriculture for employment and subsistence.
- The decline of the rural cottage linen industry, in the face of increased mechanization, removed a further source of additional income from small tenant farmers.
- Subdivision of landholdings among families into ever smaller portions was common practice, with the high yield of the potato as a subsistence crop partly sustaining this practice.
- As documented in the 1841 census, on the eve of the famine, localities in west and southwest of the country exhibited markedly lower living standards than the rest of the country.
- By 1845 the potato's share in tilled acreage was little short of one-third and about three million people were largely dependent on it for food.
- The famine resulted in an estimated 1 million famine-related deaths from a population of 8.5 million in 1845 and an emigration outflow in excess of 1 million Irish inhabitants over the period 1845–1851. Irish population decline and emigration continued well into the early decades of the twentieth century.
- Post-famine Ireland underwent a transformation of social and economic structures, as landless laborers and smallholders – those who had borne the brunt of famine-era hardship – faced further difficulties in a post-famine agricultural landscape that was shifting from tillage to pasture, undergoing a consolidation of landholdings, and becoming increasingly mechanized.

- Early famine relief efforts included temporary interventions in the grain market and the provision of food via soup kitchens. However, from 1847 onward, Irish famine relief was incorporated into the existing Poor Laws, which meant that burden of financing Irish famine relief was transferred to Irish ratepayers. From 1847, able-bodied poor could only receive relief within the workhouse system.
- Within the 1847 Poor Law Amendment, the Gregory clause has gained particular notoriety: this clause prohibited tenants with landholdings of more than one-quarter of an acre from accessing relief. The Gregory Clause triggered a wave of land clearances and evictions as landlords sought to clear indebted smallholders form their estates.
- In the aftermath of the Irish famine, nationalist narratives emphasized British culpability in exacerbating famine mortality. Subsequent revisionist narratives in turn eschewed this culpability argument. Since the 1980s, a more nuanced characterization of the Irish famine has emerged which challenges both nationalist and revisionist narratives.

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Famine in Ghana and Its Impact

3

Chih Ming Tan and Marc Rockmore

Contents

Abstract

In many developing countries, especially in Sub-Saharan Africa, agriculture plays a central role in economic life. This was particularly true in Ghana during the early 1980s, as the sector employed a little over half of the total labor force and accounted for close to 60% of gross domestic product (GDP; World Development Indicators). In the absence of any irrigation, rainfall levels largely determined agricultural output and therefore the livelihoods of much of the population. While Ghana experienced droughts throughout its history, the 1981–1983 drought stands out for its severity. This chapter examines the origins of the drought and the resultant famine before tracing out its immediate and long-run consequences on a wide range of health outcomes.

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List of Abbreviations

CRS	Catholic Relief Services
DHS	Demographic and Health Survey
FAO	Food and Agricultural Organization of the United Nations
GDP	Gross Domestic Product
GEIES	Ghana Education Impact Evaluation Survey
MCH	Maternal and Child Health
NCHS	National Center for Health Statistics
OCEAN	Openness, Conscientiousness, Extraversion, Agreeableness, and Neuroticism
SSA	Sub-Saharan Africa
WHO	World Health Organization
USAID	United States Agency for International Development

Introduction

Ghana stretches across six acro-ecological zones ranging from the more arid sudan savannah in the north to wetter coastal and guinea savannahs in the south (Antwi-Agyei et al. 2012). Rainfall in Ghana is seasonal with both a major and minor rainy season in the coastal and southern areas but only one rainy season in the North (Ghana Meteorological Agency). Although the average variability in rainfall is relatively moderate (coefficient of variation between 10% and 22.5% Ofori-Sarpong), rainfall totals can shift dramatically across years. For instance, the annual rainfall totals in the capital, Accra, have historically varied between 1,197 mm and 275 mm (Ofori-Sarponh 1986).

Agriculture in Ghana is generally inhibited by the low quality of soil, particularly in the north where the soil quality generally limits crops to maize, sorghum, and millet (Antwi-Agyei et al. 2012; Stryker 1990). Importantly, these crops require relatively high levels of water, particularly during their growth periods. Due to the almost complete lack of irrigation (only 0.2% in the early 1990s FAO 1994), agricultural production in Ghana and, in particular, the dryer north is vulnerable to droughts (Antwi-Agyei et al. 2012; FAO 1994).

The most severe drought in the modern history of Ghana began in 1981. With the exception of Accra and other parts of the South, the total annual rainfall in the country was only 70–90% of the local averages (relative to the 1931–1960 average) depending on the specific area (Ofori-Sarpong 1986. The drought further extended itself geographically in 1982 before encompassing the entire country in 1983. The rainfall levels in 1983 were particularly poor with several areas having less

than half of the normal rainfall totals. The drought was particularly severe in coastal areas where Accra had its second lowest recorded rainfall total (58% of the normal amount) with several other areas receiving their lowest recorded totals (Ofori-Sarponh 1986).

The effects of the drought were compounded by both natural and man-made events. For instance, in 1983, the dry conditions from 3 years of drought along with an extended harmattan (season with dry winds) led to extensive bush fires in the North of the country. USAID reported that up to 35% of total food production was destroyed in certain regions (USAID 194). The domestic food shortage was further aggravated by the expulsion of a large number of Ghanaian workers in 1983. Estimates range from several hundred thousand to close to one million workers. Irrespective of the true total, it was a substantial inflow as compared to the population of 11.5 million (Stryker 1990; USAID 1984; World Bank 2016).

While the food shortage could have (partially) been addressed through food imports, this was not possible as the country was in the middle of severe political and economic turmoil. Following failed coups and violent strikes in the preceding years, a new government had come to power after a New Year's Eve coup d'Etat. At the same time, the macroeconomic context had deteriorated (e.g., deficit spending of 139% of the tax revenue and triple digit inflation) and a failed attempt to borrow from the IMF (Stryker 1990). The fiscal situation was further complicated by the strong decrease in cocoa production, the main export crop and a significant source of government revenue (typically 20–50% of revenue); years of mismanagement along with the drought substantially reduced the production in 1983–1984. This harvest was only 28% of the peak production in 1964–1965 and roughly 40% of the 1972 production (Stryker 1990; Brooks et al. 2007).

The three consecutive years of drought culminated in substantial food shortfalls and domestic price increases in 1983. The tables are based on Stryker's (1990) attempt to create production and prices indices. The shortfalls in 1983 were not only substantial relative to a decade earlier (Table 1: column 1) but also relative to 1982 (Table 1: column 2), the second year of the drought. The production of almost every major crop declined compared to 1972, a normal agricultural year. While the decline was generally less steep relative to 1982, the production of certain crops notably declined between the 2 years. In particular, two of the three main crops in the North (maize and millet but not sorghum) declined by 50% from the already low totals in 1982.

The limited price data which are available suggests that the repeated years of drought and low production substantially increased food prices in 1983 (Table 2). The data show that the inflation in prices for crops outstripped general inflation. Notably, this was even true for the crops whose production increased between 1982 and 1983, such as rice, sorghum and yam (Table 1).

The prolonged drought and unfortunate combination of events greatly affected the nutritional situation. Per capita caloric availability dropped from roughly 95% of the required national total between 1961 and 1975 to only 65% in 1983, the most severe year of the drought (World Bank 1989). Unsurprisingly, per capita caloric consumption decreased from 1,900 to 1,600 between 1982 and 1983 due to a

Table 1 Production of major crops, 1983

	1983 Production relative to	
	1972	1982
Agricultural food production, per capita	64%	94%
Maize	43%	50%
Rice	57%	112%
Sorghum	57%	102%
Millet	37%	48%
Cassava	41%	47%
Yam	128%	147%
Plantain	20%	23%
Groundnuts	78%	173%

Based on Tables 6 and 7 (Stryker 1990)

Table 2 Change in nominal domestic consumer prices

	1982–1983
Inflation (consumer prices)	123%
Maize	360%
Rice	171%
Sorghum	190%
Cassava	254%
Yam	160%

Based on Table 10 (Stryker 1990) and the World Development Indicators

substantial food deficit, estimated at 378,000 tons for just maize (Stryker 1990). In fact, the 1983 daily calorie supply per capita was the lowest reported total for 1983 (World Bank 1986). The food crisis peaked in 1983–1984 and only abated with the return of rains 1984 and the arrival of food aid (Ampaabeng and Tan 2013).

While the data are not available, the poor were likely disproportionately affected. Poverty is primarily rural in Ghana where poor households predominately farm land and/or work in agricultural labor. The decreased agricultural production lowered household food availability and income precisely at the time when food prices were increasing sharply. The effect would have been aggravated by the repeated nature of the shocks. Repeated poor harvests decrease the available buffer stocks of assets which allow households to smooth consumption (i.e., to self-insure) against income and production shocks (and other covariate shocks).

The droughts and the ensuing famine affected the entire country. As noted earlier, the north was particularly vulnerable to droughts and two of three major crops declined by more than 50%. In the south, both food and cocoa (the major cash crop) production were affected. Due to the absence of regional crop and price indices, it is not possible to examine the spatial effect on production or prices. However, it is possible to obtain a sense of the regional distribution by examining the regional variation in the under-five mortality rates from trends which they obtain from DHS data by comparing deaths in 1983 to the 1985–1987 averages by

administrative region. Ampaabeng and Tan (2013) find that the Western and Central regions were the most affected with a greater than 1 standard deviation increase in deaths. With the exception of the central regions of Brong and Eastern, the rest of the country experienced roughly 0.5 standard deviation or greater mortality.

Framework

Recent developments in the Economics literature provide useful frameworks for tracing out the structural effects of initial (e.g., in utero) shocks, such as famine, on subsequent (both short-run and long-run) outcomes. A particularly influential body of work by Nobel Laureate James Heckman and co-authors (Cunha and Heckman 2007; Cunha et al. 2010; Heckman and Mosso 2014) builds on the classic family investment models of Gary Becker and Nigel Tomes (1979). These models emphasize the importance of the role of the family in developing various characteristics of the child across her lifecycle.

Formally, these models focus on the evolution of a set of child's characteristics, $\theta_{i,\,t}$, where i indexes the child and t indicates some stage in her lifecycle (e.g., early childhood, adolescence, adulthood, etc.). These characteristics may include health status, cognitive skills (as measured by IQ), personality traits (as described by the "Big Five," Openness, Conscientiousness, Extraversion, Agreeableness, and Neuroticism, for example), and so forth. The evolution of this set of skills across the child's lifecycle is modeled by a system of difference equations;

$$\theta_{i,t} = f_t\left(\theta_{i,t-1}, I_{i,t}, h_i\right)$$

where h_i denotes a vector of initial conditions for individual i; e.g., genes, initial health status at birth, parental characteristics (e.g., parental IQ, parental education, etc.), etc.; and, $I_{i,t}$ is a vector that is thought of as "investments" in the child at stage t. But, the definition of "investments" can be broad and could include parental investments in schooling and time, the nature of the family environment, any health shocks, etc. In the context of the famine in Ghana, the model therefore allows us to conceptualize the effects on birth outcomes of health shocks to the child experienced in utero (h_i), or, perhaps, a nutritional shock experienced after birth at stage t in the child's lifecycle (one element of $I_{i,t}$). The model is flexible enough to also allow for remedial interventions, for example, by the parents, in response to an in utero shock (another element of $I_{i,t}$). Cunha and Heckman (2007) demonstrate that we can obtain the following via recursive substitution:

$$\theta_{i,t} = m_t\left(I_{i,t}, \ldots, I_{i,1}, h_i\right)$$

That is, the stock of individual characteristics at stage t is determined by initial conditions and the series of investments up to that point.

Cunha and Heckman also introduced a set of useful concepts related to their model into the Economics literature. For example, they define the notion of a *critical*

(or, sensitive) development period. In the context of their model, a critical development period for an investment type j is a time s such that

$$\frac{\partial m_t\left(I_{i,j,t}, \overline{I}_{i,-j,t}, \overline{I}_{i,t-1}, \ldots, \overline{I}_{i,1}, \overline{h}_i\right)}{\partial I_{i,j,t}} > 0, \text{if } t = s \text{ and } \frac{\partial m_t\left(I_{i,j,t}, \overline{I}_{i,-j,t}, \overline{I}_{i,t-1}, \ldots, \overline{I}_{i,1}, \overline{h}_i\right)}{\partial I_{i,j,t}} \approx 0, \text{if } t \neq s$$

The sensitive period idea captures the idea that there may be particular stages in the child's development; e.g., early childhood, where certain investments or shocks (e.g., exposure to famine) may have particularly large effects. A critical period occurs when there is only one such (sensitive) period (Knudsen et al. 2006).

They also introduce the important idea of *dynamic complementarity* in the production of characteristics,

$$\frac{\partial^2 f_t}{\partial \theta_{i,t-1} \partial I_{i,t-1}} > 0$$

That is, the level of characteristics at a particular stage (*t-1*, in this case) affects the marginal returns to investment at a subsequent period. The existence of dynamic complementarities has many important implications. Crucially, it affects the optimal investment decisions of parents when deciding the allocation of resources across their children. As Heckman and Mosso (2014) point out, parental decisions to compensate (or, to reinforce) disadvantages in initial endowments of a child (e.g., as a result of experiencing famine while in utero) depends crucially on the curvature of the production function for characteristics. The stronger the dynamic complementarity between initial characteristics and investments, the more strongly incentivized parents are to actually reinforce initial disadvantages and to focus resources on their more advantaged children instead.

These family investment models generally take family structure as given. These models also ignore another important channel through which family circumstances or initial disadvantages may affect future outcomes; i.e., neighborhood or peer group effects; see, e.g., Brock and Durlauf 2001. However, if we wish to trace out intergenerational effects, we will also need to take into account models of assortative mating, and more generally, models of the marriage market that famine affected individuals will face when they reach mating age; see, e.g., Becker 1973, Durlauf and Seshadri 2003.

Physical and Cognitive Effects

Famine studies are not new in the development economics/health literature. In fact, famine incidence has been thought of as a natural "experiment" in this literature and thereby utilized to identify treatment effects on various short- and long-run

outcomes. This research agenda is deeply related to the fetal origins hypothesis (Barker 1992) that posits that exposure to shocks during critical periods in early development (e.g., in utero) result in long-run negative outcomes to recipients of those shocks.

A seminal example is the work by Stein et al. (1972) on the effects of in utero exposure to the 1944–1945 Dutch famine. In that paper, Stein et al. found no significant impact on the cognitive abilities (IQ) of male survivors at age 19 from in utero or early childhood exposure to the Dutch famine. Subsequent work, however, have found evidence of longer-term effects on outcomes such as obesity (Ravelli et al. 1999), glucose intolerance (Ravelli et al. 1998), self-reported health, coronary heart disease morbidity (Roseboom et al. 2001; Bleker et al. 2005), and psychological disorders (Neugebauer et al. 1999; Brown et al. 2000; Hulshoff et al. 2000).

Another well-studied example is the Great Chinese Famine of 1959–1961. In (rural) areas most severely affected by the famine, survivors of the 1959–1961 birth cohorts experienced significant, negative, short- and long-run effects on a range of outcomes including height/stunting (Chen and Zhou 2007; Meng and Qian 2009), obesity (Luo et al. 2006), disability incidence (Mu and Zhang 2011), mental illness (Huang et al. 2012), cognitive abilities (Tan et al. 2014) educational attainment (Meng and Qian 2009), labor market outcomes (Almond et al. 2010; Meng and Qian 2009), wealth (Almond et al. 2010), and marriage market outcomes (Almond et al. 2010; Brandt et al. 2008).

Despite the importance of famine in Africa and the increasing concentration of famines in Sub-Saharan Africa (SSA) (Devereux 2009; Ó Gráda 2007), there is relatively little research on the physical or cognitive effects of early life exposure to famines on surviving children for Sub-Saharan Africa. The most widely studied famine in SSA is the 1983–1985 Ethiopian famine where an estimated 400,000 to 1 million people died (Devereux 2000; Kidane 1990; de Waal 1991). However, we are only aware of three papers studying the health effects for survivors (Asfaw 2016; Dercon and Porter 2014; Tafere 2016). More broadly, there are only three other papers on SSA, one each for Ghana (Ampaabeng and Tan 2013), Malawi (Hartwig and Grimm 2012), and Uganda (Umana-Aponte 2011). Interestingly, neither the research in Malawi nor in Uganda finds any adverse effects on height (although they do find adverse effects on other outcomes). It is, however, possible to draw on a more developed literature on food insecurity (see for instance, Alderman et al. 2006) or even on non-food shocks, such as income shocks (see for instance, Adhvaryu et al. 2017), to predict the effects of famine exposure on health outcomes. We will explore some of these effects below.

As noted above, the literature that directly studies the impact of early exposure to the famine in Ghana is surprisingly sparse. To date, there appears to be only one such work; i.e., Ampaabeng and Tan (2013). In that work, the authors focused on cognitive outcomes as measured by an IQ test (Raven's Progressive Matrices). Specifically, they were interested in whether children who experienced famine during early childhood (aged 0–2 years during the famine) as opposed to later childhood (aged 3–8 years during the famine) did worse on the IQ test administered in 2003. Hence, they were primarily interested in the longer-term impact of the

famine on cognitive development. The authors were also interested in how the effects of early famine exposure on IQ could be quantified in terms of performance on a set of Math and English comprehension tests, also administered in 2003.

The data employed came from the GEIES. Only two waves of that data were available at the time of writing – in 2003 and in 1988/1989. The authors used the 2003 wave for data on their outcome variables. Because GEIES had data on school quality – e.g., the state of classrooms and the availability of textbooks, and the IQ of teachers – they were able to control for the schooling experience of the individuals in their sample all of whom would have been in primary (elementary) school during the 1988/1989 wave. This was an important thing to do since school quality variations during the formative years of a child may potentially (partially) remediate any negative impacts on cognitive development from famine exposure and was therefore one of the distinguishing aspects of this paper. The authors were also able to control for individual, family, and community characteristics such as respondent's age, height-for-age, gender, household size, parental schooling, rural or urban status, etc. In terms of the treatment variable, the authors employed under-5 mortality deviation from trend during the famine years calculated using data from the 1988 DHS. They also used rainfall deviation from trend during the famine years obtained using data from the World Bank's Africa Rainfall and Temperature Evaluation System as an instrument for their treatment variable.

The main finding by Ampaabeng and Tan is that early childhood exposure to the famine in Ghana did result in substantial negative consequences in terms of cognitive development for survivors. According to their benchmark specification, exposure during early childhood to a 1 standard deviation increase in famine severity led to an expected loss of 1.29 IQ points. For perspective, in terms of achievement on the Math and English comprehension tests, "the effect of such a loss on cognitive achievement test scores translates on average to a corresponding loss of around one half of a year (two-fifths in many cases) of schooling with the larger effects applying to the Math tests (Ampaabeng and Tan 2013, 1025)." Following calculations analogous to those in Maccini and Yang (2009), the authors find that such a loss translated into a corresponding reduction of 0.4% in 1997 GDP (as measured in 2000 dollars).

We next examine the effects of the famine on anthropometric measures of child development. Consistent with theory and evidence from other settings, the anthropometric data show a sharp decrease in nutritional intake for infants before a nutritional recovery. For instance, data from Maternal and Child Health (MCH) Clinics collected by Catholic Relief Services (CRS) measures weight-for-age across time. These data likely suffer from some selection bias as not all children attend MCH clinics in general or those affiliated with CRS in particular. However, these data are still suggestive as to the magnitude of the shock and its temporal nature. For instance, in the last "normal" year of agricultural production, only 35% of children fell below the 80th percentile in the NCHS/WHO standards for weight-for-age. By 1983, it had surged to 80% before returning to pre-drought and pre-famine levels (35%) in 1986. In the space of only 3 years, the number of underweight children more than doubled before returning back to normal within 3 years.

Table 3 Short health outcomes for children

Panel A	**<80% of NCHS weight-for-age**	
1986		
Aged 0–5 months	15.3%	
Aged 6–11 months	32.2%	
Aged 12–23 months	69.9%	
Aged 24–35 months	71.8%	
Aged 36–47 months	62.4%	
Aged 48–60 months	64.8%	
Panel B		
1987–1988	***Stunted (%)***	***Wasted (%)***
Boys		
Aged 12–24 months	33.3	18.8
Aged 24–60 months	39.5	4.7
Girls		
Aged 12–24 months	31.7	14
Aged 24–60 months	39.1	5

Panel A is drawn from World Bank (1989): Appendix Table 9. Panel B is drawn from Alderman (1990): Table 2

The initial effects are visible in a pair of surveys immediately following the drought (Table 3). First, a national nutritional survey in 1986 reported the proportion of children in Ghana under the 80th percentile in the NCHS/WHO reference (Table 3: Panel A). The World Bank report (1989) divides the children into six groups: the oldest three groups were affected by the drought (24–35, 36–47,48–60 months), the youngest two were not affected at all (0–5 and 6–11 months), and the last group (12–23 months) was not directly affected by the drought but potentially experienced some of its effects via food or consumption shortfalls in families affected by 3 years of droughts. The data show a clear pattern: children in the cohorts affected by the drought or immediately following it were substantially more likely to be below the 80th percentile for weight-for-age. However, children who were conceived and born after the drought were substantially healthier.

While weight-for-age provides an indication of child health, it reflects both short-run and long-run malnutrition. Short-run nutrition is typically measured by data on weight-for-height while long-run nutrition is indicated by height-for-age. These indicators are available in the 1987–1988 Ghana Living Standard Survey (GLSS 1) (Table 3: Panel B). The first column in Panel B measure the percent of children under the age of 5 who were stunted (i.e., who have abnormally low height-for-age). The older age group contains a combination of children exposed and not exposed to the drought while the younger age group was exposed (in utero or after birth) to the drought. In both boys and girls, the exposed cohort has a more than 6% point higher rate of stunting, roughly an 18–20% higher rate. Interestingly, the rate of wasting, a measure of low weight-for-height, shows the opposite effect: younger children suffer from worse short-run nutrition. The

Table 4 Height of women by age group in 2003 (Aged 19–49)

	Height (cm)	< 2 Std Dev %
1. Affected by famine (born 1979–1984)	158.4	13.1
In utero during famine (1981–1983)	157.8	16.7
Born during famine (1982–1984)	158.0	14.1
Aged 1–2 during famine (1979–1980)	158.8	10.3
2. Affected in peak year of famine (1983) (born 1981–1984)	158.2	25.4
In utero peak famine (1983)	158.9	16.2
Born peak famine (1984)	157.3	18.2
1–2 peak famine (1981–1982)	158.6	36.0
3. Women not born between 1979 and 1984 (born after 1984)	159.2	9.8

Authors' calculations based on the 2003 Demographic and Health Survey

difference between the stunting and wasting rates suggests that the higher stunting in the older age group is due to nutritional deficiencies during the drought as opposed to in later years.

Although the deterioration of nutrition was limited to several years, the effects were permanent as exposed cohorts display important physical and cognitive (as discussed earlier) deficiencies even years later. The physical consequences, already apparent as children, are clearly visible in the sample of adult women in the 2003 DHS. Table 4 presents information on adult height for a nationally representative sample of woman aged 19–49. The women are divided into three broad categories: (1) women who were conceived, born, or aged 2 or younger during the drought (1991–1983); (2) women who were conceived, born, or aged 2 or younger in the peak year of the famine, 1983; (3) all women who were not conceived, born, or aged 2 or younger during the drought. Note that the second group is a subset of the first group. Within the first two groups, the women are further divided into whether they were conceived, born, or born prior to the (peak year of) famine.

The first column reflects the average height (in cm) for each age group. The average height of women who were not affected by the drought is always higher with height "deficits" ranging from 0.3 to 1.9 cm. To put these numbers in perspective, the estimated deficits of adults who were exposed to the 1983–1985 Ethiopian Famine, which is widely recognized as one of the worst in Africa, range from roughly 0.43–5 cm (Dercon and Porter 2014; Tafere 2016). Beyond their intrinsic importance, these deficits are linked via lower earnings to lower adult economic outcomes (Case and Paxon 2008; Currie 2009). The second column reflects the percent of women who are 2 standard deviations below the median of the reference group, a similar measure to stunting. While there are important differences between groups 1 and 3, the outcomes from group 2 are particularly notable. Overall, women in group 2 are more than twice as likely to be abnormally short with those aged between 1 and 2 in 1983 almost three times more likely.

Policies and Protocols

An extensive literature links early life nutrition to adult health outcomes, from adult stature to the incidence of diseases to longevity (see Ó Gráda 2007 for a recent review). In this view, famines represent the extreme tail of a continuum of deprivation. Consequently, as with other less severe forms of undernutrition, early nutritional interventions may be particularly important. The importance of such investments is magnified by the existence of sensitive or, especially, critical periods in child development. This suggests the importance of food transfers targeted towards pregnant and nursing women as well as infants.

More broadly, two factors generally distinguish famines from less severe forms of food insecurity. First, since countries are typically able to withstand exposure to individual adverse events, such as extreme weather and associated crop failure, the more severe impacts from famines are often associated with repeated shocks (de Waal 1997; Ó Gráda 2007). It is the repetition of such shortfalls that overwhelms countries. The emerging literature on the effects of climate change on temperature extremes and the increased frequency of adverse tail events (Dell et al. 2014) suggests that repeated weather shocks may be a "new normal" that the least prepared countries may nevertheless have to face.

This ability to withstand individual shocks provides a short window during which national and international resources can be mobilized before local conditions may become aggravated by a second year of shortfalls. The inability of state actors to properly address the effects of such shocks once they have occurred is often amplified by the existing deficiencies of markets, state institutions, and production technologies. Consequently, it is important to consider the broader development framework in addressing concerns over policy (both short-term and long-term). Are food markets segmented or well-connected so that food moves to areas in need? Are government and local institutions investing in irrigation so as to lessen the reliance of agriculture on rainfall?

Similarly, households are often able to cope with one-off shocks but are very vulnerable to repeated shocks within a short period. Since developing countries are typically characterized by incomplete (or missing) formal and informal insurance markets, households frequently rely on their own asset reserves to smooth out their consumption. That is, by consuming or selling their assets, they are able to replace some of the lost income. Repeated shocks reduce or exhaust the ability of households to deal with shocks and therefore lead to sharp swings in consumption with important consequences, including the health of children. These effects are magnified when income directly depends on asset stocks (Zimmerman and Carter 2003). This suggests that particularly attention should be paid to poorer households with low asset holdings and areas with limited access to formal or informal insurance.

Second, over time, the causes of famines have somewhat shifted away from weather-created food shortages and have increasingly involved conflicts (Ó Gráda 2007). Conflict can affect food availability through a variety of channels such as when food and its availability becomes a "weapon." More recently,

research suggests that household responses to the threat of violence can shift agricultural production practices and lower dietary diversity (Dabalen and Paul 2014; Rockmore 2012). In part, this may explain the observed sharp increases in stunting in cohorts exposed to conflict in early life (Akresh et al. 2011, 2012; Minoiu and Shemyakina 2014). This underlines the importance of providing food aid in areas afflicted with violence and the threat of violence (Rockmore 2016).

Dictionary of Terms

- **Covariate shocks** – Covariate shocks which affect a large proportion of households in geographical area (such as drought, floods, or price increases). This contrasts with idiosyncratic shocks, such as sickness of a household member, which affects only the household.
- **Standard deviation** – The standard deviation is measure of the distance of a data point from the mean of a set of data points. Formally, it is defined as $\sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \bar{x})^2}$ where $\bar{x}$ is the sample mean.
- **Stunting** – Stunting is defined as being 2 standard deviations below the median height-for-age for the reference child growth standard. This is typically viewed as a measure of long-run malnutrition.
- **Wasting** – Wasting is defined as being 2 standard deviations below the median weight-for-height for the reference child growth standard. This is typically viewed as a measure of short-run malnutrition.

Summary Points

- Ghana experienced its worst drought in modern history from 1981 to 1983.
- The drought peaked in 1983 and was compounded by substantial bush fires and the expulsion of large number of Ghanaians from Nigeria in the same year.
- Food production dropped dramatically in 1983 leading to widespread food shortages.
- Effects of famine in Ghana (and in Sub-Saharan Africa) are relatively unstudied.
- Early life exposure to the famine resulted in the loss of IQ and worse performance on Math and English comprehensive exams.
- The aggregate costs of these cognitive losses are 0.4% of the 1997 GDP.
- There are important physical consequences of early life exposure to the famine. In the short-run, it is associated with higher stunting rates. In the long-run, it is associated with 2–3 times higher probability of women being of short stature.

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The Greek Famine of 1941–1942 and Its Impact

4

Sven Neelsen and Thomas Stratmann

Contents

Abstract

The Greek Famine of 1941–1942 provides a natural experiment to test the fetal origins hypothesis. This hypothesis states that exposure to detrimental conditions during the fetal stage leads to worse health and socioeconomic outcomes in adulthood. This chapter first describes the Greek famine's causes. It then reviews the impact of the Greek famine on the education and labor market outcomes of the individuals exposed to the famine in utero or in early childhood. Corroborating Barker's hypothesis, the evidence indicates that the Greek famine significantly reduced educational attainment for those who experienced it before their third year of life. The famine also reduced labor market success for those with famine

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exposure in their early childhood. This finding is partly driven by a shift towards rural birthplaces in the famine years. The sum of the findings underscores the importance of averting early childhood malnourishment.

Keywords
Greece · World War II · Famine · Malnourishment · Undernutrition · Undernourishment · Barker's hypothesis · Fetal origins hypothesis · Early childhood development · Education · Occupational status · ISEI · Natural experiment

List of Abbreviations
ISEI International Socio-Economic Index

Introduction

According to *The State of Food Insecurity in the World 2015* (FAO 2015), there are approximately 795 million people around the world who suffer from hunger, most of whom come from rural areas of developing and middle-income nations. While hunger is a problem in and of itself, a growing body of evidence indicates it also has adverse long-term consequences for health, education, and labor-market outcomes, and that this may especially be the case for those who are deprived of adequate nutrition early in life.

In 1986 epidemiologist David Barker conducted a study that found a relationship between prenatal malnutrition and coronary heart disease later in life (Barker and Osmond 1986). This led to the fetal-origins hypothesis, which proposes a causal relationship between nutrition received in utero and later health outcomes. One mechanism posited to drive these long-term effects is the development of *thrifty phenotypes* (Hales and Barker 1992; Barker and Hanson 2004). Here, the fetus develops permanent adaptations to the low-nutrient environment experienced in utero and these adaptations produce long-term adverse health consequences for the individual.

Many of the early studies following Barker's findings suffered from methodological problems that made it difficult to ascertain a causal relationship between prenatal malnutrition and later-life health outcomes. For instance, due to a lack of data on prenatal nutrition, some of these early studies used birth weight as a proxy (e.g., Launer et al. 1993; McCance et al. 1994; Barker 1995). Body weight at birth may, however, vary for many reasons other than prenatal nutrition, including genetic endowment, gestational age, and maternal behavior (Rasmussen 2001; Paneth and Susser 1995). If these factors are unaccounted for, associations between birth weight and later-life health outcomes will yield limited information on the possible impact of prenatal malnutrition.

Even if accurate data on prenatal nutritional status are available, the problem that malnutrition is endogenous to other determinants of later-life socioeconomic status

and health remains. For instance, having better-off parents likely improves later-life outcomes not only by reducing prenatal exposure to malnutrition but also through other factors such as access to better education. Therefore, comparisons of individuals with different degrees of prenatal malnutrition that do not control for confounders such as parental education and wealth will yield only biased results.

A newer strand of the fetal-origins literature therefore moved away from individual-level comparisons. Instead, famines were used as natural experiments. This approach attempts to sidestep the biases of individual-level comparisons by comparing, on the one hand, the outcomes of entire cohorts that were in the fetal stage or infancy when a famine struck, and, on the other hand, the outcomes of the surrounding cohorts without famine exposure at crucial developmental stages. Assuming the exposed and unexposed cohorts would have had the same outcomes in the absence of the famine, the approach views famine as an exogenous shock that randomly assigns cohorts to a nutrient-deprived treatment group and a nondeprived control group.

One extensively studied event in this literature is the Chinese famine that occurred during the *Great Leap Forward* period of 1959–1961. Approximately 30 million people died in its course (Yao 1999). Studies have found, for example, that individuals who experienced the famine in utero were more likely to develop adult-onset schizophrenia (St. Clair et al. 2005) and had worse literacy, labor market status, wealth, and marriage market outcomes (Almond et al. 2007). Other famines studied include the German siege of Leningrad, which took place from 1941 to 1944 (Stanner et al. 1997; Stanner and Yudkin 2001), and the Finnish famine of 1866–1868 (Kannisto et al. 1997). According to the studies neither famine caused long-lasting negative health effects.

However, the extended length of these famines as well as their severity – the Finnish famine, for instance, killed around 8% of the Finnish population – complicates attempts to infer causality. For instance, with famines lasting multiple years, cohorts exposed in utero typically also experienced malnutrition as young children. This makes it difficult to disentangle at what stage of development undernourishment matters most. Furthermore, the length and severity of the famine likely gives rise to substantive selection in reproductive behavior and survival in the cohorts exposed at early age (Song 2009). In other words, if people experiencing unobserved factors conducive to good socioeconomic and later-life health outcomes reproduce more than others during a famine, and if the least healthy children in the early exposed cohorts die, the estimated impact of famine from cross-cohort comparisons will be biased downward.

Another famine that has received significant attention is the Dutch famine, occurring from 1944 to 1945 as the result of the Nazi blockade of the Western Netherlands. This famine is better suited to studying the effects of in utero and early childhood malnutrition because it was relatively short and mild. Studies analyzing this famine find that in utero exposure was associated with impaired nervous systems and coronary heart disease (Roseboom et al. 1999, 2000a, b, 2001), antisocial personality disorder (Neugebauer et al. 1999), and glucose resistance and obesity (Ravelli et al. 1998, 1999). Less is known about the effects of the Dutch famine on socioeconomic outcomes. However, a recent study finds not only increased

hospitalization rates in the years before retirement for people who suffered middle or late gestational exposure, but also that those exposed during the first trimester of gestation were less likely to be employed at age 53 (Scholte et al. 2015).

This chapter reviews the evidence on long-run effects of the Greek famine of 1941–1942. Like the Dutch famine, the Greek famine was relatively short and mild, reducing the likelihood that selective mortality and reproductive behavior during the famine biases impact estimates. Moreover, in Greece, educational and labor-market outcomes for individuals with early childhood exposure have been observed at various points in their adulthood, so that famine effects could be tested over almost the entire duration of working lives.

Causes and Course of the Greek Famine

In early 1941, Greece came under attack by Italian and Bulgarian troops allied with Nazi Germany, in a push of the Axis powers to assert authority over South-Eastern Europe. With support from the British Army, Greek forces were initially able to halt the invasion, but on April 30, just 24 days after German troops joined the Italian-Bulgarian invaders, Greece was forced to surrender (Hionidou 2006; Mazower 1993).

The British responded to the Axis powers' occupation of Greece with a full naval blockade, which shut down all external trade. This represented a massive shock to a country which traditionally relied on foods imports – for instance, during the late 1930s, Greece imported 45% of its wheat supply (Hionidou 2006).

Actions by the Axis powers contributed to a rapid deterioration of the nutritional situation as well. The occupying armies requisitioned large amounts of foods for their own consumption. By the end of 1941, official records showed that 4,000 tons of figs, 181,000 tons of raisins, and 10,000 tons of olive oil had been confiscated (Helger 1949; Hionidou 2006). Further, they imposed on farmers a 10% in-kind tax on all agricultural production. Restrictions on internal trade and mobility proved even more harmful than the food requisitioning. The occupiers divided Greece into 13 economic zones, between which the movement of goods and people was severely restricted. To enforce the restrictions, all fuels and transport vehicles including fishing boats, pack animals, and carts were confiscated.

In an attempt to control food prices in light of diminishing supply, the occupying forces mandated farmers to sell their products well below market rates and tightened existing rationing policies. This, however, discouraged the production and sale of foods. The shortages were amplified and prices on the emerging black markets soared. Soon, the nutritional situation of the poor population in particular deteriorated, and by fall of 1941, the shortages had turned into a full-blown famine in most of the country, affecting not only the destitute, but also large parts of the urban middle-class (Hionidou 2006). Mortality rose sharply (see Fig. 1), with disproportionate increases among the youngest and elderly, as well as for male adults who had limited access to food from charitable organizations and often forwent meals in favor of their children and wives (Helger 1949). By the end of the winter of 1941–1942, the famine had caused 100,000–200,000 deaths (Hionidou 2006).

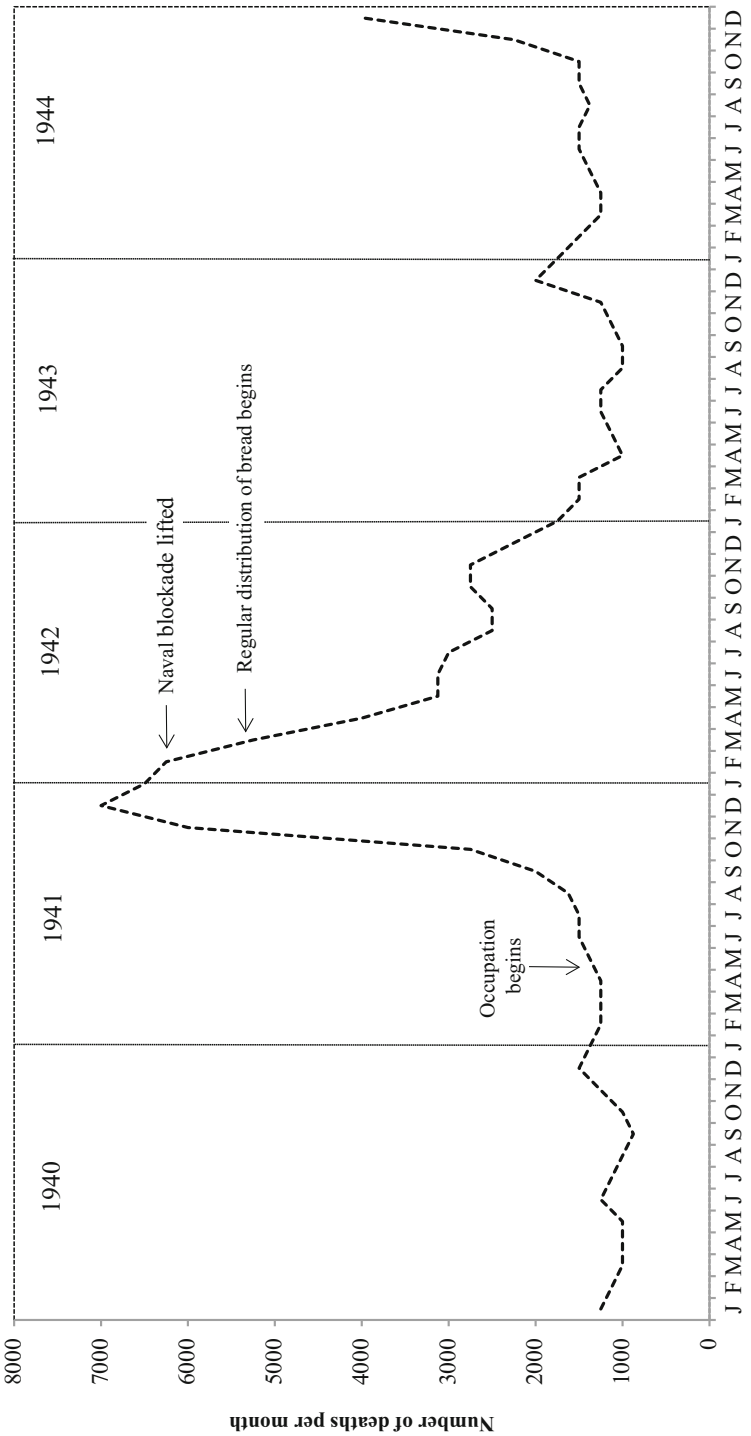

Fig. 1 **Numbers of deaths in Athens and Piraeus 1940–1944, by month**. Number of deaths in Athens and Piraeus from administrative records, showing sharp increase in mortality in the fall of 1941 shortly after the occupation began and subsequent sharp decline in the spring of 1942. Figure based on Valaoras (1946)

With large parts of the Greek population facing starvation, civil unrest increased and the occupiers grew increasingly concerned about widespread destabilization of their regime. Disagreement between Italy and Germany about who bore responsibility for food provisions for Greece, however, stalled relief efforts. The two countries eventually reached a compromise at the end of 1941, but Germany subsequently failed to meet its commitments, overstretched by attempts to control similar nutritional crises in occupied Belgium, Holland, and Norway. Ultimately, full responsibility for food provisions to Greece fell upon the Italians – a task far beyond their capacity at the time (Mazower 1993).

The Axis powers publically shifted the blame for the famine to the British naval blockade, demanding Britain to lift its blockade in order to end the suffering of the Greek population. The British initially refused. However, news of the famine leaked out of the country and media coverage received international attention. Public pressure in the Allied countries to allow international humanitarian aid to enter Greece rose. In early February 1942, the British eventually lifted the blockade and soon after, wheat shipments began. By March 1942, the Axis and Allied powers agreed to establish the Swedish-run Joint Relief Commission, which was put in charge of reorganizing food distribution in Greece. Furthermore, the Axis-power occupiers relaxed regulations on internal trade and prices, and committed to replacing agricultural products appropriated from Greek farmers with food of equal caloric value. The combined actions of the Axis and Allied powers along with the rising temperatures of spring 1942 rapidly improved food availability. By June 1942, the nutritional problems in most parts of the country had returned to acceptable levels, leading to sharp declines in mortality (Fig. 1) (Helger 1949; Hionidou 2006).

Long-Run Socioeconomic Impacts

The Greek famine's immediate effects on the health of the Greek population were devastating – beyond its estimated death toll of up to 200,000 victims, contemporary data indicate that the famine not only dramatically increased the rate of underweight children, but also led to severe, short-term growth retardation (Valaoras 1946).

Neelsen and Stratmann (2011, 2013) investigate the possibility of long-term adverse famine effects. Specifically, they examine whether exposure to the Greek famine during crucial, early life developmental periods had negative consequences on the individual's socioeconomic achievements during adulthood.

To study this question, they use data from the 1971, 1981, 1991, and 2001 decennial Greek National Population Housing Census. Their samples consist of individuals from these censuses born between 1936 and 1946 – during and shortly before and after the famine. Thus, the 1971 census sample consists of individuals aged between 25 and 36, and the 1981 census consists of individuals aged between 35 and 46, and so forth.

The medical literature suggests that the most severe long-term effects arise when malnutrition is experienced between conception and age two (Brenner and Chartow 1994; Hales 1997; Hoet and Hanson 1999; Walker et al. 2007; Victora et al. 2008).

Motivated by this literature, Neelsen and Stratmann define individuals exposed to the famine at these ages as the treatment group. The treatment group cohorts are therefore those born in 1940, 1941, and 1942, as they would have been in utero or younger than 3 years during the 1941–1942 famine. The sample cohorts without direct famine exposure – those born in 1943 or later – and those with famine exposure after the critical early life stages – individuals born in 1939 or earlier who in the majority were exposed after their second birthday – form the control group.

Neelsen and Stratmann study the effects of the Greek famine on educational attainment, and for the working subsample, job prestige. For education, impact estimates are shown for a dummy variable indicating whether an individual is literate; a dummy variable indicating whether the individual completed upper secondary school, which is the equivalent of obtaining a US high school diploma; and a variable reporting the number of years of schooling an individual has completed at the time of the census. The International Socio-Economic Index (ISEI) is used to measure job prestige (Ganzeboom and Treiman 1996; Ganzeboom et al. 1992). The ISEI ranks census occupational groups on a scale of 16–90 according to the level of education required to enter the occupation and the income associated with it. A higher rank indicates higher job prestige.

Figure 2 shows the percentage of individuals who completed upper secondary education among those born in each year from 1936 to 1946, as well as the linear trend in upper secondary school completion over this period. There is a clear upward trend in completion rates over time. However, for the 1940–1942 treatment group cohorts, completion rates are below the long-run trend, providing indication that early life exposure to the famine may indeed have harmed educational achievement.

To address potential confounders in bivariate associations of early life famine exposure and adulthood socioeconomic outcomes such as shown in Fig. 2, Neelsen and Stratmann estimate multivariate ordinary least squares regression models. Their basic specification regresses the outcome of interest on three dummy treatment variables indicating 1940, 1941, and 1942 birth cohort membership, as well as on controls for the year of birth and the year of birth squared to account for overall time-trends in outcomes, and a gender-dummy to account for outcome changes related to shifts in cohort gender composition.

The regression analysis reveals the causal effects of early life famine exposure under the assumption that the cohorts with early life famine exposure would have followed the same outcome trend as the surrounding cohorts in the absence of the famine. If the members of the famine-exposed cohort differed in unobserved physical or social endowments from other cohorts, then the estimates resulting from this regression would be biased and not capture the true causal effect of early life undernourishment. For instance, if the famine-exposed cohorts of 1940, 1941, and 1942 had better adulthood outcomes because of some form of positive selection into reproduction and survival during the famine, the estimated effects on educational attainment and job prestige would be biased downward. In this case, the impact estimates form lower bounds of any negative long-term effects of early life malnutrition.

Neelsen and Stratmann obtain the following pattern of results from their basic regression model. For the cohort born in 1940 that was in the majority exposed

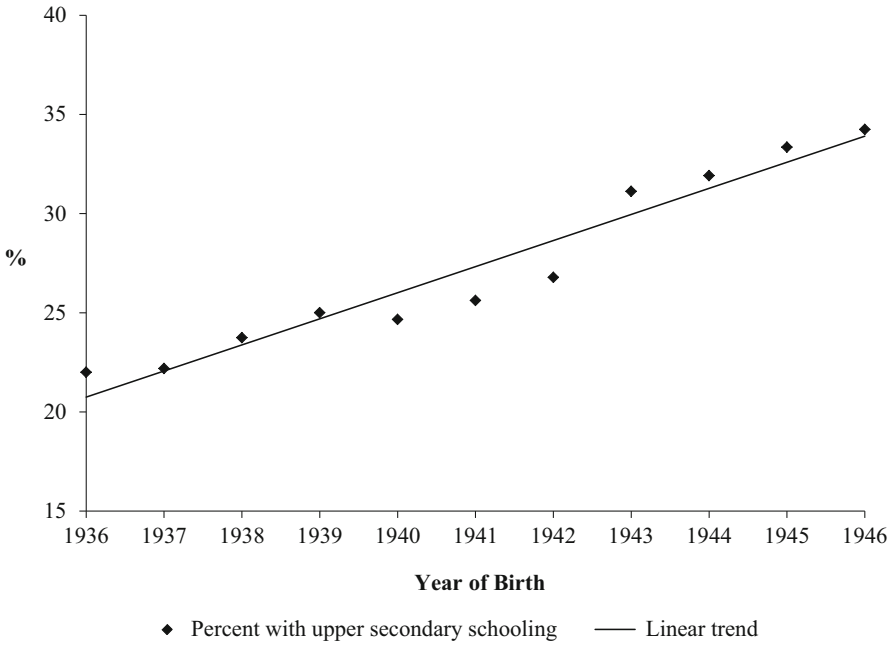

Fig. 2 Percentage of 1936–1946 Greece-born individuals in 2001 Greek census who have completed upper secondary schooling (with linear trend). Upper secondary schooling completion rates for the 1940–1942 cohorts who were exposed to the Greek famine during critical early life stages are below the overall trend

between their first and second birthday, the famine is estimated to have lowered literacy rates by about half a percentage point in the 1981 and 1991 censuses, though no reductions are found in the 1971 or 2001 census. The estimated reductions are small in relative magnitude, with mean sample literacy rates of over 95%.

Impacts are more meaningful and consistent across census waves for completion of upper secondary school and years of education. Famine effects on the 1940 cohort are negative and significant across all waves, and range between reductions of 1.3–1.8 percentage points in the probability to finish upper secondary education – large relative impacts given a sample mean completion rate of around 25% – and between 0.14–0.18 fewer years of education with a sample mean of about 7.5 years. In the 1971 and 1981 censuses, the 1940 cohort was also significantly more likely to rank lower on the ISEI job prestige score with estimated reductions of 0.5 and 0.7 points against a sample mean of about 36 points.

The group of people born in 1941, who experienced the famine during the first 12 months after birth – as infants – tend to have larger negative treatment effects than the 1940 cohort of people who were exposed at older age. With the exception of its ISEI score in the 2001 wave, the 1941 cohort experiences significant reductions across all socioeconomic outcomes. This cohort had a 0.5–1.0 percentage point reduction in literacy rates, had between a 1.8 and 2.4 percentage point less of a

chance to complete upper secondary education, had completed 0.17–0.29 fewer years of education, and had 0.5–0.8 points lower ISEI scores.

For the cohort born in 1942 that was in its majority exposed in utero, Neelsen and Stratmann do not find statistically significant reductions in literacy rates across any of the four censuses. The 1942 cohort is, however, from 2.1 to 2.6 percentage points less likely to finish upper secondary education, a similar point estimate as that for the 1941 cohort. Additionally, individuals in the 1942 cohort finished from 0.15 to 0.18 fewer years of education and scored from 0.47 to 1.1 points lower on the ISEI scale, indicating adverse effects similar in size to the effects for the 1941 cohort, which was exposed to the famine during infancy.

As discussed above, positively selective reproduction and survival among the cohorts with early life famine exposure would lead to downward bias in Neelsen and Stratmann's impact estimates. However, a factor that may cause upward bias – an overestimation of adverse effects – in their basic model estimates is that the famine was more severe in urban areas (Helger 1949; Hionidou 2006). Due to larger famine-related reductions in fertility and larger increases in early life mortality in urban areas, rural-born individuals take up a higher share of the 1940–1942 treatment group cohorts than in the surrounding control group cohorts. Thus, if rural-born individuals are less endowed with characteristics that improve their later-life socioeconomic outcomes – for instance, through having less access to higher-level education – the above results may reflect a famine-related increase in the share of those born in rural areas rather than the direct impact of early life nutrient deprivation.

Neelsen and Stratmann use birthplace information in the 2001 census to address this possible source of bias. When they add prefecture of birth dummy variables interacted with an urban birthplace indicator to their basic specification, the negative treatment effect estimates generally reduce in magnitude, and in some cases, statistical significance is lost. Among the 1940 cohort, the negative impact estimates on secondary school completion rates (see Fig. 3) and years of education diminish but remain statistically significant, and for the 1941 cohort with exposure as infants, statistical significance is maintained for all educational outcomes but not for job prestige. For the 1942 cohort the authors no longer find statistically significant famine impacts on any outcome after accounting for famine-related shifts in birthplaces. It should be noted, however, that the absence of significant negative long-term impact estimates in the in utero-exposed cohort does not amount to evidence against Barker's hypothesis. Instead, positively selective survival and, in particular, reproduction are likely greatest for this cohort that was conceived during the famine and experienced it during the earliest, most vulnerable life-stages (Hionidou 2006). With individuals with favorable socioeconomic backgrounds likely overrepresented in the in utero-exposed 1942 cohort, any downward bias in Neelsen and Stratmann's negative famine impact estimates would be larger than in the older cohorts that were exposed later in life.

To investigate if adverse long-run effects increase with the degree of early life famine exposure, Neelsen and Stratmann moreover separate the 2001 census data into subsamples of rural-born and urban-born Greeks. The split-sample analysis reveals that the significant reductions in educational attainment found for the full

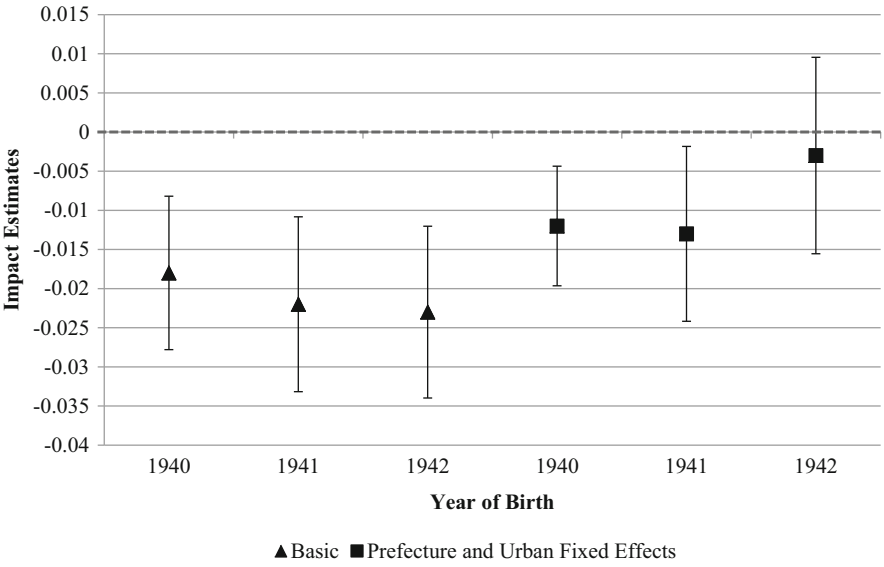

Fig. 3 Estimated impacts of early life famine exposure on upper secondary school completion and 95% confidence intervals – basic specification and specification with controls for prefecture and urban birthplace fixed effects. Graph shows famine exposure impact estimates on the probability of completing upper secondary school obtained from ordinary least squares regressions using data from Greece-born individuals in the 2001 census. The *Basic* specification includes indicators for whether an individual was born in 1940, 1941, or 1942, the year of birth and its square, and a sex indicator. The *Prefecture and Urban Fixed Effects* specification in addition includes 53 birth prefecture of birth dummies interacted with an urban birthplace indicator. Whiskers represent 95% confidence intervals based on Huber-White robust standard errors for the *Basic* specification and based on robust standard errors clustered at the prefecture of birth level for the *Prefecture and Urban Fixed Effects* specification

sample are indeed driven by those born in urban areas where the famine was most severe. For rural-born individuals, by comparison, famine impact estimates are small and largely insignificant across all three exposed cohorts.

Finally, the authors provide a falsification test by estimating famine "impacts" for the subsample of foreign-born individuals in the 2001 census who had no systematic early life famine exposure in the 1940–1942 cohorts. Reassuringly, none of the estimated coefficients significantly differs from zero.

Policies and Protocols

The Greek famine provides a good case to study the long-term effects of early life malnutrition due to its relatively short duration and relatively low mortality rate. These salient features minimize problems of endogeneity and selection bias that are more likely to be present in more severe famines, such as the Chinese famine of

1959–1961. Stratmann and Neelsen find that exposure to the Greek famine has a negative impact on educational attainment for individuals who experienced it as infants or one-year-olds. Furthermore, they find that famine exposure harms those born in urban areas more than those born in rural areas, which is consistent with the greater reported severity of the famine in the urban parts of the country. Promising policies to reduce the immediate suffering include two main aspects:

- Policies that ensure adequate nutrition for pregnant women and young children in areas affected by famine or malnutrition
- Policies that develop an institutional environment that allows for free trade and exchange

Stratmann and Neelsen's study demonstrates that the effects that famines have on young children can be severe, stretching out over a lifetime. Food aid for pregnant women and young children therefore not only has a humanitarian rationale – it is also an important means for economic development. In fact, recent papers have shown that investing in children during the earliest life stages yields the highest economic returns (Doyle et al. 2009; Currie and Rossin-Slater 2015).

Furthermore, as barriers to trade, war, and poor economic incentives were a main contributor to the Greek famine, policies that promote an institutional environment of open trade and exchange both locally and internationally can help reduce the chances of famine and famine-related problems. Economists from Adam Smith to Amartya Sen and beyond have discussed how the causes of famine are not limited to acts of god – such as weather conditions – but that famine often arises, and can be exacerbated, through trade restrictions and poor economic policy (Smith 1976; Sen 1981).

Dictionary of Terms

- **Fetal Origins Hypothesis** – A theory that proposes that events which occur during the gestational period of an individual may have lasting effects on their development and long-term wellbeing.
- **Gestational Age** – A measure of how far along a pregnancy is. Typically the end of a woman's last normal menstrual period is used to estimate the age.
- **Natural Experiment** – A natural experiment is an empirical method that claims that nature, or some other force external to the experimenter, has successfully randomly assigned individuals to different treatment groups. This allows for causal inference from observational data even though no controlled experiment has been conducted.
- **Pre-natal Nutrition** – The nutrition that a fetus receives from the mother while in utero. Insufficient nutrition during this period is hypothesized to have lasting consequences for the fetus.
- **Thrifty Phenotype** – The thrifty phenotype is a hypothesis that asserts that if a fetus' gestational stage is relatively nutrient deprived, then the fetus will develop adaptations that may result in chronic conditions later on in life. These include coronary heart disease, diabetes, and stroke.

Summary Points

- This chapter focuses on the Greek Famine of 1941–1942 and the long-term socioeconomic effects it had on individuals that were exposed to the famine during early life.
- The Greek Famine arose as a result of the combination of the occupation by the Axis powers and a naval blockade implemented by the Allied powers.
- The fetal-origins hypothesis proposes that adverse events that occur during gestation, such as significant malnutrition, may have lasting consequences for the affected individual.
- The Greek famine provides a natural experiment that allows us to study the consequences of early life exposure to undernourishment. The famine can be iewed as an exogenous shock that randomly assigns individuals into treatment groups.
- Individuals that were exposed to the Greek famine before the age of two were found to have lower levels of educational attainment and occupational status.

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Aspects of Gender in Famine: Evidence from the Chinese Great Leap Forward Famine

5

Ren Mu

Contents

Abstract

It is important to consider gender in famine studies. This chapter reviews two gender issues which affect how famine impacts women versus men. The issues discussed are female mortality advantage during famine, and son preference in intrahouse resource allocation under extreme economic constraints. Both female mortality advantage and son preference imply that female survivors would be more negatively affected by famine than male survivors. Analysis of the Chinese Great Leap Forward Famine shows a greater negative impact on disability and illiteracy for women than for men. Exploring heterogeneities in son preference

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among different ethnic groups, further analysis shows the bigger negative impact on disability for women most plausibly reflects female morality advantage, whereas a decline in female education outcomes is probably better explained by the culture of son preference. The evidence that exposure to famine in utero increases the likelihood of disability and illiteracy later in life implies the importance of timely health and nutrition interventions for vulnerable pregnant women, infants, and small children. Moreover, policies aimed to help affected people during crisis shall be designed with gender in mind.

Keywords
Chinese Great Leap Forward Famine · Gender · Female mortality advantage · Son preference

Introduction

Many rights advocates, governments, and international organizations aim to remove the gender-based disadvantages of girls and women. We have observed significant progress in narrowing gender gaps in some areas over the past three decades. For example, globally, more women attend university than men, and women's labor force participation has grown more rapidly than men's (World Bank 2012). Despite steady improvement in education and employment opportunities for girls and women, certain dimensions of gender inequality remain. One manifestation of persistent gender disparity is the higher mortality of girls and women – the underlying force driving the phenomenon of "missing women" (Sen 1990). Based on the assumption that females are born and die at the same rate relative to males, it is estimated that six million women are missing every year (World Bank 2011). Salient gender disparity in other dimensions, too, including earnings, asset ownership, and holding public office, illustrate the importance of considering gender in social and economic studies. Famine study is no exception.

One rather unanticipated and intriguing gender aspect of famine has drawn attention from demographers, epidemiologists, historians, sociologists, and anthropologists. The phenomenon dubbed "female mortality advantage" refers to the age-specific excess death rates for women that are lower than those for men during famines. As salient gender disparity in mortality unfavorable to women exists during normal times (World Bank 2012), one may expect that women would be *more* vulnerable during a famine, and more likely to suffer from the worst outcome of famine: death. Surprisingly, famine literature suggests the opposite is true: women seem to have a higher chance of surviving a famine. This female advantage in excess mortality has been identified in most historical and contemporary famines, including the Ukrainian Famine (Holodomor), the Great Irish Famine, Bangladeshi Famine, and the Darfur Famine (Table 1). Indeed, among the 15 historical famines examined by 22 studies published prior to 2000, Macintyre (2002) finds that women have a mortality advantage over men in nearly all cases. Researchers have proposed biological, behavioral, and social factors as the main drivers for the observed "female advantage" in famine survival. No single

Table 1 Sample of studies identifying female mortality advantage with mortality numbers

Famine	Year	Indicators	Indicator Value	References
Ukrainian Famine (Holodomor)	1932–1933	Male excess mortality (absolute number)	1,517,000	Mesle and Vallin (2012)
		Female excess mortality (absolute number)	1,065,000	
Great Irish Famine	1845–1852	Male excess mortality (absolute number)	511,000	Boyle and O Grada (1986)
		Female excess mortality (absolute number)	474,000	
Bangladeshi Famine	1974–1975	Neonatal odds ratio of infant mortality (boys/girls)	1.2	Razzaque et al. (1990)
Darfur Famine	1984–1986	Male excess mortality (mortality per 1000 people)	30.8	de Waal (1989)
		Female excess mortality (mortality per 1000 people)	26.2	
		Child excess mortality rate, aged 5–9 (mortality per 1000 people)	19.5	
Early English Famine	1622–1624	North region, male, number of burials	450	Healey (2015)
		North region, female, number of burials	416	

factor is believed to be the dominant cause for all observed female advantage, as each famine is likely to be context-specific.

Importantly, having a better chance of surviving famine does not mean that the famine consequences are less severe for women. Conditional on survival, the negative impacts of famine are often disproportionally borne by women. For example, adult women who were exposed to the Dutch famine during early gestation are more likely to be overweight, whereas the impact on men is relatively mild (Ravelli et al. 1999). Luo et al. (2006) and Yang et al. (2008) show that female survivors of the Chinese Great Leap Forward Famine (Chinese Great Famine) have a higher rate of overweight status. Mu and Zhang (2011) shows a higher incidence of disability and illiteracy among women born during the Chinese Great Famine. Some of the gender disparity in famine consequences is linked to the fact that households' coping strategies during and after famine are often unfavorable to women and girls, particularly in places where son preference is a social norm.

Therefore, to understand the gender aspects of famine, one has to consider these two somewhat opposite forces when appropriate: female advantage in excess mortality during famine and son preference in cultural norms. This chapter first presents a brief review of literature on gender difference in mortality in the context of famine and the consequences of son preference during crises. Then, it uses the Chinese Great Famine as a case study to highlight the role of son preference and female

mortality advantage in shaping the gender difference in the long-term impact of the famine. It also presents empirical strategies designed to separate out the two forces in estimations. The case study on the Chinese Great Famine is largely based on Mu and Zhang (2011).

Gender Specific Excess Mortality During Famine

Many famine studies conducted by historians and demographers have consistently identified female advantage in excess mortality in different famines, on nearly every continent, and across time. (For a comprehensive review of female mortality advantage during famine, please see Macintyre (2002).) Given that girls and women are, in general, disadvantaged economically and socially, their survival advantage during famines seems a rather surprising phenomenon. Various explanations have been proposed, but there is no consensus as to what causes male vulnerability during famines. The explanations largely fall into three categories, emphasizing respectively data issues, biological determinants, and gendered socioeconomic factors.

Data collection during famine can be challenging. There is a concern that women's deaths are often ignored and underreported (UN 1998), resulting in an inaccurately low female mortality rate. This explanation essentially dismisses the existence of female mortality advantage and regards it as an artifact of poor data quality. However, a growing body of literature focusing on physiological gender differences seems to support the biological underpinnings of female mortality advantage. For example, medical studies have shown that male fetuses are less physiologically robust than female fetuses and have more delayed lung development (Hassold et al. 1983; Jakobovits 1991). Consequently, male fetuses are at greater risk of death (Shettles 1961; Mizuno 2000). Boys are also subject to higher infant mortality (Wilson 1975; Waldron 1998). In addition, hormonal and chromosomal genetic differences may lead to females having a lower mortality risk for certain diseases. (For literature review on hormonal and chromosomal gender differences, see Zarulli et al. 2017.) Having smaller body sizes with a higher fat proportion may also improve women's survival chances. (For literature review on body fat hypothesis, see Macintyre (2002).) Another brand of literature focuses on gender differences in survival strategies and highlights the socioeconomic factors that give women an edge over men in famine survival. For example, as men are more likely to participate in famine-induced temporary migration or public works in return for aid, they are more exposed to infectious diseases and subjected to physical stress (Pitkänen 2002). When the survival of families depends mainly on women during a famine, a woman-mother has to resort to various coping strategies and life-saving practices (hiding food, etc.) to protect themselves and their families, which contributes to female mortality advantage (Kis 2013).

Gender difference in excess mortality during a famine has important implications for studying the impacts of famine. Excess mortality not only measures famine severity, it also captures famine selection effects. Given the same exposure to a famine, survivors are inherently stronger and healthier than those who died.

Compared to the general population in the absence of famine, survivors of a severe famine may or may not be less healthy moving forward. This is because through mortality selection they are inherently healthier on average than those not exposed to famine, but their average health outcomes are likely to be negatively affected by famine. If male excess mortality is higher relative to female excess mortality, then one would expect that the selection effect is stronger for males, hence the health advantage of male survivors over a nonfamine cohort is larger than the health advantage of female survivors. In other words, female advantage in excess mortality implies a health *dis*advantage of female survivors.

Gender Bias Under Severe Economic Constraints

In many outcomes, gender inequality is often driven by two forces working together: economic underdevelopment and the social norm of son preference. As argued in Jayachandran (2015), agriculture- and manufacturing-based economies reward physical strength, giving men a comparative advantage. Low levels of economic development are also associated with higher fertility and higher maternal mortality rates, which negatively affect women's welfare significantly (Jayachandran 2015; Duflo 2012). These features of underdevelopment exacerbate gender inequality and produce outcomes that are favorable for men. However, economic development itself does not guarantee more gender equality. This can be easily seen in India, China, and South Korea where sex ratio at birth remains male-skewed despite continued economic growth (Das Gupta et al. 2003). These countries hold deeply rooted cultural norms that perpetuate the view that girls and women are less desirable, which in turn reduces the incentive for parents to invest in their daughters. The key cultural element common to these countries is that their family structure is rigidly patrilineal and patrilocal (Das Gupta et al. 2003). Such a family system dictates that males are valued more than females, and that parents desire to have at least one son. Studies on immigrants from East and Southeast Asian countries in USA show that the sex ratio at higher birth orders is still skewed toward male (Almond and Edlund 2008; Abrevaya 2009). These results provide evidence that the culture-based son preference is persistent and does not disappear just because income is higher.

Son preference does not necessarily mean that daughters are discriminated against in everyday life. Actually, evidence on intrahousehold resource allocation often fails to show clear and strong gender discrimination under normal circumstances (Deaton 1997). But when household resources are severely limited by negative shocks (e.g., a sudden disaster or illness), gender bias is most salient and households tend toward unequal treatment of household members. As in a "lifeboat," tough decisions are made to guard certain members' welfare at the expense of other members. Family members who are perceived to be less productive or less worthy would receive less nourishment and medical care relative to others. These family members are typically female. Evidence of unfavorable treatment of girls and women during lean times has been found in many countries. For example, during

drought in India, mortality rate is much higher for girls than boys (Rose 1999). Indonesian families are more likely to reduce educational expenditures for girls than for boys when they experience crop loss (Cameron and Worswick 2001). The pollution caused by smoke from massive wildfires in Indonesia has a stronger effect on female mortality relative to male mortality (Jayachandran 2009). Baird et al. (2011) concludes that infant mortality in developing countries increases during recessions, and the increase is larger for female infants under 12 months. When investigating the effect of weather shocks around the time of birth on the adult outcomes of Indonesians, Maccini and Yang (2009) provides evidence that the impact of gender bias occurred during the negative shocks in early life continues to be felt decades later. This implies that the exposure to a negative shock early in life tends to entail more harmful long-term impacts on women.

Chinese Great Leap Forward Famine and the Gender Differences in Its Long-Term Effects

As the largest famine of the twentieth century, the Chinese Great Leap Forward Famine (1959–1961) caused 20–30 million excess deaths and was associated with elevated risks of miscarriage and stillbirths (Johnson 1998; Cai and Wang 2005). This section explores the long-term impacts of the Chinese Great Famine on the people born during the famine, with a focus on gender differences in the impacts.

Chinese Great Leap Forward Famine

A famine can be viewed as a tragic magnification of market and government failure (Ravallion 1997), and the Chinese Great Leap Forward Famine is no exception. Launched in 1958, the Great Leap Forward Movement aimed to catch up with or surpass Western countries in economic development. The initiative emphasized advancing heavy industries through mass mobilization. To facilitate mobilizing resources from rural areas to industrial production, farmers were organized into cooperatives. The shift to communes, implemented in 1958, eliminated household farming (Huang et al. 2008). In communes, material incentives and monetary rewards were rejected and an identical gender and age specific work-point standard was created for all members (Lin et al. 1996; Naughton 2007). The lack of incentivization resulted in low work effort and free riding across the agricultural sector (Lin 1990). Grain production in 1959 and 1960 dropped by 15% compared with the previous year and in 1961, it barely matched the 1960 level (Lin et al. 1996). While the organization of agricultural production can be blamed for lower production output, socialist-era pricing policy further discouraged efficient production – the price of staples was kept artificially low to allow the state to keep industrial wages low (Huang et al. 2008). At the same time, in the overheated ideological environment, local officials competed to inflate statistics and falsely reported higher levels of agricultural outputs. Driven by this same ideological fever and blinded by false

statistics, top leaders reduced resources available to agricultural production – particularly grain production – and increased the compulsory food delivery to the state. A severe famine broke out in 1959. In 1960, local food shortages escalated to regional shortages, creating a massive subsistence crisis nationwide (Naughton 2007). Through the end of 1961, this unprecedented famine resulted in 25–30 million excess deaths and an estimated 30 million delayed births (Peng 1987).

Researchers have traced the famine to policies adopted during the time. For example, excessive food consumption in collective dining halls (Chang and Wen 1998; Yang 1996; Yang and Su 1998), lower production incentives (Lin 1990), and preferential supplies of food to cities (Lin and Yang 2000) have been identified as contributing factors to the famine. Li and Yang (2005) concludes a possible mix of these factors, together with disastrous weather, led to the catastrophe.

With data on the cohorts affected by the Great Famine increasingly available, there has been an emerging body of studies on its long-term consequences. These studies commonly exploit the natural experiment aspect of the famine in their estimations to compare the outcomes between those who were exposed to the famine prenatally and those who were not exposed to the famine. Most of these studies are guided by the "Barker hypothesis" or "fetal origins hypothesis," which postulates that nutritional deprivations during the fetal period could lead to higher incidences of disease in adulthood (Barker 1992). It is expected that those who were exposed to the famine would have worse later-in-life outcomes than those who were not. These famine studies have identified significant negative impacts on outcomes across several domains: mental health, physical health, educational attainment, household wealth, and employment.

St. Clair et al. (2005) examines rates of adult schizophrenia among those prenatally exposed to the famine in Wuhu region of Anhui province. Their results indicate that the adjusted risk of developing schizophrenia in later life increased significantly among those born during the famine. Meng and Qian (2006) explores long-term impacts of the famine from an adult health and labor market perspective. Their empirical results show significant negative impacts on survivor height, weight, weight-for-height, educational attainment, and labor supply. The exposure to famine seems much worse for health outcomes than economic outcomes, consistent with the hypothesis that a reduced size for the famine cohort could benefit survivors and potentially offset the negative effects of famine through health channels. Similarly, Chen and Zhou (2007) indicates the prevalence of stunted growth among the cohort exposed to famine. There is also evidence showing negative impacts on agrarian per capita income. In a similar vein, Almond et al. (2007) finds higher famine intensity is associated with a greater risk of being illiterate, out of the labor force, marrying later (men), and marrying spouses with less education (women). An intriguing result in this study is that famine exposure lowered the sex ratio of both the famine cohort and their offspring, suggesting that prenatally exposed women are themselves more likely to bear daughters. The tendency toward female offspring found in Almond et al. (2007) is consistent with findings in Song (2012), a study exclusively focused on sex ratio at birth. Song (2012) identifies an abrupt decline in sex ratio at birth between April 1960 and October 1963. These findings provide evidence supporting

the Trivers–Willard hypothesis (1973), also known as the adaptive sex ratio adjustment hypothesis, which states that parents in poor conditions would skew the offspring sex ratio toward daughters, as daughters have higher reproductive success than sons under poor conditions. The Trivers–Willard hypothesis has been tested by examining the relationship between birth sex ratio and status, and the evidence is mixed; out of 54 analyses, 26 support the hypothesis (Lazarus 2002).

Recent studies also find the impact of famine on the offspring of the famine cohort. For example, Kim et al. (2014) finds a significant 5–7% reduction in second generation male and female entrance into junior secondary school. Li and An (2015) finds stunted growth occurs in children if both parents were exposed to the Great Famine. These findings uncover an extensive time horizon in famine impacts.

In terms of gender differences in famine impacts, Shi (2011) found that women exposed to the famine in the first year of life were, on average, less likely to complete high school, while their households had less housing areas and less number of rooms per capita. None of these effects were found among men. The following section, based on Mu and Zhang (2011), focuses on gender difference in famine impacts and introduces empirical strategies used to examine causes for the gender difference.

Gender Difference in the Long-Term Effect of the Chinese Great Leap Forward Famine

Decades after the famine experience, the gender composition of the cohort born during the famine still shows signs of the famine. Based on tabulations from the 2000 China Population Census, Fig. 1 plots the total living population in rural China and the male-to-female sex ratios by age. Due to excess mortality during the famine, the population size of the famine cohort (1959–1961) is significantly smaller than that of the neighboring cohorts. In addition, the sex ratio of the 1961 birth cohort is 100.3, signifying a substantial drop from 109.6 for the 1958 birth cohort and 103.9 for the 1962 cohort. Even though there is a considerable variation in sex ratios over birth cohorts, the drastic deviation of sex ratio in 1961 suggests that either male excess mortality exceeded female excess mortality in 1961 or the sex ratio at birth dropped dramatically that year, or both.

Like the above reviewed famine studies, Mu and Zhang (2011) is also guided by the "Barker hypothesis" hypothesizing that, compared to cohorts not born during the famine, the famine cohort exhibits worse health outcomes and consequently worse education outcomes. The famine cohort is defined as those born during the 1959–1961 period. The cohort born right after the famine (1963–1965) is chosen as the control group. The 1962 birth cohort is not included in the control group because some members were conceived during famine, and there is no way to correctly separate them into the treatment or control group.

The main dataset used in the analysis is the 1% sample of the 1990 China Population Census, which contains information about individual disability status, education, ethnicity, age, and rural or urban residential location. The urban

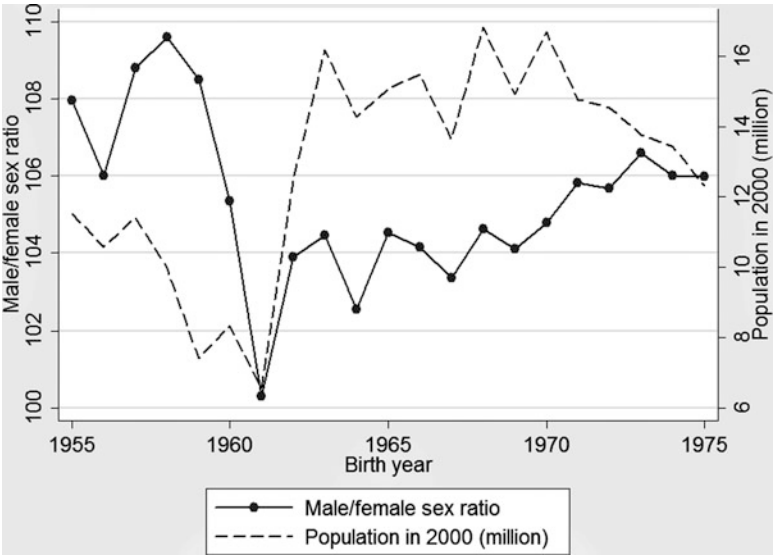

Fig. 1 Male-to-female sex ratio and population in rural China. (Data Source: 2000 China population census) (Note: The famine cohort was born between 1959 and 1961) (Source: Mu and Zhang 2011)

Table 2 Summary statistics of outcome variables

	Women		Men		Gender Difference	
	1959–1961	1963–1965	1959–1961	1963–1965	1959–1961	1963–1965
Disability rate (‰)	2.493	2.197	3.52	3.569	−1.027	−1.372
Illiteracy rate (%)	20.004	12.945	4.777	3.341	15.227	9.604
Observations	125,167	273,608	132,669	284,425		

Data Source: 2000 China population census
Source: Mu and Zhang (2011)

population is excluded from the analysis since the famine was much less severe in urban areas, and they did not suffer from as much excess mortality as the rural population (Lin and Yang 2000; Song 2009). Table 2 presents the gender-specific mean disability rate and illiteracy rate for the famine cohort and the control group. Women have a lower disability rate in both groups. The female disability rate relative to that of males is 0.10% points lower for the famine cohort, while this difference is 0.13 for the control group. Women in both groups also have a higher illiteracy rate. For the famine cohort, women are 15% more likely to be illiterate than men. The gender gap is 9.6% for the control group. These descriptive statistics suggest that, compared to the control group, women in the famine cohort may be worse off in both health and education outcomes.

Table 3 Gender difference in famine impact

	Disability Rate			Illiteracy Rate		
	Female (1)	Male (2)	(1)–(2)	Female (1)	Male (2)	(1)–(2)
Famine cohort	−0.092	−2.765[b]	2.673[a]	1.932[c]	0.345	1.587[b]
	(0.944)	(1.074)	(1.382)	(0.614)	(0.330)	(0.697)
Number of Obs	398,775	417,094	815,869	398,775	417,094	815,869

Note: The robust standard errors (corrected for serial correlation within county and arbitrary heteroskedasticity) are in parentheses. [a]Significant at 10%; [b]Significant at 5%; and [c]Significant at 1%. The famine cohort is a dummy variable coded as 1 for the age cohort of 1959–1961 and as 0 for cohorts born during 1963–1965. Other variables included in the regressions but not reported are age, polynomials of age, a dummy variable for gender-neutral ethnic groups, and county fixed effects
Source: Mu and Zhang (2011)

The regression analysis reported in Table 3, controlling for age, age polynomials, ethnicity, and county-fixed effects, further confirms that the gender difference reported in Table 2 is statistically significant. Regarding the disability rate, the impact of famine on women is not significant, whereas it is negative and significant for males. The insignificant coefficient for females implies that, conditional on survival, the female cohort is no worse than the cohort born right after the famine. The negative coefficient for males shows that the famine survivors are healthier than the control cohort. The estimated gender difference in the famine impact is 2.673. This means that, compared to the control group, women born during the famine are 2.673% more likely to be disabled than their male counterparts. Given that the gender difference in the disability rate for the nonfamine cohort is −1.372‰ (Table 2), a coefficient of 2.673 indicates that famine leads to a huge reversal of the gender gap in disability rate.

With respect to education, the coefficient for the famine cohort is 1.932 and statistically significant for the female survivors, while it is 0.345 and statistically insignificant for the males. These estimates show that the female famine survivors have higher illiteracy rates than the control group, and the male survivors also fare worse in education, although the effect is not significant. The gender difference is pronounced and statistically significant, and the exposure to famine increases the gender gap in illiteracy rate by 1.587%.

In summary, the impacts of the Chinese Great Famine on disability and illiteracy are bigger for women born during the famine than men of the same cohort.

Two Hypotheses Explaining the Gender Differences: Mortality Selection and Son Preference

The above analysis shows that the famine has impacted women more negatively. This gender difference in famine effects could be caused by two forces: mortality selection during the famine and son-preference culture. Due to mortality selection, the observed sample of survivors is likely to be the healthier portion of the total population exposed to the shock. In the case of the Great Famine, Song (2009) finds

Table 4 Heterogeneity of gender difference in famine impact

	Disability Rate			Illiteracy Rate		
	Female (1)	Male(2)	(1)–(2)	Female (1)	Male(2)	(1)–(2)
Famine cohort (α_1)	−0.139	−2.764[b]	2.625[a]	1.852[c]	0.189	1.662[b]
	(0.945)	(1.079)	(1.371)	(0.612)	(0.325)	(0.698)
Famine cohort × gender-neutral ethnic groups (α_2)	3.747[b]	0.267	3.480	0.212	2.916[b]	−2.704
	(1.548)	(1.051)	(2.444)	(1.862)	(1.174)	(2.120)
F-statistics for testing $\alpha_1+\alpha_2=0$	3.85	3.10	4.63	1.09	7.25	0.17
p-value	0.050	0.078	0.032	0.294	0.007	0.676
Observations	396,768	414,973	811,741	396,768	414,973	811,741

Notes: The robust standard errors (corrected for serial correlation within county and arbitrary heteroskedasticity) are in parentheses. [a]Significant at 10%; [b]Significant at 5%; and [c]Significant at 1%. Gender neutral ethnicity is defined as the sex ratio at birth in 2000 for the second child, and is less than 107. Other variables included in the regressions but not reported are age, polynomials of age, a dummy variable for gender-neutral ethnic groups, and county fixed effects are included
Source: Mu and Zhang (2011)

that the postnatal mortality rate among the famine cohort was lower than that among the pre- and postfamine cohorts, suggesting a strong famine-induced mortality selection. With males having higher excess mortality, as documented by literature reviewed in section "Gender Specific Excess Mortality during Famine," the mortality selection hypothesis predicts that for the cohort exposed to famine early in life, adult men may have better health outcomes than women, when compared to the cohort not exposed to famine. At the same time, the culture of son preference that persisted in many parts of China may improve the average welfare of the surviving boys relative to that of the girls, likely resulting in better outcomes for men.

Consistent with the empirical results presented in section "Gender Difference in the Long-term Effect of the Chinese Great Leap Forward Famine," both the mortality selection effect and the son preference effect predict that women would be more negatively affected by famine. Further investigation is needed in order to understand how the two effects shape gender difference in famine impacts. For this purpose, Mu and Zhang (2011) explores heterogeneities in son-preference culture across different ethnic groups in China. Some ethnic groups do not exhibit the same degree of son preference as others (Chen and Chen 2004; Zhang 2006). If son preference causes gender difference in the famine impacts, one would expect little gender difference among the ethnic groups without son preference. However, if the mortality selection effect were the main force underlying the gender difference, we would expect female survivors to fare worse, regardless of their ethnic identities.

The level of son preference is measured using the sex ratio of second births (Zeng et al. 1993; Das Gupta 2005). Given the evidence that the culture of son preference is persistent over time (Almond and Edlund 2008), the sex ratio of second births calculated from the more recent census (2000) could reasonably measure the culture of son preference at the time of famine. To assure reliability in the

measurement of the sex ratio, the 19 ethnic groups (out of 56) whose population is larger than one million are included in the analysis. An ethnic group is defined as being "gender neutral" if the sex ratio of the second births is less than 106, and "gender biased" if it is over 106. By this definition, four ethnic minorities (Uygur, Tibetan, Dai, and Bai) are identified as being gender neutral.

Table 4 presents results on heterogeneity of the gender difference in the famine impacts by gender. In the regression framework, the summation of the coefficient on "famine cohort" and the interaction term between "gender neutral" and "famine cohort" gives the famine impact for the gender neutral group, and the coefficient on the famine cohort itself is the famine effect on the gender biased group. For disability rate, the results show that in both ethnic groups, men exposed to famine have lower disability rates. For the ethnic group with son preference, the estimated gender difference is statistically significant. For the gender neutral ethnic group, the F-statistics shows that the gender difference is significant as well. Given that son preference does not apply to the gender neutral group, these results imply that son-preference culture is not the driving force for the observed gender difference, and very likely mortality selection underlies the gender difference in the famine impact on disability (Table 3).

With respect to the education outcome, the results show that men in gender neutral ethnic groups are more negatively affected by famine than men in the ethnic group with son preference. The gender difference is only significant in the group with son-preference culture but not in the gender neutral group. The results imply that son preference is likely to be the major cause for the observed gender difference in the famine impact on education.

Overall, there is strong evidence that exposure to famine in utero increases the likelihood of disability and illiteracy for women born during the famine, relative to men of the same cohort. Both female mortality advantage and son-preference culture can explain this gender difference but for different outcomes. Female mortality advantage explains the gender difference in health outcome, suggesting that female survivors adapted to a hunger environment early in life would suffer from more adverse health later in life. Whereas son preference leads to gender difference in the impact of famine on education outcomes: females of the famine cohort have a higher illiteracy rate than their male counterparts.

Policies and Protocols

To identify the long-term impact of nutrition deprivation in early life, a shock – such as a famine, pandemic, or disastrous weather – that occurs during the early life of an individual can be used as a natural experiment. With this approach, this review on studies of famine, and the Chinese Great Famine in particular, confirms the existence of such long-term impact and provides evidence on the importance of early childhood nutrition. The policy implication of this finding is clear: timely health and nutrition interventions for pregnant women, infants, and small children vulnerable to nutrition deprivation are both important and smart.

During famine, boys are likely to be favored over girls in household resource allocation. This son-preference culture, prevalent in many countries, has led to more negative famine impact on the education outcomes for Chinese women born during famine. Hence, policies aimed to help affected people during crises should be designed with gender in mind. Programs, such as conditional cash transfer programs, that can potentially change household behavior should be considered.

Lastly, understanding famine requires a gender perspective and also requires contributions from different disciplines, including anthropology, epidemiology, demography, economics, history, medicine, and sociology. Together with other chapters in this book, this chapter shows that famine study must be interdisciplinary.

Dictionary of Terms

- **Great Leap Forward Famine** – The Great Leap Forward Campaign was an economic and social movement launched in 1958 by the Chinese Communist Party. The goal was to catch up with or even surpass the Western countries in economic development through rapid industrialization and collectivization. In 1959, a severe famine broke out and through the end of 1961, the unprecedented famine caused about 25–30 million excess deaths and another roughly 30 million delayed births. Researchers have traced the famine to various policies adopted during the Campaign and the famine is thus known as the Great Leap Forward Famine.
- **Sex ratios** – Conventionally reported as the number of males per 100 females, sex ratios summarizes the gender composition of a population. A higher sex ratio is more male biased and a lower sex ratio is more female biased. Sex ratios for human populations average 105–106.
- **Excess mortality** – It is a measure of the deaths, due to a negative circumstance, which occurs over and above what the regular death rate would predict.

Summery Points

- Many famine studies have consistently identified female advantage in excess mortality in different famines, on nearly every continent, and across time.
- Evidence of unfavorable treatment of girls and women during lean times has been found in many countries.
- To understand the gender aspects of famine, one has to consider these two somewhat opposite forces when appropriate: female advantage in excess mortality during famine and son preference in cultural norms.
- Researchers have traced the Chinese Great Famine to various factors related to policies adopted during the time.
- Studies on the Chinese Great Famine have identified significant negative impacts on outcomes across several domains, particularly in mental health, physical health, educational attainment, household wealth, and employment in adulthood.

- The impacts of the Chinese Great Famine on disability and illiteracy are higher for women born during the famine than for men of the same cohort.
- Female mortality advantage explains the gender difference in health outcome, whereas son preference leads to gender difference in the impact of famine on the education outcome.
- Timely health and nutrition interventions for pregnant women, infants, and small children vulnerable to nutrition deprivation are both important and smart.
- Policies aimed to help affected people during crises should be designed with gender in mind.
- Understanding famine requires a gender perspective and also requires contributions from studies from different disciplines.

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Understanding Famine in Ethiopia: Bio-physical and Socio-economic Drivers

6

Fatemeh Taheri and Hossein Azadi

Contents

Abstract

The aim of this review study is to examine the persistence of famine in Ethiopia in the framework of sustainability which includes both bio-physical and socio-economic drivers. On one hand, the authors argue that famine occurrences are linked to drought, climatic change, and change in agricultural land use that have increased the vulnerability of Ethiopian households to hunger over time and reduced the inflexibility to environmental and economic shocks. On the other hand, the authors argue that the cause of Ethiopia's recurrent famine is not only

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natural interference, but it is also the reaction of society and governments that create a famine. Food security for Ethiopia requires an integrated long-term response to household vulnerability on the part of Ethiopian governments, civil society, and international partners by incorporating new technologies, local expertise, and active involvement of Ethiopian communities living with the realities of recurrent famine. Accordingly, the paper outlines a conceptual framework and concludes that famine cannot be explained exclusively in terms of resource shortage and politics is no less important. The framework creates a useful tool for policy makers to analyze the famine prevention approaches.

Keywords
Famine · Drivers · Natural interference · Drought · Climatic change · Land use change · Economic development · Government · Human rights · Ethiopia

List of Abbreviations

EPRDF	Ethiopian People's Revolutionary Democratic Front
MoFED	Ministry of Finance and Economic Development
CRGE	Climate Resilient Green Economy Strategy
FSNWG	Food Security and Nutrition Working Group
IPCC	Intergovernmental Panel on Climate Change
UDHR	Universal Declaration of Human Rights
ICESCR	The International Covenant on Economic Social and Cultural Rights

Introduction

Ethiopia has a long history of recurring and devastating famine. On the surface, it appears that erratic weather conditions have repeatedly triggered large-scale hunger and starvation. In recent years, famine has unfortunately become Ethiopia's trademark and even now, despite changes in regimes, the threat of famine continues. In 1973, during the Imperial regime, almost three million Ethiopians were affected by food shortages and total excess mortality in the country hovered at around 250,000. A decade later, during the "Marxist-Leninist" Derg regime, approximately 7.8 million Ethiopians were caught struggling for survival, out of which excess mortality was conservatively estimated at 700,000 (Vadala 2009). And in the year 2000, amidst the "free-market" orientation of the EPRDF (Ethiopian People's Revolutionary Democratic Front) regime, 8 million people required food aid, out of which excess mortality was estimated to be over 6000 in one district alone (Howe and Devereux 2007). Three years later, the number of Ethiopians requiring food aid rose to 14 million. For Ethiopia, 2016 was a challenging year as it faced with the worst El Niño impact in the last 50 years. The beginning of El Niño combined with failed Belg (spring harvest) and Meher (main harvest) rainfall in 2015 and resulted in need of emergency food and nutrition assistance for 10.2 million people. At the same time, Ethiopia was dealing with both residual needs from the 2015/2016 El

Niño-induced drought and new drought in lowland pastoralist areas due to below average 2016 autumn rains in the southern and southeastern parts of the country, as well as in pocket areas across the country. As a result, 5.6 million people in Ethiopia require emergency food assistance in 2017. In addition, 2.7 million children and pregnant and lactating mothers require supplementary feeding, 9.2 million people need support to access safe drinking water, 1.9 million households need livestock support, and 300,000 children between 6 and 59 months old are targeted for the treatment of severe acute malnutrition in 2017. Nutrition reports by Emergency Nutrition Coordination Unit indicate worsening nutrition situation in Ethiopia in future (FSNWG 2017).

Webb and von Braun (1994), in their discussion of famine in Ethiopia, define famine as "a catastrophic disruption of society as manifested in a cumulative failure of production, distribution and consumption systems" (p. 35). The principal consequences of famine are a concentrated decline of food consumption resulting in chronic weight losses for individuals and sharp increases in excess mortality, massive social disruption, and long-term resource depletion. Although famine has long been considered a discrete event triggered by external causes and amenable to technical solutions, researchers, and scholars have recently challenged this view, arguing that famine must be understood as a long-term socioeconomic process that accelerates destitution of a society's most vulnerable groups to the point where their livelihood systems become untenable (Baro and Deubel 2006).

Many experts relate Ethiopia's cyclical famine with the country's dependence on rainfed smallholder agriculture, drought, rapid population growth, or agricultural market dysfunctions (Vadala 2009; FSNWG 2017). Although these factors do have significant role in the matter, they tend to hide the critical cause of hunger in the country – lack of rights and accountable government. To fully understand the vulnerability of the Ethiopia population to famine, we need to understand the concurrent impact of bio-physical and socio-economic drivers. Hence, there is a clear need for research considering both bio-physical and socio-economic drivers of famine in Ethiopia. Drawing on sustainability framework, this chapter reviews drivers of famine in Ethiopia which include both bio-physical and socio-economic drivers.

Drivers of Ethiopia's Famine

Bio-Physical Interference

Famine mainly resulted from natural disasters. Natural disasters lead to reduction in food production for a particular duration and with no doubt droughts have created severe food deficiencies in the case of Ethiopia, (Vadala 2009). According to the June Food Security and Nutrition Working Group (FSNWG), food security remains a concern in north-eastern parts of Ethiopia previously hit by drought (FSNWG 2017). According to the World Bank (2015), the whole Ethiopian economy is dependent on rainfall and data on rainfall variation and GDP growth from 1982 to

2000 illustrate that there is a positive correlation between the two. Thus, natural disasters have obvious negative impacts on food production and even on the economic performance of the country. The causes for the famine can be attributed to a variety of interrelated and complex factors. The main causes include climatic change and change in land use (Shegro 2016).

Climatic Change

Climate change is widely recognized as one of the prime challenges facing Africa, and the continent is often cited as the hardest hit by potential transformations (Boko et al. 2007). Sub-Saharan Africa has been identified as one of the main parts of the world most expected to suffer from climate change due to high reliance on its agricultural sector, the main sources of people's livelihoods (Niang et al. 2014) and accounting for approximately 96% of overall crop production (World Bank 2015; Serdeczny et al. 2016). The impact varies from country to country causing substantial welfare losses and countless suffering. In East Africa, for instance, changes in rainfall patterns are expected to reduce crop yield by at least 10–20% by 2050 (Kotir 2011; Thornton et al. 2011) and will drop by 20–50% in West Africa by 2050 (Sarr 2012). Climate models suggest that Ethiopia will see further warming in all seasons between 0.7 °C and 2.3 °C by the 2020s and of between 1.4 °C and 2.9 °C by the 2050s. As a result, under moderate global warming cereal production in Ethiopia expected to decline by 10–12% (MOA 2011).

IPCC reports that rising temperature and changing precipitation patterns will likely lead to an acute decline in rain fed crop production (IPCC 2007). Temperature and precipitation alterations will also affect hydrological cycle and water availability. The groundwater levels of aquifers have also been affected by climate change due to recharge variations. Whereas the exact effects are still unknown, it is quite clear that Ethiopia will need to make far-reaching adaptations in its farming systems to accommodate changed rainfall patterns and cropping seasons by planting new crops and crop varieties and adopting new farming practices. The narrow range of agricultural products determines Ethiopian's vulnerability and Ethiopia has major heavy dependency on agricultural products to support its economies, which often fail due to pest outbreaks, climate variation, and changes in prices. Further food insecurity for rural people has increased due to climate change, inappropriate land use, or land tenure policies (Harnevik et al. 2007).

Change in Agricultural Land Use

Ethiopia's principal natural resource is its rich endowment of agricultural land. Agriculture which constitutes 46 percent of GDP directly supports about 85% of the population in terms of employment and livelihood. It contributes about 50% of the country's gross domestic product (GDP), generates about 88% of the export earnings, and supplies around 73% of the raw material requirement of agro-based domestic industries. It can be considered as the main source of food for the population and then the leading contributing sector to food security. In addition, the country's overall socio-economic development is expected to speed up by agriculture sector which plays a key role in generating surplus capital (Finnish Foreign Ministry 2009).

In the contemporary era of ecological change and global land rush, the politically contested nature of land control and land access among rural dwellers has become even more difficult a challenge across many developing countries. In the context of Ethiopia, most livelihoods are fundamentally grounded in the agricultural sector (Shegro 2016). The growth of the agricultural sector greatly determines the economic growth of the country. However, the sector is dominated by subsistence rain-fed farming systems and trapped by numerous challenges. These challenges include shrinking farm sizes, high farmland fragmentation, high population pressure, land tenure insecurity, farmland scarcity, erratic rainfall, environmental degradation, low farm income, and productivity (Nega et al. 2003; Tolossa 2005). These issues have been assumed to constrain the process of agrarian change and differentiation in rural areas. Although land remains at the center of rural livelihoods, these problems have particularly resulted in declining levels of access to this key resource by the poor, thereby affecting food security and livelihoods of most households in many rural areas (Shegro 2016). It is also plausible to decline access to land resources among the rural poor while increase access to the same resource by other actors including the state, state-owned enterprises or private corporate actors. In a new era of global land rush and climate change, the challenges of poor rural people are likely to intensify further. Many studies in rural Ethiopia (e.g., Carswell 2002; Devereux 2009; Tolossa 2005) indicate important changes in the composition and sources of rural incomes propelled by these factors.

Socio-Economic Intervention

Famine affects only certain countries while nature's forces and climatic conditions can affect any country. Drought may result in famine in many sub-Saharan countries including Ethiopia while with the same intensity in Australia causes no famine at all. It has become clear in recent years that nature's forces and climatic conditions like drought cannot solely be responsible for famine causation as was the dominant mode of thinking five decades ago. There are more events which lead to famine than just drought or other adverse climatic events (Vadala 2009). As a result, theories of famine have shifted from an emphasis on environmental causes to economic and sociopolitical causes (Baro and Deubel 2006). This section addresses the major causes and explanations of famine in Ethiopia within such a framework.

Economic Development

Out of the top ten fastest growing economies in the world since 2011 seven are African. With an average annual real GDP growth rate of 8.1%, Ethiopia ranks seven (The Economist 2011). Ethiopia's development has been articulated with the ultimate goal to become a middle income country by 2025. In order to achieve this goal, strategic sectors such as agriculture and energy will require transformation, as both sectors will have to accommodate a great demographic intensification (see Table 1) (UNESCO 2017).

Table 1 Projecting Ethiopian Demographics from 2012 to 2050 (Adapted from UNESCO 2017)

	Year		
Demographic Indicators	2012	2032	2050
Population (millions)	83.7	133.5	171.8
Employed (percentage of population)	56	61	68
Dependent population (percentage of population)	44	39	32

An old-aged (i.e., a decade) base of strong agricultural growth conclude a Ethiopia's Growth and Transformation Plan (GTP) and the forthcoming Growth and Transformation Plan II (GTP II) in order to produce a wide range of inputs (including improved seeds, fertilizer, mechanization, land), energy use (hydropower), irrigation as well as maintenance and land repossessions goals. For example, Ethiopia intends to increase cultivable land by 13%; therefore, the conversion of grazing and/or forest lands into crop lands is required in this policy. Also, it has been planned to increase irrigated land by more than 400% over the same period. Finally, in order to achieve significant productivity increases in agricultural output (30% increase in crop productivity of various crops), fertilizer use is expected to increase by approximately 100% (Ministry of Finance and Economic Development (MoFED) 2010). In addition to these traditional economic objectives of growth, GTP also outlines a National Resource Conservation Plan that seeks, among other things, to rehabilitate land and increase forest cover. These conservation targets are further detailed in Ethiopia's Climate Resilient Green Economy Strategy (CRGE) which seeks to achieve economic development in a sustainable way (Federal Democratic Republic of Ethiopia 2012).

All these transformative changes, in combination with population growth and changing consumption patterns, create an increasing demand for natural resources and ecosystem services. Along with climate change, additional pressures manifested in resource degradation result in many millions of people continue to lack basic human (food, energy, and water) securities (Karlberg et al. 2015).

Land Tenure System

According to the Constitution of the Federal Democratic Republic of Ethiopia (GOE 1997), the right of land ownership and other natural resources of the country are exclusively granted to the State and the peoples of Ethiopia. This implies that all subsidiary laws and regulations of the country which could be issued either by the Federal or Regional State bodies recognize usufructuary rights to land which can be in the form of state, communal or group, and private holdings. Private ownership of land is prohibited although the right to use and inherit land is possible (Teklemariam et al. 2017). Insecure land tenure or the lack of land ownership also restricts the farmers' access to the credits that are required for improved land practices. This lack of access to credits forces them to go for traditional land-use practices, despite their willingness to change. Thus, national policies affect the land-use systems by influencing institutional arrangements such as credit and marketing facilities, and infrastructure development.

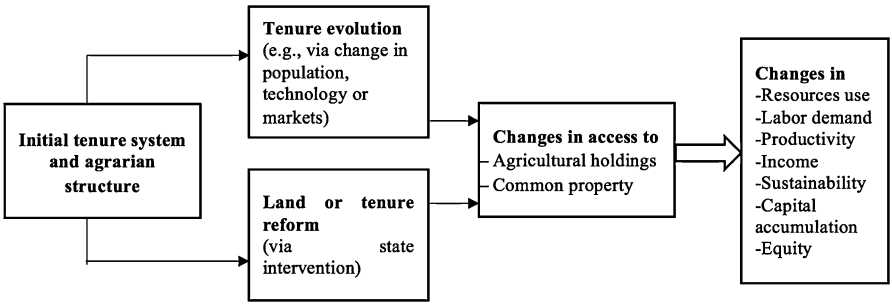

Fig. 1 Conventional characterizations of tenure's impact on agricultural production. (Adapted from Finnish Foreign Ministry 2009)

In brief, the land reforms are concentrated on food security, greater equity, productivity, better conservation practices from changes in tenure. Thiesenhusen et al. (2017) noted the following outcomes from land tenure reform: (i) food security, (ii) reduction in social polarity, (iii) increased investment, (iv) transparent production incentives, (v) poverty reduction, (vi) increased employment, and (vii) greater equity. The relationship between land tenure (either through legal changes or through institutional reforms) and agricultural productivity is depicted in Fig. 1 (Finnish Foreign Ministry 2009).

Food Availability Decline/Inappropriate Land Use

Early work on famine was heavily influenced by Malthus who proposed that famine followed excessive population growth and served to keep carrying capacity in check by reducing populations to a level consistent with food production (Baro and Deubel 2006). In his work, which dates back to 1798, Malthus entertained the notion that population growth has to be balanced with food production; failure to do so would force nature to take measures into its own hands by wiping off the "excess." There have been several critics on his work; the fact that nowadays the world is over-producing food at a time when there are almost seven times more people than the 1 billion Malthusian "limit" could be cited as an example. Malthus' analysis may have several inconsistencies, but the central theme is not so erroneous; there is indeed a limit as to the carrying capacity of the earth, though no one knows for sure how much is "full house." In the case of Ethiopia, the more relevant issue in this connection is the carrying capacity of land for agricultural purposes to a population that grows at a yearly rate of 2.3 per cent. It will be imprudent to ignore the problem of decreasing land-size holdings for agricultural purposes in the country, not least, because around 85 percent of the population is engaged in subsistence agriculture (Vadala 2009).

If people shift from agriculture to other sectors of the economy for their livelihood, then population pressure on agricultural land can be part of the explanation of famine in Ethiopia. Two thirds of households farm are on less than 0.5 hectare, a size which is known to be insufficient to support a family, at the same time high

population growth is increasingly putting a pressure on land (Ziegler 2005). Coupled with droughts and other unfavorable weather conditions, increasing population pressure on land is a challenge to famine prevention in Ethiopia.

Food Accessibility Decline

In the last two or three decades, there has been a revolution in thinking about the explanations of famines. The entitlement's approach by Amartya Sen brought the issue of food accessibility to the forefront of the academic debate on famine. Sen noted that there is often enough food available in the country during famines but all people do not have the means to access it. More specifically, famines are explained by entitlement failures, which in turn can be understood in terms of endowments, production possibilities, and exchange conditions among others (Sen 1981).

Ethiopia is a good case in point where, for instance, food was moving out of Wollo when the people in the region were affected by the 1972–1973 famine (Sen 1981), and even today some regions in Ethiopia produce surplus, while people in other regions face famine threats. There are of course infrastructural problems in the country to link the surplus producing regions to the food-deficit ones. However, the question goes beyond this simplistic level, as some people simply do not have enough entitlements to have a share of the food available in the country, a situation which can be described as a case of direct entitlement failures (Tully 2003). Or else, peasants do not find the right price for their surplus, as in the 2002 Bumper Harvest which ended up in an 80 per cent price drop, illustrated a failure in peasants' exchange entitlements. Alternatively, the most irrigated land of the country in the Awash River basin, for instance, is used primarily for cash crop production to be exported to the western world (even when there is drought) leading the vulnerability of various pastoralist groups to turn into famine or underpinned by what is known as a crisis in endowments and production possibilities.

In short, while drought and population pressure can partly explain famine threats in Ethiopia, the entitlements approach provides an explanation from an important but less visible angle. The approach points to the direction of policy failures by shifting the attention from the absence of food to lack of financial access to food. The point that only some classes in society are affected by famine clearly indicates that policy failures are central to the understanding of famine. In the next section, the success or failure of famine prevention policies and practices will be measured against internationally recognized standards, and one such standard is the right to food (Vadala 2009).

The Right to Food

The following agreements of many international accords concerning the right to food and the numerous countries who have agreed with this right have possibly created a worldwide right to food. The right to food has become internationally established due to the creation of international human rights treaties and international conventions worldwide. By agreeing to such covenants, signatories agree to live up to the covenants' purpose and procedures, which suggests that such states accept the proposition that a right to food is a basic human right that every person ought to have (Tenente 2007).

The UDHR (Universal Declaration of Human Rights) presents the right to food in article 25, which outlines the idea that everyone has the right to a standard of living adequate for the health and well-being of himself and of his family, including food. The UDHR later served as the foundation to the ICESCR (The International Covenant on Economic Social and Cultural Rights) to which Ethiopia became a state party in 1993. The covenant is legally binding on all state parties including Ethiopia. Article 11(2) of the ICESCR elaborated on the right to food. The States Parties to the present Covenant, recognizing the fundamental right of everyone to be free from hunger, including specific programs, are as follows (Vadala 2009):

(a) Improvements in methods of production, preservation, and distribution of food through full use of technical and scientific knowledge. It is achievable by spreading principles of nutrition knowledge and/or by promoting or reforming agrarian systems in such a way as to gain the most efficient development and utilization of natural resources;
(b) The problems of both food-importing and food-exporting countries must be considered to ensure an equitable distribution of world food supplies in relation to need.

This being said, the FDRE (Federal Democratic Republic of Ethiopia) Constitution is one of only 20 constitutions in the world which makes reference to food (FAO). Article 90 of the Constitution, under the banner of social objectives, states that "to the extent the country's resources permit, policies shall aim to provide all Ethiopians with access to public health education, and clean water, housing, food and social insurance." Constitutionally, food is regarded as a social objective rather than a human right; nevertheless, this is not to say that Ethiopia is not bound by the right to food. Ethiopia has been a party to the ICESCR since 1993 and has made no reservations to any articles when ratifying the covenant; hence, it is legally bound by it.

To date, there has been no court case where the right to food has been a subject of contention in Ethiopia; reference in courts to the international human rights conventions in general is "very minimal at best, nil at worst" (Rakeb 2002). To make matters more complicated, most rural citizens resort to religious, customary, or social courts at the Kebele level where the notion of human rights is unheard. There is thus a need to take all appropriate measures to make the right to food, and particularly the freedom from hunger, justiciable in the Ethiopian legal system starting from the local courts. Effective human rights in education should also be provided for beneficiaries to claim rights; unless people are aware that they have these human rights, it will be very difficult to raise the issue of freedom from hunger in Ethiopia. Defining food as a right is very important in order to prevent famine in Ethiopia, not least because the country has repeatedly been facing famines throughout the past four decades. Additionally, in order to be free from hunger ensuring direct food entitlements and legal guarantees is not enough, but agricultural development policies and land tenure systems must be ensured equally as well (Vadala 2009).

The Political Setting

In recent years, there have been attempts to determine if there is a link between the political system of a country and famine prevention, and if such a link exists, which political system can best protect the people from famine. Sen (1999: 178) asserts that "there has never been a famine in a functioning multiparty democracy," indeed, for him, it is not at all difficult to prevent famines; in addition to economic rights like the right to food, civil and political rights are of utmost importance. More and more scholars agree that recent famines, also known as new famines, are political because they are almost always preventable (Howe and Devereux 2007). In an attempt to further refine and complement Sen's theory, de Waal (2000) came up with the notion of an antifamine political contract with the objective of preventing famines. Antifamine political contracts are necessary as well as democracy, according to de Waal assumption. Such contracts aim to further politicize famine by presenting an incentive for governments to meet their responsibilities. Ineffective government action and even inaction can cause a heavy political cost through politicizing famine.

Such a political contract attempts to explain why some socio-economic rights are important enough to require a political guarantee. In fact "famine is so self-evident and so visible that it readily offers itself as a political cause" (Sen 1999). The antifamine political contract ensures a long-term solution to the problem by making the prevention of famine and starvation a priority in the governments' agenda. In the absence of civil and political rights, the government is not forced to put the fight against famine and starvation as a priority (Devereux 2000). We cannot make sure that liberal civil and political rights assist in freedom from famine unless famine is politicized (de Waal 2000). Furthermore, such political contracts could work only in democracies. Famine in this sense ceases to be the result of natural disaster or a challenge to charity and becomes a political issue. Such a political contract makes famine and starvation an electoral question (de Waal 2000). The free election of a government depends, among other things, on its agenda, and its re-election on the fulfillment of that agenda; famine therefore must appear as one government agenda in a political contract. This is instrumental in getting the attention of any government facing famine threats, and where there is free and fair election, the political contract is different from the notion of food as a right in the sense that it provides a clear incentive for a government.

Ethiopia has repeatedly been mentioned in the discussion on democracy and famine prevention. The previous regimes of Emperor Haile Selassie and the Derg serve as good examples where, respectively, the 1973–1974 and 1984 famines inter alia occurred in the absence of democracy. At present, not many people (not even the government itself) dare to assert that Ethiopia is a full-fledged democracy. In 1995 and 2000, elections were not very competitive; opposition parties that participated were weak, and election practices were not uniformly free and fair over the whole country (Pausewang et al. 2002). The last elections in 2005 were much more competitive but ended with controversial results and, among others, the main CUD opposition party leaders, most of whom were elected, found themselves behind bars. The European Union Election Observation Mission (2005) stated that overall "the elections fell short of international principles for genuine democratic elections."

Famine is still a threat in Ethiopia in part because of the lack of a functional multiparty democracy. Where opposition political parties, civil society organizations, and independent media cannot operate freely, there is no certainty that the government will put famine prevention as a priority. Democracy, according to Sen, is the one element that all famines lack; in other words, the presence of nondemocratic government is the common denominator in all famines. In Ethiopia, the issue of famine has already been politicized to some extent. The 1974 famine, for example, came at a heavy political cost for the Imperial government. However, where a full-fledged democracy is lacking, the effective politicization of famine and starvation is by no means evident (Vadala 2009).

Conclusion

Indicating the main reason or identifying one single factor which can explain the occurrence of famine in Ethiopia is very difficult. In conclusion, an important paradigm shift is underway in the field of famine and food security studies. Famine is now explained less in terms of an anomalous disaster event and more commonly as a process rooted in long term social, economic, and political inequalities. The conceptual framework (Fig. 2) emphasizes interlink ages among and between bio-physical and socio-economic intervention and famine and the need for coordination and integrated management and governance across sectors. Based on the figure, it can be argued that famine has its roots in the notion of access to resources

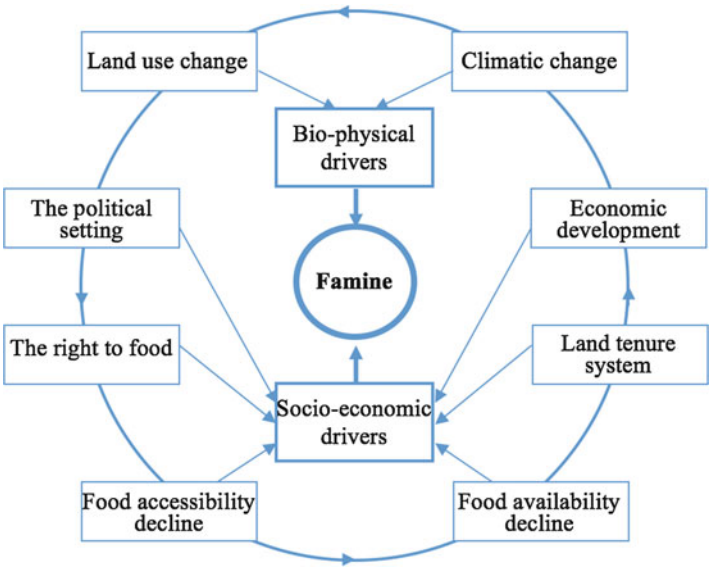

Fig. 2 Bio-physical and socio-economic drivers of famine in Ethiopia

(especially the distribution and productivity of land) and vulnerability of livelihoods to shocks (especially climate change). Therefore, the production and reproduction of rural poverty in the country cannot be de-linked from land. Climate change has also an impact on Ethiopian agriculture and especially increasing temperature is damaging. Many regions have been severely affected by climate changes in Ethiopia. Therefore, it is essential to have a policy implication worth thinking about and planning before damage occurs. Ethiopian government must design and employ adaptation policies that counteract the harmful impacts of climate change. There are numerous adaptation options such as investment in technologies, irrigation, drought tolerant, and early maturing crop varieties, promoting institutional set-ups working in research, and educating farmers and supporting ownership of livestock, as owning livestock may secure them against the effects of crop failure or low yields during harsh climatic conditions (Hassan and Nhemachena 2008).

According to the figure, nature's forces like climatic conditions and change in agricultural land use cannot solely be responsible for famine causation, but the figure also considers socio-economic drivers including economic development, land tenure system, food availability decline, food accessibility decline, the right to food, and the political setting. Economic development creates an increasing demand for natural resources and ecosystem services. The application of technologies for agricultural and natural resource management is affected by land tenure and property rights. Sufficient incentives to the farmers are provided by secured property rights which increase their efficiencies in terms of productivity and ensure environmental sustainability. The decline of food availability and accessibility caused by population pressure is the major determinant of famine in Ethiopia. There may be enough food at the national level, but still entitlement failures in some regions can cause famine. It is natural that without secured property rights farmers do not feel emotional attachment to the land they cultivate, do not invest in land development, and will not use inputs efficiently. Defining food as a right is very important in order to prevent famine in Ethiopia, not least because the country has repeatedly been facing famines throughout the past four decades. Finally, the fulfillment of the right to food also requires the respect of civil and political rights.

In developing countries where famine is a threat, a functional multiparty democracy tends to ensure that famines do not occur. Here again, democracy by itself is not sufficient; but it will render governments accountable by imposing a heavy political cost to failed famine prevention policies. Politics is therefore one major determinant in the famine equation. This approach can better provide a famine prevention strategy, and it can also shape our understanding of famine – that famine is not only the result of natural or economic problems, but that it is the result of political problems as well. In view of the fact that Ethiopia is presently not a full-fledged democracy, addressing famine requires more than just applying technical or economic fixes to a partly political problem. The protection of human rights could be considered as an antifamine political contract as well as helping in the fight against famine. In order to have a permanent solution, it is important to address the problem is an antifamine political contract, the outcome of which would inevitably depend on the strength and commitment of all contracting parties.

Policies and Protocols

- Development seems to be achievable only through damaging the environment. It is essentially required to have a new approach to resolve improvements in human securities and aspirations for better lives, with sustainable management of natural resources and ecosystems.
- The land belongs to the government is recognized as the general problem related to land policy in Ethiopia. The farmer can work on a land as long as he/she stays on the farm and neither can sell nor lease land legally. Thus, exiting tenure security is vital to have a successful agricultural development in Ethiopia, where about 85% of the population lives in rural areas.
- Future food security for Ethiopia depends on good governance, sound economic growth policies, and active preparedness. If the underlying issues of political accountability and economic disparity are adequately addressed in the context of Ethiopian governance and civil society by international humanitarian interventions and local development planning, the persistence of hunger will continue to plague most Ethiopian nations well into the twenty-first century.
- More research on the household-level impacts of famine and contextual knowledge in crisis situations is necessary to understand better the nature, scale, and history of crises and the evolution of different aspects of food security as conditions change during crises.

Dictionary of Terms

- **Bio-physical drivers** – Bio-physical drivers of famine comprise a wide range of natural disasters including climatic change and change in agricultural land use.
- **Socio-economic drivers** – Socio-economic drivers of famine comprise a wide range of economic and sociopolitical causes including economic development, land tenure system, food availability, and food accessibility decline.
- **Marxist-Leninist** – The Ethiopian Marxist–Leninist Revolutionary Organization, commonly known by its Amharic acronyms Malered or Emelared, was a communist organization in Ethiopia active from 1974 to the late 1970s.
- **El Niño** – is an irregularly occurring and complex series of climatic changes affecting the equatorial Pacific region and beyond every few years, characterized by the appearance of unusually warm, nutrient-poor water off northern Peru and Ecuador, typically in late December.
- **Kebele** – Local administrative unit, comprising about 100,000 people, was introduced during the military ("derg") regime.

Summary Points

- Famine occurrences are linked to drought, climatic change, and change in agricultural land use that have increased the vulnerability of Ethiopian households to hunger over time and reduced the inflexibility to environmental and economic shocks.

- The cause of Ethiopia's recurrent famine is not only natural interference, but it is also the reaction of society and governments that create a famine.
- Famines and food shortages in Ethiopia are linked to persistent vulnerabilities, which are often the result of bio-physical and socio-economic processes that limit the options and opportunities of households.
- Famine has its roots in the notion of having access to resources especially the distribution and productivity of land and vulnerability of livelihoods to shocks especially climate change.
- Economic development, land tenure system, food availability decline, food accessibility decline, the right to food, and the political setting are the major socio-economic determinants of famine in Ethiopia.

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Addressing Child Malnutrition in India

7

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Abstract

This chapter discusses the role of malnutrition in children under the age of 5 growing up in India. Malnutrition is an ongoing public health challenge in children around the world and specifically within this subcontinent. Malnutrition includes both undernutrition and obesity. Some common forms of undernutrition include stunting, wasting, and micronutrient deficiencies. In an effort to help address undernutrition, several studies have examined various contributing factors. Some of the challenges in addressing undernutrition have been: regional differences in the burden and determinants, rapid urbanization, and the social and economic status of families/individuals. In addition, the consequences of ongoing undernutrition in Indian children are severe. Some of the risks associated with undernutrition include: mortality, morbidity, impaired growth and development, and reduced economic productivity. Thus, addressing the biological and social risk factors contributing to poor nutrition in children under age 5 in India is a critical imperative for achieving optimal health and development of its growing population.

Keywords

Nutrition · Malnutrition · Child nutrition · India · Stunting · Wasting · Micronutrients · Mortality · Morbidity · Risk · Growth · Development · Economic · Inequality

List of Abbreviations

AHS	Annual health survey
LBW	Low birth weight
MDG	Millennium Development Goals
NCD	Noncommunicable disease
NFHS	National Family Health Survey
WHO	World Health Organization

Definitions of Words and Terms

1. Stunting: Being at a length/height below that of one's age group (minus two standard deviations from the median length for age of reference based on the WHO Child Growth Standards median).
2. Wasting: Not meeting weight expectations based on height (minus two standard deviations from the median height of reference population based on the WHO Child Growth Standards median).
3. Underweight: Not meeting weight expectations based on age. Moderate and severe underweight is being below minus two standard deviations from the median weight for age of reference population. Severe underweight is being below minus three standard deviations from median weight for age of reference population.
4. Micronutrient: A substance or chemical needed in small amounts to meet the growth and development needs of an individual.
5. Low birth weight: Being at a weight below 2,500 g or 5.5 pounds at birth.

Global Overview of Malnutrition Epidemiology, Determinants, and Consequences

Epidemiology and the Burden of Malnutrition

Malnutrition, which includes undernutrition and obesity, is one of the most widespread challenges experienced by children and adolescents worldwide (Black et al. 2013). Almost half of the deaths occurring among children under 5 years of age can be attributed to undernutrition (Ahmed et al. 2012; Black et al. 2013; UNICEF 2015). Two of the most commonly recognized forms of undernutrition are stunting (chronic, intergenerational undernutrition) and wasting (acute undernutrition). Micronutrient deficiencies are the third major form of undernutrition and are themselves a major contributor to malnutrition and possibly stunting among children. Inadequate vitamin and mineral intake can lead to deficiencies and increased susceptibility to disease. In effect, micronutrient deficiencies may also be associated with impaired fetal development, subsequent stunting, and other forms of undernutrition. Not meeting the nutrient needs of children is an indicator of the health of the population and future populations (Black et al. 2008, 2013; Ahmed et al. 2012). Unlike the prevalence of undernutrition, which has steadily decreased over the past two decades, albeit at an exceedingly slow rate, overnutrition is a rapidly growing epidemic that affects high-, middle-, and low-income countries. Therefore, many low-and-middle income countries are beginning to experience a double burden of malnutrition (Black et al. 2008, 2013; Ahmed et al. 2012; UNICEF 2015).

Stunting and other forms of undernutrition can reduce a child's chance of survival, increase the risk of infection, and hinder optimal health and growth. Stunting is also associated with suboptimal brain development, which can have long-lasting harmful consequences on a child's cognitive ability, school performance, future earning potential, and, consequently, the economic productivity of a nation (Black and Dewey 2014; IIPS 2017; John et al. 2017). Stunting in children under the age of 5 is estimated to affect as many as 178 million children worldwide. A group of 36 countries account for 90% of these cases, which is approximately 160 million children (Black et al. 2008). As of 2011, India alone accounts for 38% of stunted children under the age of 5 worldwide (UNICEF 2015).

Acute forms of undernutrition, such as moderate or severe wasting, afflict over 52 million children under-5 globally (Black et al. 2013). The prevalence of wasting in South Asia is also among the highest and is estimated to be 16%, which is one in six children. In fact, India also has the highest burden of wasting globally with approximately 25 million children wasted (UNICEF 2015).

Undernutrition and micronutrient deficiencies potentially account for 3.1 million deaths per year (Black et al. 2013). Some common micronutrient deficiencies present in low- and middle-income countries include: vitamin A, iron, iodine, zinc, and folic acid. Vitamin A deficiency in preschool-aged children is as high as 1 in 3 globally (UNICEF 2015). Subclinical vitamin A deficiency affects 90 million children under-5 worldwide, and 5–17 million children have night blindness due to this deficiency (WHO estimates (1995–2005)) (Black et al. 2013). The prevalence was found to be highest within Africa and Southeast Asia (Black et al. 2013;

IIPS 2017). Anemia due to inadequate oral intake of iron can also impede growth and development in children. Global estimates from 2011 for iron deficiency anemia due to inadequate iron consumption were as high as 18.1% for children under 5 years (Black et al. 2013). In India, The National Family Health Survey 2005–2006 indicated that 69.4% of Indian children 6–59 months were anemic and the most recent NFHS-4 data shows that 58.4% of children are currently anemic (IIPS 2017). However, the prevalence of iodine deficiency has decreased widely through the implementation of fortified salt in diets from 2003 to 2011. Although zinc deficiency is estimated to affect 17% of the population worldwide, only limited data is available for children in many regions. The prevalence of zinc deficiency is believed to be highest among children in Asia and Africa (Black et al. 2013).

Fetal growth and nutritional status at birth is an important contributor to childhood malnutrition. Globally, the incidence of low birth weight (LBW) in infants was estimated to be 15%, which means approximately 20 million children are born small (UNICEF 2015). Currently, the World Health Assembly has set out to decrease the frequency of LBW to below 30% between 2010 and 2025. India alone makes up a third of the global burden with recent estimates indicating 28% of children are born small (UNICEF 2015, 2016). Alongside suboptimal weight at birth, inadequate weight gain in children can increase the risk of infection and mortality. Worldwide estimates from 2011 indicate that as many as 101 million children were underweight. The highest prevalence of underweight children was in South-Central Asia (30%) (Black et al. 2013). Data collected in the NFHS-4 indicates that 35.7% of children in India were underweight between 2015 and 2016 (IIPS 2017). Decreasing the prevalence of underweight children was a part of Millennium Development Goal (MDG) 1 on reducing poverty. Worldwide, only 30 countries met this indicator. However, progress could be blurred by the higher prevalence of overweight children and sustained presence of stunting in children growing up in these regions within the past two decades (UNICEF 2015). The burden of overweight children under the age of 5 has grown from 28 to 42 million between 1990 and 2011. This proportion in 2011 was estimated to be 69% in low- and middle-income countries alone. Of these children, seven million are believed to be from East Asia (UNICEF 2015).

India, the Epicenter of Child Malnutrition

Historical Trends and Present Scenario

Efforts to track maternal and child health in India have focused on gathering data on the following: fertility, mortality, family planning, maternal and child health, as well as policy instruments to address countrywide nutrition and health care. Subnational data on these public health concerns have been collected using the following surveys: National Family Health Survey (NFHS), the District Level Household Survey (DLHS), and the Annual Health Survey (AHS). Specifically, NFHS provides data on national and subnational trends in child malnutrition within India in addition to a range of additional information. This ongoing national cross-sectional survey

allows us to examine the high prevalence of child malnutrition since 1992 and has had four rounds (1992–1993 (NFHS-1), 1998–1999 (NFHS-2), 2005–2006 (NFHS-3), and 2015–2016 (NFHS-4) (IIPS 1995, 2000, 2007, 2017; Pathak and Singh 2011). Data collected by NFHS includes, but is not limited to, information on state and national level estimates for nutrition, health, and healthcare (Pathak and Singh 2011). NFHS surveys indicate that the overall prevalence of malnutrition in Indian children under-5 has declined during the first two rounds of the survey, has remained stagnant during the third round, and has further declined between 2006 and 2016 (NFHS-4) (Singh et al. 2011; IIPS 2017). Three important nutritional indices that affect children's susceptibility to disease and survival are exemplified in the graph below (Fig. 1).

The data from 1992 to 2016 shows that there has been a steady decrease in stunting (58–38.4%) (IIPS 2000, 2007, 2017). Some factors that may have contributed to this decline include rapid economic growth, provision of national primary health care, and implementation of preventative nutrition programs such as Integrated Child Development Services Scheme and the National Rural Health Mission (Pathak and Singh 2011). Data on wasting for the first two rounds was not available for NFHS-1 and was only collected on children under the age of 3 years for NFHS-2 (IIPS 2000). However, NFHS-1 indicates that one in six children in India were wasted at the time of the survey (1992–1993) (IIPS 1995). Moreover, NFHS-2 indicates that approximately 16% of children under the age of 3 were wasted (IIPS 2000). In addition, during the last two rounds of NFHS, wasting has increased (19.8–21.0%) suggesting that wasting has been a persistent problem for children under-5 within this subcontinent over the past few decades (IIPS 2007, 2017). Underweight status in Indian children under-5 showed the greatest decline between NHFS-3 and NFHS-4 (IIPS 2007, 2017). Significant declines also occurred in the

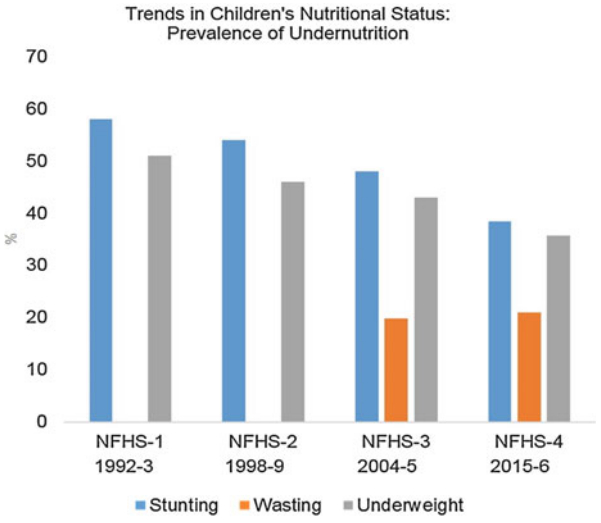

Fig. 1 Prevalence of undernutrition. Data from NFHS for percentage of children under the age of 5 who are malnourished in India from 1992 to 2016. Key: NFHS=National Family Health Survey (please note that NFHS 1 and 2 had focused on children under 3 and 4 years of age, respectively and hence the trends may not be exactly comparable)

first two rounds of the NFHS, which has often been attributed to the rapid economic growth in the 1990s (Kumar et al. 2015).

Regional differences in child malnutrition are evident from 1992 to 1998 and from 1998 to 2006. During 1992–1993, 50–60% of children in central, eastern, and western regions of India were found to be malnourished (Pathak and Singh 2011). Small declines in the prevalence of malnutrition were noted from 1992 to 1998 within different regions. In this timeframe, declines in child malnutrition were observed in most of the southern, northern, eastern, and northeastern states. During 1998–2006, several states within the western, central, eastern, and northern portions of India made significant progress towards reducing the burden of underweight children from 50–59% to 40–49%. These findings are significant as national averages from this timeframe did not depict any of these major shifts in child malnutrition (Pathak and Singh 2011). For example, certain parts of India such as the central and eastern regions have had minimal reductions in child malnutrition since 1992–2006 (IIPS 2007; Pathak and Singh 2011). Between 2006 and 2016, states progressed in remarkably different ways in stunting reduction. Analyses of publicly available summary data from the NFHS-4 (Menon et al. 2017) indicate that most states in India progressed faster than the national average, which is affected strongly by larger states with high prevalence, large under-5 populations, and slow progress. Notable progress in stunting reduction was seen in states such as Chhattisgarh, Arunachal Pradesh, and Gujarat; the limited progress in populous states like Uttar Pradesh and Bihar contributes to India's overall burden (Fig. 2).

An important opportunity offered by the NFHS-4 survey is the ability to examine the spatial distribution of malnutrition by district and not just by state. The NFHS-4 focus on district representativeness now allows national- and state-level attention to the districts that contribute the most to the high burden of malnutrition. It is notable, however, that the districts that face a high stunting burden are not the same as districts that face high wasting burdens.

Rapid urbanization in India over the past few decades is likely to pose a challenge to addressing nutritional status in children. Factors such as poor living conditions, income constraints, and higher costs of food are likely to be significant risks looking forward. The prevalence of stunting, wasting, and underweight status has mostly declined from 1992 to 2016 for urban children in India but this is not necessarily seen in their rural counterparts. Data from NFHS indicates a decline in the prevalence of stunting in children in urban areas from 40% to 36% to 31% in the first three rounds of this survey (Kumar et al. 2015). In NFHS-4, the prevalence of stunting remained at 31% indicating almost no change in stunting in urban children from 2005 to 2016 (IIPS 2017). By comparison, stunting in rural areas from 2005 to 2006 showed a greater decline (IIPS 2007). The prevalence of underweight children in urban India declined from 44%, 38%, and 37% to 29.1%, while declines in rural areas were comparatively lower (Kumar et al. 2015; IIPS 2017). In addition, current statistics from the NFHS-4 indicate that the percentage of underweight children is higher in rural areas (38.3%) than urban areas (29.1%) (IIPS 2017). Wasting had declined from 18% to 13% in the first two rounds of NFHS before increasing to 17% in the third round and 20% in the fourth (Kumar et al. 2015;

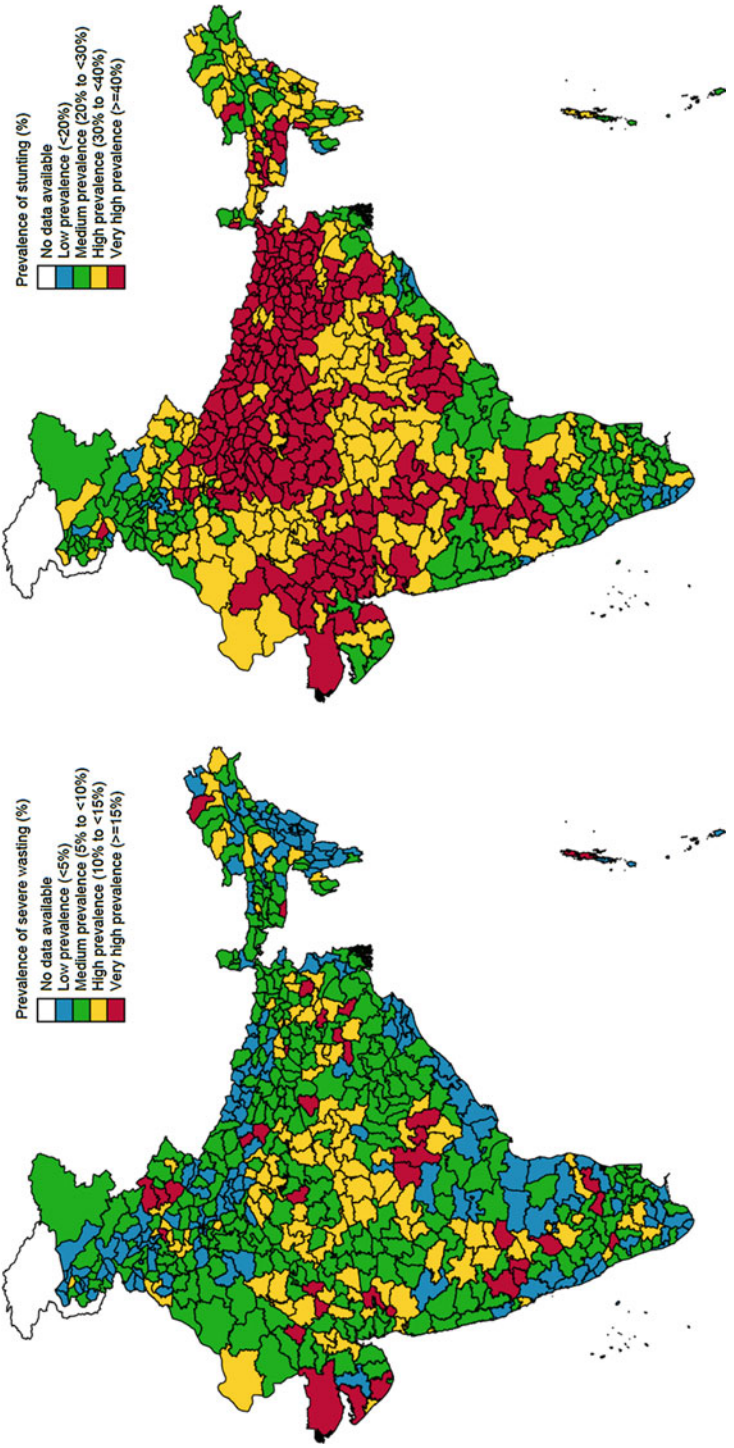

Fig. 2 **Subnational patterns of wasting and stunting for India** (Menon et al. 2017)

IIPS 2017). These increases may be attributed to population migration and urban slums (IIPS 2007, 2017). Similarly, there are trend data on linear growth and stunting from the baseline and final surveys of National Nutrition Monitoring Bureau from 1975–1979 ($N = 6043$) to 2012–2013 ($N = 11{,}910$) on anthropometry from birth to 18 years from seven states in households of rural India (Mamidi et al. 2016). The increments in height of 18+ were highest for both boys (7.4 cm) and girls (4.8 cm) in the state of Kerala followed by Tamil Nadu (boys, 7.3 cm and girls, 3.8 cm). Under-5 stunting rate reduced from 82% in the baseline survey to 45.7% in the final survey at 1.35% per year in the pooled states and was similar in both boys and girls. The recovery in stunting was highest in Tamil Nadu (1.63% per year) followed by Kerala (1.46% per year) (Mamidi et al. 2016).

Determinants of Malnutrition in India

The determinants of malnutrition among children range from immediate determinants like poor child feeding and childhood illness to underlying determinants such as the health, economic and educational status of women, the economic status of households, and sanitation and food environments (Black et al. 2013). In India, these determinants play out in different ways across the country. In recent years, several studies based on the NFHS and other data sets from India have focused on identifying the major determinants of poor nutrition. Studies have focused on the role of infant and young child feeding, especially complementary feeding (Menon et al. 2014), on sanitation (Hammer and Spears 2016), on gender and women's roles (Bose 2011; Coffey et al. 2017), and on the role of income and growth (Subramanian et al. 2016). Over two decades ago, Ramalingaswami and colleagues had identified the important role that female empowerment had played in determining high rates of low birth weight in India (Ramalingaswami et al. 1996). These associations of malnutrition with women's living conditions and empowerment have been underscored in a range of studies from various settings in India (Sethuraman et al. 2006; Ghosh-Jerath et al. 2013; Kshatriya and Acharya 2016), and the impact of various grass root nutrition education interventions at impacting outcomes has also been shown to affect women's empowerment and participation (Kadiyala et al. 2016).

The data from NFHS-4 suggest an uneven spread of these determinants across the country, as well as uneven change in several determinants over time and by geography (Menon et al. 2017). Among the determinants of most concern are complementary feeding, and issues surrounding the status and health of women (early marriage, poor women's nutrition, uneven and inadequate access to antenatal care during pregnancy), and sanitation. Indeed, there is now an increasing understanding (also articulated in India's new nutrition strategy) that action on malnutrition must address these multiple determinants in a localized fashion – district-by-district and state-by-state (NITI Aayog 2017).

Poor complementary feeding has been recognized as an important correlate of undernutrition in India. Patel et al. (2012) reviewed data on 15,028 last-born children aged 6–23 months from the National Family Health Survey 2005–2006.

They examined inappropriate complementary feeding indicators against a set of child, parental, household, health service, and community-level characteristics. The prevalence of timely introduction of complementary feeding among infants aged 6–8 months was 55%. Among children aged 6–23 months, minimum dietary diversity rate was 15.2%, minimum meal frequency was 41.5%, and minimum acceptable diet was 9.2%. Children in northern and western geographical regions of India had higher odds for inappropriate complementary feeding indicators than in other geographical regions. Richest households were less likely to delay introduction of complementary foods than other households. Other determinants of not meeting minimum dietary diversity and minimum acceptable diet were: no maternal education, lower maternal body mass index (BMI) (<18.5 kg/m(2)), lower wealth index, less frequent (<7) antenatal clinic visits, lack of postnatal visits, and poor exposure to media (Patel et al. 2012).

Economic status of households is a major determinant of children's nutritional status as indicated by differences between wealth quintiles in NFHS surveys over the last few decades where the rate of malnutrition has been shown to be higher among the poorest quintiles (Pathak and Singh 2011; Kumar et al. 2015). Although the national prevalence of child malnutrition has declined from 1992 to 2006, inequality has grown and children from the poorest wealth quintile do not seem to have fared as well as their counterparts in wealthier quintiles. Child malnutrition in the poorest wealth quintile has declined by an average of 6% compared to an average 27% reduction among the wealthier quintiles – a gap that indicates widening economic inequality with respect to child malnutrition. In addition, the poor-rich ratio increased from 1992 to 1998 (1:8 to 2:4) and remained at 2:4 ratio from 1998 to 2006. These wide ratios indicate that there is a significant income gap and infer that food security inequalities remain in India (Pathak and Singh 2011).

Consequences of Malnutrition

Undernutrition impacts children's survival, susceptibility to disease, physical and cognitive development, as well as the country's economic productivity. Although, the prevalence of undernutrition has decreased over the past decade, it remains a persistent problem in India. The impact of undernutrition can be divided into short- and long-term consequences. In the short-term, children are at increased risk for mortality, morbidity, and disability. In the long-term, children do not develop to their full potential. Thus, they may become stunted, have impaired cognition, and women may have increased risk for poor pregnancy outcomes. Poor cognitive development can lead to poor school performance, less schooling, and reduced economic productivity and earning potential. Additionally, these children are at increased risk of becoming overweight and developing noncommunicable diseases (NCDs), such as hypertension and cardiovascular disease (UNICEF 2015). Thus, it is important to address the nutrient needs of children within the first 1,000 days of life as many of these risk factors can be potentially irreversible (Black et al. 2013; UNICEF 2015).

In the context of India, the relationship of early childhood and adolescent nutrition with long-term outcomes and the risks of NCDs has been underscored in several settings with gradual equalization between urban and rural populations (Tripathy et al. 2016). These risks have been specifically underscored among poor adolescents in South India (Panuganti et al. 2017) and have also been the subject of food policy analysis for reducing the double burden of malnutrition (Thow et al. 2016).

Mortality

Under-5 mortality in children worldwide was 6.9 million in 2011. One-third of these deaths were related to undernutrition (Bhutta and Salam 2012). Almost half of these deaths were found in India, Pakistan, China, Nigeria, and the Democratic Republic of Congo. Most of these deaths were preventable. In 2015, over 5.9 million children died before their fifth birthday. The majority of these deaths occurred within Sub-Saharan Africa and South Asia (Liu et al. 2016). The most current national statistics from India (NFHS-4) estimates that the rate of mortality for children under the age of 5 to be as high as 50% (IIPS 2017).

The most common causes of mortality among children growing up in India are: pneumonia, preterm birth complications, diarrhea, intrapartum-related complications, and malaria. Targeting the main causes of under-5 mortality includes treating disease states and taking measures to promote healthy pregnancies in expectant mothers. Addressing the underlying risk factors of mortality such as undernutrition is key to helping reduce mortality rates in children under-5 in India (Caulfield et al. 2004).

Morbidity

Children who are stunted, underweight, or wasted have impaired immunity and are at increased risk for infectious diseases including measles, pneumonia, meningitis, and malaria (Black et al. 2008). Infection, in turn, further aggravates undernutrition – a cycle that increases the likelihood of mortality for a child (Ramachandran and Gopalan 2009; Ahmed et al. 2012). Immune system impairment is particularly problematic when an undernourished child suffers from repeated infections and is unable to meet their nutrient needs as repeated exposure can have a negative impact on a child's ability to meet their growth needs for their age or even regain a normal growth pattern at a later stage. Persistent exposure to infection can even lead to death (Ahmed et al. 2012). In addition, many infections such as measles or helminthic infection increase a body's demand for proteins and calories (Black et al. 2008; Ahmed et al. 2012). The increased macronutrient demand only further impairs growth and development in malnourished children as well as their susceptibility to other infections.

In particular, malnourished children are at increased risk for developing diarrhea which can further affect the nutritional status of a child. Persistent diarrhea can damage the lining of the small intestines and negatively impact absorption and

utilization of nutrients needed for healthy development and growth in undernourished children. As this process persists, the infected child becomes increasingly malnourished and increasingly susceptible to other diseases as even deficiencies in micronutrients such as, iron, vitamin A, zinc, and iodine can have serious consequences. Iron is needed for hemoglobin synthesis, oxygen delivery to tissues, brain and motor development, and protection against infections like malaria (IIPS 2007; Ahmed et al. 2012; John et al. 2017). A diet low in vitamin A can increase susceptibility to infection, cause preventable diseases, such as measles and diarrheal diseases, and slow one's ability to heal from illness (IIPS 2007; Ahmed et al. 2012; Black et al. 2013). Severe deficiency in vitamin A can also lead to permanent eye damage. Iodine deficiency can impair psychomotor development, speech, and hearing. It also decreases the amount of energy available to developing children and can cause goiter and mental retardation, both of which are preventable (IIPS 2007; Ahmed et al. 2012; John et al. 2017). Other micronutrient deficiencies such as zinc can lead to increased risk of diarrhea, pneumonia, malaria, dermatitis, retarded growth, mental disturbance, delayed sexual maturation, and recurrent infections (Ahmed et al. 2012). In light of all this, NHFS data indicating 58% of Indian children are anemic (NHFS-4) and almost 71 million people suffer from iodine deficiency (NFHS-3) is alarming (IIPS 2007, 2017). Not meeting micronutrient needs during critical years of development decreases an individual's immune response and decreases linear growth (Black et al. 2013). All of which combined, increases morbidity and mortality (Singh et al. 2011; Bhutta and Salam 2012).

Risks of Noncommunicable Diseases

Alongside the increased risk of infection, undernutrition can lead to inappropriate weight gain and susceptibility to noncommunicable disease. Undernutrition prevents children from meeting their weight- and height-for-age. Normally, weight gains within the first 1000 days of life are associated with the development of adult lean mass. Weight gains after the first 1000 days are associated with the development of adult fat mass. Undernourishment early in life prevents children from meeting their length-for-age and therefore leads to stunted growth. Thus, undernourished children have less lean body mass and shorter stature (Black et al. 2013). Not meeting nutrient needs early in life puts children at increased risk of being overweight later in life (Ahmed et al. 2012; Black et al. 2013). As a consequence to not meeting nutrient needs and carrying excess fat mass, these children have increased susceptibility to noncommunicable diseases, such as diabetes, metabolic syndrome, cardiovascular disease, and obesity (Black et al. 2013; UNICEF 2015).

Obesity in low- and middle-income countries has increased from 4.2% to 6.7% from the early 1990s to 2010 (Black and Dewey 2014). It is expected to continue to increase as children in India still are unable to meet their nutrient needs during critical times of growth and development. In addition, most nutrition interventions are not focused on preventing obesity but instead focus on undernutrition and stunting. Many of these programs target availability, accessibility, and acceptability

of food and nutrients from conception to early childhood. Healthy portion sizes, key nutrients, and healthier food choices are not directly incorporated into integrated interventions - all of which could help prevent and proactively treat overweight/obesity in Indian children (Black and Dewey 2014).

Developmental Outcomes and Economic Productivity

In the first few years of life, children undergo rapid brain development. Development in children can be broken down into several domains: sensory-motor, cognitive, and social-emotional. Poor health, nutrition, and disease can lead to poor brain development in the short- and long-term (Grantham-McGregor et al. 2007; Black and Dewey 2014; Prado and Dewey 2014).

Children develop crucial neuronal connections and have high nutritional needs, which are critical to cognitive development. Not only do these changes occur at a fast rate but continue to build on each other as the child grows. Inability to form these connections impacts the brain's structure and functional capacity. As the child develops, these deficits become more pronounced (Grantham-McGregor et al. 2007; Black and Dewey 2014; Prado and Dewey 2014).

Poor cognitive development also perpetuates the child into a cycle where diminished learning ability leads to poor school achievement, delayed school entry, early school termination, and diminished ability to continue education, which limits employment opportunities and earning potential later in life (Grantham-McGregor et al. 2007; Black and Dewey 2014). Studies from over 51 countries indicate that every year of schooling leads to a 9% difference in wages (Grantham-McGregor et al. 2007). Thus, on a societal level, there is a significant economic cost associated with malnutrition's impact on children's developmental outcomes. Not meeting the nutrient needs of Indian children handicaps a future generation and impacts national development.

Implications for Policies and Interventions

India's malnutrition burden affects the world's malnutrition burden. More importantly, it affects millions of children, who are India's future. In recent years, there has been an increasing understanding of the scale and scope of India's malnutrition burden, and this is reflected in India's new nutrition strategy (NITI Aayog 2017). India's policy environment for nutrition has been robust for many years, however, with large-scale delivery platforms that include most evidence-based nutrition-specific interventions (Avula et al. 2017) and several social welfare programs that address the underlying determinants of malnutrition – including those related to food security, poverty, gender issues, and sanitation. Nevertheless, given the diversity in context and capacity across India, implementation and quality of programs are India's most pressing challenges. Despite improvements in the coverage of both delivery platforms and interventions in the last decade, many gaps persist, especially

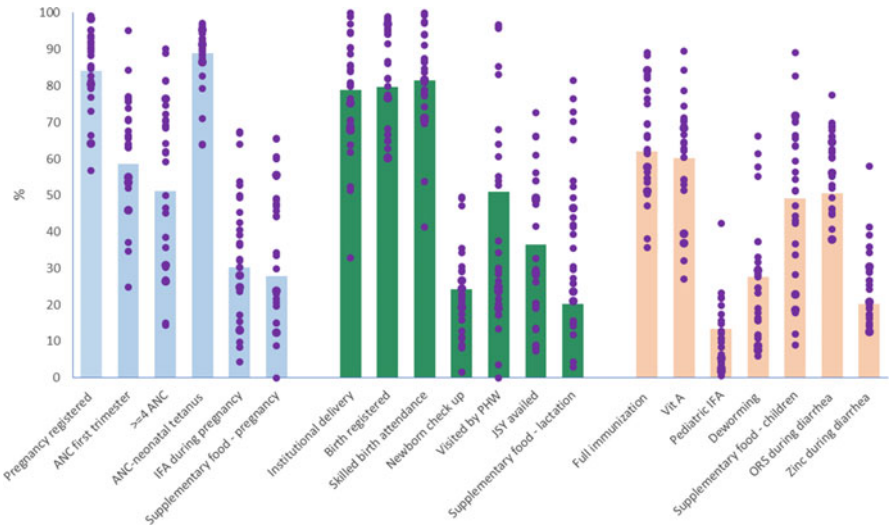

Fig. 3 Comparison of nutrition-specific intervention coverage in India. Data presented as median coverage with each dot representing a state (Menon et al. 2017)

in relation to health and nutrition services for children (Fig. 3), and there are wide differences between states.

What is encouraging is that some poor states (like Odisha and Chhatisgarh) have been exemplars for scaling up health and nutrition services in recent years (Kohli et al. 2017 for Odisha) and thus set the tone for what is possible within India's existing policy instruments. However, much more is needed to address further issues of quality of services and commodities within the health system and the ICDS.

Looking forward, addressing India' nutrition challenges in the future will require a close look at the health and nutritional status of adolescents – both boys and girls. Although these groups are recognized as being vulnerable, as well as offering a window of opportunity to intervene, there is limited data on the health and nutritional status of adolescents, and of the reach of interventions to these groups. Investments will be needed, therefore, to close some of these gaps and to explore the potential of programs like the Midday Meal Scheme to deliver more nutrition interventions to school-based children. In addition, given India's growing challenges of overweight and obesity, engaging and working with school-age children to strengthen healthy diets and physical activity will be essential to secure the health of future generations.

Conclusions

Undernutrition has a significant effect on a child's survival and development. Undernutrition in all of its forms is associated with almost 45% of the current burden of under-5 child deaths (Black et al. 2013). In countries like India, the prevalence of

stunting, wasting, and micronutrient deficiencies is very high. Progress has been made in addressing malnutrition concerns such as stunting; however, many children continue to not meet their nutrient needs for growth and development. Not only is undernutrition substantially high in India, it differs in populations affected throughout the subcontinent. For example, the prevalence of stunting varies according to region, is higher among rural areas compared to urban areas, and disproportionately affects the poorest population quintiles. Risk factors associated with undernutrition can lead to significant consequences for children. Risks include mortality, morbidity, poor growth and development, and economic inequality. This impact is evident in a child's physical development, mental development, educational attainment, and economic productivity later in life. These consequences have a significant impact on affected individuals and families – impacts that persist within families from one generation to the next and leave large numbers of our next generation unable to meet the nation's developmental and workforce needs. Meeting nutrient needs of children within the first 2 years of life is crucial. Meeting nutrient needs not only benefits a person's health and development but has a positive impact on a person's cognitive abilities and economic opportunities later in life. Investing in child nutrition is vital to the health of India and its future generations (Grantham-McGregor et al. 2007).

Summary Points

- There is a particularly high burden of child malnutrition in India.
- 28% of newborns in India are low birth weight.
- 21% of children under-5 have moderate or severe wasting.
- 38% of children under-5 have stunting.
- Micronutrient deficiencies, particularly vitamin A, iron, iodine, zinc, and folic acid, are common and contribute to child malnutrition.
- Malnutrition in children varies according to region and is impacted by rapid urbanization and economic status within households.
- Undernutrition increases the risk of infectious diseases.
- Undernutrition impacts growth and development in children.
- Malnutrition affects the economic productivity of individuals and the growing population in India.
- Despite several programs, rate of progress is relatively slow with much national disparities.

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Part II

Effects of Famine

The Effects of Prenatal Exposure to the Dutch Famine 1944–1945 on Health Across the Lifecourse

8

Tessa J. Roseboom

Contents

Abstract

Poor nutrition during critical periods of early human development has lasting negative consequences for growth, development, and health. This chapter summarizes the evidence from studies investigating the effects of prenatal exposure to the Dutch famine on later mental and physical health. The Dutch famine was remarkable in several ways, and its unique features have contributed to the fact that it has most often been used in studies examining the long-term consequences of prenatal undernutrition. The Dutch famine was an acute period of undernutrition that was clearly circumscribed in time and place; it had an abrupt beginning

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and end and struck a population that was previously and subsequently well nourished. Also, the administration was well organized and records were kept allowing researchers to investigate the consequences of starvation in the decades that followed. All these characteristics make the Dutch famine uniquely suited for such studies, and allow researchers to take a quasi-experimental design to address a question that would otherwise be impossible to answer in a human setting.

The effects of famine depended on its timing during gestation and the organs and tissues undergoing critical periods of development at that time. Early gestation appeared to be the most vulnerable period which may not be surprising considering the fact that all organs are laid down within 12 weeks after fertilization. The effects of famine were widespread and affected structure and function of organs and tissues, resulted in altered behavior and increased disease risks, which in turn led to reduced participation in the labor market and increased mortality. The effects of famine were apparent in the absence of any effects on size at birth. Some effects of prenatal undernutrition were more pronounced or even limited to one sex, but generally the effects applied to both men and women. There is preliminary evidence to suggest that the effects of famine may not to be limited to those affected prenatally, but were passed on to the next generation, both through the maternal and paternal line.

Studies in other settings show similar effects and suggest that the findings are not uniquely linked to the characteristics and setting of the Dutch famine, but reflect biologically fundamental processes that describe human plasticity. We expect that adequately feeding women before and during pregnancy will allow future generations to reach their full potential and lead healthier and more productive lives, ultimately leading to healthier and more equal future.

Keywords

Hunger · Undernutrition · Pregnancy · Fetus · History · Development · Growth · Health · Cardiovascular · Metabolic · Mental · Chronic degenerative diseases · Aging · Plasticity · Global health

List of Abbreviations

BMI	Body mass index
DOHaD	Developmental Origins of Health and Disease
HDL	High density lipoprotein
LDL	Low density lipoprotein
WHO	World Health Organization

Introduction: Hunger in an Age of Plenty

Good nutrition is fundamentally important for maintenance, growth, development, reproduction, and health. Poor diets, due to insufficient, imbalanced, or excessive intakes of nutrients, can impair growth and development and induce disease.

Today the world faces a double burden of malnutrition that includes both undernutrition and overnutrition. Never before in history have there been so many people with hunger worldwide. It is estimated that nearly one billion people worldwide go hungry each day. While at the same time more than one billion people are overweight or obese. Malnutrition – in any form – poses threats to health. Counterintuitive as it may seem, both undernutrition and overnutrition are reducing the health of millions of individuals around the globe. Hunger and inadequate nutrition contribute to early deaths for mothers, infants, and young children and account for more losses of life than AIDS, malaria, and tuberculosis combined (WHO nutrition facts). Deficiencies in the diets of children can impair growth and development and lead to stunting and wasting in childhood, while deficiencies in adulthood can lead to blindness, scurvy, or anemia. Such cases are most often seen in developing countries, while in developed countries the consequences of imbalanced or excessive diets lead to obesity, diabetes, and cardiovascular diseases.

The evidence presented in this chapter indicates that the consequences of undernutrition may not be limited to the individuals suffering from undernutrition, but that the offspring, especially if they have been exposed to undernutrition while in their mothers' womb may be negatively affected as well. These consequences are apparent throughout the lifecourse and might even extend into the following generation. Similarly, imbalances in prenatal nutrition caused by maternal obesity and gestational diabetes are known to negatively affect offspring's health.

The Long-Term Consequences of Poor Diets in Early Life

Studies across the world have consistently shown that babies who were small at birth have increased rates of chronic degenerative diseases. These associations cannot be explained by prematurity, but rather reflect variations in early growth to be associated with later disease risk. This led to the hypothesis that a limited supply of nutrients to the developing fetus leads to adaptations that increase its chances of survival in the short term but increase its risk of disease in later life. This is thought to reflect developmental programming: early environmental cues induce anatomical, physiological, and biochemical changes that have lasting consequences for the structure and function of organs and tissues and permanently affect the physiology. Although the adaptations may be beneficial for short-term survival, the adaptations may come at a cost in later life, especially if the environment into which the individual is born is very different from the environment in which the individual developed. The greater the degree of mismatch between the prenatal environment and the environment in adult life, the greater the disease risk associated with it. This hypothesis is known as the Developmental Origins of Health and Disease hypothesis (DOHaD).

The Dutch Famine as a Model to Test the DOHaD Hypothesis

The DOHAD hypothesis suggests that undernutrition in utero permanently changes the body's structure, function, and metabolism in ways that lead to chronic degenerative disease in later life. The hypothesis was formulated based on epidemiological studies, which have subsequently been confirmed in populations across the world. These observational studies in humans consistently showed that small size at birth was linked to greater disease risk in later life. Small size at birth was taken as an indication of reduced fetal growth due to limited supply of nutrients to the fetus. Animal studies have experimentally shown that restriction of fetal nutrition indeed induces adaptations that lead to altered structure and function of organs, increased rates of disease, and shortened lifespan. But the experimental evidence for the DOHAD hypothesis in humans is impossible to obtain.

While famine is sadly not uncommon in many parts of the world, studying effects of undernutrition during pregnancy is hampered by the fact that undernutrition is usually not restricted to pregnancy alone, and effects of chronic undernutrition and accompanying problems of infection complicate the situation. The tragic circumstances of the Dutch famine of 1944–1945 created a unique opportunity to assess the effects of prenatal famine exposure on health in later life. The Dutch famine has been used by various investigators as an equivalent to an experimental setup to investigate the effects of prenatal undernutrition in humans. What is unusual about the Dutch famine is that the famine was imposed on a previously well-nourished population; there was a sudden onset and relief from the famine; and, despite the adversities of the war, midwives and doctors continued to offer professional obstetric care and kept detailed records of the course of pregnancy, the delivery, and the size and health of the baby at birth. Furthermore, detailed information is available on the weekly rations provided during the famine, and in several inflicted cities birth records were kept which allowed researchers to trace those born around the time of the famine and thus to study the long-term effects of prenatal famine exposure (Figs. 1 and 2).

The Historical Course of Events That Led to the Dutch Famine 1944–1945

After the invasion of the Allied forces on the 6th of June 1944, a few weeks of heavy fights followed. Then, the Allied forces finally broke through German lines. Quickly, the Allied troops took possession of France, Luxembourg, and Belgium. By the 4th of September 1944 the Allies had the strategic city of Antwerp in their hands, and on the 14th they entered the Netherlands. The Dutch expected that the German occupation would soon be over, and so did the commanders of the Allied forces. Hoping to capture strategic bridges across the river Rhine to open a pathway for rapid invasion into Germany, the Allied forces launched a parachute attack behind the Nazi forces near the city of Arnhem. However, the operation failed with major losses. Operation Market Garden had failed. Subsequently, the Dutch government called for a strike of the Dutch railways in order to support the Allied offensive. As a

Fig. 1 Famine pamphlet 1944 ("Famine in Holland")

Fig. 2 Ration provided to adults in Amsterdam, the Netherlands April 1944, consisting of 2 slices of bread, 2 potatoes, and half a sugar beet

reprisal, the Germans banned all food transports. The food situation in the western part of the Netherlands worsened dramatically. Food stocks ran out rapidly, and soon rations for adults dropped to below 1000 calories a day. The embargo on food transports was lifted in early November 1944, when food transport across water was permitted again. But because most canals and waterways were frozen due to the early and extremely severe winter, it had become impossible to bring in food from

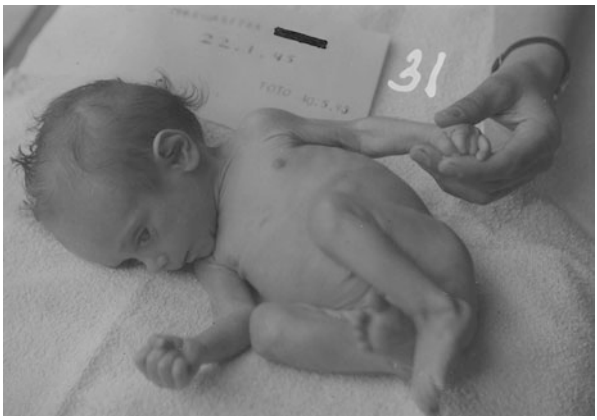


Fig. 3 Baby born in Wilhelmina Gasthuis during the Dutch famine 1944–1945

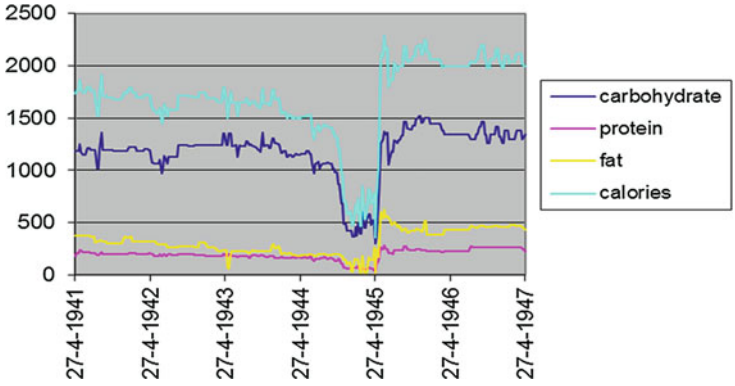
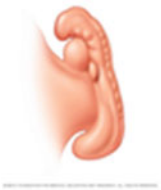
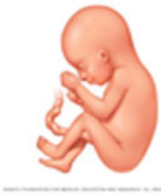
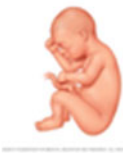


Fig. 4 Official rations for adults in Amsterdam over time

the rural east to the urban west of the Netherlands. Food rations declined to extremely low levels between February and May 1945, with daily rations varying between 400 and 800 calories a day. During the famine, infants were relatively protected, because their official daily rations never fell below 1000 calories. Pregnant and lactating women were entitled to an extra amount of food, but at the peak of the famine these extra supplies could not be provided anymore. Also, extra food came from the black market, central kitchens, church organizations, and foraging trips to the countryside. The period of famine ceased in early May 1945 immediately after the final surrender of the Germans. The food situation quickly improved and within a month rations were above 2000 calories (Figs. 3 and 4).

In addition to the immediate provision of food after the war, medical aid was a top priority for the Netherlands. The famine had a profound effect on the general health of the population. In Amsterdam, the mortality rate in 1945 had more than doubled compared to 1939, and it is very likely that most of this increase in mortality was attributable to undernutrition. Doctors from the UK and USA were sent to survey

early	mid	late
glucose tolerance	glucose tolerance	glucose tolerance
Perceived health	obstructive airways disease	
cardiovascular disease	microalbuminurea	
breast cancer		
stress responsiveness		
depression		
schizophrenia		
addiction		
food preference		
cognition		
participation labour market		
mortality		

Fig. 5 The effects of prenatal exposure to famine depend on its timing during gestation and the organs and tissues developing at that time

medical needs. Clement Smith from Harvard Medical School was among the first to witness the effects of the famine on the health of the Dutch population. He immediately saw the opportunity to obtain information that would help resolve important questions on how poor maternal nutrition affects pregnancy and the development of the fetus before birth. Using obstetric records from Rotterdam and The Hague, he studied effects of prenatal exposure to famine on pregnancy and the fetus which he described in his paper "*The effect of famine on pregnancy and its product*" (Smith et al. 1947). This paper describes that babies born during the famine were lighter at birth.

Since the Dutch famine lasted 5–6 months (from late November 1944 until early May 1945), investigators have been able to not only assess effects of prenatal undernutrition per se but also to differentiate between effects of undernutrition according to its timing during gestation and the organs and tissues developing at that time. Although the exact definitions differ between studies, all studies assessing effects of prenatal famine exposure have differentiated between effects of famine in early, mid, or late gestation (Fig. 5).

In general, the Dutch famine studies have provided support for the fetal origins hypothesis. Studies in different cohorts and with different setups have shown differences in outcomes between those who had been exposed to the Dutch famine during gestation and those who had not been exposed to famine. The effects of famine depend on its timing during gestation and the organs and tissues developing at that time.

Consequences of Prenatal Famine Exposure

In general, the Dutch famine studies have provided support for the fetal origins hypothesis. Studies in different cohorts and with different setups have shown that those who had been exposed to the Dutch famine during any stage of gestation were found to have raised glucose levels as adults. One study found increased blood pressures and rates of hypertension among those who had been exposed to famine during any stage of gestation (Stein et al. 2006), while another study could not confirm effects of famine on blood pressure (Roseboom et al. 1999). Glucose problems and diabetes are more common among individuals in their 1950s who were exposed, for any gestational stage of exposure.

Early Gestation

Studies on the long-term consequences of prenatal exposure to famine on later health started with the landmark studies of Stein and Susser performed in the early 1970s following the increasing awareness in the late 1960s that early nutritional deprivation might cause irreversible damage to the brain (Stein and Susser). This study among military conscripts did not demonstrate any effect of undernutrition during gestation on the adult mental performance. Those exposed in early gestation had a twofold increase in risk of schizophrenia (Neugebauer et al. 1999) and antisocial personality disorder (Hoek et al. 1996), as well as increased rates of addiction in later life (Franzek et al. 2008). Prenatal famine exposure has also been associated with affective psychoses and depression, though not all studies replicated this finding (Stein et al. 2007; de Rooij et al. 2008). Some studies have found indications of effects of exposure to famine in early gestation on cognition (De Rooij et al. 2010) though other studies did not detect such effects (Stein and Susser; Stein et al.). Imaging studies of the brains have shown lasting effects of famine exposure on brain size and structure (De Rooij et al. 2016; Hulshoff Pol et al. 2000). Men who had been exposed to famine in early gestation had smaller intracortical volumes and total brain volumes than unexposed men. They also had smaller volumes of total cortical gray matter, white matter, cerebellar gray matter, thalamus, caudate nuclear, and accumbens area and a large number of more specific cortical white and gray matter areas. The overall reduction in brain size after prenatal famine exposure was ~5%. A decreased intracortocal volume was also reported in a smaller study of schizophrenic patients (Hulshoff Poll et al. 2000), which suggests that prenatal undernutrition has lasting effects on brain size and structure.

Stein and Sussers landmark study found that 19-year-old conscripts exposed to famine in early gestation were more likely to be obese (Ravelli et al. 1976). These effects on obesity were also found in two other studies which examined effects on anthropometry in adulthood (Ravelli et al. 1999; Stein et al. 2007) with the effects being more pronounced among women than men. Women who had been exposed to famine in early gestation had higher BMI and appeared to be more centrally obese than those who had not been exposed to famine prenatally. These differences in

adiposity could at least in part be mediated by differences in food preferences and food intake as there are indications that those exposed to famine in early gestation had higher energy intakes, higher protein intakes, and higher intakes of fat (Lussana et al. 2008; Stein et al. 2009). They also had more atherogenic lipid profiles, with higher LDL and lower HDL cholesterol levels (Roseboom et al. 2000c; Lumey et al. 2009), again the effects being more pronounced in women than in men. There were indications that those exposed to famine in early gestation more often had disturbed blood coagulation (Roseboom et al. 2000b) and were more responsive to stress (Painter et al. 2008). Exposure to famine in early gestation was furthermore associated with increased rates of cardiovascular disease (Roseboom et al. 2000), which occurred at an earlier age (Painter et al. 2006) and led to increased mortality (Ekamper et al. 2014; van Abeelen et al. 2012).

Although based on small numbers, there is evidence to suggest that women exposed to famine in early gestation have increased rates of breast cancer (Painter et al.). Analyses of mortality data suggest that those exposed to famine in early gestation have increased mortality, which is mainly found in women and mostly due to cardiovascular causes and cancer. The famine affected mortality in the short term and also increased childhood mortality. Subsequent studies initially did not detect any effects of famine exposure on mortality before the age of 50 and 58 years (Roseboom et al. 2000; Painter et al. 2006). More recently, studies with longer follow-up have shown that exposure to the Dutch famine in early gestation is associated with increased mortality (Van Abeelen et al. 2012; Ekamper et al. 2014). Uniquely, Ekamper used a large national birth cohort to assess effects on mortality up to age 63 years and found a 12% increase in mortality among those who had been exposed to famine in early gestation.

The effects of famine exposure in early gestation are not limited to health but appear to have economic consequences too. The most striking finding was that the probability of being employed was significantly lower among those who had been exposed to famine in early gestation (Scholte et al. 2015). This result fits with findings of poorer performance on cognitive tasks in men who had been exposed to famine in early gestation. It seems that the effects of famine on employment are at least partly explained by effects on cognition. Mental disorders such as schizophrenia and antisocial personality disorders which were more common after exposure to famine in early gestation may contribute to this, as well as the physical health. It could be argued that the effects of famine exposure on health reduced individual productivity and hence employability.

Mid Gestation

Exposure to famine in mid-gestation was linked to an increase in occurrence of microalbuminurea in adulthood and a decrease in creatinine clearance (Painter et al. 2005). It may be that mid-gestational exposure to famine – the period of rapid increase in nephron number – may prevent formation of sufficient glomeruli and thus increase the risk for microalbuminurea and deteriorated renal function in

adulthood. This supports the concept that intrauterine conditions during distinct, organ-specific periods of sensitivity may permanently determine health outcome in later life. Another example of this phenomenon is the finding in the same study that people who had been exposed to famine in mid-gestation had an increased prevalence of obstructive airways disease (Lopuhaa et al. 2000). These observations were not paralleled by reduced lung function or increased serum concentrations of IgE. This suggests that the increased prevalence of symptoms and disease may be attributable to increased bronchial reactivity rather than to irreversible airflow obstruction or atopic disease. Because the bronchial tree grows most rapidly in mid-gestation, our findings support the hypothesis that fetal undernutrition permanently affects the structure and physiology of the airways during "critical periods" of development that coincide with periods of rapid growth.

Late Gestation

One study found an association between prenatal exposure to famine and later hypertension,[22]

which may be due to an insulin secretion defect.[16]

These findings provide further evidence that the timing of exposure in relation to the stage of pregnancy may be of critical importance for determining health outcomes.

Epigenetics

The Dutch famine families study provided the first direct evidence for epigenetic programming through prenatal famine exposure. Men and women who had been exposed to the famine periconceptionally had less methylation of the IGF2 gene compared with their unexposed same-sex siblings (Heijmans et al. 2008). Further studies from the same group provided additional evidence that the early gestation period is a critical time window during which the prenatal environment may affect the human methylome, with direct evidence for effects in blood, which may suggest that other tissues are effected as well (Tobi et al. 2009). The functional implications of these findings need further exploration. The studies do suggest that the effects of famine exposure on methylation are specific – i.e., are present in some genes but absent in others. The mechanisms underlying DNA methylation differences from prenatal exposure await elucidation but the observed timing-specific associations in blood may hint at an intrinsic sensitivity of newly developing tissues.

Conclusions

Findings from the Dutch famine studies – despite slight differences in individual findings between the different studies – in general suggest that maternal nutrition before and during pregnancy play an important role in later disease susceptibility.

They have shown that maternal undernutrition during gestation has lasting negative consequences for the offspring's health. Many chronic diseases that plague our society may originate in the womb. The effects seem to be large and depend on the timing during gestation and the organs and tissues developing at that time. Also, the effects are independent of the size of the baby at birth. Most notably, those exposed to famine in early gestation did not have lower birth weights than those who were not exposed to famine prenatally, but did have worst health outcomes as adults. This may imply that adaptations that enable the fetus to continue to grow may nevertheless have adverse consequences for health in later life. Chronic degenerative disease may be viewed as the price paid for adaptations made to an adverse intrauterine environment.

These findings confirm experimental evidence from studies in animals showing that undernutrition during gestation permanently affects the structure and function of organs, thereby affecting behavior as well as disease risk, and ultimately shortening lifespan (Ozanne et al. 2004). Moreover, the findings from the studies of the long-term consequences of the Dutch famine have now been replicated in other settings in which the effects of famine have been examined. Studies in other settings of famines with different durations and severity affecting different populations support these findings and suggest that the results of studies on the Dutch famine are not uniquely linked to the characteristics and setting of the Dutch famine, but rather reflect biologically fundamental processes that describe human plasticity . A study in Nigeria showed that prenatal undernutrition also affects later health in African populations (Hult et al. 2010). People who had been exposed to the Biafran famine during the Nigerian civil war (1967–1970) in utero were found that have increased rates of hypertension and type 2 diabetes at the age of 40 compared to those who had not been exposed to the Biafran famine in utero. Similarly, studies of people exposed to the Great Leap Forward famine in China have shown similar effects of prenatal famine exposure in later life risk of diabetes, hypertension, and schizophrenia. Undernutrition in early life contributes significantly to the increasing prevalence of hypertension and glucose intolerance. Therefore, prevention of fetal and infant undernutrition should be given high priority in national health, education, and economic agendas to limit the increase of noncommunicable diseases in many developing countries.

Policies and Protocols

The knowledge presented here has been taken up into policy actions.

Evidence for the importance of early nutrition for later health has come from many cohort studies across the globe. Pooled analyses of several cohorts from low and middle income countries have shown that not only are babies who were larger at birth more likely to be healthy, they are also more likely to complete secondary school (Adair et al. 2013). These findings have clear implications for public health policy and nutrition interventions. A focus on improvement in nutrition in pregnancy and the first 2 years after birth could lead to substantial reductions in stunting and

improved survival (Bhutta et al. 2008). These improvements form the basis for the emphasis on the first 1000 days of life. The 1000 days between a woman's pregnancy and her child's second birthday offer a unique window of opportunity to shape healthier and more prosperous future. The right nutrition and care during this 1000 day window can have a profound impact on a child's ability to grow, learn, and rise out of poverty. It can also shape a society's long-term health, stability, and prosperity. The 1000 Days Initiative was born in 2010 in response to groundbreaking scientific evidence that identified a powerful window of opportunity from a woman's pregnancy to a child's 2nd birthday when nutrition had a long-term impact on the future health and development of both children and societies. With the backing of the US Government, the Government of Ireland, the Bill & Melinda Gates Foundation, and several nonprofit organizations, 1000 Days began its work as a partnership to drive greater action and investment to improve nutrition for women and young children throughout the world.

Investing in the early years is one of the smartest investments a country can make to break the cycle of poverty, address inequality, and boost productivity later in life. Today, millions of young children are not reaching their full potential because of inadequate nutrition, lack of early stimulation and learning, and exposure to stress. Investments in the physical, mental, and emotional development of children – from before birth until they enter primary school – are critical for the future productivity of individuals and for the economic competitiveness of nations.

Hunger is caused by poverty and inequality, not scarcity. For the past two decades, the rate of global food production has increased faster than the rate of global population growth. The world already produces more than enough food to feed everyone on the planet. We should prioritize a more equal distribution of food across the world so that both the consequences of poor diets due to undernutrition and overnutrition will be prevented. Priority should be given to women of reproductive age. Based on the findings of the Dutch famine studies, we expect that adequately feeding women before and during pregnancy will allow future generations to reach their potential and lead healthier and more productive lives, ultimately leading to healthier and more equal future. Breaking the vicious cycle of poverty and undernutrition will most likely succeed if we provide women with sufficient food to provide their children a good start in life.

Malnutrition kills millions of children every year and robs millions more of the opportunity to reach their full potential. This global crisis requires global action in order to give every child a fair start to life. In 2012, world leaders committed to reaching six global nutrition targets by 2025. Yet, reaching these targets in the next decade will require increased investment. World leaders must act now to fulfill their promises and save millions of lives. Attempts to divide the available food around the world in a more equal manner seem a very important issue to address. Giving women of reproductive age priority in allocating food in times of food shortage seems important to try and break the vicious cycle of undernutrition and poverty. In recognition of this, WHO and UN policies have prioritized nutrition during the first 1000 days of life, from conception to the 2nd birthday as one of the most important investments we can make in human development and in the health of future generations.

Dictionary of Terms

- **Prenatal undernutrition** – undernutrition before birth
- **Plasticity** – the ability of living organisms to adapt to the environment
- **Programming** – nutrition and other environmental factors acting in early life permanently affect the way in which the organism grows and develops with lasting consequences for later health.
- **Epigenetics** – refers to changes in/regulation of the expression of genes without alterations to the DNA sequence. These changes in expression of genes have consequences for phenotype and explain why one genotype can result in various phenotypes.

Summary Points

- Poor nutrition during critical periods of early human development has lasting negative consequences for growth, development, and health.
- The effects of prenatal famine exposure depend on its timing during gestation and the organs and tissues undergoing critical periods of development at that time.
- Early gestation seems to be the most vulnerable period.
- The effects of prenatal undernutrition are widespread and affect structure and function of organs and tissues, result in altered behavior and increased disease risks, which in turn increases mortality.
- The effects of famine exposure are apparent in the absence of any effects on size at birth.
- Some effects of prenatal undernutrition are more pronounced or even limited to one sex, but generally the effects apply to men and women.
- The effects of famine pass on to the next generation, both through the maternal and paternal line. This is likely to involve epigenetic regulation mechanisms.

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Impact of Childhood Experience of Famine on Body Composition: DEX and Beyond

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Contents

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Abstract

Evidence has shown that growth patterns in early life are associated with the diseases risk in adulthood. This reflects the concept of programming in which environmental factors generate long-lasting variability in phenotype for individual to adapt to an adverse environment. Low birth weight (BW) and poor growth rate are associated with obesity, diabetes, and cardiovascular disease in later life. Animal and epidemiological studies have linked BW and growth rate with the changes in body composition. Prenatal and early postnatal undernutrition can affect fat distribution, reduced bone mineral content, and muscle mass in later life in animals. Individuals who were exposed to the Dutch Famine, the Great Leap Famine in China, World War II, or Nazi occupation have a higher body weight, poor grip strength, and physical performance, and/or poor bone density and higher risk of osteoporosis. Epidemiological studies also show that infants with lower BW or poor growth rate were associated with changes in body composition. Recent animal studies, however, demonstrate that this developmental programming is potentially reversible. Nevertheless with the current evidence, appropriate dietary advice can be given to promote optimal fetal and infant growth and lower diseases risk in the offspring in later life.

Keywords

Adaptive change · Aging · Body composition · Famine · Fetal origins hypothesis · Growth pattern in early life · Malnutrition · Osteoporosis · Postnatal nutrition · Prenatal nutrition · Sarcopenia · Suboptimal growth · Undernutrition

List of Abbreviations

BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BW	Birth weight
SD	Standard deviation
SES	Socioeconomic status
TBBMC	Total body bone mineral content

Introduction

Poor accessibility of food and insufficient caloric intake could result in starvation. About 800 million people in the world were suffering from chronic undernourishment in 2014–2016 (Kueper et al. 2015). Famine can be declared when mortality, malnutrition, and hunger are not met. It is defined as at least 20% of households in a particular area facing extreme food shortages with a limited ability to cope, acute malnutrition rates exceeding 30%, and the death rate exceeds two persons per day per 10,000 persons (UN 2017). Children are the most

vulnerable victims in famine. Globally 161 million under 5 years old were estimated to be stunted in 2013 (World Hunger 2017).

Famine is known to affect the nutritional status at gestation and postnatal period. Fetal origins hypothesis proposes that the exposure to poor nutritional conditions in utero and/or during postnatal life leads to alterations in the development of vital organs, tissues, and body systems. These alternations are advantageous for survival in short term but may permanently predispose the fetus to structural, physiological, and metabolic adaptations, and lead to an increased risk of chronic degenerative diseases in later life (Barker et al. 2002). Various studies suggested the association between impaired intrauterine and early postnatal growth and increased risk of development of obesity, diabetes, and cardiovascular disease at adult life (Barker et al. 2002). There is also evidence showing that early life nutrition can affect the process of aging, frailty, and ultimately life span (Bleker et al. 2016).

Several animal studies and case series studies have examined the effect of famine during prenatal and postnatal period on adult health. Epidemiological studies on the impact of low BW and adult health have also been reported. This article aims to summarize the impact of famine in childhood on adult health with a focus on the changes in body composition including fat, muscle, and bone mass.

Evidence from Animal Model Regarding the Effect of Famine In Utero and during Early Life on Body Composition

Animal studies have examined the effect of prenatal and early postnatal undernutrition on body composition in later life. The results are summarized in Table 1. These studies provide insights that early life environment is critical for optimal health and ameliorates chronic diseases in adulthood.

Fat Mass

Development of adipocyte is highly influenced by the nutritional environment at prenatal and early postnatal life (Eleftheriades et al. 2016). The fat cell size in later life was increased in female and male rats who were born from mothers that were undernourished for the first 2 weeks of gestation. Female rats did not become obese, while male rats became hyperphagic and obese in adults (Jones and Friedman 1982). Such gender-specific changes have also demonstrated in Engelbregt et al. (2004). Intrauterine growth retardation was induced by ligation of the uterine arteries from day 17 of gestation to mimic prenatal malnutrition. Rats were postnatally starved by litter enlargement to 20 pups per mother from day two after birth until weaning. The total fat mass in female rats at 6 months old in either prenatal or postnatal starvation groups did not differ from the control group. However, significant result was found in male postnatal starvation rats when compared with the control. Starved male rats had a lower total fat mass and body fat percentage while a higher lean mass percentage.

Table 1 Summary of animal studies on early life undernutrition and its effect on body composition in later life

Outcome studied	Exposure to undernutrition	Effect of early life undernutrition on body composition in later life
Fat mass	Prenatal	- male offspring became hyperphagic and obese; increased fat cells in both female and male offspring as adults (Jones and Friedman 1982) - rat offspring born heavier at birth showed greater value of abdominal and total fat than offspring born with a lighter birth weight at 26 days old (Eleftheriades et al. 2016)
	Postnatal	- Total fat mass for male rats was significantly lower compared to the control group at 6 months old (Engelbregt et al. 2004)
	Prenatal and postnatal	- no difference was observed in abdominal and total fat in offspring that were born with a heavier birth weight compared to the control group at 26 days old (Eleftheriades et al. 2016)
Bone health	Prenatal	- lower TBBMC compared to rats fed with a normal pre or postnatal diet at day 26 of life (Eleftheriades et al. 2016) - TBBMC was significantly lower in both male and female rats compared to controls at the age of 6 months; reduced in trabecular bone and cortical content in both male and female offspring at 6 months old. Trabecular bone density was found to be lower in female rats only (Engelbregt et al. 2004) - maternal protein restriction resulted in reduced bone area and BMC in the offspring later in life (Mehta et al. 2002)
	Postnatal	- lower TBBMC in male rats compared to the control but no such difference in female rats at the age of 6 months (Engelbregt et al. 2004)
Muscle mass	Prenatal	- reduced muscle fiber numbers at term guinea pig fetus (Dwyer et al. 1995) and in near-term ewes fetus (Demirtaş and Özcan 2012)
	Postnatal	- percentage of lean mass in male rats at the age of 6 months was significantly higher compared to control (Engelbregt et al. 2004)

Another study found that prenatal food restriction had a significant impact on abdominal adiposity later in life (Eleftheriades et al. 2016). Pregnant rats were randomized to control diet or 50% of normal diet throughout gestation. The prenatally starved offspring were further divided into two groups based on BW, either with a heavier BW (a mean BW at or above two SD of the control group that received standard prenatal diet) or a lower BW (less than two SD of the control) and lactated by either mother with standard prenatal diet or restricted diet. Heavier offspring in the prenatal starved groups fed with a standard postnatal diet resulted in a greater value of abdominal and total fat than those with a lower BW and those in control group at 26 days old. However, this difference was not observed when the prenatally starved rats were continuously postnatally starved. These observations indicated that BW can be a factor contributing to the increased adipose tissue in rats that had suffered in utero undernutrition.

Bone Health

Several animal studies have demonstrated that prenatal and postnatal undernutrition affect fetal bone development and body mineral content (BMC) in adulthood. Eleftheriades et al. (2016) found that rats fed with standard prenatal and postnatal diet had a significant higher total BMC when compared to prenatal growth restricted rats at day 26 of life. In another study, adult rats exposed to either prenatal or postnatal malnutrition resulted in persistent growth retardation in terms of body weight and total body bone mineral content (TBBMC) at age of 6 months (Engelbregt et al. 2004). TBBMC was significantly lower in both male and female rats that had experienced prenatal malnutrition compared to control ($p < 0.01$ and $p < 0.05$). When these rats experienced postnatal food restriction, TBBMC was found significantly lower in male rats ($p < 0.01$) whereas no such difference was observed in female rats (Engelbregt et al. 2004).

Romano et al. (2010) used bilateral uterine vessel ligation in pregnant rats to induce uteroplacental insufficiency and found that both male and female rats were born 14% lighter compared to the control group ($p < 0.05$) and remained smaller at 6 months of age ($p < 0.05$). Trabecular bone and cortical content was reduced when compared to the control in both male and female rats. Trabecular bone density was found to be lower in growth restricted female rats ($p < 0.05$). When the rats were fed with a constant high calcium diet since adolescence, there was an increased in cortical bone mineral density (BMD) in both sexes ($p < 0.05$). However, it was not sufficient enough to recover bone content and strength even with calcium supplementation when compared with rats that did not suffer from in utero undernutrition. Adequate protein intake may also contribute to bone health development. Mehta et al. (2002) demonstrated that maternal protein restriction resulted in reduced bone area and BMC in offspring later in life.

Muscle Mass and Strength

Prenatal undernutrition is associated with reduced neonatal muscle weight in sheep and a reduction of postnatal muscle fiber in pig (Dwyer et al. 1994; Greenwood et al. 2000). In a guinea pig experiment, a 40% restriction in maternal feed intake through gestation resulted in reduction of fetal body weight and a 20–25% reduction in muscle fiber number at term (Dwyer et al. 1995). Furthermore, early gestational undernutrition that lasted for the first 25 days of gestation did not affect muscle fiber number development of the placenta, whereas gestational undernutrition period that extended to 25 days and longer affected development of secondary fibers (Dwyer et al. 1995). The results indicated that the stages of undernourishment during gestation had different effects on muscle fiber development.

Demirtaş and Özcan (2012) investigated the effect of mild and severe nutrient restriction during early gestation on fetal muscle development in ewes. Ewes from the mild group received 85% of daily nutritional requirement and ewes from the severe group received 50% of daily nutritional requirement from the first day of

gestation and throughout the gestational period. The total number of muscle fibers, primary and secondary fiber numbers had decreased in ewes with either mild or severe undernutrition during early gestation. Moreover, the more severe the nutrient restriction during in utero, the greater decrease in the total fiber numbers (Demirtaş and Özcan 2012). Skeletal muscle is vulnerable to the availability of nutrients during particular period in fetal development.

Evidence from Case Series Studies and Epidemiological Studies Regarding the Effect of Famine during Childhood on Body Composition

The Dutch Famine

The Dutch Famine took place in the winter of 1944–1945 in the western Netherlands. The general daily calorie intake of the inhabitants decreased gradually from about 1800 calories to 1000 calories and further to a daily intake between 400 and 800 calories. It liberated after 6 months and the daily intakes increased to more than 2000 calories. The famine had a profound effect on the health of the population. The mortality rate was more than doubled compared with 1939 (Roseboom et al. 2001). The effects of exposure to the Dutch Famine during gestation on health in later life are summarized in Table 2.

Higher chronic diseases risk and impaired physical function have been found in individuals exposed to the Dutch Famine. Women who had been exposed to the Dutch Famine aged 0–9 years had 25% higher risk of being overweight (95% CI 1.05–1.50), higher BMI by 0.48 kg/m^2 (95% CI 0.13–0.83), and 1.02 cm more of waist circumference (95% CI 0.17–1.88) compared to unexposed women after adjustment for potential confounders (van Abeelen et al. 2012). Men exposed to prenatal undernutrition during the Dutch Famine had lower grip strength

Table 2 Summary of disease risk associated with the Dutch Famine (Painter et al. 2005; Portrait et al. 2007; Roseboom et al. 2000, 2001, 2006, 2011)

Period of starvation	Effects
Famine in early gestation	- more atherogenic lipid profile - higher fibrinogen concentrations and reduced plasma concentrations of factor VII - higher BMI - higher risk of coronary heart disease, dyslipidemia, more stress responsive - increased risk of schizophrenia and antisocial personality disorder
Famine in mid gestation	- increased prevalence of obstructive airways disease - greater prevalence of microalbuminuria - reduced glucose tolerance - decreased in creatinine clearance
Famine in late gestation	- reduced glucose tolerance - highest mortality rate

($\beta = -5.0$ kg; 95% CI -9.2 to -0.8, $p < 0.05$) and physical performance score ($\beta = -0.8$ points; 95% CI -1.6 to 0.0, $p < 0.05$) than unexposed men after adjusted for BMI, birth weight, socioeconomic status (SES), and various lifestyle factors (Bleker et al. 2016). These studies provide evidence that poor nutritional status in prenatal and early postnatal is a predictor of various health outcomes.

During the World war II

During World War II, Japanese women who experienced low nutrient intake when they were 5 years old showed a significantly lower BMD than unexposed women. By comparing women aged 50–54 years in 1992–1993 and women who reached the same age in 1999–2002, the older women group was relatively shorter and had significantly lower BMD (0.86 ± 0.15 g/cm^2 vs. 1.02 ± 0.16 g/cm^2) ($p < 0.001$) (Yoshimura et al. 2005).

Chinese adults who suffered from famine during this period showed a poor bone health. Chinese adults aged 65 years and above who had experienced famine (defined as a caloric restriction for at least 1 year) were shorter (OR 0.92; 95% CI 0.89–0.99), had a higher BMI (OR 1.11; 95% CI 1.03–1.19), and higher prevalence of recurrent falls (defined as two or more falls in the previous 12 months) (OR 1.67; 95% CI 1.17–2.37) (Woo et al. 2010). Women who were exposed to famine also had higher rate of developing osteoporosis ($p = 0.049$), lower femoral neck BMC ($p = 0.031$), and lower femoral neck BMD ($p = 0.034$). In addition, women who had experienced famine had a higher loss in femoral neck BMD (percentage change -1.38; 95% CI -2.72 to -0.03) than those who had not been affected (Chan et al. 2007). However, these associations were attenuated when education was entered into the analysis model. Famine exposure had little impact on BMC, BMD, and osteoporosis status in men (Chan et al. 2007). These studies showed that poor growth during infancy and childhood has impact on bone health and the effect may be gender-specific.

Holocaust Survivors

The incidence of osteoporosis and osteopenia was found to be high in Holocaust survivors. Holocaust survivors are those who were in Europe under Nazi occupation during the years 1939–1945 and were constrained in concentration camp, ghetto, labor camp, or in a children's institution. Female Holocaust survivors were significantly associated with greater likelihood of osteoporosis (OR 1.39; 95% CI 1.00–1.93, $p = 0.03$) (Werner 2003). In addition, female survivors at age 60 years or older had 2.88 times higher risk of osteoporosis than those in the control group after adjustment for various covariates ($p = 0.014$) (Marcus and Menczel 2007). The incidence of osteoporosis and osteopenia was significantly higher in those who exposed to unfavorable condition at 17 years old or younger. They showed a 3.47 higher risk of having osteoporosis than the control group ($p = 0.019$). In contrast,

the lack of difference in the osteoporosis risk was seen among those exposed at age of 17 years and/or older and the control (Marcus and Menczel 2007). These observations further support the period of nutrients restriction is a determinant of bone health later in life.

Great Leap Famine in China

The Great Leap Famine (Great Chinese Famine) that occurred in 1959–1961 resulted in death and malnutrition of millions of people including babies. It had been estimated that the daily per capita availability of food energy during 1959–1961 decreased dramatically to 1500 calories and this level was well below the average food energy requirement of about 2100 calories (Ashton et al. 1984; Chen and Zhou 2007).

The health impacts of the Great Chinese Famine are summarized in Table 3. Studies showed that early life exposure to famine was associated with increased risk of overweight later in adult life (Luo et al. 2006). Yang et al. (2008) compared BMI data based on 2012 China Health National Survey of rural residents born during the famine years of 1959–1961 with those born after the famine, 1964 (control group). The mean BMI and the prevalence of overweight of the women who were born during the famine years were significantly higher compared to the controls ($p < 0.01$). Yang et al. (2008) concluded that higher risk of overweight and obesity in women were caused by malnutrition in fetal life. However, this difference was not observed in men. Similarly, Wang et al. (2010) found that the effect of famine and body weight in adulthood might be gender-specific. Wang et al. (2010) examined the body weight and height of adults who had suffered from famine either as a toddler or during gestational stage in the Chongqing region. Fifty years after exposed to famine, those female exposed to famine during toddler or gestational showed an increase in weight, consequently, leading to overweight and obesity compared to the control. However, males that were exposed to the famine as a toddler showed a decreased in body weight in adulthood and did not affect the males that were exposed to the famine during gestation.

In addition, bone health may also be affected as a consequent of this famine. Chen et al. (2016) found that male survivors of the Great Chinese Famine who were born in 1961 (i.e., those with fetal development in the most severe period of the famine) had a highest risk of developing osteoporosis in adulthood compared to males that did not suffer from the famine (control).

Association of Growth Patterns in Early Life on the Body Composition

Substantial evidence has suggested an association of growth patterns during early life with later health outcomes. The changes of body composition in response to the early environmental conditions may be the determinants of the increasing prevalence

Table 3 Study outcomes of the Great Chinese Famine

Publication	Study data/ population	Study outcome	Famine exposure (birth year)[a]	Age at examination (years)[a]	Results
Chen et al. 2016	2013 health examination at a health screening center of a hospital in Chongqing	Osteoporosis	Exposed to famine (1959–1962); Mean age 0–3 years Control (1956–1958) and (1963–1962) combined as control	48–57	Male survivors of the great Chinese famine who were born in 1961 had a highest risk of developing osteoporosis in adulthood compared to males that did not suffer from the famine (control)
Luo et al. 2006	The China health and nutrition Survey 1991, 1993, 1997, and 2000	Overweight	Famine group (1959–1962); Mean age 0–3 years Control (1963–1966)	–	The probability of being overweight was higher in the provinces that experienced more severe famine compared to provinces that were less severely affected. Such differences were more pronounced in women
Wang et al. 2010	2006–2008 annual physical evaluation data of a hospital in Chongqing	Body weight Overweight Obesity	Toddler exposed group: (1956–1958); mean age 1–3 years Gestational exposed group (1959–1961); mean age	42–52	Female subjects: a significant increase in the body weight of female subjects exposed to famine during toddler stage

(*continued*)

Table 3 (continued)

Publication	Study data/ population	Study outcome	Famine exposure (birth year)[a]	Age at examination (years)[a]	Results
			0–2 years Control (1962–1964)		and gestational stage ($p < 0.05$ vs. control) Male subjects: Exposure to famine during toddler stage reduced body weight ($p < 0.05$ vs. control). No impact on the adulthood male body weight during gestational stage
Yang et al. 2008	2002 Chinese nutrition and health Survey	BMI	Faming group (1959–1961); mean age 0–2 years Control (1964)	38–43	The mean BMI of rural residents born during the famine years 1959–1961 were significantly higher than the control group ($p < 0.01$)

[a]approximate age estimation

of obesity, sarcopenia, and osteoporosis (Sayer and Cooper 2005). Epidemiological studies have investigated the association between BW and lean mass or fat mass in adult (Table 4). Studies suggest that BW or weight at 1 year old is associated to adult weight. Three studies have further examined the association between BW and body composition at young adult and elderly subjects. BW was associated with thigh muscle and bone area in young adults (Kahn et al. 2000) and total lean mass and muscle mass in elderly group (Gale et al. 2001; Kensara et al. 2005; Patel et al. 2011). More body fat and more central distribution of fat with a greater trunk to limb fat ratios was found in low BW group ($\leq$3.18 kg) at the age above 64 years old (Kensara et al. 2005). These studies suggest a positive association between BW and subsequent lean body mass and a negative association between BW and relative adiposity.

Table 4 Association between weights measured at birth and at infancy with fat mass and/or muscle mass

Number of subjects	Study population	Assessment age	Results
153 women	Resident in Bath UK	21	No significant association between BW and adult weight Weight at 1 year old was a significant predictor of adult weight ($r = 0.35$, $p < 0.001$) (Cooper et al. 1995)
224 men, 189 women	Born in Hertfordshire	63–73	BW was associated with adult weight in men only ($p < 0.001$) Weight at 1 year old was associated with adult weight in both sexes ($p < 0.001$) (Cooper et al. 1997)
102 men, 41 women	Living in Sheffield	70–75	BW was negatively associated with whole body total fat (23.09 kg for BW $\leq$ 3.15 kg vs. 21.03 kg for BW $\geq$ 3.64 kg, $p = 0.03$) BW was positively associated with whole body total lean mass (45.02 kg for BW $\leq$ 3.15 kg vs. 46.88 kg for BW $\geq$ 3.64 kg, $p = 0.002$) Data adjusted for age, sex, height, and weight (Gale et al. 2001)
192 men	White and black male for military service	17–22	For every kg increment in BW, BMI increased by 1.66 units ($p < 0.001$), thigh muscle and bone area increased by 16 cm^2 ($p = 0.0029$), waist circumference increased by 3.9 cm ($p = 0.0014$) No significant association with thigh subcutaneous fat area (log_{10} of cm^2) ($p = 0.086$) Data adjusted for race and height (Kahn et al. 2000)
32 men	Born in Hertfordshire	64–72	Low BW group ($\leq$3.18 kg) had a higher percentage of body fat (29.31% vs. 25.33% in high BW group ($\geq$3.86 kg), $p = 0.029$), fat mass (24.49 kg vs. 21.67 kg, $p = 0.039$) but a lower fat free mass (59.12 kg vs. 61.95 kg, $p = 0.039$) and muscle mass (27.23 kg vs. 29.22 kg, $p = 0.022$) Data adjusted for weight and height (Kensara et al. 2005)
105 men	Born in Hertfordshire	68–76	Low BW group ($\leq$3.18 kg) has a lower total lean mass ($p = 0.04$). However, results attenuated after adjustment for age, height, and physical activity ($p = 0.06$) (Patel et al. 2011)

Table 5 Association between weights at birth and at infancy and skeletal status

Number of subjects	Study population	Age	Results
153 women	Resident in Bath UK	21	BW and weight at 1 year old were not associated to BMD Weight at 1 year old was positively correlated with lumbar spine BMC ($r = 0.32$, $p < 0.01$) and femoral neck BMC ($r = 0.26$, $p < 0.01$) (Cooper et al. 1995)
224 men, 189 women	Born in Hertfordshire	63–73	BW and weight at 1 year old were not associated with BMD in both sexes Weight at 1 year old was associated with BMC in men at lumbar spine ($p = 0.03$), in women at lumbar spine ($p = 0.01$), and at femoral neck ($p = 0.01$) Data remained significant after adjusting for age, SES, smoking, alcohol consumption, calcium intake, activity level, and years since menopause (Cooper et al.1997)
55 men, 57 women	Resident in Finland	71	Poor growth rate was associated with hip fracture risk. The risk was 1.9 times higher in the lowest quartile in the growth rate of height ($p = 0.006$) and 1.7 times higher in the lowest quartile in the growth rate of weight ($p = 0.009$) (Cooper et al. 2001)
498 men, 468 women	Resident in Hertfordshire	60–75	BW was associated with BMC in men (proximal femur: $r = 0.16$, $p = 0.003$; lumbar spine $r = 0.10$, $p = 0.03$) and women (proximal femur $r = 0.16$, $p = 0.0008$; lumbar spine $r = 0.11$, $p = 0.03$) Weight at 1 year old also associated with BMC in men (lumbar spine $r = 0.17$, $p = 0.0001$; proximal femur $r = 0.22$, $p < 0.0001$) and women (lumbar spine $r = 0.13$ $p = 0.01$; proximal femur $r = 0.14$ $p = 0.002$) Data remained significant after adjusted for age, BMI, cigarette, alcohol consumption, physical activity, social class, years since menopause, and the use of hormone replacement therapy in women BW was associated with BMD in men in proximal femur only ($r = 0.10$, $p = 0.03$) Weight at 1 year old was associated with BMD in men in lumbar spine only ($r = 0.11$, $p = 0.01$) (Dennison et al. 2005)
102 men, 41 women	Living in Sheffield	70–75	BW and BMC was associated in men at lumbar spine (69.61 g for BW $\leq$ 3.15 kg vs. 78.23 g for BW $\geq$ 3.64 kg) ($p = 0.012$) and femoral neck (4.4 g for BW $\leq$ 3.15 kg vs. 4.73 g for BW $\geq$ 3.64 kg) ($p = 0.024$). No association with BMD BW and BMC was associated in women at lumbar spine (46.52 g for BW $\leq$ 3.15 kg vs. 56.41 g for BW $\geq$ 3.64 kg) ($p = 0.003$) and femoral neck (3.14 g for BW $\leq$ 3.15 kg vs. 3.72 g for BW $\geq$ 3.64 kg) ($p = 0.004$)

(*continued*)

Table 5 (continued)

Number of subjects	Study population	Age	Results
			BW was associated with BMD in women at lumbar spine (0.84 g/cm^2 for BW $\leq$ 3.15 kg vs. 0.96 g/cm^2 for BW $\geq$ 3.64 kg) ($p = 0.004$) and femoral neck (0.65 g/cm^2 for BW $\leq$ 3.15 kg vs. 0.75 g/cm^2 for BW $\geq$ 3.64 kg) ($p = 0.011$) Data adjusted for age (Gale et al. 2001)
866 men, 1282 women	Born in Hertfordshire	59–73	Men who have a fall history had a lower weight at 1 year old ($p = 0.04$) and poor conditional infant growth ($p = 0.02$) Poor conditional infant growth was associated with fall history in men only (OR 1.27, 95% CI 1.04–1.56 $p = 0.02$). Results attenuated after adjustment for adult grip strength, height, age, walking speed. No association was found in women (Sayer et al. 2006)

Osteoporosis is a skeletal disorder characterized by low bone mass and strength, deterioration of bony tissues, and increase risk of fracture. BW and weight at 1 year old as well as a poor growth rate are correlated with decreased BMC and increased risk of hip fractures in later life (Table 5). BW was associated with BMC in men and women at lumbar spine and femur neck (Dennison et al. 2005; Gale et al. 2001). Associations were also found with infant weight at 1 year old (Cooper et al. 1995, 1997; Dennison et al. 2005; Gale et al. 2001). However, the correlation between BW and BMD was less prominent in these studies. Low childhood growth rate in weight and height was associated with an increased hazard ratio for hip fracture at older age (Cooper et al. 2001) and it is usually found in men who had fall history in past years (Sayer et al. 2006). The strong association of BW with BMC suggests that there are prenatal and postnatal programmed factors in determining bone mineralization and risk of osteoporosis in later life (Dennison et al. 2005).

Muscle strength has a major impact on the age-related decline in physical performance and independence. It is generally studied by using hand grip strength (Kuh et al. 2002). The association of BW and grip strength in later life was studied (Table 6). Lowest BW ($\leq$2.5 kg) was associated with reduced grip strength (28.5 kg vs. 32.4 kg in the highest BW group, $p = 0.01$) (Sayer et al. 1998). Similarly, lowest weight at 1 year old was also associated with reduced grip strength ($p = 0.02$) (Sayer et al. 1998). In another study in which grip strength was assessed at age 53 years old, there was a positive relation between BW and adult grip strength in both sexes. For a 1 kg increase in BW, there was an estimated mean increase in grip strength of 1.83 kg in men (95% CI 0.52–3.19, $p < 0.01$) and 1.2 kg in women (95% CI 0.29–2.11, $p < 0.01$) when adjusted for adult body size, weight, and height (Kuh et al. 2002). These data suggested that children born into an adverse environment in which they had not gained weight in infancy would result in persisting consequences through later adult and such poor adult environment could possibly lead to more rapid aging as well (Sayer et al. 1998).

Table 6 Association between weights at birth and at infancy and grip strength

Number of subjects	Study population	Age	Results
1371 men, 1404 women	Birth in England, Scotland, Wales	53	Lower BW had a lower grip strength in men (45.9 kg at BW $\leq$ 3.06 kg vs. 50.6 kg at BW $\geq$ 3.87 kg p < 0.001) and in women (26.9 kg at BW $\leq$ 2.95 kg vs. 29.5 kg at BW $\geq$ 3.65 kg, $p < 0.001$) BW was associated with grip strength in both sexes. An extra kg of birth weight was associated with 1.83 kg difference in men and 1.27 kg difference in women ($p < 0.01$) Data adjusted for adult body size, body weight, and height (Kuh et al. 2002)
411 men, 306 women	Birth in Hertfordshire	65–74	BW was associated with grip strength (28.5 kg at BW $\leq$ 2.5 kg vs. 32.4 kg at BW $\geq$ 4.31 kg, $p = 0.01$) Weight at 1 year old was associated with grip strength (29.8 kg at weight $\leq$ 8.16 kg vs. 34.2 kg at weight $\geq$ 11.79 kg, $p = 0.02$) Data adjusted for age, sex, current social class, SES at birth, and height (Sayer et al. 1998)

Potential Mechanisms Linking the Effect of Famine during Childhood to the Body Composition

Phenotypic "plasticity" enables one's genotype to give rise to a range of different physiological or morphological states in response to different environmental conditions (Barker et al. 2002). It enables the production of phenotypes that are better matched to the environment. The mechanisms underlying the changes are still unknown. It has been postulated that genetic, physiological, behavioral, and environmental factors influence permanently in the cell proliferation and differentiations, heritable changes in gene expression, and alter the neuroendocrine systems which are crucial in the regulation of the body composition and physiological processes (Vickers 2011).

When the food intake is limited, the amount of glucose utilized by the body is reduced to a minimum since brain, kidney, and erythrocytes require glucose for their metabolism. The metabolic rate of the cells decreases significantly to allow for the subsistence of the organism as a whole (Kueper et al. 2015). Both the reduction of the basal metabolic rate and the nutrient deficiency contribute to the stunted growth. In addition, lower muscle mass resulted in reduced ability to metabolize calories and more likely to have positive calorie balance (Kahn et al. 2000). Stunted children had impaired fat oxidation and preferential oxidation of carbohydrate. The tendency to store fat was enhanced with this adaptation (Kulkarni et al. 2014) and permanently altered the number of adipocytes in the body (Sayer and Cooper 2005).

Furthermore, there are leading hypotheses that changes in stress hormones which could result in changing body composition (Kulkarni et al. 2014). Undernutrition is a powerful stimulator of stress leading to a reduction in growth hormones, insulin-like

growth factor-1, and thyroid hormones, resulting in impaired growth, lower energy expenditure, and a higher risk of positive energy balance (Fall et al. 1998; Kulkarni et al. 2014). These also alter the protective effect of bone health, reduce bone mineralization, and predispose an individual to an accelerated rate of bone loss during life (Cooper et al. 2002). The poor protein intake during famine could also reduce the intestinal calcium absorption as the results of secondary hyperparathyroidism and lead to a lower BMC (Matinolli et al. 2015).

Poor growth in early life is associated to lower muscle mass and poor muscle strength later in life and may lead to sarcopenia. The alteration of skeletal muscle morphology including the decrease in muscle fiber score and myofiber density may explain this association (Patel et al. 2011). Moreover, low BW was associated with increased proportion of type IIX fibers and deceased type IIA fiber. The increased in type IIX fiber may influence the whole body and muscle glucose metabolism, a situation which is similarly observed among patients with diabetes (Jensen et al. 2007).

Reversibility of the Developmental Programming

Fetal origins hypothesis proposes that alternations in fetal and postnatal nutrition and endocrine status result in the developmental adaptation that permanently predisposes the individual to higher diseases risk in adulthood (Barker et al. 2002). However, recent animal studies have suggested that developmental programming is potentially reversible by nutritional or targeted therapeutic interventions and may be able to ameliorate or reverse the consequences associated with developmental programming (Vickers 2011). Administration of leptin to rats that were undernourished in utero reversed the metabolic phenotype of insulin resistance and obesity that would otherwise develop in these animals (Guilloteau et al. 2009). Leptin given orally in rats resulted in lower body weight, lower fat content, and consumed fewer calories than their untreated controls at 6 months of age (Guilloteau et al. 2009). Another study in adult female rats also showed that IGF-I infusion led to a complete normalization of adiposity, fasting plasma insulin, and leptin concentration in the offspring (Vickers 2011). Furthermore, the effects of hyperleptinemia and increased fat mass development in rat were completely ameliorated by a diet high in omega-3 (Wyrwoll et al. 2006). These animal studies provide evidence for the possibilities of dietary manipulation on exacerbating programming effects. However, more research is needed to translate these animal studies to human settings.

Implementation

Animal studies and epidemiologic studies of human cohorts showed an association between undernutrition during gestation and at postnatal stages and future adult health. The process of adaptation that enables the infants to continue to grow may have adverse consequences for their health in later life. This association is likely to be reversed as demonstrated in animal studies recently. More work is needed to elucidate the mechanisms and identify the potential biomarkers responsible for the

adaptation process. With this information, it is useful to identify susceptible patients for dietary or nutritional interventions. A public health approach to promote optimal fetal and infant development through providing appropriate dietary advice is necessary. Reinforcing the awareness of health should be considered in terms of maternal and neonatal health, the growth of infant, their health as an adult, and even the health of subsequent generations (Gluckman et al. 2008). In addition, preventive strategies against obesity, osteoporotic fractures, and sarcopenia may be implemented at early life to improve BW and allow linear growth in children, and to promote optimal body composition and muscle strength, and minimize the potential loss (Kulkarni et al. 2014).

Conclusion

Evidence has shown that early nutrition factors may influence the long-term health and consequently the aging process. Under condition of suboptimal postnatal nutrition, infant must adapt to its environment and survive through such poor condition. However, such adaptations can lead to poorer muscle mass and higher fat mass (Gale et al. 2001, Kensara et al. 2005); lower grip strength (Kuh et al. 2002; Sayer et al. 1998), reduced BMC (Cooper et al. 1995, 1997; Dennison et al. 2005; Gale et al. 2001), and higher bone fracture and fall risk (Cooper et al. 2001; Sayer et al. 2006). Case studies on individuals in the Dutch Famine, the Great Leap Famine, and Holocaust survivors also supported the findings. The mechanisms for these adaptive changes are still unknown but it has been postulated that they are related to genetic, physiological, behavioral, and environmental factors (Vickers 2011). Recent findings on animals showed that the adverse consequences can be reversed by nutritional and pharmacological interventions. More research is needed to elucidate the underlying mechanisms for these adaptations and to identify the critical window period to reduce the associated diseases risk later in life.

Policies and Protocols

This chapter highlights the importance of early life nutrition on adult health, in particular the impact of famine in childhood on adult health with a focus on the changes in body composition including fat, muscle, and bone mass. In this chapter, we have described that BW and growth rate are both associated with the changes of body composition and skeletal status. We have also summarized that malnutrition or undernutrition in infants and early childhood leads to a disturbance in energy balance. Such energy imbalance could interact with the genome and lead to complex mechanisms which result in reduced body size and muscle mass, lower BMC and higher fat store. These changes can persist into aging which increase the risk of fall, osteoporosis, sarcopenia, and poor quality of life. To promote healthy aging, one should not only focus on the health status at mid-age adult but also the nutritional status at early ages. Therefore, nutrition intervention programs during

pregnancy and lactation are undoubtedly important to optimize the intrauterine environment and postnatal nutritional status from the public health perspective. However, current evidence is still lacking to justify any changes in dietary guidelines and dietary recommendations in these groups. More studies are needed to identify the factors that are associated with the delivery of nutrients in placenta, infant's adaption to a limited nutrient supply environment, and the possible mechanisms of this adaptive change on gene expression, body structure, and physiology. Further advances in this field may be able to reduce the prevalence of age-related diseases which represent the major healthcare issues in the aging population.

Dictionary of Terms

- **Aging** – Aging results in changes in body composition including increased total and abdominal fat accumulation, decreased lean muscle mass and bone mineral density. These changes predispose individuals to higher diseases risk, such as diabetes, heart disease, sarcopenia, and osteoporosis.
- **Famine** – Famine is a scarcity of food leading to malnutrition, starvation, and increased in mortality. Pregnancy and the first few years of life require adequate amount of food intake to support the growth and development of the infant. Limited food availability may lead to poor pregnancy outcomes and poor growth rate and may cause adverse health effects of the offspring in adulthood.
- **Fetal origins hypothesis** – It proposes that the period of gestation and early life has great impact on individual's development and health outcomes.
- **Optimal nutritional status** – It is a result by taking adequate amount and various types of nutrients so that the body can achieve the best performance and possibly a good health in lifetime.
- **Sarcopenia** – It is the loss of muscle mass, quality, and strength due to aging. It leads to higher risks of falls, mobility limitations, disability, and fractures. Exercise, in particular the resistant exercise is currently considered to be the best approach to delay its onset.

Summary Points

- Fetal origin hypothesis proposes that fetal growth condition is associated to the health outcome in adulthood. Epidemiological studies have shown that low birth weight and poor growth rate increase risk of obesity, diabetes, and cardiovascular diseases, possibly mediated through the changes in body composition.
- Evidence from animal model suggests that in utero or early life exposure to malnutrition affect body composition later in life including altered fat distribution, and reduced bone mineral content and muscle mass. However, translating such evidence to human setting is still difficult.
- People who were exposed to the Dutch Famine, the Great Leap Famine, World War II, or Nazi occupation generally showed higher body weight, poorer grip

strength, and physical performance, and/or lower bone density and higher risk of osteoporosis.

- Epidemiological studies support a link between infant weight and adult weight. Lower birth weight is associated with decreased lean muscle mass and increased fat mass.
- Infant weight is an important determinant of later bone health. Weight at 1 year old was associated with bone mineral content in women. Poor growth rate was related to a history of fall or hip fracture risk in individuals age 60 and above.
- Infant weight can influence the muscle strength in midlife which is associated with quality of life and independency in aging.
- Evidence of animal studies suggests that the developmental programming is potentially reversible.
- Dietary interventions for pregnant and lactating women allow optimal fetal growth and hence could lower the diseases risk in offspring in later life.

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Famine and Bone Metabolism

10

George M. Weisz and Ruth M. Hadfield

"Never has a generation fallen from such intellectual heights as ours, to such a depth."
Stefan Zweig,
The World of Yesterday, 1942

Contents

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Abstract

Throughout history, populations have been exposed to periods of severe food shortage, famine, and starvation. Famine continues to be a major world health issue today with an estimated 794.6 million people (10.9% of the world's population) undernourished in the 2012–2014 period. Historical episodes of famine have sometimes allowed the effects on subsequent health to be studied in detail. For instance, during WWII, the studies of starved children in the Warsaw Ghetto documented bone softening, osteoporosis, and osteomalacia. Histologically the bone marrow was replaced with connective tissue and few myelocytes or chondrocytes were visible. Infants born to women who experienced famine during pregnancy may have increased risk for certain health conditions and there is evidence that musculoskeletal conditions may originate in the intrauterine period. Fetal programming is likely to increase the risk of osteoporosis when the environment during pregnancy is lacking in essential minerals and vitamins. The interplay between the three most important components of bone metabolic health, calcium, vitamin D, and vitamin K, often occur together in communities affected by hunger. Lack of adequate nutrition and vitamin D may lead to Rickets in children. Vitamin K deficiency can lead to intrauterine epiphyseal ossification and adolescent osteoporosis. Folic acid deficiency during pregnancy is also a key factor in neural tube anomaly development. Lathyrism due to famine-related dietary restriction to predominantly one food type, the lathyrus legume species, can lead to osteolathyrism. Symptoms include bone tissue hemorrhage, collagen disease causing bone softening, osteoporosis, and frequent fractures. Famine osteopathy is a conglomerate of metabolic aberrations resulting from nutritional deprivation. Developed countries that welcome immigrants and refugees from famine hit regions should make provision for screening and early detection of metabolic deficiencies such as osteoporosis.

Keywords

Bone metabolism · Osteoporosis · Spina bifida · Lathyrism · Rickets · Osteomalacia · Vitamin K

List of Abbreviations

ACTH	Adrenocorticotrophic hormone
IU	International units
MK	Menaquinone
NTA	Neural tube anomaly
ODAP	L-amino acid alanine derivative
WWI	World War I
WWII	World War II

Historical Background

Famine as a socioeconomic phenomenon was known in antiquity and the Latin term *famina* evolved later on in history into the French and English term famine. The quote by Zweig, a pacifist and pan-European writer, applies to both the ideological and social aspects of famine. In the twentieth century food production reached an all-time high, with sufficient levels for the entire world population. It is in this century that the unnecessary occurrence of famines is *one of terrible and inexcusable failure* (Devereux 1988). Despite high economic achievement *it is this century's shame* that over 75 million people perished during the twentieth century as a result of nutritional deprivation (Devereux 1988).

Developmentally, famine may be either acute or chronic. The syndrome of famine can also be categorized according to intensity into three distinct types: (i) mild, namely hunger; (ii) moderate, also called destitution or inanition; or (iii) extreme famine, leading to mortality. Howe and Devereux (2004) have called for a scale for famine rather than a binary, famine or no famine, categorization (Howe and Devereux 2004). The staggering figure of 75 million deaths due to famine in the twentieth century can be attributed to natural disasters, such as flood, drought, or earthquake; government or societal repression of populations; political or military invasion; or genocide.

Both World War I and II resulted in widespread destitution due in part to the destruction of crops and farmland. There was famine in Europe during WWI, and in Russia millions of people were victims of the 1917 October Revolution. There was the catastrophic Soviet food confiscation that led to over 6 million Ukrainian deaths between 1932 and 1934 (Davies et al. 2004). Famine also emerged in Europe following WWII and in Asia hunger spread to include India (3 million victims), Southeast Asia, Bangladesh (1.5 million victims), and Cambodia (2 million). Later on, famine was documented in Eastern and Central African countries such as Ethiopia (with 1 million victims) and Somalia and the Sudan (each with 0.5 million). However, by far the most catastrophic figures were revealed by China later in the century with as many as 33 million deaths due to the "Great Leap Forward" between 1959 and 1961. More recently there has also been famine in North Korea with a reported 3.5 million deaths between 1995 and 1999 (Goodkind and West 2001; Owen-Davies 2001). Famine remains a major world health issue today with an

estimated 794.6 million people undernourished in the period 2012–2014, accounting for approximately 10.9% of the world's population (World Hunger News 2016).

Pathology of Famine

The literature on socioeconomic aspects of famine is voluminous. Apart from widespread mortality, famine may also result in clinical sequelae for survivors. In the young and very old, the effect of famine on the immune system leads to vulnerability to infectious diseases, further increasing the risk of mortality (Barbosa-Saldivar and Van Itallie 1979). Famine may also lead to delayed symptomatology in the early adult lives of survivors.

Following the Siege of Leningrad (1939–1943), the population was exposed to prolonged famine; however, the wider world did not learn the full extent of the hardship until sometime later. During World War II the invading German army instituted a siege around Leningrad from September 1941 to January 1944 which lead to a reported 830,000 deaths as a result of the causes related to the population-wide famine. The population of Leningrad (now St Petersburg, Russia) at that time was 2.9 million of which 0.5 million were children (Sparen et al. 2004). Infection was often the main complication with diseases such as tuberculosis, dysentery, scarlet fever, and pneumonia the prevalent cause of death (Brozek et al. 1946). During the siege, severely restricted diets during pregnancy resulted in an estimated daily sustenance of only 300–800 calories. The rate of premature births was as high as 41.2%, with neonatal mortality rates of 9% for full-term births and 30.8% for premature births (Antonov 1947).

Some 40 years after the Siege of Leningrad, epidemiological studies of children born at the time of the siege were conducted at Stockholm's Karolinska Institute (Sparen et al. 2004). The authors concluded that:

> *Starvation, or accompanying chronic stress, particularly at the onset of or during puberty, may increase vulnerability to later cardiovascular disease.*

Figure 1 illustrates the increase in risk of cardiovascular mortality for males who were children during the siege compared to controls not exposed to famine.

Earlier studies had reported that adults who had been exposed to malnutrition or starvation *in utero* during the siege did not appear to be at increased risk of glucose intolerance, dyslipidemia, or cardiovascular disease, yet they were more likely to have endocrine dysfunction, and high blood pressure, if obese (Stanner et al. 1997). However, it is well-established that restricted growth *in utero* increases the risk of Type II diabetes, coronary heart disease, and hypertension in adult life (Barker 1998).

In November 1944, as a reprisal for an anti-German railway bridge strike, the occupying army instituted a food embargo over the western Netherlands. By April 1945, nutrition had been gradually reduced to 400 calories a day, with a reported 18,000 deaths in the Dutch famine (known as the "Hongerwinter") (van der Zee 1998; Susser and Stein 1994).

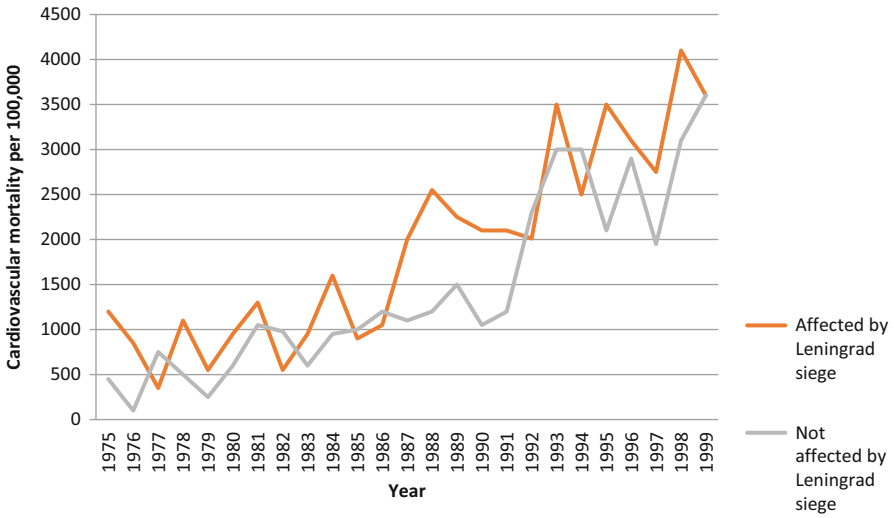

Fig. 1 Cardiovascular mortality in males exposed to famine during the Leningrad siege versus controls (Adapted from Sparen et al. 2004)

Records, studied some 40 years later at Amsterdam University, showed that small size and low birth weight were related to metabolic aberrations (in glucose and lipid levels), obesity, and increased mortality rate (Roseboom et al. 2006). Changes in reproductive function, early menopause, and increased breast and colon malignancy were also observed, all related to nutritional deprivation in pregnant women (Dirx et al. 2003; Elias et al. 2004).

During World War II, there was also severe food shortage on the Channel Islands, including Guernsey and Jersey, when they were demilitarized by the British in 1940 and then occupied by the German military. The invasion of Normandy in 1944 cut off the food supply to the 60,000 inhabitants of the five Channel Islands. Only a fifth of the population was evacuated, but those that remained endured a five-year long critical food shortage and famine. Islanders exposed to malnutrition as infants were later found to have increased cardiovascular morbidity, delayed puberty, and an increase in breast cancer, although statistically nonsignificant (Fentiman et al. 2007; Head et al. 2008).

Pathogenesis of Famine-Induced Metabolic Bone Disease

The concept of trimester-based intrauterine development has become essential for an understanding of fetal programming, including the programming of bone development.

Intrauterine conditions during the first trimester (the embryonic period) affect neuronal and mesenchymal-cartilaginous tissue development. The following 6 months of pregnancy, namely the second and third trimesters, are critical to the multiplication and growth of existing cells (Weisz and Albury 2014).

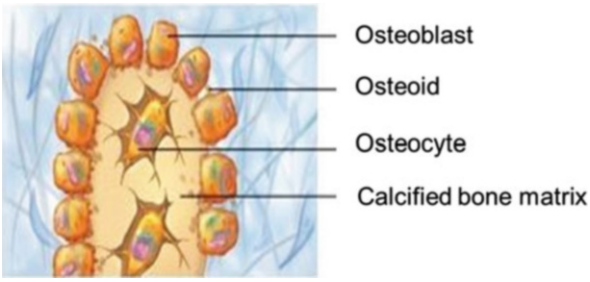

Fig. 2 Bone matrix, or osteoid, is secreted by the osteoblasts which then become calcified and develop into osteocytes

The second trimester is the time when the fetus is most sensitive to maternal metabolic status and dietary intake; direct effects on fetal adipose, muscle and bone formation have been observed. In the third trimester glucose and cortisol levels are critical, exerting effects on kidney formation, birthweight, limb length, and head circumference at birth, as well as postpartum maternal weight changes (Weisz and Albury 2014).

Maternal and fetal nutrition are in delicate balance and the state of maternal nutrition influences the fetal state by enforcing functional changes. Studies have shown that the second trimester is the most strongly affected by maternal metabolic intake. At this time, the adipose, muscle, and bone tissue formation takes place. An adaptation or reprogramming to the food shortage is applied: the number of cells formed is adjusted down; oxygen and nutrition are deviated toward the vital organs (brain, heart, arteries). The baby is at increased risk of being small in size with low birthweight and the foundation is laid for potential future neonatal, childhood, or adult diseases (Fig. 2).

Effect of Famine on Bone Metabolism

At this stage it is necessary to review normal fetal osteogenesis (Fig. 3).

The recognition of intrauterine starvation and its effect on bone metabolism followed on from studies on general metabolism. Numerous studies have observed that intrauterine skeletal development is under hormonal control, as well as dependent upon the availability of minerals and the vitamins D, B9 (Folate), and K (Weisz and Albury 2013).

Bone formation, or osteogenesis, is already evident by the fourth week of the embryonic period, but the bone is still a soft structure. In the developing fetus, mesenchymal cells are transformed into osteoblasts, and eventually further into osteoclasts, with two types of ossification: intramembranous axial ossification (skull, mandible, clavicle, ribs, and sternum genesis), or endochondral appendicular ossification (long bones, pelvis, and vertebrae) (Fig. 4). During the second and third trimesters, limb development continues with nonmineralized bone matrix being secreted by osteoblasts and then transformed into osteocytes.

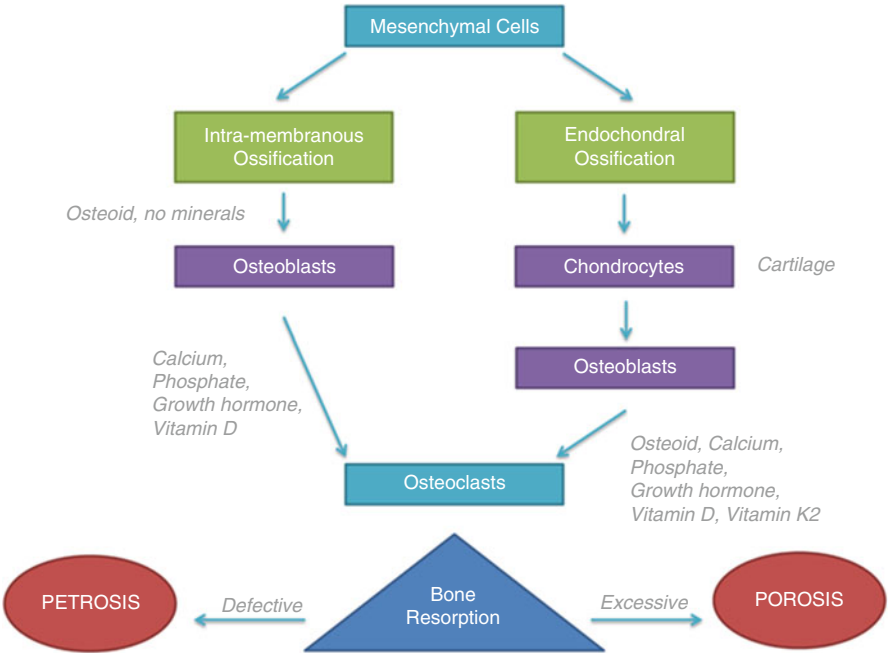

Fig. 3 Normal and pathological bone formation in the developing fetus (designed by author, G.M.W). Mesenchymal cells are pluripotent cells capable of differentiating into various cell types including osteoblasts, chondrocytes, myocytes, and adipocytes.

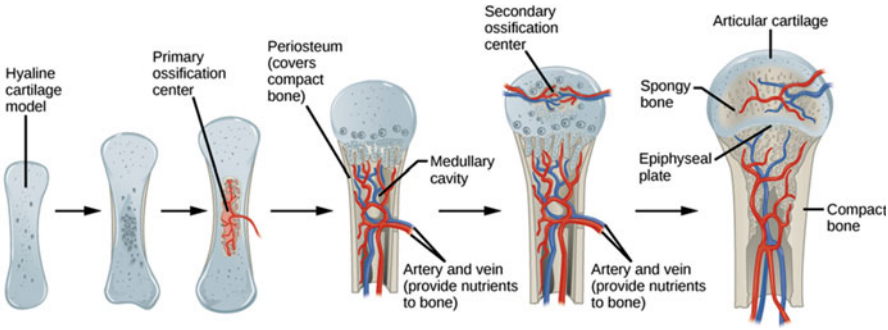

Fig. 4 Bone development [adapted from copyright free image http://cnx.org/contents/qmrJu64i@3/Bone (Accessed 21 Mar 2017)

In the bone marrow, the osteoblasts contribute to destruction of the central bone matrix. The matrix continues to absorb calcium, and the central bone space is emptied, while the bone grows externally toward the cortex/periosteum (Gilbert 2000).

A balance is maintained between osteoblast and osteoclast activities, under the influence of minerals (sulfur, calcium, phosphates, magnesium, selenium, strontium,

and zinc) and the vitamins D, B9, and K. This balance is also regulated by hormones (including pituitary, growth and parathyroid hormones); as well as glucose, cortisol, and the important osteoproteins. Vascularization of the bone leads to the blast/clast equilibrium, producing normal bone architecture. A disturbance in the blast/clast equilibrium can lead to osteopenia or osteoporosis if there is excessive osteoclastic activity, while reduced osteoclastic activity can lead to osteopetrosis.

Pathology of Famine Osteopathy

The effects of famine could result in either cortex trabecular decalcification or diminished osteoid/matrix formation, which then leads to rickets in children or osteomalacia in adults.

The Acute Effect of Famine on Bone Metabolism

The acute clinical signs of famine were detected in most tragic circumstances during WWII in the Warsaw Ghetto where over 200,000 people were incarcerated, with decreasing food supply which progressively diminished.

Teaching was considered unnecessary and was forbidden by the occupying authorities, but permission was given for a *Medics Course for Prevention of Epidemics*, which was transformed into a clandestine Medical School, conducted within the confining walls of the Ghetto. The faculty was comprised of 27 detained professors from various Polish universities. They conducted the teaching of medicine and also research into hunger. The preclinical studies were conducted in makeshift laboratories, while the clinical studies were conducted within six hospitals with mainly nocturnal lectures. The results of the research on hunger physiology were recorded with remarkable accuracy given the circumstances, and were smuggled out or buried in metal containers. These "archived" records were recovered after the war by the five surviving teachers, formulated into a book and published initially in Polish then in French, and finally in the USA, with commentaries by the Columbia University staff in New York (Winick 2005).

The researchers commented on the effects of hunger on metabolic pathways, as well as on cardiac, renal, pulmonary, and hormonal activities. They also described the softening and bowing of bones and the loss of bone fracture healing, making surgery impractical. Osteomalacia and osteoporosis were documented on autopsies performed on swollen, edematous children, abandoned and left dying on the streets. This was a rather rapid effect of inanition, the second effect of famine, which often progressed to the third effect – fatality. Histology obtained from autopsies showed the replacement of bone marrow with connective tissue, with a few myelocytes and chondrocytes remaining.

> *"Scientifically it still remains the most detailed study of semi-starvation ever carried out. It has had a profound influence on the way we treat this disease."* Winick 2005

The Chronic Effect of Famine on Bone Metabolism

According to the 1962 classification of Casuccio, which is still applicable today, osteoporosis develops in several ways (Casuccio 1962). These are:

(i) Primary osteoblastic deficiency, which may be congenital (*osteogenesis imperfecta*)
(ii) Reduced osteoblastic activity in the absence of trophic stimuli (inactivity, ovarian agenesis, testicular agenesis, menopause)
(iii) Reduced osteoblastic activity from inhibitory stimuli (Cushing's disease, excess adrenocorticotrophic hormone (ACTH), thyrotoxicosis)
(iv) Normal osteoblastic activity, but insufficient bone construction material (due to malnutrition, disturbances of the digestive system, insufficiency of vitamin C, diabetes, thyrotoxicosis, cortisone, ACTH, stress, Cushing's disease)
In view of recent developments in the late twentieth century, the above classification requires an additional category, namely:
(v) Fetal programming as a mechanism of osteoporosis development

Development of the Theory of Fetal Programming

The relationship of the theory of fetal programming to adult metabolic disease was first identified in the 1980s, after initially being analyzed from a pediatric perspective. The theory of fetal programming is often referred to as "The Barker Hypothesis" as the epidemiologist Dr David Barker initially identified a direct link between prenatal nutrition and late-onset coronary heart disease in poor areas of England (Barker and Osmond 1986). This theory has since been expanded to include additional conditions, with evidence of increased risk of stroke, hypertension, and diabetes due to impaired growth and development during fetal life and infancy (Barker et al. 2009; Barker 1997). Soon, the theory was extended to include skeletal pathology, with the risk of developing osteoporosis later in life being linked to undernutrition in fetal or early postnatal life (Cooper et al. 2000). The use of rat models showed the effect of altered nutrition *in utero* on bone biochemistry and strength, supporting the importance of the intrauterine environment on skeletal growth and later disease incidence (Lanham et al. 2008a, b).

Intrauterine Starvation and Bone Metabolism

It is unknown at what point in development the effect of starvation influences adult human development. However, epidemiological reviews have provided evidence that adult musculoskeletal conditions may be founded in the intrauterine period (Blyth et al. 2009). There is also evidence that epigenetic changes may affect placental transfer of nutrients, including calcium and vitamin D (Bocheva and Boyadjieva 2011).

In the 1990s, Cooper and associates developed the theory that compromise of the early stages of bone development due to reduced provision of nutrition, minerals,

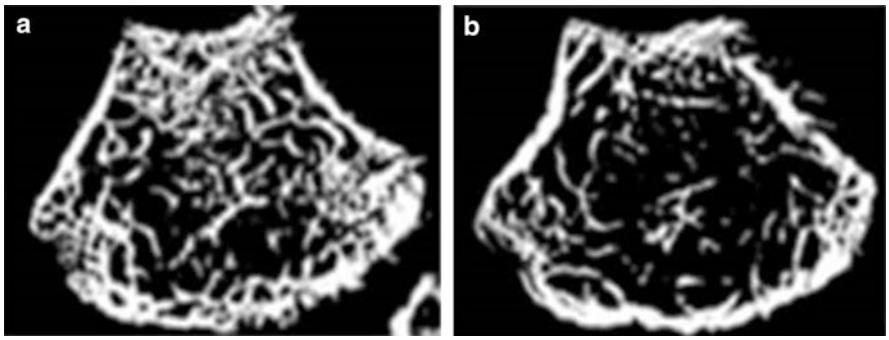

Fig. 5 Bone architecture seen in transverse cross-section (**a**) normal bone architecture. (**b**) osteoporotic bone. Note scarcity of bone trabeculi (computer design by author, GMW)

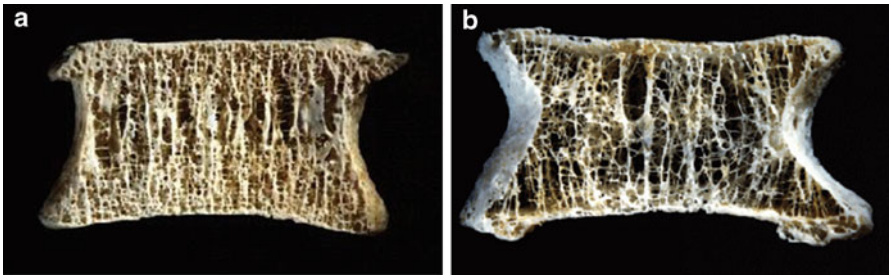

Fig. 6 Histology of normal and porotic bone

and growth factors could lead to changes in bone metabolism in adults.(Fall et al. 1998; Gale et al. 2001) The pathogenesis of osteoporosis was determined and a role was attributed to intrauterine programming (Cooper et al. 2000).

Further descriptions were published giving clear confirmation of the bone metabolic aberrations that result from intrauterine macro- and micronutritional deprivation (Weisz and Albury 2014, 2016). It was proven that even with the "catch-up phenomenon," whereby normal nutrition is achieved, not all bones achieve normal developmental growth (Weisz and Albury 2013; Weisz et al. 2012). Regardless of the mechanism, the end result is low bone tissue density, i.e., osteoporosis (Figs. 5 and 6). Famine therefore affects neonates, children, adolescents, and early adult life.

The Famine-Related Metabolic Bone Diseases

Rickets and Osteomalacia

The childhood form of osteomalacia, or rickets, is a condition resulting from a lack of vitamin D, which is essential for calcium absorption and bone mineralization. It is the result of a lack of ultraviolet sun ray exposure and of malnutrition. Vitamin D is

present in an inactive form in the skin and is partially activated by ultraviolet rays, a process completed in the liver and essential for the absorption of calcium and phosphates in the small intestine. Vitamin D, calcium, and phosphate are all necessary for calcification of the osteoid matrix of the bone tissue. Paradoxically, during starvation it has been observed that bone marrow adipose tissue increases and contributes to the weakening and softening of the bone and the lack of ability for fractures to heal (Devlin 2011).

Nutritional rickets may result from a lack of ingestion of eggs, fish oil, milk, or margarine; none of which are available in famine-affected areas, in squalors or natural disaster areas, and in prisons, ghettos, or concentration camps. The effect in childhood is on the epiphyseal growth area of the bones, which becomes low or completely devoid of matrix calcification, and the bone has no lateral expansion. In rickets, joint pain and swelling is clinically apparent, most evident at the wrist and ankle. Also apparent is the obvious lack of growth of long bones, spinal deformities, and bowing of long bones with fractures at the ends. Radiology of the lower ends of the radius and ulna shows cupping and fraying of the metaphysical regions. Blood tests reveal low levels of vitamin D and calcium. Histologically, unmineralized areas of matrix, surrounded by mineralized trabeculae, are detectable.

Osteomalacia, the adult form of induced-metabolic bone disease, resulting from lack of vitamin D has similar clinical joint signs. The histology is also similar, namely islands of unmineralized osteoid areas (Fig. 7).

Vitamin K Deficiency

Vitamin K was discovered incidentally in 1929 by Henry Dam in Denmark and was published as the K-oagulation vitamin in 1934 ("K" is derived from the German word "koagulation"); it was further chemically identified by E.A. Doisy. Both scientists were awarded Nobel Prizes in 1943, which were collected postwar in 1946.

Vitamin K consists of a group of three protein components:

(i) Vitamin K2, the most potent, has subtypes MK4 and MK7 (menaquinones). While absorbing calcium, MK4, and MK7 activate the osteoid in the matrix of bone by enzymatic carboxylation.
(ii) Vitamin K1 (or Matrix Gla protein) is protective against arterial wall calcification and cartilage ossification.
(iii) Vitamin K3 (or Protein S) promotes coagulation and its deficiency leads to childhood osteopenia and vertebral osteoporosis (Figs. 8 and 9).

Vitamin K deficiency leads to early intrauterine or early postnatal epiphyseal ossification (detectable by ultrasound), which may cause adolescent osteoporosis of the long bones and vertebrae, and also lead to early adult arterial wall calcifications. The early adult arterial wall calcification is paradoxically paralleled by osteoporosis.

The syndrome of vitamin K osteopathy is due to either malnutrition or malabsorption. This hemorrhagic syndrome, catastrophic during the early fetal intrauterine

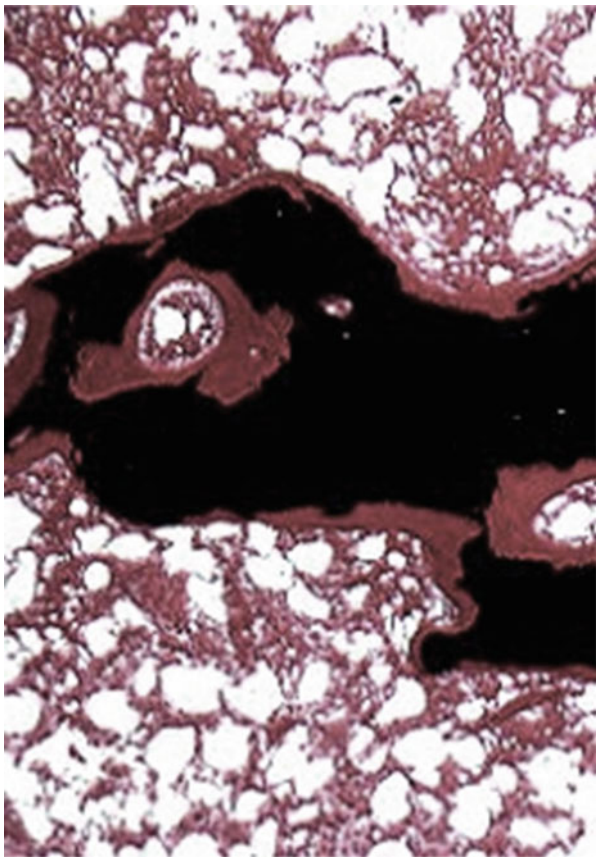

Fig. 7 Histopathology of osteomalacia showing islands of noncalcified bone

Fig. 8 Osteogenesis – the roles of Vitamin K and D in bone formation (Prepared by author, G. M. Weisz)

period, is at times also present in adults. Although vitamin K was discovered in 1929–1930, the osteopathic syndrome was only described in the late twentieth century and in more detail in the twenty-first century.

The condition is a famine-related syndrome that is typically found in regions affected by famine. The interplay between the three most important components of bone metabolic health: calcium, vitamin D, and vitamin K often occur together in communities affected by hunger (Dam 1946; Zetterstrom 2006).

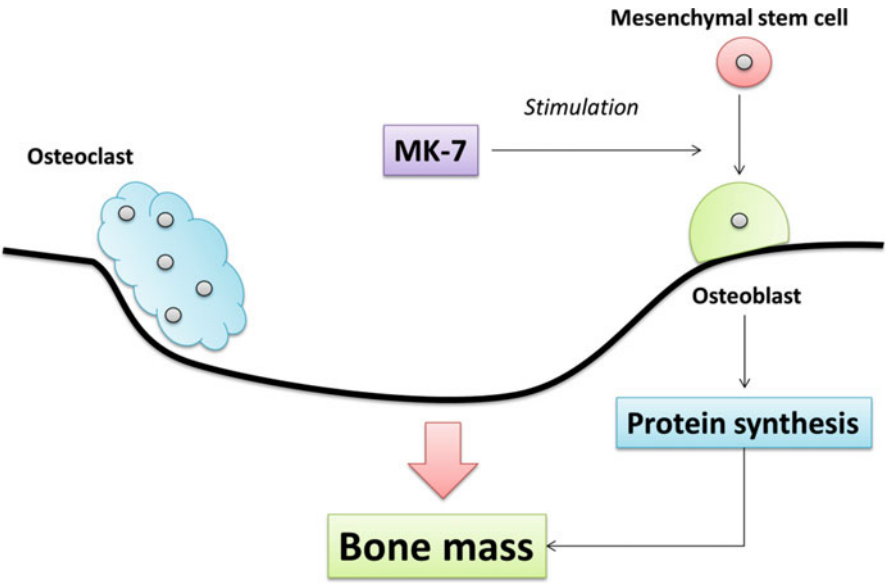

Fig. 9 The role of menaquinone 7 (MK-7) in bone formation (Prepared by author, G. M. Weisz)

Folic Acid Deficiency and Skeletal Malformations

Nutritional deficiency of folic acid leading to anemia was studied in 1930 by Lucy Wills in the subcontinent of India. Wills observed the high frequency of neural tube anomalies (NTA): spina bifida, meningocele, myelomeningocele, and also cranial abnormalities such as facial split, hydrocephalus, and anencephaly (Hoffbrand and Weir 2001).

Perhaps the geographical distance of the publication (in the Indian Journal of Medical Research) caused the considerable delay in recognition of this important observation in Western Medicine. Indeed, it was not until the late twentieth century that research in the UK, Europe, and the USA was directed towards understanding the interdependence of folate and NTA.

Numerous publications described the correlation without being definitive about a direct causal relationship (Smithells et al. 1976, 1977). It was in 1988, in Western Australia, that Bower and Stanley established a definite causal relationship (Bower and Stanley 1989). In the 1980s, it was the contribution of Wilson (Wilson et al. 2003), Smithells (Smithells et al. 1980), Schorach (Schorah et al. 1983), Laurence (Laurence and Campbell 1981; Laurence et al. 1981), and others that raised awareness of the relationship between folate (vitamin B9) and NTA. This was followed by an authoritative editorial in The Lancet in 1991 (MRC Vitamin Study Research Group 1991), further reinforced in a detailed monograph by Bannister (Bannister and Tew 1991). In 1996, the mechanism of pathogenesis resulting from folic acid deficiency and the parallel increase in homocysteine was suggested by Rosenquist (Rosenquist et al. 1996). In 1998, the Academy of Pediatrics, USA, determined the

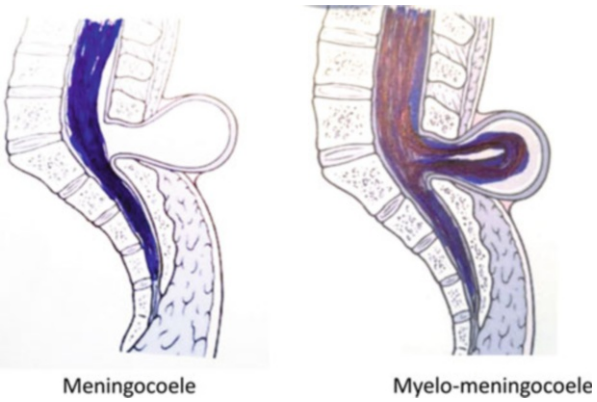

Fig. 10 Spina bifida meningocele and myelomeningocele (Drawn by author, G. M. Weisz)

prophylactic, preconception, primary, and secondary treatment for recurrent cases and this was further reinforced by the Royal College of Obstetricians & Gynaecologists, UK, in 2004 (Clarke 2004; Evans et al. 2004).

The pathology of folate deficiency is anemia, fatigue, gastrointestinal disturbances, hearing loss, and cardiac anomalies. The impact on development of the central nervous system ranges from asymptomatic, to moderate, to catastrophic. The neural canal, an invagination of the ectoderm, develops into the spinal canal to be closed posteriorly by pedicles and laminae. This will form a tube containing the dural (neural) sac, with the spinal cord, from the base of the skull to lumbar two vertebral level and containing the cauda equina nerve conglomerate down to the sacral canal.

Failed closure of the tube leaves the L4-5 and sacral areas open most frequently, exposing the nerves to external pressure. This spina bifida is not infrequent, is mostly asymptomatic, discovered incidentally, and at times covered by a hairy formation over the regional skin area. A more serious cystic bulging may emerge externally, the meningocele and occasionally with nerve content, the myelomeningocele. Surgical correction is possible at times leaving neurological signs of paresis, paralysis, sphincter incontinence, and erectile dysfunction (Fig. 10).

Nutritional folate deficiency is almost inexistent in developed countries, except perhaps due to malabsorption following surgical excision. However nutritional deficiency is present in famine hit areas of sub-Saharan and the East-Asian desert regions.

Metabolic Bone Disease due to Famine-Imposed Lathyrus Ingestion

Mesenchymal membrane changes leading to osteoblastic malfunction and ending in disturbed bone metabolism have been observed following the ingestion of toxic substances, in a similar way to the teratogenic effect of thalidomide on limb development and the effect of alcohol on the development of the corpus callosum in the brain.

The toxic effect of grass pea variants of *Lathyrus sativus* or *Lathyrus odoratus* was known to Hippocrates and was also described by Ramazini in seventeenth century

Modena. In Bavaria, it was forbidden to consume Lathyrus by the Duke of Wittenberg in the eighteenth century, its availability was widespread in India in the nineteenth century. Lathyrism was a condition reported in Spain during the Civil War in the 1930s.

During WWII, the ingestion of grass pea was imposed by the Romanian authorities as the sole source of food in the Vapniarca concentration camp (Transnistria, Ukraine) on a population of some 100,000 incarcerated people (Weisz 2016). On observing the symptoms, an ingenious prisoner physician, Dr Arthur Kessler (1903–2000), unlikely to have had any former knowledge of this historical condition which did not exist in Europe at that time, recorded some 1,800 patients with various degrees of paralytic signs. This eventually forced the authorities to allow access to alternative food types. Two hundred survivors of this camp were examined after the war in Tel Aviv's Ichiloff University Hospital (Cohn and Streifler 1981a, b, 1983) and together with the prisoner physician's notes, the condition was revived in the literature.

It was a group of Belgian researchers, led by Lambein, who eventually discovered that the toxic compound in the grass pea was ODAP (L-amino acid alanine derivative), and was the cause of what became known as the complex condition **neurovasculo-osteo-lathyrism** (Haque et al. 1996; Ngudi et al. 2012; Riepe et al. 1995).

The Pathology of Lathyrism

Neurolathyrism, a motor neuron disease of the thoracic spine, demyelination, and degeneration of the anterior horn cells, was described clinically and reproduced experimentally (Getahun et al. 2002; Belanger 1959). Changes were localized to the thoracic cord using MRI studies. Neurolathyrism was characterized by symptoms including wobbling, scissor walk, collapsing gait, spastic paralysis of the legs, and incontinence.

Vasculo-lathyrism is characterized by fragility of blood vessels and connective tissue, thrombosis, gangrene of lower limbs, and development of aortic aneurism.

Osteolathyrism results from ingestion of both lathyrus legume species, but mostly *L. odoratus*, which influences bone turnover, softening the bone due to collagen disease. Hemorrhage in the bone tissue is also observed, due to fragility of arterioli, eventually developing into osteoporosis and frequent fractures. These symptoms occur as a result of the second identified toxic amino acid (beta-amino-propio-nitrite), promoting the formation of exostosis and development of kyphoscoliosis.

As an aside, it is interesting to recall the Spanish painter, Francesco Goya's graphic illustration of the crippled woman lying on the floor, in an early nineteenth-century print, with the title of *Gracias a la almorta* (Fig. 11).

Famine Osteopathy and the Medical and Legal Implications

Famine osteopathy is a conglomerate of metabolic aberrations resulting from nutritional deprivation with a clear clinical symptomatology and distinct histopathological features. The pathogenesis is clearly established. The outcome in general is

Fig. 11 'Thanks to the millet (grass pea flour)', ca. 1810, aquatint print by Francisco Goya [public domain]

described as a benign, nonfatal condition, except for embryonic vitamin K deficiency and lathyrism. The main malignant complication of the bone disease is major fractures and their consequences.

In developed countries accepting immigrants and refugees from famine-hit countries, the medical community has an obligation to facilitate early detection of metabolic deficiencies. The diagnosis should be based on clinical examination and serum analysis for early detection of metabolic deficiencies, testing that (in the Australian healthcare system) would cost less than $50.The respective health authorities also have an obligation to expedite treatment of any diagnosed conditions, particularly in light of the fact that all diseases, except lathyrism, are curable. Similarly, all the discussed metabolic bone diseases are preventable.

Hypocalcemia and Osteomalacia

The famine osteopathy induced by hypocalcemia, namely osteoporosis, is curable with calcium supplementation or calcium-rich diet. Calcium is present in food such as meat, eggs, and dairy products. The daily recommended intake is between 700 mg and 1200 mg a day (as recommended by the National Institutes of Health, USA) a dosage also accepted by the British Health Authorities and the Australian Dietary Guidelines of the Department of Health, 2013. See Appendix A.1.

Rickets and Osteomalacia

In rickets and osteomalacia, mineral homeostasis can be achieved with double or triple doses of vitamin D, namely up to 1000–2000 IU per day. It is a preventable and treatable metabolic bone disease. The treatment is successful with the daily

requirement of 50 nmol per litre and is obtained from ultraviolet skin exposure and consuming milk products, meat, fish, and eggs (Munns et al. 2016).

Vitamin K Deficiency

Vitamin K deficiency requires consumption of 1 mg per day of vitamin K, although less is required in childhood (around 90 mcg a day). Apart from bone health, supply of the vitamin is beneficial in blood clotting and in prevention of arterial wall calcifications. It is available from foods such as greens (turnip, spinach broccoli, kale, etc.). It is also found in meat (chicken, beef), butter, and cheese, which is unlikely to be available in the famine-ravaged regions. Deficiencies caused by malabsorption and malnutrition are curable and preventable.

Folate Deficiency

The recommended dosages differ according to the severity of the pre-existing condition. For those in the low-risk category (no prior case of neural tube defect in the family of either parent), 0.4 mg folic acid a day starting 2 months preconception (in a planned pregnancy) to be continued until the end of lactation is recommended. In those with a moderate risk (with history of prior incident in either of the parent's family), the recommended daily dose increases to 1 mg a day, starting at three months preconception and lasting until the end of lactation. For those at high risk (previous child with NTA born to the same parents), the recommended dosage is 4 mg for the same period of time. Folates are readily present in meat, milk, and liver, which are all rarely available in famine ravaged regions. Folates are also found in green leaves, spinach, beans, turnips, etc.

Lathyrism

Lathyrism requires palliative treatment for instability, immobility, and/or incontinence. Prophylactic measures were outlined at the "Workshop on toxiconutritional neurodegenerations konzo and lathyrism," held at Ghent University in September 2009. Various dilutions of the legumes by water bleaching reduce the toxicity of ODAP. Combining grass pea with grain reduces the frequency of the condition. Lathyrism is an incurable condition. However, the condition is preventable and requires education of often illiterate and/or poorly educated people living in famine-ravaged regions of Asia (India, Nepal, Bangladesh) and in the Horn and Sub-Saharan regions of Africa (Ethiopia, Sudan, etc.). Australian researchers intend to improve the quality of grass pea crops by reducing ODAP toxin levels, thus making this highly drought resistant and nutritious crop safe for human consumption in drought-ravaged areas of the globe (Dixit et al. 2016).

Policies and Protocols

Clinical examination and serum analysis for early detection of metabolic deficiencies should be considered, particularly in immigrants and refugees who have been exposed to famine.

Vitamin and mineral supplementation that could be considered in famine-exposed areas, for prevention of deficiencies resulting in bone disease:

- **Calcium** (daily recommended intake is between 700 mg and 1200 mg)
- **Vitamin D** (1000–2000 IU per day)
- **Vitamin K** (1 mg per day)
- **Folic Acid** (0.4 mg per day during pregnancy and 2 months prior to conception)

Dictionary of Terms

- **Lathyrism** – Osteolathyrism occurs during times of famine when the diet is predominantly reliant on Lathyrus species of legume (grass pea). Symptoms include neurological symptoms and collagen cross-linking abnormalities leading to weak bones and frequent fractures.
- **Neural tube anomaly** – Neural tube anomalies, including spina bifida and anencephaly, may result from a diet deficient in folic acid during pregnancy. This deficiency can affect the development of the brain and spinal cord.
- **Osteoblast** – Osteoblasts are cells which produce bone.
- **Osteomalacia** – Osteomalacia is a deficiency in the bone-building process resulting in bone softening. Osteomalacia is usually a result of calcium or vitamin D deficiency.
- **Osteoporosis** – Osteoporosis is a condition in which bones become more brittle and less dense due to a loss in minerals such as calcium. People with osteoporosis have an increased risk of fractures.
- **Rickets** – Rickets is a bone disease occurring in infants and children usually caused by a lack of vitamin D. Rickets results in soft, weak bones and may lead to skeletal deformities, muscle weakness, and delayed growth.

Summary Points

- Famine affects an estimated 794.6 million people worldwide (2012–2014), accounting for approximately 10.9% of the world's population.
- Throughout history there have been opportunities to study the effect of starvation and famine on populations including the Leningrad siege, the Dutch Hongerwinter, and the Warsaw ghetto.
- In the Warsaw ghetto, one of the most detailed studies of starvation was conducted by a group of imprisoned physicians. Severe food restriction led to bowing of bones, osteomalacia, and osteoporosis in children with histological evidence of bone marrow being replaced with connective tissue.

- The risk of developing osteoporosis in later life has also been linked to undernutrition during the fetal period.
- During periods of famine, there is evidence that maternal epigenetic changes may affect placental transfer of essential nutrients for bone development including calcium and vitamin D.
- Osteomalacia, or rickets, is a condition resulting from a lack of vitamin D. Vitamin D is essential for calcium absorption and bone mineralization, and its deficiency may occur due to a lack of ultraviolet sun ray exposure or malnutrition.
- The interplay between the three most important components of bone metabolic health, being calcium, vitamin D, and vitamin K, often occur together in communities affected by famine.
- Vitamin K deficiency can lead to intrauterine epiphyseal ossification and adolescent osteoporosis.
- Folic acid deficiency during pregnancy is a key risk factor for neural tube anomaly development.
- Lathyrism due to famine-related dietary restriction to predominantly one food type, the lathyrus legume species, can lead to osteolathyrism. Symptoms include bone tissue hemorrhage, collagen disease causing bone softening, osteoporosis, and frequent fractures.

All Metabolic Bone Disorders Resulting from Malnutrition Are Preventable and All Osteopathies, Except Lathyrism, Are Curable

Daily Requirements (Nat Inst Health USA)

Life stage group	Calcium recommended dietary allowance (mg/day)	Vitamin D recommended dietary allowance (IU/day)
0–6 months	a	b
6–12 months	a	b
1–3 years old	700	600
4–8 years old	1,000	600
9–13 years old	1,300	600
14–18 years old	1,300	600
19–30 years old	1,000	600
31–50 years old	1,000	600
51–70 years old	1,000	600
51–70 year old females	1,200	600
71+ years old	1,200	800

[a]For infants, Adequate Intake is 200 mg/day for 0 to 6 months of age and 260 mg/day for 6 to 12 months of age

[b]For infants, Adequate Intake is 400 IU/day for 0 to 6 months of age and 400 IU/day for 6 to 12 months of age

The British Nutrition Requirements

Reference nutrient intakes for vitamins (μg/day)			
Age	Vitamin B12	Folate	Vitamin D
0–3 months	0.3	50	8.5–10[c]
4–6 months	0.3	50	8.5–10[c]
7–9 months	0.4	50	8.5–10[c]
10–12 months	0.4	50	8.5–10[c]
1–3 years	0.5	70	10
4–6 years	0.8	100	10
7–10 years	1.0	150	10
11–14 years	1.2	200	10
15–18 years	1.5	200	10
19–50 years	1.5	200	10
50+ years	1.5	200	10
11–14 years	1.2	200	10
15–18 years	1.5	200	10
19–50 years	1.5	200	10
50+ years	1.5	200	10

Sources: British Nutrition Foundation 2016, Nutrition Requirements, Reference Nutrient Intakes for Vitamins. https://www.nutrition.org.uk/attachments/article/261/Nutrition%20Requirements_Revised%20Oct%202016.pdf. Accessed 16 August 2017
Institute of Medicine of the National Academies, November 2010, Dietary Reference Intakes for Calcium and Vitamin D. http://www.nationalacademies.org/hmd/~/media/Files/Report%20Files/2010/Dietary-Reference-Intakes-for-Calcium-and-Vitamin-D/Vitamin%20D%20and%20Calcium%202010%20Report%20Brief.pdf. Accessed 16 August 2017
[c]Safe intake

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Suffering the Great Hunger: Scurvy and Tuberculosis as Reflected in Skeletons of Victims of the Great Irish Famine (1845–1852)

11

Jonny Geber

Contents

Abstract

The Great Famine in Ireland between 1845 and 1852 was one of the worst health calamities of the nineteenth century. In recent years, palaeopathological analyses of human skeletal remains from archaeologically excavated famine mass burials from Ireland have revealed the physical impact of health deprivation due to starvation and infectious disease. Vitamin C deficiency and tuberculosis were two conditions that caused immense suffering during this period, and which are observable in the skeletal remains of the victims of the Famine. The manifestations of these reveal aspects of physical exhaustion but also that of recovery. Bioarchaeology – which is the study of human remains from archaeological contexts – provides a unique insight how the human experienced famines in the past. In the context of the Great Famine, it has provided means to acknowledge

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the lives of those who were lost and forgotten during a very dark and relatively recent period in the history of Ireland.

Keywords
Bioarchaeology · Clones · Consumption · Infectious disease · Ireland · Kilkenny · Mass burials · Mortality · Nineteenth century · Osteological paradox · Palaeopathology · Vitamin C deficiency · Workhouse

Introduction

Health conditions in human populations change with societal transformations. This occurred, for instance, alongside and following the agricultural transition about 10,000 years ago (Cohen and Armelagos 2013). An increasing dependency on agricultural produce made populations more vulnerable to crop failures with famines occurring as a consequence. Famines and human starvation are mentioned in some of the most ancient literary sources in the world, such as, for instance, the *Epic of Gilgamesh* dating to c. 2000–1400 BC (Geber 2015a). In addition to written sources, archaeology also provides the means to study how these conditions may have been expressed in past societies. A particularly valuable insight is gained from direct studies of the people themselves, by assessing the evidence of disease and starvation through bioarchaeological analyses of human bones and teeth. Using the most commonly accepted definition, bioarchaeology is the study of human skeletons from archaeological contexts in a broad anthropological sense that takes the social and cultural context in which they lived into account (Buikstra 2006). By investigating how the skeleton adapts to social, cultural, and ecological environments, bioarchaeology enables a unique and direct insight into the living conditions people experienced. This insight is yielded in particular from palaeopathological research, which aims to assess and describe the evidence and consequence of disease in past societies.

While bioarchaeology and palaeopathology have the means to detect the effects and consequences of famine and starvation in past societies, it is only in recent years that these topics have been studied (e.g., Beaumont and Montgomery 2016; Geber 2015b; Yaussy et al. 2016). A characteristic feature of famine, in general terms, is an excess death rate that is leaning towards particular age groups and social levels of a given population – namely, the weakest and most vulnerable such as the young, the old, and the poor (Ó Gráda 2009). This pattern is potentially discernable from a contextual analysis of archaeological burial grounds and by assessing the mortality profile of the dead (based on the estimated age-at-death) who were interred therein (see Chamberlain 2006; Margerison and Knüsel 2002). The human skeletal response to starvation is potentially detectable by observing elevated nitrogen levels ($\delta^{15}N$) in bone and teeth collagen as a consequence of a prolonged period of severe malnutrition that has resulted in the body attempting to regain energy by obtaining it from its own protein tissue (Beaumont and Montgomery 2016). Likewise, sudden dietary shifts due to the consumption of so-called "famine foods" may also be detectable in stable isotope analysis of skeletal tissue (Beaumont and Montgomery 2016).

In addition, the bone remodeling process might become altered and result in decreased bone mass and osteopenia (Brickley and Ives 2008; Ortner 2003). However, in general death by famine and starvation is analogous with death by disease, in particular, infectious ailments, but also metabolic diseases due to malnutrition. Most infectious and metabolic conditions will not manifest skeletally, but when observed in the human remains they provide the most tangible insight into the nature of suffering disease for the people who lived before the advent of antibiotics.

Death by Famine: The Kilkenny and Clones Union Workhouse Mass Burials

An intense level of suffering is evident from the skeletal remains of victims of the notorious Great Famine in Ireland (1845–1852) that have been discovered during archaeological excavations in recent years. Two such important discoveries are the mass burial grounds at the former workhouse institutions in Kilkenny City in County Kilkenny (Geber 2015b; O'Meara 2006) and Clones in County Monaghan (O'Donovan 2012; Fig. 1). In both of these cases, the local awareness or archival records relating to these mass burial grounds did not exist and they appear to have been intentionally repressed from communal memory in the years and decades that followed the Famine. A total of 970 skeletons were unearthed from the Kilkenny mass burials and 35 individuals from Clones (Fig. 2). They constitute the remains of individuals on the lowest rank of the social scale who were incarcerated in institutions that through their very function exposed them to further demeaning treatment. Although there are physical testimonies to a horrid period in Irish history, the analysis and study of the remains has been able to bring some humanity and dignity back to these people who were treated extremely badly in life, by telling their story and acknowledging their lives and what they experienced in the final months leading up to their deaths.

The Great Famine in Ireland – also known as the Great Hunger or *An Gorta Mór* – has been described as "the greatest social disaster of nineteenth-century Europe" (Eagleton 1995, 22). It is in terms of relative mortality outcome one of the worst subsistence crises in human history (Ó Gráda 2007). The Famine was the result of a potato blight *(Phytophthora infestans)* that arrived in Ireland in the late summer of 1845 and then quickly spread across the country (Zadoks 2008). A vast proportion of the population, who were completely dependent on the potato crop for their subsistence, suffered famine as a consequence. The estimated numbers of famine deaths have ranged from about 800,000 to one million, which comprised over 10% of the total population of Ireland at the time (Boyle and Ó Gráda 1986; Cousens 1960). A great proportion of these, about 20% (c. 200,000), took place in the notorious workhouse institutions (Guinnane and Ó Gráda 2002). These establishments (Fig. 3a), which were operating on the notion that only those "deserving" of relief (i.e., the most desperate, impaired, and critically ill) would be allowed access, were operating under severe financial and logistical strains during the Famine period (O'Connor 1995, 121–143). Originally intended to provide accommodation for

Fig. 1 County map of Ireland, with the geographic locations of Kilkenny and Clones. Based on template by Alan O'Rourke (©www.toodle.com), used by permission

80,000–100,000 people across all of Ireland (Kinealy 2006; O'Connor 1995), by September 1849, about 250,000 were dependent on indoor relief (Donnelly 2001). Overcrowded conditions meant that the spread of infectious disease became rampant and the mortality rates were exceptionally high. This, in turn, resulted in considerable logistic (and economic) difficulties relating to the burial of the dead, who in many places were interred in non-consecrated grounds, in mass burials, with no ceremony (Geber 2012).

The Kilkenny Union Workhouse (Fig. 3b) opened in April 1842 and was built to accommodate 1,300 inmates. During the Famine, however, the institution became severely overcrowded, developed into a hotspot for infectious disease, and there

Fig. 2 In-situ photographs of skeletons of victims of the Great Irish Famine at the Kilkenny and Clones Union workhouses. (**a**) The remains of a 2–3 year-old-child (left) and an older adult (≥46 years) female (right) buried at the Kilkenny Union Workhouse (Photo: Margaret Gowen & Co. Ltd). (**b**) The skeleton of an adolescent aged approximately 13 years at the time of death from Kilkenny (Photo: Margaret Gowen & Co. Ltd). (**c**) The burial of an older adult (≥46 years) female from Clones Union Workhouse (Photo: Edmond O'Donovan & Associates)

were mass deaths as a result. Between August 1847 and March 1851, inmates were buried in mass burials within the grounds of the workhouse at the back of the establishment, a short distance from the infirmary block (Geber 2015b). A similar situation appears to have taken place at the union workhouse in Clones. Mass burials adjacent to where the boundary wall and the mortuary building of the institution once stood were discovered in 2012, and the dead had, just as in Kilkenny, been interred by being placed in the graves on top of each other (O'Donovan 2012). The workhouse in Clones first opened in February 1843 and was constructed to provide relief for 600 inmates (O'Connor 1995). In June 1849, however, over 1,600 people were dependent on indoor relief in Clones, and hundreds of people were housed at auxiliaries at various locations in the town and its vicinity (MacDonald 2000).

Suffering the Great Irish Famine: The Palaeopathological Evidence

It is not possible to determine the direct cause of death of the individuals who were interred in the Kilkenny and Clones mass burials from their skeletons, but the historical sources reveal that it was most likely due to infectious disease (Census of Ireland Commission 1856). These were generally described as "famine fever" and

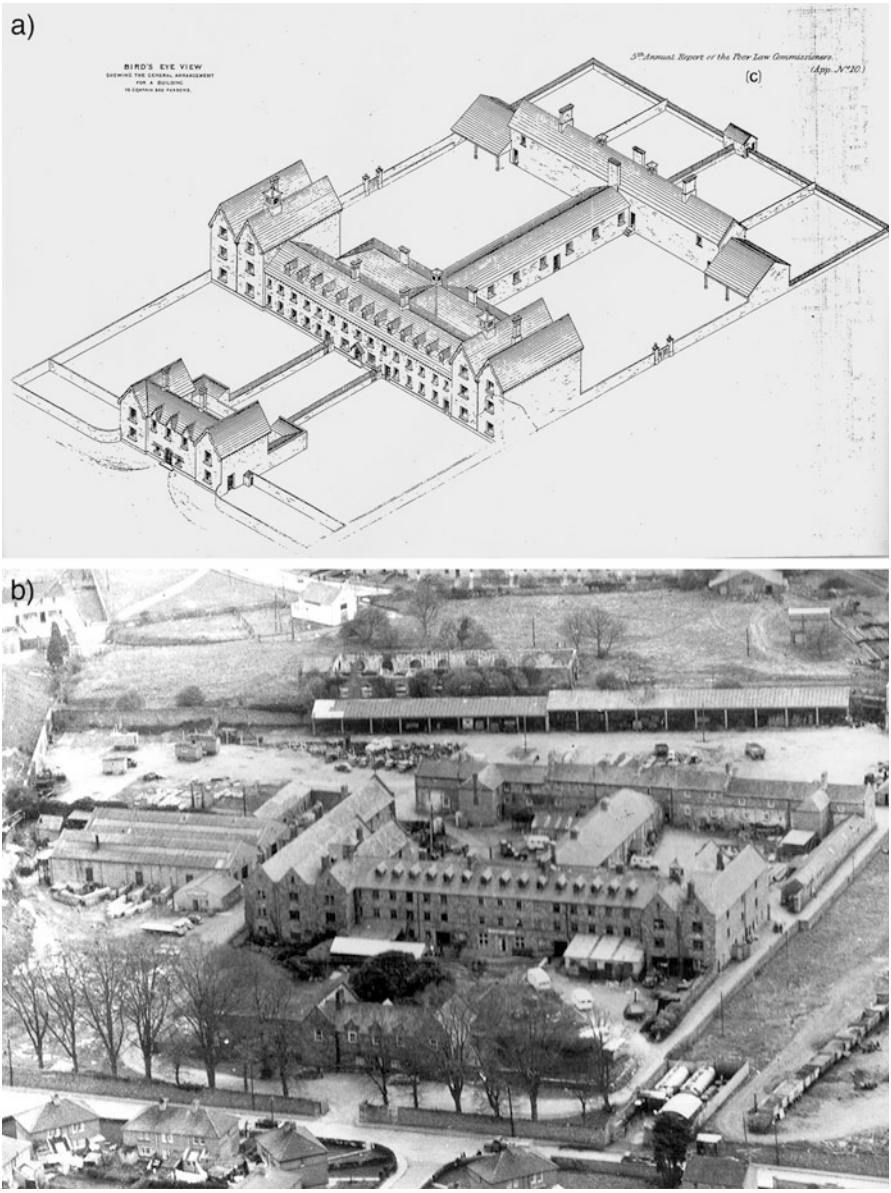

Fig. 3 (**a**) The general layout of the typical Irish union workhouse institution, as designed by the English architect George Wilkinson (1814–1890) (Source: *5th Annual Report of the Poor Law Commissioners, Appendix no. 10*). (**b**) Aerial photograph of the Kilkenny Union Workhouse, when the buildings functioned as a depot for the Kilkenny County Council during the 1960s (Source: Kilkenny County Library, Local Studies, courtesy of Karyn Deegan)

would have included conditions such as typhoid, typhus, and relapsing fever (Mokyr and Ó Gráda 2002). Infectious diseases spread quickly across Ireland during these

years, as the population was weakened by severe malnutrition and starvation. The highest famine-induced mortality was seen in the winter of 1846 and 1847 when an epidemic of typhus – and other diseases – tragically resulted in the deaths of an estimated 400,000 men, women, and children (Kinealy 1997, 79). The year 1847, which would later be called "Black '47," was the year when the highest relative numbers of excess deaths were recorded in both County Kilkenny and County Monaghan, as well as most of Ireland (Cousens 1960). It was during 1847 that the intramural burial ground in Kilkenny was taken in use, and it is likely that the mass burials discovered at Clones include interments of inmates who perished during this specific period of mass mortality.

Vitamin C Deficiency

Pathological changes in the Kilkenny and Clones skeletons reveal that they suffered from severe metabolic stress in the period leading up to their deaths. The most evident of these were the widespread manifestation of osseous lesions indicative of Vitamin C deficiency or scurvy. This debilitating and often extremely painful disease became very common in Ireland following the destruction of the potato crop in the 1840s, and it would have been synonymous with the physical experience of the Famine for those who suffered the most. Prior to the blight, the required daily amount of Vitamin C that is required to sustain good health would have been more than plentifully provided for by the potato (Crawford 1988). Scurvy was therefore rarely encountered amongst the agricultural populace in Ireland prior to the blight, and in the early years of the Famine it was often misdiagnosed (ibid.). To diagnose scurvy in skeletal tissue is not straightforward, as many of the lesions it produces are nonspecific and can occur in other conditions (Brickley and Ives 2008). What is considered a virtually pathognomonic skeletal marker of scurvy is the manifestation of porotic lesions at the site of the attachments of the masticatory muscles on the temples of the skull (Fig. 4) and of the lower jaw, as well as abnormal porosity around the sockets of the teeth (Fig. 5) and of the hard palate (Ortner et al. 2001; Ortner et al. 1999). Layers of new bone may also form at the site of muscle attachments elsewhere on the skeleton, i.e., the shafts of the long bones and on the scapulae (shoulder blades) (Brickley and Ives 2008). These manifestations are the result of hemorrhage, which in turn is linked to micro-trauma of soft and hard tissues that is weakened due to impaired collagen formation in the body and the skeleton (Ortner 2003).

Nearly 52% of all skeletons from Kilkenny and 29% from Clones showed evidence suggestive of scurvy (Table 1). This is an exceptionally high rate in comparison to other bioarchaeological samples that have rarely revealed crude prevalence rates exceeding 5% (Mays 2014). The true rate of individuals suffering from the disease in Kilkenny and Clones cannot be ascertained however; the skeletal manifestation of Vitamin C deficiency is a paradox as it is only evident once some Vitamin C has been reintroduced to the system. Without the vitamin, the osseous lesions from which the disease is diagnosed skeletally cannot form (Brickley and Ives 2008). The high rate of scurvy in both the Kilkenny and Clones is instead a

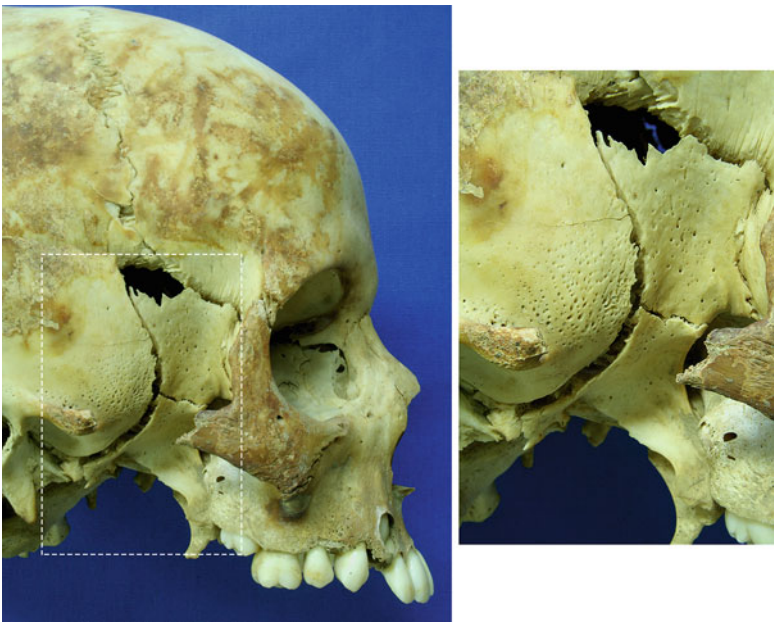

Fig. 4 Porotic lesions on the ectocranial surface of the greater wing of the sphenoid and the anterior portion of the temporal bone on the right side of the skull of a 10–11-year-old child from the Kilkenny Union workhouse mass burials. The lesions are indicative of micro-trauma affecting the mastication musculature which in turn would have been a consequence of scurvy that affected this child (Photo: Jonny Geber)

reflection of the desperately poor state of health these individuals would have suffered prior to having been granted access to the workhouse institutions. They evidently recovered from their scurvy – some Vitamin C would have been provided from the daily meal rations in the workhouse – but due to a weakened immune system they had little chance of surviving once they entered institutions ridden with infectious disease (Geber and Murphy 2012). The skeletons are therefore a physical testimony to the reality faced by many in Ireland during the Famine to either seek admission in the union workhouses and risk death from infectious disease or avoid it and risk death by starvation (Fig. 6). The social shame of entering the workhouse would have forced many to the latter action. In other accounts, it was said that people who had avoided the workhouses would ultimately seek relief when they were nearing death. They might then, at least, be buried in a coffin (Cohen 2002, 125).

The manifestation of scurvy in these samples have allowed for a comparison of how the disease is likely to have affected different sections of the population. Palaeopathological scurvy is easier to diagnose in children, as the manifestations of the disease are more evident in the growing skeleton. In adults, the changes are more subtle and less evident, which explains why the adult prevalence is substantially lower than the non-adult rate in both the Kilkenny and Clones samples. In the

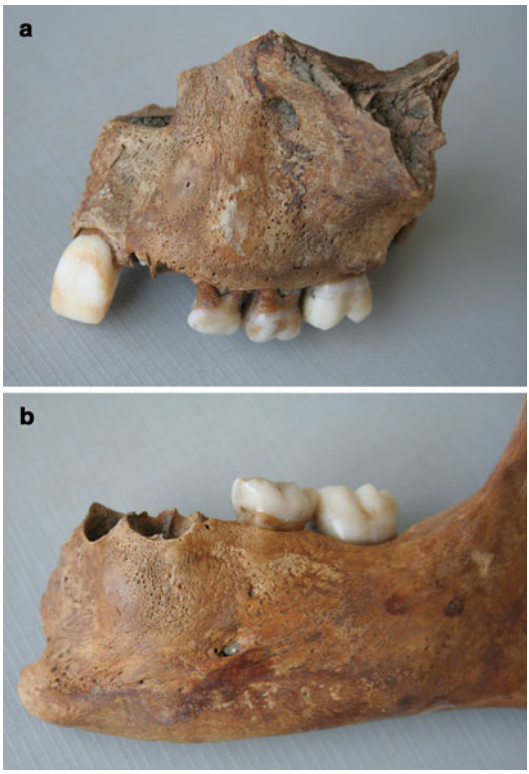

Fig. 5 Porous and porotic lesions on the left maxilla (**a**) and mandible (**b**), and around the alveolar margins, of an approximately 9-year-old child from Clones Union Workhouse, indicative of scurvy (Photo: Jonny Geber)

Table 1 Prevalence rates of scurvy, diagnosed skeletally in victims of the Great Irish Famine from the Kilkenny and Clones Union workhouses

	Kilkenny Union Workhouse		Clones Union Workhouse	
	Non-adults	Adults	Non-adults	Adults
Vitamin C deficiency[a]	60.8% [330/543]	40.1% [169/421]	41.2% [7/17]	16.7% [3/18]

[a]See Geber and Murphy (2012) for information on how the diagnoses were quantified

child sample, lesions suggestive of scurvy are present in all the age groups, from infants (<1 year) to adolescents (13–18 years). In adults (>18 years), it was most commonly observed in the skeletons of males (Table 2), and in particular those of taller stature (Table 3). This is an interesting observation, as it does indicate that physical build and sex were factors that influenced the ability to cope with famine stress. During the Irish Famine, it is estimated that the excess death ratio between males and females was 1.1:1, and that about 37,000 more males than females died (Boyle and Ó Gráda 1986). The discrepancy in the rates of scurvy between the sexes would imply that the Vitamin C deficiency in males was more pronounced than females during the Famine, at least for the section of the population that were admitted into the workhouse institutions. As stated above, scorbutic lesions in the

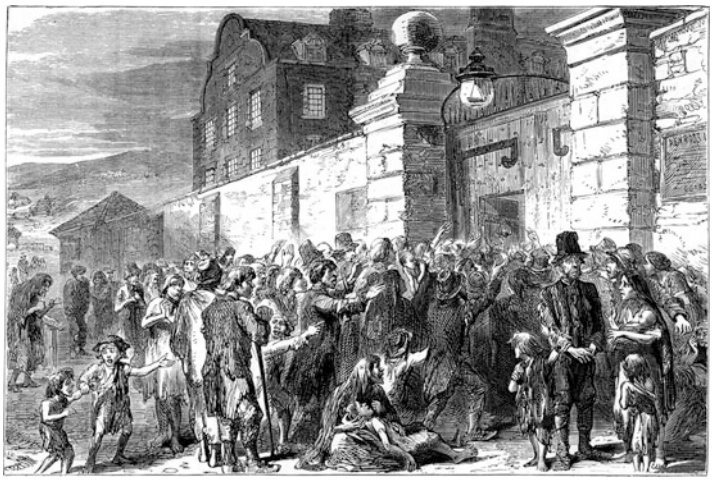

Fig. 6 "The Famine in Ireland – Peasants at the Gate of a Workhouse." Illustration of a scene from Ireland in 1846 (Source: *The Life of the Right Honourable William Ewart Gladstone, Volume 1*, by Smith 1880)

Table 2 The prevalence rate of lesions indicative of scurvy in skeletons of adult males and females from the Kilkenny Union Workhouse mass burials

	Males	Females
Vitamin C deficiency[a]	50.9% [107/210]	29.6% [58/196]

[a] $\chi^2(1) = 19.175$, p = <0.001

Table 3 The difference in estimated living statures (mean) in scorbutic and non-scorbutic male and female adults skeletons from the Kilkenny Union Workhouse mass burials

	Scorbutic	Non-scorbutic
Male statures (cm) (n = 186)[a]	172.23 (SD = 5.48)	170.43 (SD = 6.16)
Female statures (cm) (n = 159)[b]	159.00 (SD = 6.30)	157.88 (SD = 5.26)

[a] $t(184) = -2.290$, $p = 0.023$
[b] $t(157) = -1.176$, $p = 0.241$

skeleton will only be apparent once Vitamin C has been reintroduced to the diet, which would have been the case once inmates had access to the diet provided for them in the workhouse. At the Kilkenny institution, the quantities of the food rations were the same for both males and females, and the higher nutritional requirement of males was not taken into consideration. This may explain why the male rates were higher. It would also explain why the taller built individuals in this sample appear to have been more affected, as those of shorter stature would have had a lesser biological requirement of the vitamin to sustain an adequate health status (see FAO 2001). Recent research has also suggested that Irish males are genetically

predisposed to develop scurvy compared to other populations (Delanghe et al. 2013), and this possibility further highlights the complexity of the range of factors that determined the outcome for those who suffered during the Great Famine.

Tuberculosis

While typhus, dysentery, and cholera were some of the infectious diseases that were the main cause of death during the Irish Famine (Kennedy et al. 1999), they leave no markers on bones. Other "famine diseases" on the other hand, such as typhoid fever, small pox, and tuberculosis, are potentially manifested in the skeleton (see Aufderheide and Rodríguez-Martín 1998; Ortner 2003), although it is only the latter that leaves specific osseous changes. In the context of a famine, the likelihood of detecting skeletal markers of these conditions is reduced due to the immediate death and demise of those who are most affected; it is only in its most chronic state that infectious disease affects the skeleton, and then in individuals who were "strong" enough to survive the disease to such an advanced stage (this notion is generally referred to as the "osteological paradox," see Wood et al. 1992). The skeletal manifestations of the infectious diseases that were observed in the victims from Kilkenny highlight the extent of this suffering for only a few, but it indirectly suggest the agony these diseases would have caused to thousands of people suffering the Famine in Ireland in the middle of the nineteenth century.

Of the seven cases diagnosed as tuberculosis in the Kilkenny sample, six were active at the time of death (Geber 2015b). Of these, three adults displayed vertebral involvement, which was manifested as characteristic psoas abscesses of the vertebral bodies in the lumbar spine. This pathological change, which is a typical skeletal manifestation of the disease, can potentially result in severe back pain and eventually spinal deformity with further negative health consequences (Garg and Somvanshi 2011). Two children, aged five to 6 years and 9 years, were affected by cranial tuberculosis, which had resulted in large lytic lesions on their cranial vaults and mandible. One adolescent female, aged between 14 and 17 years, was affected by a severe active infection of the left hip joint, which was probably due to tuberculosis. This is a common joint affected by the disease, especially in young individuals, and can result in a considerable painful disability (Tuli 2016, 71–115). In addition, there were 32 adults and 29 children from Kilkenny and Clones that displayed lesions on the visceral surface (facing the lungs) of their ribs (Table 4). This is indicative of a pulmonary infection and may potentially relate to tuberculosis (Kelley and Micozzi

Table 4 Crude prevalence rates of active cases of tuberculosis and rib lesions diagnosed skeletally in victims of the Great Irish Famine from the Kilkenny and Clones Union workhouses

	Kilkenny Union Workhouse		Clones Union Workhouse	
	Non-adults	Adults	Non-adults	Adults
Tuberculosis	0.6% [3/545]	0.7% [3/425]	0.0% [0/17]	0.0% [0/18]
Rib lesions	5.8% [28/479]	9.3% [32/343]	12.5% [1/8]	0.0% [0/7]

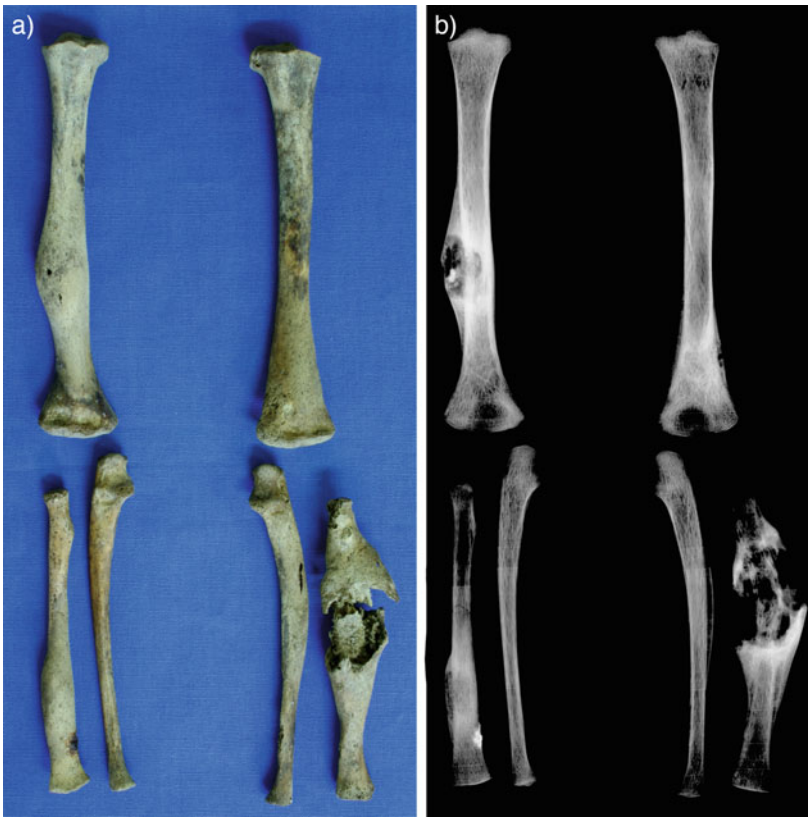

Fig. 7 Photograph (**a**) and radiograph (**b**) of the arm bones of a 5–6-year-old child from the Kilkenny Union Workhouse. The bones are severely affected by osteomyelitis caused by tuberculosis

1984; Roberts et al. 1998), although a definite diagnosis based on these osseous lesions alone is not possible (Mays et al. 2001; Rogers and Waldron 1989).

Tuberculosis in children has a different skeletal manifestation than in adults. One gross deformity which is more commonly observed in their skeletons in chronic cases of tuberculosis is osteomyelitis. This condition, which is a severe infection that affects the bone marrow and bone cortex, was observed in a 5- to 6-year-old child (Fig. 7). This individual also displayed evidence of a bilateral pulmonary infection (probably tuberculous) and Vitamin C deficiency. Osteomyelitis is, however, not exclusively associated with tuberculosis, and in the context of these skeletons, typhoid fever is a condition that also needs to be considered. Typhoid osteomyelitis is generally more common in children than in adults, with a multifocal manifestation that often attacks the ribs, tibiae, and spine (Adler 2000, 154; Aufderheide and Rodríguez-Martín 1998, 191). This possibility highlights the likelihood that the experience of famine and starvation in Ireland during the 1840s and early 1850s

involved comorbidity of conditions, and whatever ailment that ultimately caused the death of those who died was only one of the many illnesses suffered at the time.

According to Steinbock (1976, 175), tuberculosis is only detectable in the bones and joints of approximately 5–7% of those who suffer the disease, and it would then only appear when it has reached a chronic stage. Other authors have suggested that the percentage is much lower, from 1% to 3–5% (see Roberts and Buikstra 2003, 89). The skeletal evidence, as observed in the bones of the workhouse inmates assessed in this study, is, therefore, likely to be a drastic underrepresentation of the true prevalence of the disease in these two population samples. Tuberculosis was not an uncommon disease in Ireland before the Famine, far from it (see Breathnach and Moynihan 2011; Jones 2001). As in most parts of the world, the disease was associated with poverty and poor living conditions (Roberts and Buikstra 2003). The deteriorating health conditions due to malnutrition and living conditions during the Famine, however, meant that the disease took hold on a much broader level, and it caused approximately 107,200 deaths in Ireland between January 1846 and the end of March 1851 (Census of Ireland Commission 1856, 661). According to the 1851 Census, it was the fifth most common cause of death (excluding natural causes due to old age) in the Irish union workhouses between January 1846 and the end of March 1851, with 10,377 individually recorded deaths attributed to the disease (ibid., 116–117).

Perceiving of the Impact of the Great Irish Famine

Between 1841 and 1851 Ireland saw a population loss of almost 20% (Kennedy et al. 1999) that was approximately equal parts the result of famine-related deaths and emigration. It is a tantalizing thought to consider what Ireland was like immediately after the subsistence crisis had ended, and how people coped with adjusting to a post-Famine society. In some places, whole local communities had been destroyed (Kinealy 1997). Expressions of rural Irish folk culture, such as the use of Irish language, which at the time was viewed as an expression of inferior and of low social status, declined more rapidly than it had done previously, and it would eventually be largely lost as a consequence (Hindley 1990). The Irish rural landscape must have been perceived as completely desolate in places; an emptiness and silence that marked a striking contrast to what had been before. There does also appear to have been a silence in terms of what experiences and witness accounts were preserved in communal memory (see Ó Gráda 1999, 194–225; Ó Gráda 2001). It is clear that selective memories were in place – much of it was probably relating to the social shame of poverty, and that some stories were never told or passed on to the following generations. This evidently occurred at Kilkenny and Clones, where the location and awareness of the mass burials adjacent to the workhouse institution were eventually forgotten.

There is a vast collection of contemporaneous written sources relating to the Famine, from government reports and newspaper articles to recorded witness accounts and folklore. But these sources also have limitations, and they rarely give

an ample insight into the experience of the Famine from the perspective of those who were worse off and of those who vanished. While these people may not be that visible in the written records, the traces of their lives are present in the archaeological record – in the Irish landscape itself through the environment in which they lived and the fields in which they worked, to the remains of the cabins in which they lived. And they are physically present in the burial record, which occasionally is discovered in Ireland during archaeological excavations prior to road or building developments (Geber 2015b, 1–10). The potential of using archaeology to expose this dark period in Irish history has been recognized for a long time (see O'Sullivan 1997; Orser 1996), but it still remains a largely unexplored research topic. Ireland's Famine heritage is of international significance; it tells not only the story of Ireland and the nations to which hundreds of thousands of people were forced to flee to, but it is also the story of the ultimate consequence of a strictly hierarchical society and social injustice in nineteenth-century Europe and elsewhere. The people who died in the Irish Famine were primarily those who had lived on the social margins, and they tend – as a consequence – to be largely invisible in the historical records.

One of the most invisible population subgroups in the historical sources are the children. However, in the bioarchaeological record from this period they are the most apparent, particularly in Kilkenny where 56.2% of all 970 individuals interred in the mass burials were aged less than approximately 18 years. The largest age group of these comprised of young children (n = 239) aged between 1 and 5 years of age at the time of death (Geber 2015b). This fact highlights an aspect of Famine history which tends to be forgotten: the impact it had on the youngest generation. While it is well-known that children would have comprised over half of all the victims of the Great Irish Famine (see Boyle and Ó Gráda 1986), their story is still largely untold (see Geber 2014, 2016a, b; Jordan 1998). Even for those who survived, the long-term consequences of having experienced a childhood during the Famine should not be underestimated (see Galler and Barrett 2001; Lumey et al. 2011). For a child (and even fetus) to have experienced malnutrition and severe health deprivation would have resulted in a negative effect on their physical growth and eventually physical health as an adult, as well as possibly their cognitive and behavioral development (see Walsh 2012, for a discussion on the potential increase in schizophrenia during the second half of the nineteenth century in Ireland, as a consequence of in utero stress during the Great Famine). In that sense, the Famine may have affected the Irish population beyond the substantial social, political, economic, and cultural consequences that have been discussed by numerous historians and other famine scholars (e.g., Ell et al. 2014; O'Rourke 1994; Smith 1993; Stout 1996; Whelan 1995).

Acknowledging Lives Lost

The human reality of the Great Hunger, as indicated from the bioarchaeology of its victims, gives a direct insight into the calamity which is unique in famine studies. The extensive manifestation of skeletal lesions indicative of Vitamin C deficiency and scurvy does not only confirm the historical accounts of the period of the

widespread occurrence of a painful and debilitating disease, it also allows an insight into how the condition affected different sections and members of the population. The extent and severity of the skeletal lesions caused by tuberculosis give a similar insight. Death by famine in Ireland during the second half of the 1840s and early 1850s would have been extremely painful. Those who perished experienced levels of suffering and privation that are probably too difficult to fully comprehend today. The archaeological discovery of the mass burials at Kilkenny and Clones have provided a poignant reminder of a very dark period in the relatively recent history of Ireland, which not only relates to the apparent hardship that people endured during the Famine but also the demeaning manner in which the poor and the lowest social strata were treated by contemporaneous Victorian society. In both Kilkenny and Clones the local community expressed a sincere concern and wish for the remains to be returned to their parish and respectfully reinterred after the scientific analysis of the skeletons had been completed. These reburials took place in May 2010 and March 2014, respectively. In Kilkenny and Clones, the rediscovery of these burials have enabled the story of these people to be told, their lives to be acknowledged, and for them to have finally been given dignified treatment in death.

Policies and protocols

The skeleton adapts and responds to biological, environmental and cultural factors (Larsen 2015). Bioarchaeology assess morphological features in the skeleton and employ metrics to determine and estimate sex, age at death, physical built and assess evidence of disease and trauma in ancient human remains. With this information, and interpreted in the cultural and social context the once living population the human remains derive from, the discipline attempts to reconstruct how life was experienced by the people who lived in the past. The historical records relating to the Great Irish Famine of the 1840s and early 1850s are extensive, however, it often lacks to provide insight of those who suffered most from this catastrophe. Recent bioarchaeological research of the Great Irish Famine has enabled a unique historical perspective of the human experience of famine and starvation. While the historical and archival records—almost all written by the societal élite or middle classes—enable historians to gain a detailed insight into the social construct of mid-nineteenth-century Ireland, the skeletons of the people of the labouring "lower" classes who lived during this time are able to tell their stories from their perspective. The physical experience of disease, malnutrition and physiological stress can be discerned from their bones and teeth. The high prevalence of skeletal evidence of scurvy has, for instance, revealed the great level of suffering endured before people were granted access to the notorious workhouse institutions. Males suffered from scurvy to a greater degree than females, possibly due to a relatively lesser and inadequate intake of nutrients as they biologically required. Infectious diseases, such as tuberculosis, are likely to have been much more prevalent than what the skeletal evidence suggests. Few people who experienced famine and starvation would have survived an infectious

disease long enough for it to manifest itself on bone tissue. Social and cultural context is therefore of essential importance when studying famine and starvation from human skeletal tissue, in both past and present societies. The archaeological research of the famine-period mass burials at Kilkenny and Clones has highlighted the importance of recognising human skeletons from archaeological contexts as remains of people, of individuals; men, women and children with names and personalities who lived and worked in the very landscape that people and communities call home today. Archaeological discoveries such as these enable communities to make an emotive connection to a very poignant and difficult past. This was a past that for a very long time was ignored, not talked about and eventually forgotten. About one million people are believed to have perished as a consequence of the Great Irish Famine. The bioarchaeology of the Kilkenny and Clones Union Workhouse mass burials has enabled the stories of some of these people to be told.

Dictionary of Terms

- **Bioarchaeology** – The study of ancient or historic human remains from archaeological contexts, which takes social, historical, and cultural frameworks into account.
- **Mass burials** – Multiple interments of corpses within a single grave, which are generally occurring during catastrophic mass mortality events.
- **Osteological paradox** – The idea that the skeletal evidence of disease and health in bioarchaeological samples represent a paradox, as pathological conditions in bones may only be evident in "healthy" individuals who have survived long enough for these manifestations to occur. As a consequence, the skeletons of "unhealthy" individuals who died during the early onset of an infection may display no evidence of disease at all.
- **Palaeopathology** – The study of disease in the past from the analysis of ancient or historic human remains.
- **Victorian** – A term relating to the historical era between 1837 and 1901 which is defined by the reign of Victoria (1819–1901), Queen of the United Kingdom of Great Britain and Ireland.
- **Workhouse** – A house of industry, which in Britain and Ireland related to social policies deriving from the enactment of the British and Irish Poor Law Acts of 1834 and 1838, where government-aided relief to the poor in terms of basic shelter and food were given in exchange of hard and often degrading labor. The living conditions in these institutions were notoriously harsh and intentionally kept to a low standard to "encourage" those in need of help to avoid seeking "indoor relief" and thereby be forced to improve their situation by themselves. Access to the workhouses was determined by a range of set criteria that were intended to ensure that only those that were considered most destitute, desperate, and of immediate risk of death were granted access.

Summary Points

- Starvation and famines are of great antiquity in human history.
- Bioarchaeology – the study of archaeological human remains – provides unique insights into how famine affected human societies in the past.
- In recent years, skeletal remains of victims of the Great Irish Famine have been discovered in mass burial grounds associated with workhouse institutions across Ireland.
- The skeletal evidence of scurvy is a direct reflection of the impact the potato blight – which was the principal cause of the Irish Famine – had on the physical health on those who died.
- Skeletal manifestations of scurvy are only apparent during the convalescent or healing stages when Vitamin C has been reintroduced to the diet.
- The so-called "osteological paradox" will result in low rates of infectious disease in human skeletons, as death by infectious disease during a famine is relatively rapid and skeletal manifestations of infectious disease generally only occur in chronic cases.

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The Barker Hypothesis

12

Matthew Edwards

Contents

Abstract

The Barker hypothesis proposed that adverse nutrition in early life, including prenatally as measured by birth weight, increased susceptibility to the metabolic syndrome which includes obesity, diabetes, insulin insensitivity, hypertension, and hyperlipidemia and complications that include coronary heart disease and stroke. Periods of rapid postnatal growth associated with high-energy intake seem to be risk factors, along with a high-energy western diet. Theories proposing the mechanism of this association include the thrifty gene, bet-hedging, fetal predictive adaptive response, and drifty phenotype hypotheses. The cause of metabolic syndrome is likely to be multifactorial, with many nuclear DNA and cellular RNA sequences acting in concert with environmental influences.

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Epidemiological data in humans and experimental data indicate that transgenerational epigenetic inheritance is a possible mechanism where a history of starvation or deprivation during early life is seen in a grandparent and transgenerational effects are seen in their grandchildren. It remains to be seen whether this is mediated by heritable RNA sequences, or by acquired, possibly mosaic mutations in DNA coding for example for regulatory RNAs. Recent research has raised the possibility that the nature and quantity of gastrointestinal microorganisms (microbiota) can be modified by diet and conversely can modify an animal's metabolic program. As the microbiota is inherited largely from the mother, modification of her nutrition, health before and during pregnancy, and mode of delivery could influence the child's microbiota, introducing further potential avenues to improve the prevention, reduction of complications, and treatment of malnutrition and metabolic syndrome.

Keywords

Barker hypothesis · Thrifty phenotype · Genotype · Fetal programming · Intrauterine malnutrition · Epigenetic inheritance · Somatic mutation · Microbiota · Gastrointestinal microorganisms · Developmental origins of health and disease

List of Abbreviations

siRNA Small or short interfering RNA	targets messenger RNA (mRNA) of specific sequence for inactivation or degradation
miRNA MicroRNA	small noncoding RNA 22 nucleotides long which regulates gene expression by targeting mRNA
XIST X-inactive specific transcript	a gene located on the X chromosome that encodes RNA, which inactivates the same X chromosome

The Barker hypothesis proposed that obesity, hypertension, hyperlipidemia, and type 2 diabetes, combinations of which have been called the metabolic syndrome (Hales and Barker 1992), were associated with malnutrition or some other cause of growth restriction during early development, if there was later exposure to a high-energy diet (Fig. 1). The deleterious effects of rapid "catch-up" gain in weight during early childhood were observed later. More complex multifactorial contributors to the syndrome could include the relationship between nutrition during pregnancy, maternal metabolic status, and the socioeconomic and even psychosocial status of the mother and child (Barker 2002). There was an inverse correlation between birth weight and the severity of several measures of the metabolic syndrome. Barker based his hypothesis on epidemiological research by his unit and elsewhere. Some areas of Norway had a high prevalence of poverty in the past, associated then with a relatively high child mortality rate and poor nutrition (Forsdahl 1977). Although

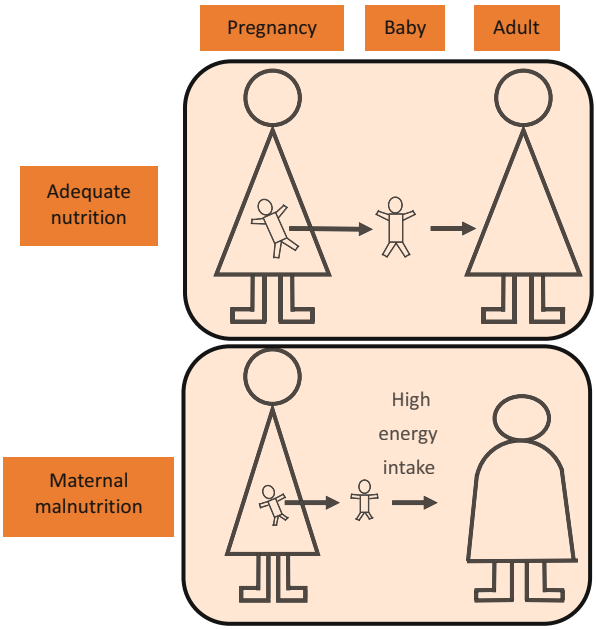

Fig. 1 Barker hypothesis. Two different scenarios for the same mother and baby, therefore irrespective of genotype in both situations. Normal maternal nutrition would result in a normal-sized baby who will be a healthy adult. Exposure of the same mother and child to famine during the pregnancy would be followed by growth restriction of the child who then develops metabolic syndrome as an adult

these areas became as prosperous as the rest of the country and their child mortality dropped to the national level by the 1970s, adults exposed to poverty in early childhood had an increased incidence of heart disease compared to those living in areas that had always been prosperous.

Based on epidemiological studies correlating birth weight with components of the metabolic syndrome that developed in adulthood, Hales and Barker proposed that fetuses survived malnutrition by irreversible adoption of a thrifty phenotype, which they called the fetal programming or thrifty phenotype hypothesis (Hales and Barker 1992) and later broadened to developmental origins of health and disease (Gillman 2005). Studies of adults who were subjected to a variety of causes of intrauterine growth retardation or restriction confirmed a relationship between reduced birth weight and adult metabolic syndrome (Varga et al. 2010). By limiting the growth or function of some organs such as the kidneys, liver, and pancreas that were inessential to immediate survival and adopting a state of insulin resistance, crucial glucose or other limited energy supplies would be diverted to the heart and brain which were crucial for survival, so the fetus survived the period of malnutrition. The irreversible reduction of hepatic, pancreatic, and renal size and/or function was assumed to be chosen by the fetus as a protective mechanism in response to malnutrition. This would improve the survival of fetuses or older children programmed this way as long as they were exposed to malnutrition (Stanner and Yudkin 2001; Victora et al. 2008). On the other hand, it would mean they would be poorly suited to any subsequent period of high-energy consumption as the permanent reduction of hepatic, renal, and pancreatic capacity

and peripheral insulin resistance would result in the metabolic syndrome. A number of refinements have been made, but most of the experimental and epidemiological evidence since has enabled its reinforcement and refinement.

The Dutch hunger winter of 1944–1945 presented a "natural" experiment comparing the adult health of people exposed to famine at different intrauterine ages to controls born elsewhere or before or after the famine. Effects of intrauterine malnutrition at different stages of gestation were found on rates of congenital abnormalities (Stein 1975), obesity (Ravelli et al. 1976), permutations of the components of the metabolic syndrome (Roseboom et al. 2006), disorders of brain growth, schizophrenia (Hulshoff Pol et al. 2000), and abnormal cognitive test results (de Rooij et al. 2010). Similar effects on incidence of schizophrenia were seen in Chinese adults exposed to famine in utero during the Great Leap Forward (Hulshoff Pol et al. 2000; St Clair et al. 2005). Other epidemiological evidence supporting the Barker hypothesis came from the appearance of metabolic syndrome in Pima, Arizona, and Nauruan Islanders after the adoption of a high-energy western diet (Dowse et al. 1991; Diamond 2003; Stoger 2008; Khambalia et al. 2011).

Thrifty Gene Hypothesis

An association between the metabolic syndrome and a high-energy western diet was observed in populations in which previous generations had been exposed to malnutrition (Neel 1962). Survival during famine would be more likely in those with DNA sequences that conferred a thrifty phenotype (Fig. 2). As the survivors with these sequences (especially women of child-bearing age) were more likely to reproduce successfully because of their favorable energy reserves, the mutations were therefore more likely to be passed on to descendants, increasing their frequency in populations prone to famine. This hypothesis was presented to explain why the metabolic syndrome more commonly affected populations exposed to a western diet relatively recently. One argument against it was that the selection of genetic thrift should have applied to every modern population as all ancestors would have been malnourished, rather than just the ancestors of groups in which metabolic syndrome is most prevalent (Speakman 2008). If everyone has thrifty genes, how can they explain the high prevalence of metabolic syndrome only in those newly exposed to a western diet? There has also been debate about when a universal ancestral exposure to famine would have occurred. Earlier assumptions about this being in the Paleolithic period have been questioned (Neel 1989). Famines and severe malnutrition were more likely after the later dependence on agriculture for nutrition than was the case for hunter-gatherer cultures (Prentice et al. 2008). There has been insufficient time since agriculture evolved for malnutrition to select favorable gene sequences. A more plausible selective effect could be the influence of malnutrition on fertility, rather than mortality, in the time elapsed since the evolution of agriculture (Prentice et al. 2008).

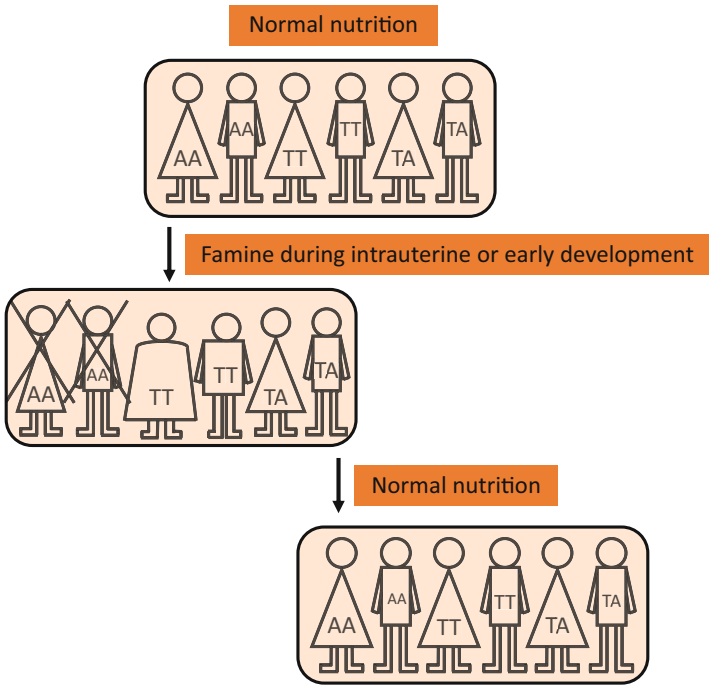

Fig. 2 Thrifty gene hypothesis: if T (Thymine) in a DNA sequence is associated with "thrift," e.g., lipogenesis during periods of adequate nutrition which increases length of survival during famine, it confers a survival advantage on carriers, compared to a sequence containing A (Adenine) at that position. During periods of plenty, it might not confer any advantage, so the frequencies of A and T return to the previous equilibrium. The increase in frequency of polymorphism A in times of adequate nutrition would take many more generations than the one shown here

Postzygotic Somatic and/or Germ Line Mutation

Postzygotic mutation occurs after conception and can affect genes that encode proteins, miRNA, or other regulatory sequences (Fig. 3). Inherited Mendelian variation in the frequency of genomic mutability might determine a higher rate of postzygotic mutation in some individuals. The potential cost of a highly mutable phenotype might be a predisposition to malignancy, autoimmunity, or degenerative disease. Postzygotic mutation could increase the frequency of embryonic cells with a mutation that is favorable to survival and mitosis under certain intrauterine conditions, which are constrained at the best of times (Edwards 2012). That cell with a competitive advantage would divide faster and colonize a tissue or the whole embryo, including much or all of its germ line. In males with continuous division and opportunity for mutation in germ cells, this process might not be limited to

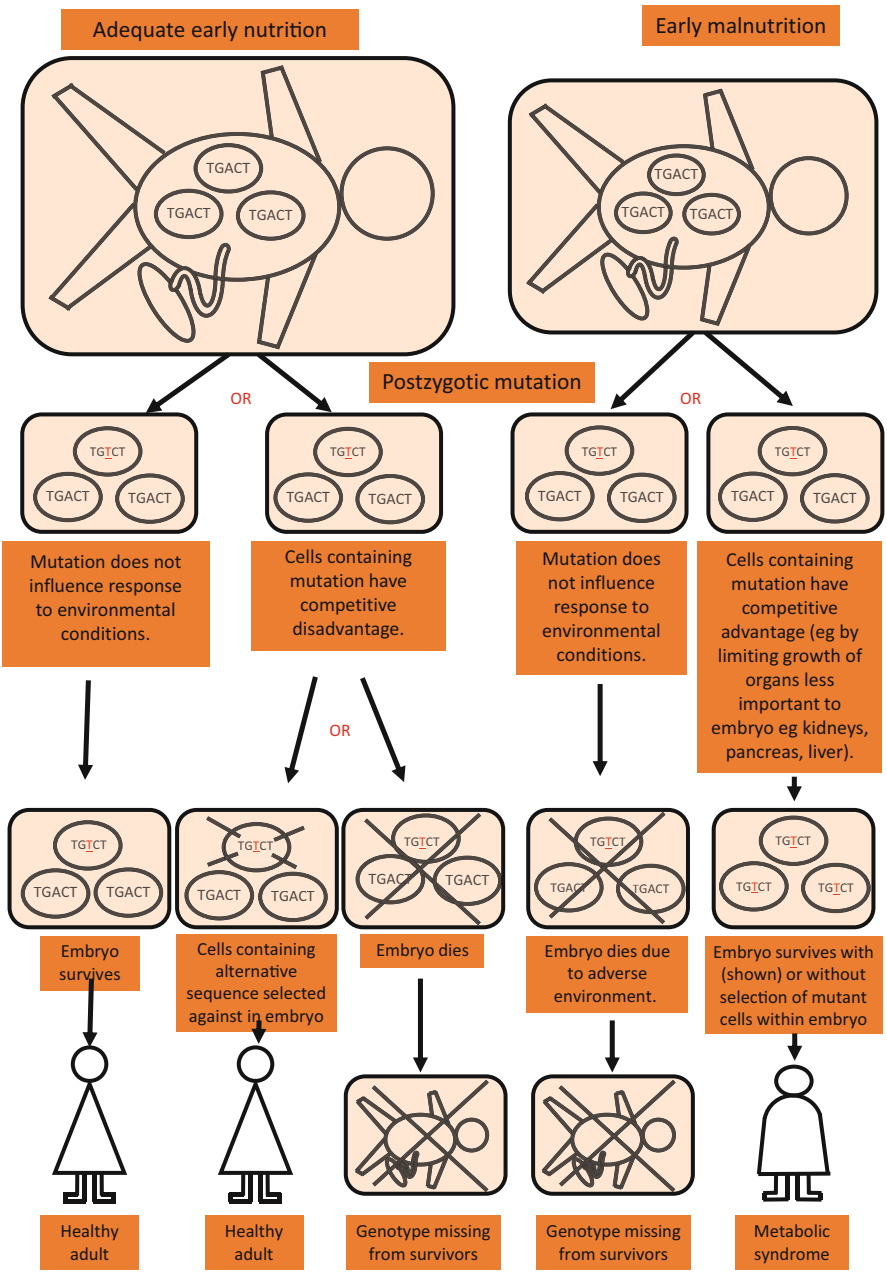

Fig. 3 Postzygotic mutation

embryonic and childhood development. If a postzygotic mutation occurs early enough in development to involve the germ line and produces a thrifty phenotype in those cells, or in tissues those cells will found, it could be a transmissible and

heritable cause of metabolic syndrome. Although Mendelian variation and Darwinian selection were thought to act very slowly over many generations, a similar process occurring within each individual human body between conception and death might explain the appearance of a cellular genetic mechanism contributing to the metabolic syndrome within a relatively short evolutionary time span. An example of this type of Darwinian process occurring in the body is the immense and random somatic mutability in the developing immune system, associated with allele silencing and cellular selection that only leaves cells whose random immune receptor gene mutations are tolerant to self-antigens. With whole genome sequencing of single cells, it will be possible to assess the contribution of this mechanism by comparing the genomic sequences of different cells from different tissues and stages of development, in patients or laboratory animals exposed to intrauterine malnutrition and those with the metabolic syndrome, parents, and controls. If specific gene sequences appear early in development and the cells or embryos containing them die and the sequences are lost in survivors exposed to malnutrition, comparing the genotypes of survivors with parents, unexposed siblings, or exposed siblings without metabolic syndrome might identify more genes of importance to the Barker hypothesis.

Drifty Gene Hypothesis

Genetic drift, the random and continuous accumulation of mutations in the absence of any selective environmental pressure, has been proposed as a mechanism for the increasing incidence of obesity (Fig. 4). A need for agility and mobility, favoring escape from predators, or successful migration in prehistoric hunting and gathering cultures would select against mutations that promote conservation of energy and predispose to obesity. The evolution of agriculture and civilization and a sedentary life would relax this selection against obesity and allow bearers of random spontaneous mutations that promote obesity to survive and pass these mutations on to their descendants (Speakman 2008).

Bet Hedging

The previous theories were based on the concept that selection favored genes for obesity during famine (genetic thrift) or that selection against genes for obesity was relaxed after agriculture developed (genetic drift). Bet hedging referred to the evolutionary advantage of flexibility in reproductive capacity and performance in different environmental conditions (Schaffer 1974). The term refers here in a genetic sense to the preservation within the population, of different DNA sequences having different, opposite, or even mutually exclusive effects. Passage of one DNA sequence to the next generation would be more likely if the prevailing environmental conditions at the time favored it (Fig. 5). If environmental conditions were changeable, as in agricultural cultures with excess food production interspersed with famines, a population could benefit from retaining both DNA sequences (Wells

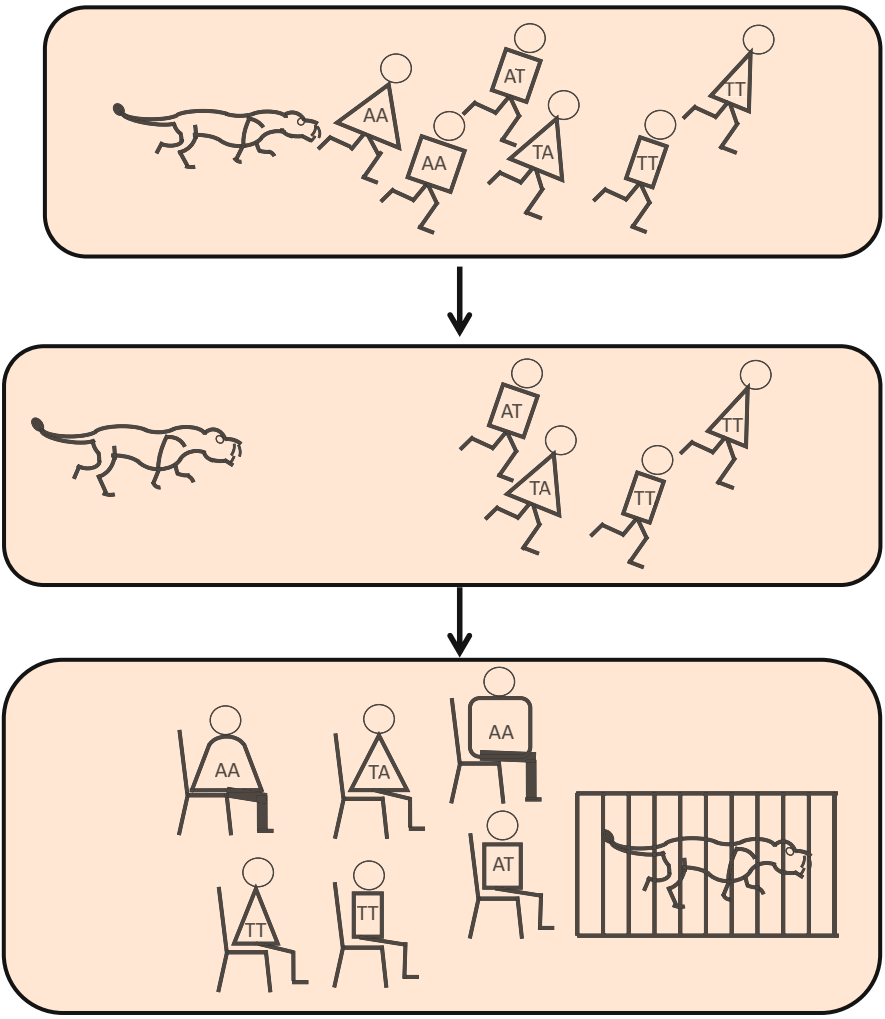

Fig. 4 Genetic drift: new A mutations appear randomly and selection against their effects on activity, consumption, and obesity alters depending on environmental conditions

2009). A thrifty genotype that promotes a positive energy balance with reduced metabolic rate and/or activity, increased consumption of food, avid assimilation of scarce nutrients, and the storage of fat during adequate nutrition might be advantageous to the population. It could ensure the passage of a repertoire of gene activities to the next generation after famines or other causes of fetal malnutrition. The population has multiple sequences at the same or other loci that have opposing effects on thrift. In addition to these, other sequences could be associated with a higher metabolic cost but come into play during periods of surplus nutrition. They could confer evolutionary advantages because as their limitation of appetite or early

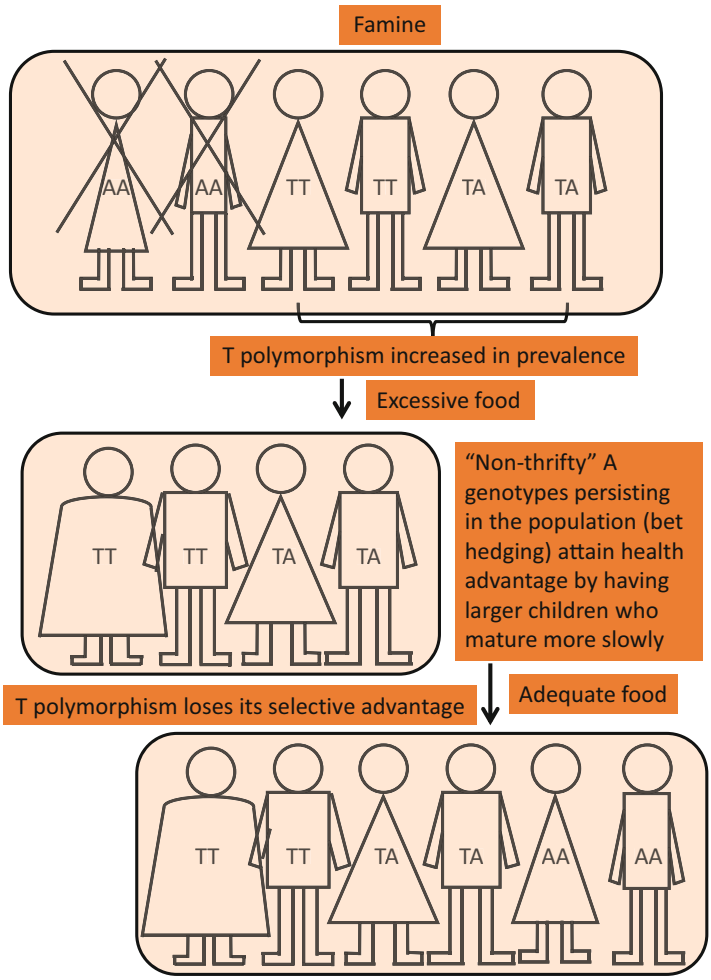

Fig. 5 Bet hedging. If T (Thymine) in a DNA sequence is associated with "thrift," e.g., lipogenesis during periods of adequate nutrition which increases length of survival during famine, it will confer a survival advantage on carriers, compared to a sequence containing A (Adenine) at that position. With adequate nutrition, it might not confer any advantage, so the frequencies of A and T return to the previous equilibrium

growth rate during periods of plenty delays maturation and reduces demands on a lactating mother, they result in slower but continued growth with delayed cessation at puberty, later age of reproduction, larger eventual lean mass and size of the adult brain, and longer life span. When environmental conditions for mothers and/or fetuses enable adequate or excessive energy supply, a denser population and increased competition for mates and territory would favor larger, stronger experienced adults whose parents and grandparents have survived to pass on genetic, social, or behavioral investments to the population. Gene sequences that influence

susceptibility to the metabolic syndrome and are inherited within populations could be sought by whole genome sequencing in adults with metabolic syndrome associated with early malnutrition and compared to parents and unaffected siblings to establish a database of sequences that could be compared in different populations.

Epigenetic Inheritance

The X chromosome contains *XIST*, an X-inactivating gene whose product diffuses along the same chromosome to initiate the inactivation of that copy of the X in each cell (Fig. 6). The inactivation of the X chromosome is an early example of epigenetic modification by methylation of DNA and histones and deacetylation of histones, associated with heterochromatic changes in the structure of the inactive chromosome. Epigenetic changes do not involve changes in the underlying DNA sequence. In female embryos one of the two X chromosomes is randomly inactivated (lyonization) and all mitotic descendants of that cell form a clone inheriting the inactivation of the same X chromosome. Females therefore consist of two clones of cells with respect to their X chromosomes: one with their paternal X chromosome inactivated and another clone has the maternal X inactivated. X inactivation occurs at the time the epiblast is formed in the gastrulating mammalian embryo, while the paternal X is nonrandomly inactivated in the extra embryonic tissues, possibly

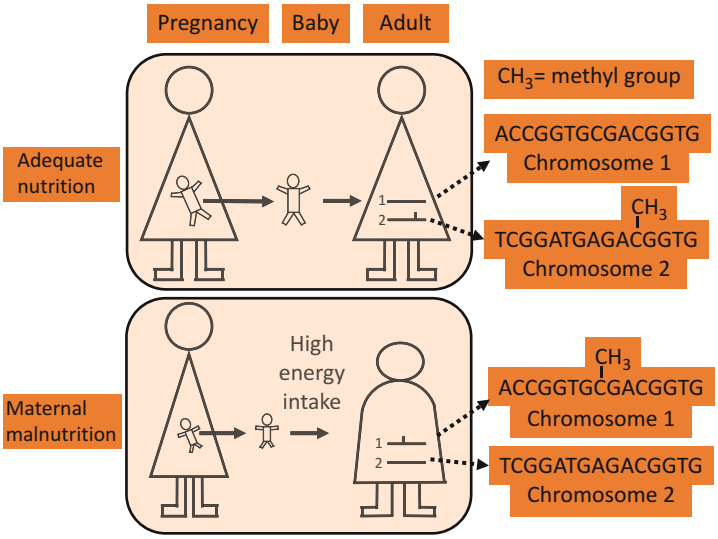

Fig. 6 Epigenetic programming of fetus is influenced by conditions during growth and development. Hypothetical genes on chromosomes 1 and 2 have different lifelong methylation patterns depending on exposures during development. Methylation pattern and other changes in histone structure affect gene expression

reflecting an evolutionary benefit in the activity just of the maternal copies of X chromosome genes at the maternal interface.

Epigenetic imprinting mechanisms inactivate some autosomal genes or chromosomal segments inherited from a parent of one sex, but not those inherited from the parent of the other sex. Sex-specific imprinting evolved independently in placental mammals and flowering plants and is thought, in the case of mammals, to enable a compromise between the competing evolutionary benefits of paternal gene sequences and maternal gene sequences. Specific paternal sequences promote fetal, placental, and childhood growth and might seem in the context of this chapter to be tailored to adverse environments. In contrast, specific maternal sequences favor smaller babies with smaller heads which present less liability for both mother and child before, during, and after the birth to equip both the mother and baby better for long-term survival and further reproduction. As the alleles segregate independently in the formation of germ cells, sex-specific imprinting needs to be reset in the embryo so that, for example, the mother's chromosome 15 imprinting pattern can be reversed from a paternal to a maternal imprint when her ovum contains the copy she inherited from her father (Lewis et al. 2015). Some genes are only imprinted in specific tissues or organs, resulting in a basic phenotype if a mutation in the gene is inherited from either parent, plus additional manifestations if the mutation is on a parental allele that is normally active in the affected tissue and the other parental allele with the normal sequence is inactivated by imprinting (Weksberg et al. 2010). Disorders of imprinting appear to be more frequent in identical twins (Fahrner and Bjornsson 2014), but studies of metabolic syndrome in twin pairs have not uniformly shown effects on some diseases associated with metabolic syndrome (Bo et al. 2000; Iliadou et al. 2004; Wannamethee et al. 2004; Petersen et al. 2011; Frost et al. 2012). Imprinting disorders appear more frequent in babies conceived by certain types of in vitro fertilization (Ventura-Junca et al. 2015), and so does monozygotic twinning. Whether the twinning process predisposes to later metabolic syndrome or both twinning and metabolic syndrome are both secondary to some other cause (Hall 1996) is unknown. Parent-specific imprinting is intimately associated with fetal and childhood growth and metabolism (Hattersley et al. 1998; Constancia et al. 2002), development, and behavior, so the epigenetic mechanisms involved could be relevant to the Barker hypothesis.

A similar process of methylation at the CpG islands of genes, associated with changes in chromatin structure, selects which genes are inactivated in different tissues during developmental differentiation, so that in each tissue only the necessary genes are active. This process does not necessarily or entirely depend on the sex of the parent contributing the allele, but is replicated at each mitosis so that all cells of the tissue usually have the same transcription signature. Genes that are not inactivated long term by epigenetic mechanisms can interact with transcription factors or other modifiers of activity to respond to short-term changes in demand on the tissue. For that to happen the transcription machinery requires access to the gene, and the epigenetic patterns of methylation and histone changes prevent this by changing the coiling and shape of the DNA. Once established, the epigenetic profile of a clone of cells is copied in all daughter cells at mitosis to direct the growth and

specific functions of an organ. The body might retain stem cells with different epigenetic patterns that enable growth and repair of tissues with more than one cell type if needed, but the epigenetic pattern of terminally differentiated cells that undergo mitosis remains stable for the life of the individual. Regional alteration of epigenetic pattern within the body with age and environmental exposure could contribute to autoimmunity (Issa 2003), neoplasia (Jones and Laird 1999), and degenerative disease (Brunet and Berger 2014). Which genes are methylated in the orderly process of differentiation appears to depend on the interplay between the temporal and spatial effects of specific genes and the levels of diffusible growth factors or morphogens.

Environmental changes are thought to influence the epigenetic status of cells by direct effects on methylation. Some drugs can directly influence methylation, and indirect mechanisms include local deficiency or excess of folic acid, which is a cofactor in methylation reactions, or alteration of the activity of enzymes involved in DNA methylation, or histone methylation or acetylation. Exposure of agouti mice to promoters of methylation such as folate or phytoestrogens (Dolinoy et al. 2006) or bisphenol A that reduces methylation (Dolinoy et al. 2007) modifies the development of metabolic syndrome in offspring. Expression of the agouti gene is determined by methylation of a transposon that acts as an ectopic promoter. Transposons comprise 45% of the human genome, and retrotransposons comprise 3%, and many varieties of noncoding RNA molecules are responsible for their regulation or inactivation, commonly by methylation. Polymorphisms in imprinted genes, including the peroxisome proliferator-activated receptors, appear to be involved in diverse processes that could explain the mechanism behind the Barker hypothesis including lipid and carbohydrate metabolism (Ahmadian et al. 2013), inflammation and immune function, atherogenesis, neuronal plasticity, memory (Roy et al. 2013), thermogenesis, healing, and myelination. Interaction between the common P12A polymorphism in the PPARγ protein and birth weight influences susceptibility to insulin resistance and hyperinsulinemia in adults (Roy et al. 2013). Genes associated with lipogenesis and appetite have a similar spectrum of actions relevant to metabolic syndrome (Ellis et al. 2014).

Retrospective epidemiological studies in the UK (Pembrey et al. 2014) and Scandinavia (Kaati et al. 2002; Pembrey et al. 2006) have revealed associations between various measures of malnutrition affecting prenatal or postnatal growth and outcome variables relevant to metabolic syndrome in grandchildren. These identified sex-specific effects on grandchildren removed at least one generation from the exposure factors and exposures as late as preadolescence. Environmental changes can alter the levels of small RNAs, or small double-stranded RNAs can be acquired from the environment. Elaborate mechanisms that regulate the transmission and subsequent replication of small interfering RNA (siRNA) for multiple generations in *C. elegans* have been reviewed (Houri-Zeevi and Rechavi 2017). These regulate not only translation but also transcription by selectively altering chromatin in the vicinity of specific genomic loci. Heritable RNA interference was only transmitted past meiosis for three to five generations, and there is a very short generation time and a limited number of cells in these animals. It is not certain if this will be found in

larger animals in which development of the germ line involves a greater number of mitotic rounds of cell division and there is a much longer time span between generations. This type of inheritance could last long enough to equip several generations of a plant or animal like *C. elegans*, with limited migratory capacity, to deal with forecast prevailing environmental conditions. This process and the epidemiological studies that appear to support its existence in humans might be relevant to the Barker hypothesis and modifications that consider the "weather forecast" model as predicted in 2001 (Bateson 2001), maternal fitness (Wells 2003), and short-term versus long-term changes in environmental conditions (Kuzawa 2008). It is now possible to study the epigenetic signature of cells by analysis of methylation at multiple gene promoter sites, to identify how transgenerational effects can be mediated, although studies on identical twins related to metabolic syndrome have been negative (Tan et al. 2014).

There is evidence for a "Darwinian" process of selection of cells that have particular epigenetic patterns. In the female rabbit embryo, there is stochastic inactivation of the X chromosome so that equal quantities of cells are formed with inactivation of neither X, inactivation of both X chromosomes, inactivation of the paternal X, and inactivation of the maternal X. The cells that have both or neither X are active and then die, leaving only those with an inactivation pattern favorable for survival and division to form a clone that populates the embryo (Okamoto et al. 2011). The pattern of organ growth seen after fetal malnutrition could just be a reflection of the differing susceptibilities of those organs to irreversible damage due to cell death followed by reduced growth rather than anything more programmed and coordinated, and the metabolic changes seen in this situation might just be "making the best of a bad job" (Bateson 2001; Jones 2005).

The epigenetic effects of environmental influences such as malnutrition, psychological stress and behavior, drugs, toxins, and xenobiotics are the subject of much recent research (Thomson et al. 2014; Jimenez-Chillaron et al. 2015). Epigenetic effects include alteration of DNA structure (e.g., histone acetylation or deacetylation) and insulator proteins, methylation of CpG dinucleotides, and the quantity and activity of regulatory molecules such as noncoding RNAs. Drugs, xenobiotics, or psychological stresses alter methylation of different genes or chromosome segments, resulting in a specific phenotype such as metabolic syndrome. Fetuses with resulting nonspecific epigenetic changes that reduce the activity of genes essential for survival to reproductive age would be lost from the population so that the survivors appear to have made a logical choice. In fact the ones that have survived might have done so because the timing is specific or the region of exposure in their case meant that some cells or organs were exposed to more severe environmental conditions or were at a more vulnerable stage of development than others, and cells with changes favorable to survival in those prevailing conditions populated the embryo, not directly because of their genomic sequences but because of their epigenetic patterns. A plausible mechanism has yet to be explained for a xenobiotic, behavioral, or other nongenetic or environmental agents to aim specifically at a particular metabolic program that perpetuates the pharmacological, psychological, physicochemical, or endocrine effects of the environmental agent in subsequent

generations. In biological systems the most parsimonious explanation for the appearance of a specific phenotype is its random appearance in a proportion, but not the whole population, followed by environmental selection for cells or organisms with favorable traits and against those with traits that do not suit the current environment. A plausible mechanism that is known to cause the random appearance of a phenotype is either somatic or germ line genomic mutation. Random nongenetic variations in epigenetic pattern might occur because the actions of agents that affect the epigenetic pattern are not uniform in time or space. Their persistence in a population would suggest they confer no competitive disadvantage under the prevailing environmental conditions.

A number of causes, plus or minus mechanisms, for epigenetic change causing thrift have been postulated. The fetus was proposed to make a predictive adaptive response, a protective "decision" to adopt an epigenetic program that resulted in a thrifty phenotype predicted by current circumstances to be beneficial when the animal was old enough to reproduce (Gluckman and Hanson 2004; Ellis et al. 2014). The thrifty phenotype did not reduce reproductive fitness so it could be transmitted to subsequent generations if it was somehow not erased in embryonic stem cells. If it escaped erasure, it might not influence fetal survival in times of adequate intake, improve survival in fetuses that had retained it and were in turn exposed to malnutrition, and only cause problems if energy consumption became plentiful.

Gastrointestinal Microorganisms (Microbiota)

Viruses incorporate parts of their genomes into host genomic DNA and RNA from viruses, although it was horizontally transmitted initially and can be replicated in mitosis and meiosis to be transmitted vertically. Bacteria and humans share nutrients and synthesize some necessary nutrients for each other. Regulatory nucleic acid sequences, known to be shared between bacteria, can also be shared between them and their human host. A significant proportion of human DNA can be cloned in fungi but not in *E. coli* (Negri and Jablonka 2016). Some of these human genomic sequences might maintain the symbiotic balance between host and enteric bacteria by coding for RNA or protein that regulates bacterial metabolism or growth. They could either promote the production of host toxins that are taken up by bacteria, or the nucleic acid sequences themselves are taken up and transcribed by bacteria. The population of gut microorganisms is established by age of 3 years, and the variety and quantity of species are influenced by the diet of the child including breast milk or other foods. Most of this population is established by colonization from the maternal enteric, vaginal, and cutaneous populations, although it possibly includes a prenatal intrauterine population (Ardissone et al. 2014; Stinson et al. 2017). Maternal malnutrition, metabolic syndrome, or other diseases predisposing to metabolic syndrome in the child might therefore be associated with particular populations of microorganisms passed on from mother to child and subsequent generations. This could contribute to the difference seen between effects of maternal malnutrition

during pregnancy and paternal malnutrition during childhood (Pembrey et al. 2006; Pembrey et al. 2014). The long generation time of humans and their slow rate of germ line evolution argue against the Darwinian selection of new mutations that could affect such large proportions of populations and appear as quickly as the metabolic syndrome. If the genetic material carried by the human is actually considered to be both the DNA in the human cells plus that of the trillions of symbiotic organisms living in or on the body, the evolution of this entity as a whole could be so rapid it could appear to have a Lamarckian mechanism (Osmanovic et al. 2016). Fascinating research involving twins discordant for obesity showed that germ-free isogenic mice receiving fecal bacteria from the obese twin developed an obese metabolic pattern that was rescued by cohousing with mice fed bacteria from the leaner twin (Ridaura et al. 2013). Children delivered by Caesarian section were more likely to develop obesity after controlling for their mothers' BMI (Mueller et al. 2017).

Conclusions

The cause of metabolic syndrome is likely to be multifactorial, with many nuclear DNA and cellular RNA sequences acting in concert with environmental influences. The Barker hypothesis has enabled new approaches to the study and management of different types of intrauterine malnutrition and metabolic syndrome. These will hopefully lead to control of this major worldwide health problem. It has not been resolved whether the Barker hypothesis affects subsequent generations, after one episode of growth retardation at one stage of development. Epidemiological data in humans and experimental data suggest that transgenerational epigenetic inheritance is possible. It remains to be seen whether this is mediated by heritable RNA sequences analogous to transposons or RNA viruses that have entered the germ line, or by acquired, possibly mosaic mutations in DNA coding for regulatory RNAs. The association of enteric microbiota with human metabolism raises the fascinating possibility of new treatment or even prevention.

Dictionary of Terms

- **Agouti** – banded lighter and darker pattern of hair pigmentation in mammals caused by pigmentary genes being turned on or off at stages of hair growth. These genes are normally only expressed in hair follicles, but a transposon can result in their expression in other tissues, leading to metabolic syndrome.
- **Chromatin** – the DNA of the chromosome is coated with RNA and proteins, especially histones, to form chromatin which coils to produce the typical structure of chromosomes that are visible through the microscope just before the cells divide. Banding of the chromosomes with a stain called Giemsa (G-banding) produces a bar-code appearance which aids in numbering and identification of deletions or duplications. The light bands were called euchromatin and dark

bands heterochromatin which largely contain active and inactive genes, respectively.

- **Clone** – all cells descended in the body that have descended, by mitotic cell division, from one ancestral cell.
- ***Caenorhabditis elegans*** – C. elegans is a tiny transparent worm which is valuable experimentally because of its short developmental period, 3-day life cycle with rapid growth to maturity resulting in a short generation time, and its fecundity, 300 offspring for each worm. Its embryonic development was studied by Sulston and colleagues so the division and migration of every embryonic precursor and eventual predictable position could be identified for every 1 of the 959 cells comprising the adult worm's body.
- **Epigenetic (adj, n epigenesis)** – the alteration of gene activity without alteration of DNA sequence; by alteration of chromosome structure, relationship between DNA and histones, histone methylation or acetylation, or DNA methylation. The nucleotides cytosine and guanine, found together on the phosphate (p) backbone of DNA, are called a CpG dinucleotide and are very frequently found in the promoter of genes, and the cytosines in this dinucleotide are methylated to inactivate the gene by preventing access of RNA polymerase to the promoter. This prevents transcription, the synthesis of messenger RNA from the DNA template.
- **Expression** – the transcription from a DNA sequence of RNA which has some metabolic or epigenetic effect in the cell.
- **Genome** – the DNA sequence of an organism. This does not include the sequence of RNA that might not be copied from genomic DNA but does include the sequence of ancestral viral RNAs that have been copied in the reverse direction into genomic DNA. Whole genome sequencing is the sequencing of all known chromosomal and mitochondrial DNA sequences, now possible for a single cell. Whole exome sequencing just included the 1% of DNA that coded for proteins, but that misses the huge majority of DNA that codes for regulatory sequences within genes (intronic sequences) and outside genes.
- **Germ line** – sperm, ova, and their precursors.
- **Imprinting** – inactivation of genes, not by altering their sequence (mutation) but by methylation of DNA and histones, deacetylation of histones, and structural changes to the supercoiled chromosome to limit access to gene promoters by RNA transcription molecules.
- **Lamarckian** – a form of inheritance proposed by Jean-Baptiste Lamarck in the 1800s to occur by transmission of acquired traits to offspring. Inheritance of epigenetic traits is an example of a Lamarckian mechanism.
- **Mendelian** – a form of inheritance proposed by Gregor Mendel in the 1800s but rediscovered in the early 1900s. It suggested that genes were in pairs and that members of gene pairs segregate independently. Mendelian inheritance is the inheritance of alleles now known to be sequences in genomic DNA. Mendelian variation is based on the inheritance of different alleles. Superimposed on this is genomic variation due to new mutation.
- **Methylation** – the addition of CH_3 (methyl) groups, often to cytosine where it is found paired with guanine in genomic DNA.

- **Peroxisome** – a tiny cellular organelle bounded by a lipid membrane within body cells in which, among other actions, very long chain fatty acids are oxidized to release energy, components of cell membranes called plasmalogens, and enzymes, such as catalase that protects against free radicals, are synthesized.
- **Polymorphism** – a DNA sequence found in more than 1% of healthy members of a population. There might be one of more variants of the same sequence. Polymorphisms are considered not to cause disease (as do mutations), but for various reasons they might predispose to disease.
- **Postzygotic mutation** – this occurs after conception so it just affects the first cell to acquire it and all its descendants which receive copies of its DNA. If the mutation occurred before the differentiation of the germ line, it could affect both germ cells and somatic cells, but if it occurred after separation of the germ line, it would only affect the germ line or only somatic cells but not both. As only some of the embryo cells have the mutation, the embryo has *mosaicism*, more than one population of cells, each with a different genome sequence.
- **RNA interference** – RNAs introduced from the environment, or synthesized from endogenous DNA or RNA sequences, bind to complementary nucleic acid sequences of other RNA or DNA molecules and in association with proteins such as dicer or argonaute inactivate or destroy the other nucleic acids (such as viruses, transposons, or messenger RNAs) or are transferred to the nucleus, possibly also in the germ line.
- **Somatic mutation** – mutation just affecting somatic cells which are body cells other than the germ line. These mutations occur after conception and just affect a proportion of body cells: the first cell to acquire the mutation and its descendants which receive copies of its DNA.
- **Transcription** – the copying of a genomic DNA template in the synthesis of RNA.
- **Translation** – the copying of a messenger RNA sequence to synthesize a protein
- **Transposon** – a DNA sequence that was introduced into the ancestral genome of an organism. They can shift from one chromosomal location to another, resulting in new activation or inactivation of genes, or new gene functions, but most commonly when this happens it is deleterious causing disease. They are tolerated in the genome because most are inactivated by various mechanisms including RNA by a process called RNA interference, and because their changes in location (transposition) have contributed to genetic variation, and thus evolution. Retrotransposons were originally part of an RNA viral genome, incorporated into the host's DNA by a process called reverse transcription, where a viral enzyme called reverse transcriptase synthesises a DNA copy of the viral RNA.

Summary Points

- The *Barker hypothesis* linked early childhood malnutrition to adult-onset diseases comprising the metabolic syndrome. Synonyms include the *thrifty phenotype* hypothesis, *developmental origins of health and disease*, or the *fetal programming* hypothesis.

- Mechanisms postulated to explain this association included:
  - Refinements of the earlier thrifty gene hypothesis which claimed that early malnutrition selected for genes for obesity. It was discarded because metabolic syndrome appeared or disappeared too quickly from populations to be caused by genetic selection based on mortality.
    - The bet-hedging hypothesis suggests that genes for obesity or genes favoring leanness can both be retained in the population by selection under different environments at different times.
    - Genes predisposing to the metabolic syndrome might appear by new mutation somatically or by selection of inherited polymorphisms. Their contribution could be assessed by comparing the whole gene sequences of survivors of malnutrition in early life who now have metabolic syndrome and controls including parents and unexposed siblings.
  - The drifty gene hypothesis means release from selection of genes for obesity associated with civilization, agriculture, and freedom from predation.
  - Maternal well-being and fetal programmed adaptive response hypotheses are modifications of the Barker hypothesis. The former involves fetal programming based on the mother's past and present health and nutritional status, while the latter involves fetal programming that integrates information from the past and postulated future environmental conditions.
- Epigenetic gene inactivation programs have been implicated in fetal programming and metabolic syndrome:
  - These could be transmitted to subsequent generations, and there is molecular evidence for this in laboratory models of metabolic syndrome.
  - There is epidemiological evidence for inheritance of effects associated with malnutrition in early life in humans for at least one generation.
  - RNA interference is a postulated mechanism for transgenerational passage of epigenetic information.
- Gut microbiota is passed from mother to child. There is evidence that diet and other factors modify the population of gastrointestinal microorganisms, and in turn these can modify the metabolic program of the human host.

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Part III

Food Insecurity, Security, and Waste

Food Security and Nutritional Health of Newcomer Children

13

Hassan Vatanparast, Christine Nisbet, and Rashmi Patil

Contents

Abstract

The socioeconomic growth of some countries is impacted by immigration. For example, Canada has sheltered numerous refugees and promptly responded to the global refugee crisis. Food insecurity, dietary acculturation, inadequate access to healthcare, and poor nutrition are major contributing factors to newcomer health. For refugees, compared to immigrants, barriers to health include pre- and post-migration factors such as language, trauma, social stratification, and lack of access to healthcare. Newcomer children can be negatively affected by these inequities. Refugee children, specifically, are at higher risk of poor nutritional health. Studies on refugee children showed they were vulnerable to excess weight, chronic conditions, mental health issues, poor education, and poverty. Newcomer

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health decreases over time, which can increase the burden on the Canadian healthcare system. Limited information is available regarding the relationship between food security and social determinants of heath and how this affects unique subgroups of newcomers. Research is required to identify culturally appropriate and cost-effective ways to promote traditional food, and to assess nutritional quality and safety in Canada. Although Canada is used as an example, there is wide applicability to other countries where refugees have similar experiences and data regarding children is lacking.

Keywords
Acculturation · Canada · Child · Culture · Food security · Health · Immigrant · Refugee · Newcomer · Nutrition

List of Abbreviations

BMI	Body mass index
DFE	Dietary folate equivalents
HEI	Healthy eating index
HEIC	Canada's healthy eating index
TBBMC	Total body bone mineral content
UNHCR	United Nations High Commissioner for Refugees
WHO	World Health Organization

Introduction

International migration occurred at a faster rate than the world's population growth (United Nations 2016). As of 2015, there were approximately 244 million migrants worldwide (United Nations High Commissioner for Refugees [UNHCR] 2017). There were 65.3 million people forcibly displaced from homes around the world, among them, nearly 21.3 million were refugees, which is more than the number of people displaced after World War II (UNHCR 2017). More than ten million of these refugees were children and youth (UNHCR 2017). Ten million people worldwide have been denied a nationality and lack access to basic necessities including employment, food, education, and healthcare (UNHCR 2017). Immigrant is often used for immigrants and refugees. However, these two populations differ due to cause of migration, different countries of origin, cultures, pre- and postmigration factors, and socioeconomic status, all of which affect quality of life and integration into a new society (Nisbet 2011). Immigrants choose to migrate for a better future while refugees are forcefully displaced from their homeland.

Canada is thought of as the land of immigrants since the majority of its population is new immigrants or descendants of previous immigrants (Fig. 1) (Government of Canada 2011). People migrate to Canada for multiple reasons including job opportunities, health, and ultimately a desire for a better life. Canada's first Governor

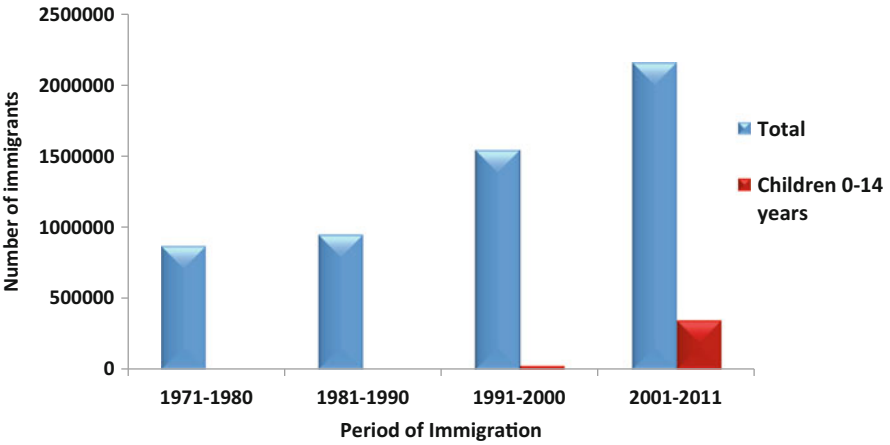

Fig. 1 The number of immigrants and immigrant children to Canada according to period of immigration. This figure indicates a rise in the total number of people who have immigrated to Canada as well as the number of children who have immigrated to Canada from 1971 to 2011. (*Data retrieved from Statistics Canada (2017))

General, John Buchan, stated that immigrants "should retain their individuality and each make its contribution to the national character" (Government of Canada 2011). This philosophy has been maintained throughout the years in Canada's Multiculturalism Policy (Government of Canada 2011). Immigration has had a major impact on the population and socioeconomic growth of Canada. Statistics Canada (2011) states the level of education is higher among immigrants than Canadian-born. Politicians at all levels of government believe immigration is necessary for economic growth and stability (Statistics Canada 2011). The racial diversity of Canada's immigrants may be responsible for the superior social cohesion compared to other developed nations (Statistics Canada 2011).

According to the 2011 census, 6.8 million people living in Canada are foreign born, which accounts for 21% of the total population. People migrate to Canada as individuals or families and are grouped into four categories: economic, families, refugees, and others (Pottie et al. 2015). Based on available data, as of 2011, there were more than one million newcomers in Canada who immigrated between 2006 and 2011, out of which 19% were children aged 14 years and under (Fig. 1) (Statistics Canada 2016). Another 15% were youth between the ages of 15 and 24 years (Statistics Canada 2016).

Refugees entering Canada come with hopes, experiences, and skills, which contribute to a wealthy and affluent society. Canada, in the past, has responded on various occasions to the global refugee crisis and sheltered numerous refugees. From November 2015 to January 2017, approximately 40,081 Syrian refugees arrived in Canada (Government of Canada 2017a). In 2016 (January–September) alone, there were 16,369 in-person refugee claims (Government of Canada 2017b).

Health of Newcomers to Canada

Newcomer health is influenced by culture including knowledge, skills, practices, beliefs, and experiences associated with traditional, complementary, or alternative medicines (World Health Organization [WHO] 2000). Social determinants of health include income, education, employment, social structures, culture, gender, and child development. They play an important role in the health status of newcomers, particularly regarding food security, which is discussed later in this chapter.

Immigrants with a higher socioeconomic status possess more knowledge about health and follow the Western medicine system (Rothstein and Rajapaksha 2003). Highly educated and wealthy immigrants are more likely to maintain a healthy body weight, adhere to preventive health advice, and exercise more frequently (Rothstein and Rajapaksha 2003). Those who are not satisfied with Western healthcare, whether due to high costs, long wait times, or perceived discrimination, have a tendency to use traditional or complementary medicine more than those who are satisfied (Ku 2007). Exposure to factors such as past harmful exposure from war, lack of nutritious food, violence, trauma, poor sanitation, socioeconomic status, English- or French-language proficiency, limited access to preventive services, and specific disease vectors exposure, put refugees at higher risk of ill health and poor nutrition when compared to immigrants (Ng et al. 2011). English language proficiency was greater in immigrant children compared to refugees (Hoover et al. 2016). Refugee newcomer children to Canada were significantly more likely to come from families where neither parent had a secondary level education and where their main source of income was social assistance compared to immigrants (Nisbet 2011). Newcomer immigrant and refugee families with children found themselves in the lowest income category at 49% and 62%, respectively (Nisbet 2011).

The Healthy Immigrant Effect

Previous literature suggests the health status of immigrants upon arrival is high, eventually deteriorating over a period of 5 years postmigration before matching that of native-born Canadians (Newbold 2009). This healthy immigrant effect is associated with three hypotheses, selective immigration, better access to healthcare services over time, and acculturation (Antecol and Bedard 2006; Gushulak 2007). In order to understand the healthy immigrant effect, these hypotheses need to be investigated further.

Selective Immigration

Self-selection is a term that describes individuals who are economically affluent and healthy in their home countries and more likely to migrate due to better opportunities. Immigrants must pass a screening process, including a medical examination, which removes those with severe medical conditions who pose a danger to public health or safety and strain the healthcare system more than the average Canadian, which was $5,292USD per capita in 2014 (Antecol and Bedard 2006; WHO 2014). However, refugees are "protected by law from exclusion on the basis of noninfectious

burden of illness" (Government of Canada 2017c). Immigrants from Asia, Africa, and South America were not as likely to indicate excellent/very good health compared to immigrants from Europe, the United States, Australia, and Mexico combined (Ali et al. 2004). The definition of health changes for immigrants and refugees over time due to acculturation, as they compare themselves to the general population, which affects their perceived health (Newbold 2005).

Although self-selection exists, it is likely only one contributing factor to the healthy immigrant effect and may not be applicable to all immigrants. This theory does not consider the weakening health of refugees after migration. Investigation of other hypotheses is necessary.

Healthcare

Large scale migration inevitably impacts migrants' health as well as the healthcare system of the host country. Compared to native-born Canadians, immigrants and refugees healthcare needs can vary drastically, and they seek access to medical care less, although, they do not necessarily have better health even though their self-perceived health is higher upon entry (Gushulak 2007). Although self-assessed health of immigrants to Canada decreased, their utilization of hospitals did not increase from 1994/1995 to 2000/2001 (Newbold 2009). Barriers to healthcare affecting immigrants can include poor socioeconomic status, loss of social networks, use of traditional medicine, familial and cultural responsibilities, hesitation providing personal information, perceived discrimination, weak language skills, and poor working environments related to "deskilling" (Newbold 2009; Canadian Paediatric Society 2013). The Canadian healthcare system can be difficult to understand as it is not consistent throughout the country. Some provinces have a waiting period of 3 months, which may contribute to lower usage of the healthcare system upon entry (Government of Canada 2016a). For protected persons like refugees, a temporary health card and programs are available (Government of Canada 2016b).

The decline of newcomer health after migration is not limited to poor healthcare access. Healthcare services utilization by immigrants increases with length of stay. This could possibly increase identifying, diagnosing, reporting, and treating health-related issues (Newbold 2009). Certain groups of refugees are given immediate access to healthcare services upon arrival in Canada; hence the hypothesis of self-selection and poor access to healthcare does not apply to them. Therefore, the healthy immigrant effect could be associated with several other factors.

Dietary Acculturation

Adaptation of an individual of a particular ethnicity to a new culture is known as acculturation. Acculturation in itself is a combination of various stages. Typically, the last stage is "assimilation," which occurs when someone from a different ethnicity completely adopts the norms of their new culture, leaving their own behind (Kittler and Sucher 2012). Those who migrate at an older age are less likely to adopt the new culture when compared to those who migrate at a younger age (Kaushal 2009). It is important to note that acculturation is complex and nonlinear, occurring along a spectrum (Berry et al. 2006). A balance should be maintained between the traditional culture and the new culture.

Obesity is a serious condition that can increase the risk of chronic conditions including, certain cancers, coronary heart diseases, hypertension, elevated blood cholesterol, and type 2 diabetes. These conditions could be attributed to poor dietary choices. The possibility of becoming obese increases during the process of acculturation (Kittler and Sucher 2012). When an individual or group of immigrants adopts the eating patterns of the host country, dietary acculturation occurs (Satia-Abouta et al. 2002). Dietary acculturation is important among immigrants to developed countries as diets are high in fat and sugar, which negatively affects their health as their length of stay increases in the host country (Satia-Abouta et al. 2002). Poor accessibility to traditional food items, lack of nutrition education, and convenience of Western food results in increased consumption of prepackaged items or fast food and can be attributed to dietary acculturation (Kittler and Sucher 2012). Dietary acculturation has been associated with increased chronic conditions among adults; hence health could be negatively affected among those children who experience dietry acculturation.

Food Security

One of the most common approaches to evaluate income-related household food security is using a questionnaire developed by the United States Department of Agriculture (2016) and adapted by Statistics Canada (Tarasuk et al. 2014). The United States classifies responses to these questions into four categories whereby households, adults, or children with high or marginal food security are considered food secure and those with low or very low food security are food insecure. Canada uses the same questionnaire and classifies households as food secure, marginally, moderately, and severely food insecure. Food security is directly related to income. Nonvisible minority Canadians are more likely to receive a higher wage job when compared to that of visible minority immigrants (Simich and Jackson 2010). Higher income, greater food security, and better opportunities are typically associated with higher education levels. However, for immigrants, higher education levels do not result in better job opportunities due to deskilling. This explains why immigrants have lower levels of food security when compared to native Canadians. Recent immigrants, even those with postsecondary education, were more likely to be underemployed, low-income, and need housing compared to the Canadian-born (Simich and Jackson 2010).

Since 2004, Statistics Canada has collected information on the food security status of Canadians by means of the Canadian Community Health Survey. Almost 13% of Canadian households (four million individuals) experienced some form of food insecurity, which included 16% (1.15 million) of children (Tarasuk et al. 2014). Food insecurity was more prevalent in households with low income, children, and single-mothers, and where the respondent was Black or Aboriginal (Tarasuk et al. 2014). Recent immigrant households had a higher prevalence of food insecurity (20%) compared to nonrecent immigrants (11.8%) and Canadian-born (12.4%) (Tarasuk et al. 2014). A pilot study, Healthy Immigrant Children (HIC), examined newcomer immigrant and refugee children in Saskatchewan, Canada, and found the

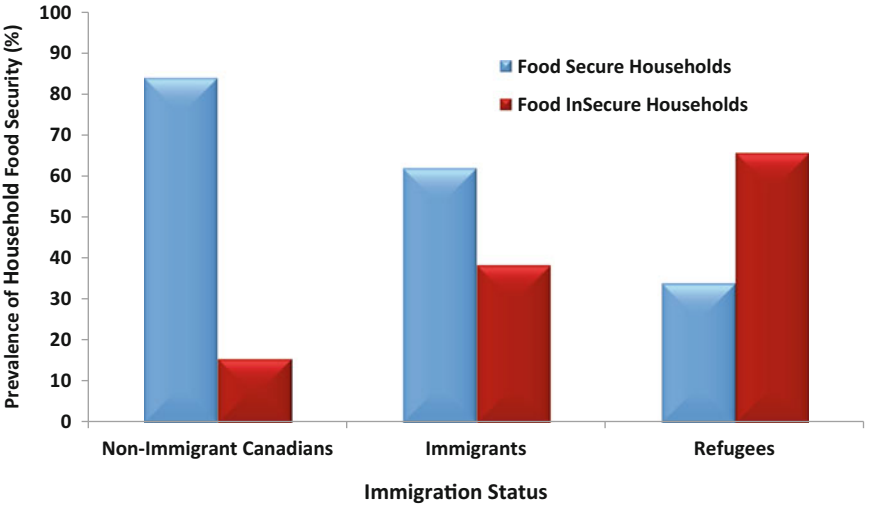

Fig. 2 Prevalence of household food security among households with children. This figure compares national data (Tarasuk et al. 2014) to local data from Saskatchewan in immigrant and refugee children in the Healthy Immigrant Children study (Nisbet 2011) (*Data retrieved from Tarasuk et al. (2014) and Nisbet (2011))

prevalence of household food security was 62% and 34%, respectively (Fig. 2) (Nisbet 2011). Child food security status was 51% compared to household food security at 46% (Nisbet 2011). Although a higher prevalence of household food insecurity was observed in refugee newcomer families, child food security was not significantly different between immigrant and refugee families (Nisbet 2011). As the duration of stay in Canada increased, the risk of food insecurity in children decreased (Nisbet 2011). The study also noted that children living in households with more than two children were more likely to be food insecure (Nisbet 2011).

Food insecure individuals can binge eat, consume high-calorie food, and, therefore, develop overweight/obesity (Che and Chen 2001). Children living in households where high school is the highest level of education are at greater risk of being overweight/obese in comparison with those living in households with a post-secondary degree (Shields 2005). Studies also showed that 21% of people in food insecure households had at least three chronic conditions and 15% of these people were obese (Che and Chen 2001).

Nutritional Health of Newcomers

The health status of individuals is strongly affected by the food they consume. Culture strongly influences people's food choices. Healthy food choices can be limited by the ability to purchase expensive food items (Kittler and Sucher 2012). Data gathered by Statistics Canada (2014) using body mass index (BMI) showed nearly 20% (5.3

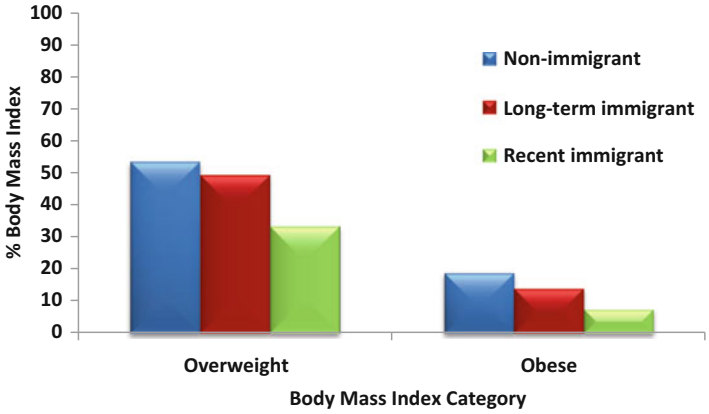

Fig. 3 Classification of overweight and obese according to immigration status. Overweight and obesity is shown for adult non-immigrants, long-term immigrants, and recent immigrants. This figure supports the healthy immigrant effect, as overweight and obesity increases with length of stay in Canada (*Long-term immigrants were in Canada 11 years and longer and recent were in Canada less than 11 years. Data retrieved from Tremblay et al. (2005))

million) of Canadian adults were obese (self-reported height and weight). Estimates suggest that Canadians consume about 13% of their caloric intake from added sugar. High sugar consumption is frequently associated with adverse health outcomes including cancer, dental caries, diabetes, heart disease, high blood cholesterol, overweight/obesity, and stroke. Figure 3 portrays overweight and obesity according to immigration status. Culture influences newcomer food choices, possibly due to lack of access to traditional food items. Newcomers also witness differences in schedules, such as working long hours, which results in minimal time to prepare food in a traditional manner. Immigrant children tend to prefer Western food, while parents particularly prefer traditional food items (Gray et al. 2005). Income, country of origin, city of relocation and comprehension of its primary language all affect the likelihood of food habits changing postmigration (Gray et al. 2005).

Health of Newcomer Children

Detailed information on health and nutritional status of newcomer children is not available in Canadian literature. A large number of youth (37%) acculturate by being involved in activities of both traditions (Berry et al. 2006). The Canadian Institute of Child Health reported that immigrant children and youth were less likely to consume alcohol, smoked less, and had lower suicidal behavior when compared to native-born Canadian children (Pottie et al. 2015). A huge proportion of immigrant youth reported being in good stable physical and mental health (Kukaswadia et al. 2014). Also, 88% of immigrant youth aged 12–14 years reported having a strong sense of belonging to their community compared to 82% of native-born Canadian youth

(Pottie et al. 2015). Canada's vision is dependent on the well-being of children and youth who are the future of the country, and the diversity of immigrant and refugee children plays a vital role.

Refugees, particularly children, are prone to infection, which is only heightened by unsanitary living conditions and malnutrition (Food and Agriculture Organization 1995). Unfortunately, many immigrant and refugee children face serious issues with poverty, education, dietary needs, and emotional changes while coping with North American culture (Tienda and Haskins 2011). Immigrant children and youth are subjected to a wide range of mental health issues. Causes for mental health issues were a combination of several complex factors including language barriers, loss of friends or family members, living in poor neighborhoods, and cultural discordance or dissonance (Canadian Paediatric Society 2016). Cultural discordance occurs when a child perceives divergence between two cultures. This discordance is greater for children who migrate at a younger age and can have a negative impact on various aspects of their lives including relationships with peers, mental health, and tension with family (Rohmann et al. 2006).

Prevalence of nutritional anemia, growth abnormalities, malnutrition, dental caries, enteric parasites, and psychiatric disorders among the children of recent immigrants is high (Pottie et al. 2015). The term "malnutrition" comprises both under- and overnutrition and develops due to poor nutrient absorption within the body and inadequate or excess intake of protein, energy, and micronutrients (WHO 2001). Malnutrition causes deficiencies, which can affect children's physical development/growth, increase their risk of diseases, and decrease their concentration levels/performance at school (International Development Committee 2008; WHO 2001). The likelihood of being malnourished increases among families who have lower household incomes and poor health education (e.g., newcomer refugees) (WHO 2001). A study conducted in Toronto, Ontario, showed 7% of immigrant children were below the third percentile for height-for-age and 12% for weight-for-age (Salehi et al. 2015). The same study also showed the prevalence of anemia and iron deficiency among immigrant children was 23% and 53%, respectively (Salehi et al. 2015). Health issues in children postmigration can arise because of poor dietary choices leading to overweight/obesity, and eventually chronic diseases (Shields 2005).

Nutritional Health of Newcomer Children

Diversity in diet is important to ensure adequate intake of all nutrients for optimal growth and development. Children have specific requirements as they go through this particular stage of life. Over the last three decades, the prevalence of overweight/obesity has increased among children in Canada. The possibility of being an obese adult increases if obesity is acquired during childhood. Research on immigrant and refugee children in Canada is lacking in the literature.

The predisposition to numerous negative health outcomes associated with obesity makes childhood obesity a serious public health concern. From 2005 to 2014, 29% of boys and 17% of girls were classified as overweight/obese (Statistics Canada

Table 1 Indices of disease in newcomer Canadian children. Considering the paucity of data, information was included from a pilot study in Saskatchewan to show a small picture of the health status of newcomer children

Characteristics	Immigrants n = 33 (45.8%)	Refugees n = 39 (54.2%)	All participants n = 72 (100%)
Blood cholesterol in mmol/L (Mean ± SD)	4.6 ± 0.7[a]	5.3 ± 0.9	5.0 ± 0.9
Borderline high ≥4.4 mmol/L High ≥5.2 mmol/L	10 (34.5%) 7 (24.1%)	10 (26.3%) 20 (52.6%)	20 (29.9%) 27 (40.3%)
Total serum vitamin D in nmol/L (Mean ± SD)	45.7 ± 13.9[a]	37.8 ± 15.5	41.2 ± 15.2
Deficient and insufficient < 50 nmol/L Sufficient >50 nmol/L	19 (63.3%) 11 (36.7%)	31 (79.5%) 8 (20.5%)	50 (72.5%) 19 (27.5%)
Total body bone mineral content (TBBMC) in grams (Mean ± SD)	984.9 ± 245.0	947.8 ± 208.8	964.6 ± 224.9
Low TBBMC	13 (41.9%)	14 (35.9%)	27 (38.6%)
Percentile BMI (Mean ± SD)	62.9 ± 27.6	62.4 ± 29.4	62.7 ± 28.4
World Health Organization criteria			
Normal Overweight Obese	24 (72.7%) 6 (18.2%) 3 (9.1%)	27 (69.2%) 10 (25.6%) 2 (5.1%)	51 (70.8%) 16 (22.2%) 5 (6.9%)

Data retrieved from Nisbet (2011)
[a]Significant difference between immigrants and refugees

2014). In 2011–2012, immigrant youth were less likely to be overweight (15%) when compared to their Canadian counterparts (19%) (Pottie et al. 2015). The chance of being overweight/obese was higher among refugees compared to immigrants (Nisbet 2011). Hypertensive levels were reported in 26% of overweight/obese newcomer children (Nisbet 2011). Table 1 displays indices of disease in newcomer children to Canada.

In 2004, 71% of Canadian children 4–8 years and 62% of girls and 68% of boys 9–13 years were lacking the daily recommended servings of fruit and vegetables (Garriguet 2007). This value jumps to 80% for newcomer children 7–11 years (Nisbet 2011). Reduced intake of fruit and vegetables depletes nutrients from the body; this decreases immune system function and increases the chance of developing chronic conditions (Kirkpatrick and Tarasuk 2008). Also, 37% of children 4–9 years and 61% of boys and 83% of girls 10–16 years were not getting the daily recommended milk and alternatives servings (Garriguet 2007). When examining newcomer children 7–11 years of age, 76% did not meet recommendations for milk and alternatives (Nisbet 2011). Over 25% of children aged 4–8 years and 89% of newcomers aged 7–11 years were not consuming enough whole grains (Garriguet 2007; Nisbet 2011). Another 14–18% of girls aged 9–18 years were not getting the recommended amount of meat and alternatives (Garriguet 2007).

Prevalence of food insecurity negatively impacts nutrient consumption among Canadian children. Food insecure children were more likely than food secure

children to consume energy-dense food and have lower protein, milk, and vegetable and fruit intake (Kirkpatrick and Tarasuk 2008). In 2004, the average HEIC score for children aged 4–8 years was 65.4, 59.7 for boys aged 9–13 years, and 60.0 for girls in that same age range (Garriguet 2009). More than 90% of Canadian children aged 4–13 years and newcomer children aged 7–11 years had diets classified as needing improvement (Garriguet 2009; Nisbet 2011). Newcomer refugee children to Saskatchewan had a significantly lower mean score (60.4) compared to newcomer immigrant children (65.4) (Vatanparast et al. 2013). Refugees had a poor diet at 14% compared to zero immigrants (Vatanparast et al. 2013).

Nutrients of Concern

Immigrant and refugee children can be at greater risk for nutrient intake inadequacies due to previously discussed pre- and postmigration factors compared to Canadian-born children. Prevalence of intake inadequacy was <10% for all nutrients for children 1–8 years of age (Kirkpatrick and Tarasuk 2008). Among children aged 9–18 years who were food insecure, the prevalence of nutrient intake inadequacy for protein, vitamin A, and magnesium was greater compared to food secure children (Kirkpatrick and Tarasuk 2008). Newcomer children to Canada are particularly at risk for nutrient inadequacies of folate (DFE), iron, vitamin B12, zinc, calcium, and vitamin D as found in HIC study (Fig. 4) (Nisbet 2011). The prevalence of intake inadequacy of both folate (DFE) and iron was significantly greater among refugee compared to immigrant children (Nisbet 2011).

Vitamin D is important during the developmental stage of childhood. It is a major nutrient for bone mineral accrual. The majority of requirements, 80–90%, must be synthesized from the sun (Hintzpeter et al. 2008). It is available through consumption of food such as oily fish, cod liver oil, and egg yolk. A deficiency in vitamin D involves deterioration of cortical bone, which progresses to osteoporosis (Institute of Medicine 2010). Some noncalciotropic functions of vitamin D, primarily those with an immune regulatory role, are associated with cancer, multiple sclerosis, and types 1 and 2 diabetes (Whiting and Calvo 2005). Calcium deficiencies are often found among those who are also vitamin D deficient (Nellen et al. 1996). Hyperparathyroidism has been noted in individuals whose regular diets contain high amounts of unrefined cereals. These cereals have a high content of phytates, which hinder calcium absorption (Pettifor 2004).

Immigrants to developed countries, including Canada, are at greater risk for vitamin D and calcium deficiencies. Vitamin D deficiency is often found in female immigrants and refugees. Some females have minimal sun exposure due to cultural beliefs associated with full-body veils, darker skin pigmentation, which decreases the dermal synthesis of vitamin D, and higher parity (number of births) (Nellen et al. 1996). Other risk factors for vitamin D deficiency, relevant to the general population, include genetics, use of sunscreen, living in areas of high latitude (around 37° latitude in the winter months), and insufficient dietary vitamin D (Hintzpeter et al. 2008).

A vitamin D deficiency less than 25–27.5 nmol/L is considered representative of rickets (Roth 2007; Ward et al. 2007). Individuals with darker skin represented 89% of children with rickets while 24% were immigrants (Ward et al. 2007). As children

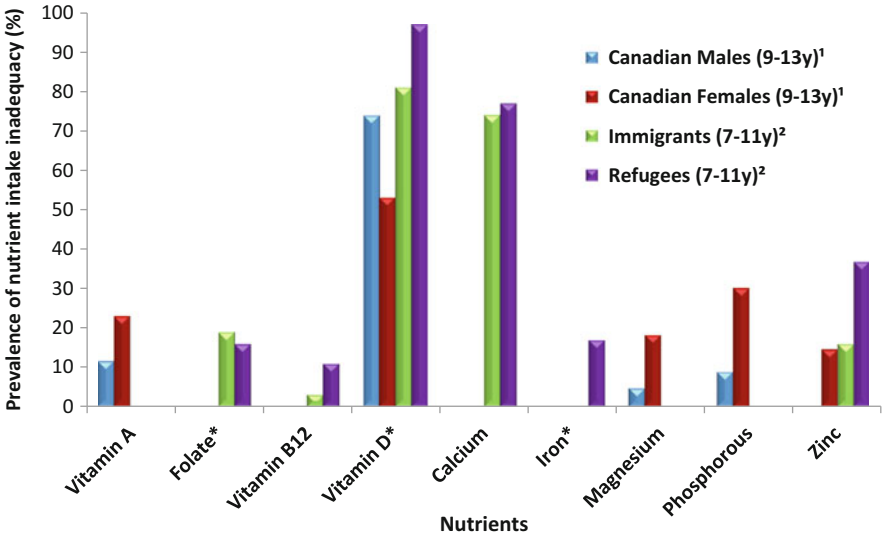

Fig. 4 Prevalence of nutrient intake inadequacy in Canadian and newcomer children. Prevalence of nutrient intake inadequacy for vitamin A, folate, vitamin B12, vitamin D, calcium, magnesium, phosphorous, and iron are compared amongst Canadian children 9-13 years of age and immigrant and refugee children to Canada, 7-11 years of age. (*Significant difference between immigrant and refugee children. [1]Canadian data obtained from Health Canada (2008). [2]Newcomer data obtained from Nisbet (2011))

age, vitamin D concentrations decrease. This may be associated with poor milk consumption, higher consumption of sugar-sweetened beverages, low sun exposure, and decreased activity levels (Roth 2007). In Canada, children who live at greater than 55 degrees latitude are more likely to be vitamin D deficient (Ward et al. 2007). A study using data from 2005 to 2010 in Calgary showed 42% of newcomer refugee children had lower than adequate vitamin D and 10% were deficient (Aucoin et al. 2013). It also showed the refugee population had lower levels of vitamin D when compared to the general Canadian population (Aucoin et al. 2013). Prevalence of intake inadequacy was significantly higher in newcomer refugee (97%) children to Saskatchewan compared to newcomer immigrant children (81%) (Vatanparast et al. 2013). Females were at greater risk of deficiency, likely due to cultural practices limiting skin exposure to the sun (Vatanparast et al. 2013). Prevalence of calcium intake inadequacy in newcomer immigrant and refugee children was 76%. This could be associated with only 24% meeting Canada's Food Guide recommendations for milk and alternatives (Vatanparast et al. 2013). Total serum 25(OH)D (nmol/L) was significantly higher in newcomer immigrant children compared to refugees (Table 1) (Nisbet 2011). Low levels of vitamin D were noted among individuals who had been living longer in Canada, particularly females (Nisbet 2011). Height and serum vitamin D status were significant determinants of TBBMC in newcomer children (Nisbet 2011).

Research Gaps and Recommendations

The health of immigrants decreases over time. However, many factors come into play and must be considered when investigating this population's health. In order to appropriately influence policy, we must turn to research to understand how and why this decline in health manifests. Research has shown that certain measures of health are poor for newcomers, particularly refugees compared to the Canadian-born. The Canadian health system could be negatively impacted. Therefore, evidence-based interventions should be carried out to mitigate the growing burden of disease. In Canada, little is known regarding the dietary transition that newcomers face post-migration. Information is particularly sparse when it comes to children and differentiating between varied experiences of immigrants and refugees.

Further investigation is required to identify culturally appropriate and cost-effective ways to promote traditional food and to assess their nutritional quality and safety in Canada. It is also imperative to identify the most important influential factors in nutrition-specific health promotion as well as priority subgroups such as culture, gender, age, and immigration status.

The relationship between food security and the social determinants of health is important to determine how they affect acculturation, immigrant dietary behaviors, and resultant health outcomes. The impact of food security on health and diet in the newcomer population is unknown as is the extent of its reach.

Literature is lacking any sort of measurement for acculturation, which would be beneficial to understand where along the acculturation spectrum immigrants find themselves and how that relates to dietary behaviors (Table 2). Further research is required when it comes to how, and to what extent, Canadian culture influences the nutrition knowledge, perceptions, beliefs, and behaviors of immigrants. The challenges of accessing and maintaining a traditional diet and how to incorporate nutrition knowledge into a traditional diet should be explored.

Nutrient deficiencies specific to immigrants and the role they play in diet-related health outcomes requires further investigation. With a wide range of cultural groups, it is important to identify the role of genetics and if these groups have unique

Table 2 Factors that increase dietary acculturation. Lending to recommendations for policy, possible measurement tools to assess acculturation were tabulated

Single item measures	Examples	Dietary acculturation
Residence	Increased length of stay	Increases
Language	Ability to speak host country's language	Increases
Generational level	Younger	Increases
Social circles/community	Work outside the home	Increases
Self-identification	Willingness to blend in with new culture	Increases
Pre-arrival exposure to the Western diet	Accelerated nutrition transition and globalization	Increases

Adapted and modified from Satia-Abouta et al. (2002)

standards in regards to health measurement tools. Further exploration is required into dietary risk factors for chronic conditions, especially among newcomer children, and how they vary across age groups, cultures, immigration status, gender, and time since migration. When it comes to providing culturally appropriate nutritional care and services, cultural competency skills among registered dietitians and the care they provide is essential.

Protocols

Anthropometry: Anthropometrics included measured height, weight, and waist circumference. BMI was assessed using the age- and sex-specific BMI calculator from the WHO (2007).

Blood Pressure: A child with a blood pressure measurement greater or equal to the 95th percentile on three occasions was deemed hypertensive (NHBPEP 2004).

Dietary Intake: The average of three 24-h dietary recalls was used to obtain usual dietary intake. Food was entered into a diet analysis program and classified in groups according to Eating Well with Canada's Food Guide (Health Canada 2007). For nutrient intake inadequacy, see the Mini-Dictionary of Terms. The Canadian Healthy Eating Index was based on recommendations for age and sex from Eating Well with Canada's Food Guide as illustrated by Garriguet (2009). Based on a total score out of one hundred, a person's diet was classified as poor (<50), needing improvement (50–80), and good (>80).

Food Security: Food security was assessed using a validated questionnaire, adapted from Health Canada (2007), whereby participants were food secure (0–1), moderately food insecure (2–5), and severely food insecure (6–10).

Serum Measures: Serum was collected on blood spot cards using a finger prick. Nonfasting total cholesterol was classified as acceptable (<4.4 mmol/L), borderline (4.4–5.1 mmol/L), and high (≥ 5.2 mmol/L) (American Heart Association 2011). Vitamin D was defined as deficient (<30 nmol/L), inadequate (30–50 nmol/L), and sufficient (>50 nmol/L) according to the IOM (2010).

TBBMC: Values were obtained using dual-energy x-ray absorptiometry and compared to estimated normal values for each child's age, sex, and ethnicity based on longitudinal studies (Baxter-Jones et al. 2009).

Dictionary of Terms

- **Deskilling** – Deskilling occurs when the host country does not acknowledge immigrants' education from their native country (Newbold 2005).
- **Food Insecurity** – The Food and Agricultural Organization (2003) states "Food insecurity exists when people do not have adequate physical, social or economic access to [sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life]."

- **Food Security** – The Food and Agricultural Organization (2003) states "Food security [is] a situation that exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life."‖
- **Health** – The World Health Organization (2004) defines health as "the state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity…and [it] is largely culturally defined."
- **Healthy Eating Index** – "The Healthy Eating Index (HEI) uses a point system on a scale of 1–100 to evaluate certain aspects of a person's diet to conclude if their diet is good (>80pts), needing improvement (50-80pts), or poor (<50pts)" (Garriguet 2009). The HEI was originally developed by the United States Department of Agriculture and adapted by Canada (HEIC) (Garriguet 2009).
- **Healthy Immigrant Effect** – Immigrants are healthier upon arrival to the host country compared to the native-born population (Newbold 2009; Kaushal 2009). The healthy immigrant effect is influenced by self-selection, access to healthcare, acculturation, and other factors (Newbold 2009; Kaushal 2009).
- **Immigrant** – "A person who has come to a different country in order to live there" (UNHCR 2017).
- **Newcomers (recent immigrant/refugees)** – People who migrated to their host country less than 5 years ago.
- **Prevalence of Inadequacy** – The prevalence of inadequacy is the percentage of a group's intake below the estimated average requirement (EAR) for a specific nutrient (Health Canada 2012). The EAR is the "average daily nutrient intake level that is estimated to meet the requirement of half the healthy individuals in a life-stage and gender group" (Health Canada 2012).
- **Refugee** – A refugee is someone who "…owing to well-founded fear of being persecuted for reasons of race, religion, nationality, membership of a particular social group or political opinion…is unable or unwilling to return to his/her country of birth due to fear for their safety" (UNHCR 1951).

Summary Points

- In 2011, 6.8 million people living in Canada were foreign born with more than one million being newcomers. Children aged 14 years and under made up 19% of the total newcomers and youth aged 15–24 years made up 15%.
- Canada has welcomed several refugees in the past, responding to the global refugee crisis. In the last 1.5 years, 40,081 Syrian refugees arrived in Canada and in 2016 (January–September), there were 16,369 in-person refugee claims.
- The health status of immigrants upon arrival is high. However, it eventually deteriorates over time before matching that of native-born Canadians.
- Refugees are at greater risk of poor nutrition and health compared to immigrants due, in part, to premigration exposures. Refugee children, specifically, are at greater risk.

- Dietary acculturation could be due to expensive or less available traditional food items, a lack of nutrition education, and convenience in preparation of Western food, resulting in increased consumption of prepackaged energy-dense items, which contribute to elevated overweight and obesity levels.
- Newcomer households had a higher prevalence of food insecurity, less access to healthcare, and lower than recommended levels of required nutrients.
- Immigrant and refugee children face issues with poverty, education, food security, nutritional requirements, and mental health while acculturating to North American culture, which results in poor health outcomes.
- Canada lacks comprehensive information on the health and nutritional status of immigrant and refugee children. However, immigrant and refugee children were found to have higher rates of food insecurity and be at greater risk for vitamin D and calcium deficiencies.
- Continued research on food security and the identification of culturally appropriate food with consideration for availability, accessibility, quality, and affordability is important to address these issues.
- Further investigation is required to identify culturally appropriate and cost-effective ways to promote traditional food and assess their nutritional quality and safety in Canada.

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Household Food Insecurity and Child Nutritional Status: Pattern, Causes, and Relationship

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Abstract

Food is generally known to be one of the basic necessities of life and is known to be crucial to the survival and well-being of individuals, family, community, and the nation at large. Food security covers a wide scope and is beyond food available to individuals, but includes access to kinds of food desired at all times and how well it is utilized. Household food insecurity is defined as the inability of household members to have access to the quantity and quality of food required at all times for their daily energy needs and maintenance of good health.

Studies have shown that the causes of hunger and household food insecurity are a mix of economic, social, poor agricultural practices, and absence of community-driven food assistance program. These factors are those that influence food production and availability of food at markets and stores referred to as supply factors and those that will affect the ability of individuals and household members to be able to purchase food for household consumption, referred to as demand factors. Household food insecurity influences nutritional status of children and is usually assessed using anthropometric methods. Anthropometry is the use of body measurements to assess and classify nutritional status of children 5 years and below. These measurements include age, sex, weight, and height (or length in children 6–23. 9 months or under 87 cm in height). When two of these variables are used together they are called an index. Mid-upper arm circumference (MUAC) is used for individuals aged 6 months and older. The common anthropometric parameters are **Height-for-age** used to assess stunting and is used to detect children who have been undernourished for not less than 6 months otherwise referred to as chronic malnutrition; **Weight-for-height** which detects children suffering from current or acute under-nutrition and is usually referred to as wasting and **Weight-for-age** used to identify underweight. The factors found to be associated with nutritional status of children less than 5 years are household food insecurity, feeding practices, family wealth status, and education of caregiver. This chapter explained the effect of household food insecurity on the nutritional status of children less than 5 years and measures designed to address both household food insecurity and identified child nutritional problems. Conclusively causes of household food insecurity and child nutritional problems were used as a guide to their solution.

Keywords

Household food insecurity · Child nutritional status, pattern, causes and relationship

List of Abbreviations

FAO	Food and Agriculture Development of the United Nations
FAP	Food assistance programme
HDDS	Household dietary diversity scale
HFIAS	Household food insecurity access scale
MUAC	Mid-upper arm circumference

NCHS GR	National Center for Health Statistics Growth Reference
SD	Standard deviation
UNICEF	United Nations Children's Fund
WFP	World food program
WHO	World Health Organization

Introduction

Household Food Insecurity, Hunger and Child Nutritional Status

Food is generally known to be one of the basic necessities of life and is known to be crucial to the survival and well-being of individuals, family, community, and the nation at large (Allmer 2011). Food security covers a wide scope and is beyond food available to individuals, but includes access to kinds of food desired at all times and how well it is utilized. **Household food insecurity** is the inability of household members at all times, to have access to the quantity and quality of food required for their daily energy needs and maintenance of good health. It is associated with either absolute lack of food, or food available in insufficient quantity with or without poor quality. Household food insecurity can also occur when food is available both in quantity and quality but access is denied to some significant members of the household. This may be due to inappropriate intra-household food distribution which is common in household with internal crises or disunity where those favored by the primary caregiver/household head are given more food, even in period of food sufficiency. This scenario occurs frequently in African countries where polygamy is widely practiced with poor birth control and its attendant large family members (Adebayo et al. 2012). Food and Agriculture Organization of the United Nations (FAO), 2010 define **Food security** as "when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food which meets their dietary needs and food preferences for an active and healthy life." Household food insecurity occurs in the absence all the parameter mentioned by FAO in their definition of food security.

Hunger is defined as insufficient intake of food substances or absolute lack of food, either as a result of household food insecurity, poor feeding practices, or reduced access to the consumption of adequate food substances. Household food insecurity is a precursor to most cases of hunger in the absence of self-induced denial to food consumption or an aftermath of being restricted to food consumptions by forces beyond one control even in a situation of food availability.

Children less than 5 years nutritional status is the nutritional adequacy or inadequacy of nutrition of children less than 5 years and is usually measured using anthropometric measurement which compares weight and height of the children against standards, using World Health Organization (WHO) parameters as shown below:

1. Underweight as weight for age < -2 standard deviations (SD) of the WHO Child Growth Standards median.

2. Stunting as height for age < -2 SD of the WHO Child Growth Standards median.
3. Wasting as weight for height < -2 SD of the WHO Child Growth Standards median.
4. Overweight as weight for height $> +2$ SD of the WHO Child Growth Standards median.

Household food insecurity has various scope and its impact on nutrition, especially in children less than 5 years depends on which dimension is predominant. The scope of household food insecurity is food availability, access to food, food supply stability, and food consumption pattern.

Assessment of Household Food Insecurity

There are various methods used by various researchers for the assessment of household food insecurity (Sanusi et al. 2006; Swindale and Bilinsky 2006; Coates et al. 2007; Ajao et al. 2010; Ijarotimi and Odeyemi 2011; Coleman-Jensen et al. 2013).

However, the parameters mostly used are Household Food Insecurity Access Scale (HFIAS) for measurement of food access and Household Dietary Diversity Scale (HDDS) for measurement of different food groups. However, the HFIAS which use series of questions about conditions and behaviors that characterize households which occurred in the last 12 months when they were having difficulty meeting basic food needs is preferred by this author. Each question asks whether the condition or behavior occurred at any time during the previous 12 months and specifies a lack of money and other resources to obtain food as the reason. The series includes 10 questions about food conditions of the household as a whole and of adults in the household and, if there are children present in the household, an additional 8 questions about their food conditions were added.

The food security status of each interviewed household is determined by the number of food-insecure conditions and behaviors the household reports (Wunderlich and Norwood 2007; Coates et al. 2007).

1. Food secure were households that denied all items or affirmed one or two items
2. Foods insecure without hunger were households that affirmed three to seven items
3. Food insecure with moderate hunger were households that will affirm eight or more items
4. Foods insecure with severe hunger were households that affirmed five or more foods insecure condition among children.

However, the limitation to HFIAS as a means of assessing household food insecurity is recall bias, nevertheless, it is still the most universally acceptable methods for assessing household food insecurity.

Causes of Household Food Insecurity

Causes of hunger and household food insecurity is a mix of economic, social, poor agricultural practices, and absence of community-driven food assistance program. There is a complex interplay of these factors in many households and any measure directed at reducing household food insecurity must look at these factors singly and in combination (Bhattacharya et al. 2002; Clover et al. 2003; Chaparro 2012; Coates et al. 2007; Coleman-Jensen et al. 2010, 2011; Dean et al. 2011; Diouf et al. 2010; Donna et al. 2002 and Frehiwot 2007).

During our various studies on household food insecurity and nutrition factors of children under-fives, both quantitative, observational checklist and qualitative methods were used to explore various causes of household food insecurity and malnutrition. The findings have been summarized into supply factors and demand factors. The supply factors address issues that will make food commodities available at markets, groceries stores, supermarkets, and other retail food stores, while the demand factors address those factors that will reduce the ability of individuals and household to access and purchase food commodities for consumption (New South Wale Centre (NSW) for Public Health Nutrition 2003).

These facts have been used to build an evidenced-based Conceptual Framework for Household Food Insecurity in rural and urban areas of Ondo State, Nigeria. This same scenario might operate in other settings outside Ondo State, Nigeria (Fig. 1).

This conceptual framework shows that the food security status of the household depends on factors that influence food supply as well as those factors that enhances or decreases the ability of household to demand (have access) for food as explain further below.

Supply Factors Influencing Household Food Insecurity

Household food insecurity is a function of many factors among which is the amount of food supply available for purchase by individuals, families, and households. The supply of food is influenced by macroeconomic policies of the national government, level of unemployment, transportation cost, and cost of fuel or energy.

Macroeconomic Policy of National Government

The overall management of a nation's economy by its economic team depends on its macroeconomic policies, which influence the various economic activities of that country. It influences the gross domestic products, prices of goods and services, supply of food materials, clothing, medicine, and housing. Macroeconomic policy determines the exchange rate of a country currency in relation to those of other countries, and this could either lead to devaluation or increase in the value of that

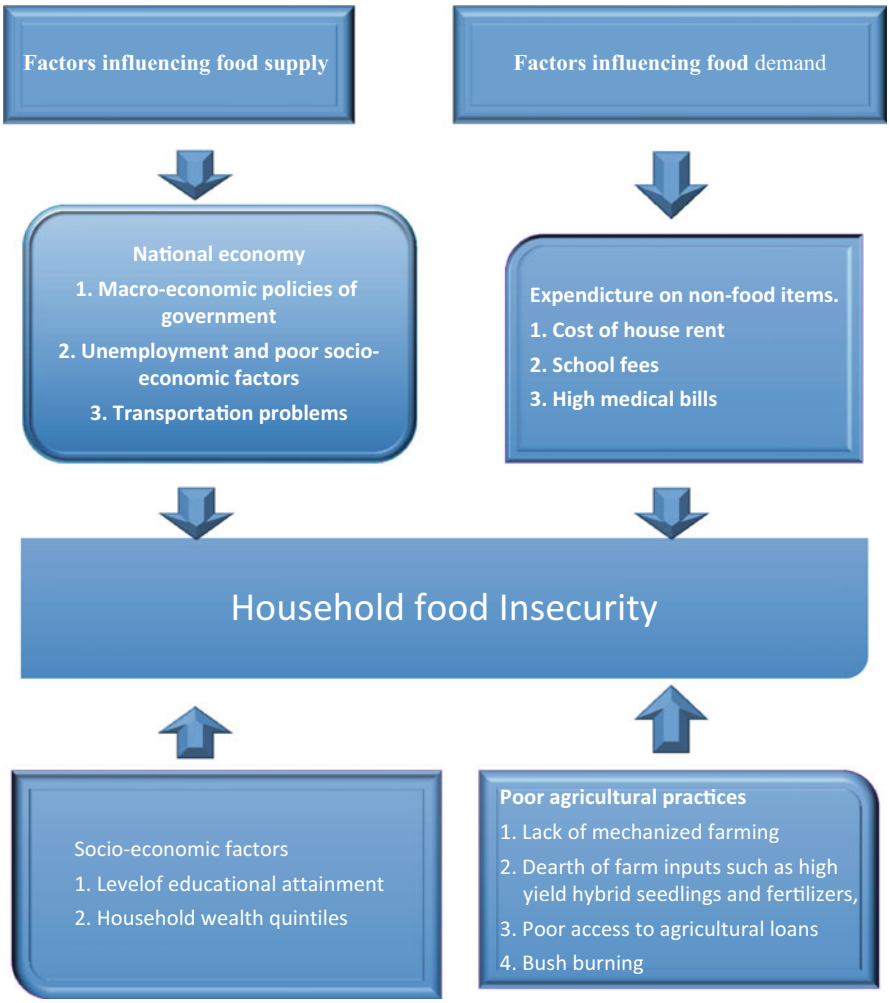

Fig. 1 The conceptual frameworks for household food insecurity (Source: Dr. Akanbiemu et al. 2012: Studies on Household Food Insecurity in Rural and Urban Commuities of Ondo State, Nigeria)

currency. Currency devaluation in countries which depends mainly on the importation of staple food, especially where food production is far below the national demand will tend to have food shortage as more money will be required to import fewer food commodities which will make demand higher than food supply and will invariably lead to increase in prices of food commodities. In a country where the currency is devalued and household income is relatively unchanged or lesser in value even in period of food price increase as is the case in Nigeria, food insecurity and hunger will become more prevalent.

Level of Unemployment and Poor Socioeconomic Factors

A country wealth and that of its citizens depends on the level of production of goods and services and this is a function of the proportion of its population engaged in economic activities, i.e., those employed by both formal and informal sectors. Employment generates income and income will lead to economic multiplier effects which could further stimulate the growth of the economy. The amount of income available to individuals and households will determine their ability to purchase the quantity and types of food required for consumption or storage. In areas of a country especially rural communities with high unemployment rate and lowest wealth quintiles, household food insecurity is as high as 90% (Akanbiemu et al. 2016b). Barriers to food security are diverse and include broadly; socioeconomic and cultural factors. Low income has been recognized by other researchers as the primary risk factor for food insecurity. Other factors acting as barriers to food security are household, community, and social and physical environments (Olson et al. 2004; Lee 2001; Sultana and Kiani 2011; Mahgoub et al. 2006; Hackett et al. 2010).

Transportation-Related Problems

The quantity of food supply to homes, groceries stores, and markets depends on critical issue affecting transportation of food products within and outside various communities. Majority of agricultural activities resulting in food production occurs in rural areas, especially the hard to reach areas. During raining seasons, many hard-to-reach rural communities are cut off from having easy access to semiurban and urban areas as roads become unmotorable. In such situations, food produced are either wasted to natural spoilage or are transported using extreme cost in such countries. This situation occurs in Nigeria where there are poor storage facilities for agricultural products especially in rural farms (subsistence farming) where over 70% of food production occurs, cost of transportation results in inability of farmers to transport their farm products to towns and urban markets, such that many of these goods are lost to spoilage. In other words, even with good food production, transportation remains a great challenge to rural farmers as many lack the means or money to move their products outside production sites and this greatly impedes food supply and increase food insecurity.

Fuel and Energy

The emerging rise in the cost of fueling small-scale farm machines and poor energy supply to local farms have of recent become a major hindrance to agricultural outputs in Nigeria. The cost of fuel has increased since January 2012, and has continue to increase thereafter, while electricity supply has continued to decreased in the mix of fuel (petrol and diesel) price increases. The implication is that majority of farmers can no longer power those critical machines used for farm grazing and

food processing. This has resulted in decrease production of agriculture products with the resultant low supply to homes, groceries store and markets.

This attendant poor supply of farm products will increase household food insecurity as the case in Nigeria.

Demand Factors Influencing Household Food Insecurity

Household food insecurity occurs even in situation of adequate food supply to food outlets. This occurs when households are unable to demand for these food products due to many interrelated factors. The two main demand factors militating against access and food utilization are expenditure on nonfood items and socioeconomic status of household members. These demand factors are further explained below.

Expenditure on Nonfood Items

Non-food expenditure competes with other expenses for limited family or household income and the proportion of non-food expenditure on the household income determine the amount available for food purchase. Money spent on house rent, school fees and medical bills were reported as some of the causes of household food insecurity by participants in a qualitative aspect of a study on determinants of household food insecurity in rural and urban districts of a Southwest State, Nigeria, by Dr. Akanbiemu et al. (2016b). These are:

(i) **Expenditure on house rent**: Significant proportion of household monthly or annual income is spent on rent for residential purpose. In many rural and urban dwellers in Nigeria, income is limited and in many cases mortgage facilities that will make house ownership affordable for both rural and urban dwellers are lacking. Families and households use part of their annual income to pay owners of houses built for residential profit-making purposes, making such expenditure a major hindrance for household food purchase.

(ii) **Expenditure on school fees**: Money spent on school fees has increasingly become a big drain on families and household income. In Nigeria, failure of public school system especially primaries and secondary schools have led the majority of parents or guardians to send their children to private schools which are often more expensive. This form of household expenditure competes with other needs for the scarce resources available for household such that money that would have been used for food purchase are purposefully used for their children education.

(iii) **Expenditure on medical expenses**: household expenditure on medical bills greatly impair their ability to have enough money to buy food for household consumption. Majority of the households spent more than 60% of their income on medical treatment when they or members of their household are ill. The National Health Insurance scheme in Nigeria caters only for few of the former

sector staff and even for those that benefit, the scope of treatment is greatly limited to disease that is acute in nature, while treatment of chronic diseases and cancers are yet to be widely covered. In addition, the scope of diagnostic investigation is also limited to those that are of minimal cost, while expensive ones are presently being excluded. In countries with low health insurance coverage, majority of those that seek medical care rely on out of pocket spending on health which could be higher than 40% of their total income (catastrophic health expenditure). Catastrophic health expenditure greatly compromises resources available for food purchase and use.

Socioeconomic Status of Household Members

Many sociocultural factors act singly or in combination to influence household ability to demand and utilize food. Poor demand for food will influence household food security status. The common factors identified in a study on Determinants of Household Food Insecurity in Rural and Urban Districts of a Southwest State, Nigeria, by Dr. Akanbiemu et al. (2016b) are a level of educational attainment and household wealth quintiles.

(I) **Level of Educational Attainment**
Households where the primary caregiver has low or no formal education are likely to be more food insecure than families with better educational attainment (Akanbiemu et al. 2016b) and are also more likely to be malnourished especially among children less than 5 years as shown in a study by Akanbiemu et al. (2016a) on the nutritional status of under-fives in rural and urban communities of Southwest, Nigeria. Education influences perception and feeding practices of household members by influencing the types and food consumption pattern. This is particularly important when household members consume poor quality food due to ignorance or preference for foods with poor nutritional values. Education may determine the type of paid income for household members and highly educated members are more likely to secure better-paid jobs that will make enough money available for food purchase for household consumption.

(II) **Household Wealth Quintiles**
The occurrence of household food insecurity is a function of wealth status of households. Study has shown that households with lower wealth quintiles were more likely to be food insecure than those with higher wealth quintiles (Akanbiemu et al. 2016b). Wealth status determined by principal component analysis of ownership of household items clearly show this important predictor of household food insecurity. In addition, study also shows that those with income higher than the national monthly minimum wage were less likely to be food insecure than those with lower income (Akanbiemu et al. 2016b). Income and wealth are important determinants of household food insecurity. This is important because the ability to purchase food items for household consumption is a function of the amount of income and other resources available to household members.

Table 1 Adjusted odds of predictors for household food insecurity

Variables for total population	Odds ratio	95% confidence interval	p-Value
Primary caregiver educational status			
No formal education	1.000		
Had formal education	0.547	0.407–0.737	<0.001
Occupation of household head			
Other occupations	1.000		
Farming	2.363	0.826–6.761	<0.001
Household wealth quintiles			
Quintile (5)	1.000		
Quintile (1)	10.340	3.632–29.438	<0.001
Variables for rural population			
Primary caregiver educational status			
No formal education	1.000		
Had formal education	0.362	0.215–0.610	<0.001
Household wealth quintiles			
Quintile (3)	1.000		
Quintile(1)	8.288	2.677–25.657	<0.001
Variables for urban population			
Primary caregiver educational status			
No formal education	1.000		
Some education	0.636	0.452–0.896	0.010
Household wealth quintiles			
Quintile (5)	1.000		
Quintile (3)	2.782	1.067–7.254	0.036

Table 1 shows the predictors of food insecurity as extracted in a study on household food insecurity and nutritional factors of under-fives in the rural and urban district of a Southwest State, Nigeria (Akanbiemu et al. 2016a).

Table 1 shows the odds for household food insecurity. In the total population, household where the primary caregivers had formal education was 0.5 times less likely to be household food insecure compared to those with no formal education (95% CI 0.4–0.7; $p < 0.001$). In addition, household with wealth quintiles (1) was 10.0 times more likely to be household food insecure compared to household with wealth quintiles (5) (95% CI 3.6–29.4; $p < 0.001$). Households where the household heads were farmers were 2.4 times more likely to be food insecure than households where the household heads belong to other occupational groups (95% CI 0.8–6.8; $p < 0.001$).

Household Food Security and Nutritional Status of Children Less Than Five Years

The survival and well-being of children depend on adequate and proper nutrition, and this is particularly important in growing children especially those less than 5 years. Nutritional status of children is usually assessed using anthropometric methods.

Anthropometry is the use of body measurements to assess and classify nutritional status of children 5 years and below (Cogill 2003). These measurements include age, sex, weight, and height (or length in children 6–23. 9 months or under 87 cm in height). When two of these variables are used together they are called an index. Mid-upper arm circumference (MUAC) is used for individuals aged 6 months and older.

In using the anthropometric method, the 2006 WHO Growth Standards is now recommended for the use of the 1978 National Center for Health Statistics growth reference (NCHS GR-WHO 2008). The WHO Child Growth Standards were developed by WHO based on a sample of children from six countries: Brazil, Ghana, India, Norway, Oman, and the United States of America. Three factors are generally considered: Height for age for assessing **stunting,** Weight for height for **wasting**, and weight for age for **under-weight**. Nutrition indices are best presented as Z-scores as opposed to a percentage of the median. Percentage of the median is no longer recommended for use in classification of individual nutrition status (WHO 2008). The indexes are used to classify children into stunting, underweight, and wasting as described by Cogill (2003).

Height-for-Age

Height-for-age index is used to detect children who have been undernourished for not less than 6 months otherwise referred to as chronic malnutrition. It is not valuable in identifying brief changes in nutritional status or malnutrition. For children below 2 years of age, the term is length-for-age; above 2 years of age, the index is referred to as height-for-age. Any deficit in length-for-age or height-for-age is referred to as stunting. Stunting is an indicator of past growth failure. It is associated with a number of long-term factors including chronic insufficient protein and energy intake, frequent infection, sustained inappropriate feeding practices, and poverty. In children over 2 years of age, the effects of these long-term factors may not be reversible. For evaluation purposes, it is preferable to use children under 2 years of age because the prevalence of stunting in children of this age is likely to be more responsive to the impact of interventions than in older children. Stunting, based on height-for-age, can be used for evaluation purposes but is not recommended for monitoring as it does not change in the short term such as 6–12 months (Cogill 2003).

Weight-for-Height

This identifies children suffering from current or acute undernutrition and is useful when exact ages are difficult to determine. The term Weight-for-length is used for children under 2 years of age and weight-for-height (in children over 2 years of age). Any deficit in this index is referred to as wasting. Wasting indicates current or acute malnutrition resulting from failure to gain weight or actual weight loss. Causes include inadequate food intake, incorrect feeding practices, disease, and infection or, more frequently, a combination of these factors. Wasting in individual children and population groups can change rapidly and shows marked seasonal patterns associated with changes in food availability or disease prevalence to which it is

very sensitive. Since it is responsive to short-term influences, wasting is not used for evaluation but is useful for screening or targeting purposes in emergency settings. Weight-for-height is not advised for evaluation of change in nonemergency situations since it is highly susceptible to seasonality (Cogill 2003).

Weight-for-Age

This index is used to identify underweight. Any deficit in this measurement reflects both past (chronic) and/or present (acute) undernutrition but may not distinguish them. It is a composite measure of stunting and wasting and is recommended as the indicator to assess changes in the magnitude of malnutrition over time (Cogill 2003).

Mid-Upper Arm Circumference (MUAC)

This is a good predictor of immediate risk of death. It is used for rapid screening of acute malnutrition from the 6–59-month age range. MUAC overestimates rates of malnutrition in the 6–12-month age group. MUAC can be used for screening in emergency situations but is not typically used for evaluation purposes (Cogill 2003).

Factors Associated with Under-Fives Nutritional Status

These parameters for assessing nutritional status as described by Cogill (2003) and are important for the overall assessment of nutritional status of children as well as for evaluating nutrition intervention programs.

Many factors have been identified as important determinants of child nutritional status (Morrow et al. 2008; Nakabo-Ssewaryana 2003; Nyaruhucha et al. 2006 and Mondal et al. 2009). Study by Akanbiemu et al. (2016a) shows that the occurrence of stunting in children less than 5 years was significantly associated with household food insecurity, primary caregiver body mass index, household wealth quintiles, and educational status of household head. The study shows that children in food insecure households were found to be significantly more stunted than those in food secure household. The binary logistic regression of factors associated with stunting of children less than five year show that household food insecurity was an important predictor of stunting both in the total population, rural and urban areas. In the total population, children of food secure households were 0.2 less likely to be stunted compared to those who are in food insecure household, while in the rural and urban areas, children in households that were food insecure were 3 and 8 times more likely to be stunted compared to children in household that were food secure respectively. This finding agrees with that of Helen Keller International, Nepal, 2010, which showed that stunting and underweight, was significantly associated with household food security status; in order words, the more food insecure the household, the higher the prevalence of stunting and underweight.

Wealth quintiles as measured by Principal Component Analysis were significantly associated with stunting. This association was significant for both the total and

urban population where regression analysis for covariates factors showed that households with lower wealth quintiles were 12 times and 18 times more likely to be stunted than those children in higher wealth quintiles respectively.

Household head educational status was found to be significantly associated with stunting in the urban population with children in households where household heads had some level of formal education being 0.731 times less likely to be stunted compared to those children where the household had no formal education.

In terms of **under-weight**, Akanbiemu et al. (2016a) show that household food insecurity and household wealth index were the only predictors.

Wasting is a measure of acute hunger or food shortage and is one of the most hazardous nutritional problems among the three of stunting, underweight, and wasting. Wasting is also amenable to food intervention problem. Its prevalence is more in regions with socioeconomic disruption, such as civil wars, religious conflict, armed insurrection, and natural disasters. Wasting is currently a major problem in North-Eastern Nigeria, the hub of terrorist insurgency which has claimed over a million lives and created the largest humanitarian and severe acute malnutrition crises in Nigeria history. Hunger among internally displaced persons at risk of wasting is estimated to be as high as 49,0000 (UNICEF 2016).

It is important to note that a child can manifest with one or a combination of stunting, underweight, and wasting. This point is very germane because a child who is chronically malnourished can develop both stunting and underweight, while a child who is stunted can also manifest with wasting at the same time. It is therefore imperative to bear this in mind when planning nutritional intervention programs. Measures design to tackle any form of malnutrition in under-fives should be holistic and should on the long run be able to eradicate any form of under-fives malnutrition.

Table 2 shows the odds for stunting. In the total population, children of food secure households were 0.2 less likely to be stunted compared to those who are in food insecure household (95% CI 0.1–0.4; $p < 0.001$). Also, children in households with wealth index quintiles (1) were 11.7 times more likely to be stunted compared to those in household with wealth index quintiles (5), (95% CI 5.6–24.7; $p < 0.001$).

In rural population, only household food insecurity was significantly associated with stunting using binary logistic regression analysis. In other words, children in households that were food insecure were 2.6 times more likely to be stunted compared to those children in food secure household (95% CI 1.1–6.2; $P = 0.028$)).

In urban population, children in household where the household head has some level of formal education were 0.731 times less likely to be stunted compared to those children where the household had no formal education (CI 0.5–1.0; $p = 0.048$). In addition, children in food insecure households were 7.297 times more likely to be stunted compared to children in household where households that were food secure (95% CI 3.5–15.0; $p < 0.001$). Also, children in households with wealth index quintiles (3) were 18.9 times more likely be stunted than those in household with wealth index quintiles (5), (95% CI 8.0–44.5; $p < 0.001$).

Table 3 shows the odds for underweight. In the total population, children in households that were food secure were 0.3 times less likely to be underweight compared to those children in households that were food insecure (95% CI 0.1–0.6; $p = 0.00$). Also, children in households with wealth quintiles (1) were

Table 2 Adjusted odds of predictors for stunting

Variables for total population	Odds ratio	95% Confidence interval	p-Value
Household food security status			
Household food insecure	1.000		
Household food secure	0.201	0.115–0.351	<0.001
Household wealth index			
Quintile (5)	1.000		
Quintile (1)	11.705	5.554–24.669	<0.001
Variables for rural population			
Household food security status			
Household food secure	1.000		
Household food insecure	2.625	1.112–6.196	0.028
Variables for urban population			
Household head educational status			
No formal education	1.000		
At least primary education	0.731	0.535–0.998	0.048
Household food security status			
Household food secure	1.000		
Household food insecure	7.297	3.539–15.046	<0.001
Household wealth index			
Quintile (5)	1.000		
Quintile(3)	18.888	8.015–44.513	<0.001

27.8 times more likely to be underweight compared to those children in households with wealth quintiles (5) (95% CI 8.3–92.6; $p < 0.001$).

In the rural population, children in households that were food insecure were 3.3 times more likely to be underweight compared to those children in households that were food secure (95% CI 1.2–8.9; $p = 0.020$). Also, children in household with wealth quintiles (3) were 0.614 times less likely to be underweight compared to those children in households with wealth quintiles (1), (95% CI 0.4–0.8; $p < 0.001$).

In urban area, children in households that were food insecure were 2.7 times more likely to be underweight compared to those children in households that were food secure (95% CI 1.1–6.6; $p = 0.03$). Also, children in household with wealth quintiles (3) were 0.4 times less likely to be underweight than those children in household with wealth quintiles (5) (95% CI 0.3–0.7; $p < 0.001$).

The findings as shown above indicate that the most common determinants of stunting and underweight are household food insecurity and wealth status of the households.

Correcting Household Food Insecurity and Child Malnutrition

The predictors of child malnutrition are attainment of some level of education by household heads, food insecure households, and wealth status of household members. Education and household food insecurity will be addressed further here.

Table 3 Adjusted odds of predictors for underweight

Variables for total population	Odds ratio	95% Confidence interval	p-Value
Household food security status			
Household food insecure	1.000		
Household food secure	0.290	0.147–0.575	<0.001
Household wealth index			
Quintile (5)	1.000		
Quintile (1)	27.803	8.348–92.596	<0.001
Variables for rural population			
Household food security status			
Household food secure	1.000		
Household food insecure	3.283	1.210–8.912	0.020
Household wealth index			
Quintile (1)	1.000		
Quintile (3)	0.614	0.447–0.845	0.003
Variables for urban population			
Household food security status			
Household food secure	1.000		
Household food insecure	2.679	1.091–6.581	0.032
Household wealth index			
Quintile (3)	1.000		
Quintile (5)	0.443	0.294–0.666	<0.0/01

Educational Attainment

There is a compelling need for individuals, household members, community, and government at all levels to work together to promote educational development for all if we must improve nutritional conditions of under-fives. Education has a multiplier effect on all facet of human life; education will help improve on safe-feeding practices such as exclusive breastfeeding in the first 6 months of life, and avoidance of harmful feeding practices such as depriving children of eating protein-rich diet by preventing them from eating fish, egg, and meat considered only good for adults. Perception is a function of knowledge and attitude, and it affects feeding practices. Individuals with education are better placed to discard culturally based poor feeding perception and practices that negatively affect child nutritional status. In addition, a good education will enhance the chances of a better-paid income and improve the household wealth.

Food Insecurity

Food insecurity is the most recurrent predictor of all forms of malnutrition in children less than 5 years and measures directed at improving household food insecurity will greatly improve the status of under-fives nutrition. The study has shown that education of primary caregivers, household head occupational status, and

household wealth status are the common predictors of household food insecurity (Akanbiemu et al. 2016b). Therefore, for a holistic approach to solving child malnutrition, these factors must be taken into consideration when planning nutritional intervention programmes.

As explained earlier, education cuts across all activities of life and there is a strong interrelationship between education of individuals, families, and household income as well as household wealth quintile.

Occupation of household members, especially house head, influences household food insecurity. Farmers and other low-income earners must be empowered by government through improving job conditions, enhancing farming income through better pricing of agricultural products, and eliminating middle-men phenomena. It is also imperative to provide farmers the skills and resources to be involved in agricultural products processing, which is a better way of improving farming income.

Food assistance programme(FAP), a concept not common in Nigeria, was seen by many participants in a qualitative study on improving household food insecurity as a veritable means of improving food security status of individuals and households. Many participants in the qualitative study expressed readiness and eagerness to participate in any community-driven food assistant program. However, it is important that technical support from experts on FAP from both local and international organizations, e.g., Civil Society Organizations, the World Food Program of UN, etc., assist such community-driven FAP in building the skills and the initial trust if it must succeed and produce the desired outcome.

Therefore, addressing hunger, starvation, food insecurity, and child malnutrition will be better achieved if all the interrelated factors are addressed in an all-inclusive manner.

Policies and Protocols

This chapter has shown that household food insecurity is the most recurrent predictor of all forms of malnutrition in under-fives children, as well as the major cause of food deprivation and hunger. Household food insecurity is strongly associated with economic, social, poor agricultural practices, and absence of community-driven food assistance program. Household food insecurity is usually measured by various methods of which HFIAS is the most validated and mostly used by various researcher including the author of this chapter. It uses a series of questions about conditions and behaviors that characterize households which occurred in the last 12 months when they were having difficulty meeting basic food needs. Each question asks whether the condition or behavior occurred at any time during the previous 12 months and specifies lack of money and other resources to obtain food as the reason. The series includes 10 questions about food conditions of the household as a whole and of adults in the household, and if there are children present in the household, an additional 8 questions about their food conditions were added. The food security status of each interviewed household is determined by the number of

food-insecure conditions and behaviors the household reports (Wunderlich and Norwood 2007; Coates et al. 2007). Food secure were households that denied all items or affirmed one or two items; foods insecure without hunger were households that affirmed three to seven items; food insecure with moderate hunger were households that will affirm eight or more items; foods insecure with severe hunger were households that affirmed five or more foods insecure condition among children. Therefore, HFIAS is strongly recommended for the measurement of household food insecurity by researchers and government at all levels when assessing and planning intervention programs in situations of food insecurity crises.

Nutritional status of children less than 5 years is globally assessed using the anthropometric method. The child's weights are measured in kilograms to one decimal and the child's length in centimeters to one decimal. Weight measurements are better obtained using UNICEF-SECA mother-infant scales with a digital screen (this author preference). Height measurements are carried out using a measuring scale produced by Pfizer Nutrition, though other standardized height measurement scale will suffice. Children younger than 24 months are measured lying down on the board, while children aged 24–59 months are measured standing. The height and weight measurements are converted into Z-score using WHO Anthroplus software and are used to estimate the nutritional status of children less than 5 years (WHO 2007): **Height-for-age** is used to identify stunting; **Weight-for-height to** identify **wasting** and **Weight-for-age** to identify underweight. These three indices are expressed in standard deviation units from the median of the WHO Multicentre Growth Reference Standard. Any Z-score below minus two standard deviations (−2 SD) from the median (0 SD) are considered stunted, wasted, and underweight respectively and below minus three standard deviations (−3 SD) as severely stunted, severely wasted, and severely underweight respectively.

The predictors of stunting and underweight among the under-fives are the attainment of some level of education by household head, food insecure households, and wealth status of household members. It is imperative that nutritional assessment of children less than 5 years should be carried out following the above protocols, and all nutritional interventions by local, regional, and national health/agricultural authorities should consider addressing the predictors identified above and any other as may be identified by other credible researchers.

Dictionary of Terms

- **Food security** – When all people, at all times, have physical, social, and economic access to sufficient, safe, and nutritious food which meets their dietary needs and food preferences for an active and healthy life.
- **Household food insecurity** – Is the inability of household members at all times, to have access to the quantity and quality of food required for their daily energy needs and maintenance of good health. It is associated with either absolute lack of food, or food available in insufficient quantity with or without poor quality.

- **Household Food Insecurity Access Scale (HFIAS)** – Is a widely validated tool for Measurement of Household Food insecurity recognized by the Food and Agriculture Organization (FAO) of the United Nations and the United States Agency for Agriculture Development (USDA).
- **Overweight** – Weight for height plus 2 standard deviations (SDs) of the WHO Child Growth Standards median for the same age and sex.
- **Stunting** – Height for age minus 2 standard deviations (SDs) of the WHO Child Growth Standards median for the same age and sex.
- **Under-fives** – Children male or female aged 0–59 months of life.
- **Underweight** – Weight for age minus 2 standard deviations (SD)s of the WHO Child Growth Standards median for the same age and sex.
- **Wasting** – Weight for height minus 2 standard deviations (SDs) of the WHO Child Growth Standards median for the same age and sex.

Summary Points

- This chapter tried to give a simple definition of household food insecurity, hunger, and under-fives malnutrition.
- It also describes the conceptual framework of household food insecurity, which was used to explain the supply and demand factors responsible for household food insecurity.
- The supply factors are those issues that affect food production and availability in the markets, groceries shops, supermarkets, and retail shops.
- The demand factors address those factors that will influence household and individuals to be able to access or purchase food items for household use. It went further to explain in details each of the factors using evidence-based information.
- The concept of child nutritional status was also described and the methods for assessing nutritional status using anthropometric methods was provided.
- The factors associated with various forms of malnutrition were discussed.
- Methods designed to address hunger, starvation, food insecurity, and under-fives malnutrition were provided.
- The approach adopted by this author was based strictly on his understanding, knowledge, and findings from research on household food insecurity and nutritional status of children less than 5 years in a Southwest state of Nigeria and is by no means exhaustive, but meant to add to body of knowledge on the topics, provide insight on possible methods of food insecurity and hunger intervention programs, as well as to stimulate more research on food insecurity, hunger, and under-fives malnutrition.

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Food Insecurity, Nutritional Programs, and Educational Achievement

15

Simone Angioloni, Allison J. Ames, and Glenn C. W. Ames

Contents

Abstract

This book chapter examines the relationship between food insecurity, education, and federally supported school lunch programs, with a focus on elementary education in the United States of America. Previous studies indicated that there is a strong inverse relationship between food insecurity and educational outcome: malnutrition and poor diet generate physical and psychological problems that impair the student's ability to learn. In this chapter, qualification for school lunch programs is considered an indicator of food insecurity, primarily because school lunch participation is based on family income which has been found to be the main factor associated with food insecurity. The National School Lunch Program,

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the largest school lunch program in the USA, is the focus of the chapter. Specifically, Georgia's public schools, a state in the Southern USA with a strong presence of food insecurity, are used as a representative case study. Descriptive simple statistics indicate that the negative relationship between food insecurity and academic success does not just reduce educational performance on standardized assessments, but it appears to be one of the causes that reinforce inequality and segregation, thus undermining the development of effective social mobility.

Keywords

Achievement gap · Achievement scores · Childhood development · Elementary schools · Food insecurity · Inequality · National School Lunch Program · Outcomes of education · Public schools · Segregation · Social mobility

List of Abbreviations

AMA	Atlanta metropolitan area
CRCT	Criterion referenced competency test
NCES	National Center for Education Statistics
NSLP	National School Lunch Program
SAT	Scholastic attitude test required for the college admission in the USA

Introduction

A strong inverse relationship between food insecurity and educational achievement outcomes has been found in previous studies (Jyoti et al. 2005; Frongillo et al. 2006). The reasons behind this are numerous. First, poor nutrition contributes to developmental problems, possibly fostering attention deficit and hyperactivity disorders. This directly reduces the student's capacity to learn, lowering educational performance. Food insecurity is also empirically associated with higher drop-out rates and fewer years of schooling. Students have difficulty studying and retaining information in the presence of malnutrition and a poor-quality diet. This makes learning more challenging, increasing school drop-out rates, and reducing the number of years of completed education (Alaimo et al. 2001).

This chapter also examines how qualification for school lunch programs, such as the National School Lunch Program (NSLP) in the United States of America (USA), can be considered an indicator of food insecurity. This proxy is possible because participation in NSLP is based on the income. In general, when a family's income does not exceed a given threshold, the student is eligible for free or reduced price school meals. This is typical of the National School Lunch Program (NSLP), the largest school meal program in the USA (USDA, 2014). Similarly, in the United Kingdom (UK) a child is eligible for free school meals if at least one parent receives income support or income-based jobseeker's allowance (UK Government 2017).

A representative case study is used in this book chapter to illustrate and define the inverse relationship between food insecurity and academic achievement.

Specifically, the NSLP and Georgia's public schools, a state school system in the Southern USA with a strong presence of food insecurity, are presented. Georgia schools with a high percentage of students that qualified for the NSLP tend to have a low proportion of students that pass the mathematics exam. The opposite is also seen: Georgia schools with a low percentage of students that qualified for the NSLP tend to have a high proportion of students that pass the mathematics exam. Moreover, the inverse relationship between food insecurity and educational outcomes shows a strong spatial pattern. Regions characterized by prevalent poverty, such as the inner part of the Atlanta Metropolitan Area, are also characterized by limited school performance. This problem is common to several metropolitan areas in the USA where the inner cities are often characterized by poor socio-economic conditions (Chetty et al. 2014). Food insecurity appears related to not just poor nutrition and lower academic performance, but it can involve a higher engagement of parents, lower level of criminality in the community and, consequently, higher chances to complete schools and find a better job (Reardon 2011). Thus, the negative relationship between food insecurity and academic success is not limited to reduce the educational performance, but it appears to reinforce inequality and segregation, thus undermining the development of a more effective social mobility.

Relationship Between Student's Performance and Family Income

Student academic performance can be affected by numerous socio-economic factors which include school quality, the family's economic status, and the student's personal characteristics (Hoxby 2000). Previous studies have indicated that the family's economic background plays an important role in determining a student's performance. For instance, Dahl and Lochner (2012) analyzed a sample of 9796 US children and their families from 1998 to 2000. They found that a $1000 increase in family income raised combined math and reading test scores by 6% in the short run. Moreover, test gains were larger for children from disadvantaged families. Similarly, Henderson and Berla (1994) reviewed 66 research studies on the factors shaping student academic performance and found that low-income was one of the crucial drivers for educational success.

Among the indicators of household socio-economic status, eligibility for free school meals plays a fundamental role. The first such reason is that eligibility is usually based on income of the family, providing a gauge of its living standards (Food Research and Action Center 2013; USDA 2014). In the USA, the National School Lunch Program (NSLP) is a federally assisted program that targets students whose family's income does not exceed threshold values based on the poverty line (USDA 2014). Specifically, children from families with incomes below 130% of the poverty level are eligible for free meals. Children from families with incomes between 130% and 185% of the poverty level are eligible for reduced-price meals. In 2017, the year of this volume's publishing, the poverty line for a household of three persons was equal to $20,420 (Federal Register 2017). Thus, children of families with an income below $26,546 were eligible for free school lunch, while

families with income below $37,777 were eligible for reduced price lunches. Similarly, in the UK a child is eligible for free school meals if at least one parent receives income support or income-based jobseeker's allowance (UK Government 2017). In the UK, the job seeker's allowance is a form of unemployment benefit paid by the Government to people who are unemployed and are actively seeking work (UK Government 2017).

Another reason for this proxy is that low-income families are negatively and significantly associated with food insecurity, which directly impacts student academic achievement. Several studies have indicated that children who are hungry are less likely to be ready to learn, translating into reduced academic performance. For instance, Jyoti et al. (2005) have shown that by the third grade, children who had been food insecure in kindergarten incurred a 13% lower achievement scored in their reading and math tests compared to their food-secure peers. Similarly, Hirsch (2007) summarized findings on the impact of poverty and educational disadvantage in the UK. He concluded that "low income is a strong predictor of low educational performance . . . and [that] children in poverty have on average lower educational achievement and are more likely to continue to under-achieve" (Hirsch 2007, 1). This is especially true among children from disadvantaged backgrounds that qualify for free school lunches in the UK and Scotland. He concluded that disadvantaged socio-economic circumstances in a child's early years may result in lower adult qualifications on the job market and adult educational opportunities, possibly perpetuating poverty across generations.

In general, the negative relationship between food insecurity and future academic performance in early childhood is three-fold in its effect. First, food insecurity can cause permanent, physical damage to children's developing brains without a proper diet. Nutritional deficiency includes severe acute malnutrition, chronic under-nutrition, iron deficiency, and iodine deficiency. Such deficiencies impair physical and cognitive development (Prado and Dewey 2014). Moreover, the rapidly developing brains of children are more vulnerable to the effects of insufficient nutrients such as protein, energy, certain fats, iron, zinc, copper, iodine, selenium, vitamin A, choline, and folate (Georgieff 2007).

Second, food insecurity can also cause long-lasting cognitive problems, including attention deficit and hyperactivity disorders. Melchior et al. (2012) examined a longitudinal studying using data collected from 2120 children born in the Québec region, Canada, in 1997–1998. Children's mental health symptoms were assessed longitudinally using validated measures of behavior at ages 4.5, 5, 6, and 8 years. Family food insecurity was determined when children were 1.5 and 4.5 years old. Results indicated that family food insecurity predicts many symptoms of children's developmental difficulties, particularly hyperactivity and inattention. Moreover, the authors suggest that addressing food insecurity and associated problems in families could help reduce the burden of developmental problems, having the potential to reduce inequalities in student physical and social development.

In addition to physical and cognitive developmental impairment, food insecurity may also reduce a student's academic performance during the food-insecure time. This has the potential to have lasting and negative repercussions on future

educational success. Beside, Bratti and Staffolani (2013) found that the measurement of students' previous educational outcomes is the most important indicators of students' future achievement. Specific studies such as Reynolds et al. (2001) studied the long-term effects of early childhood educational programs for 1580 African-American students from low-income families over 25 years in Chicago, Illinois, USA. They found that participation in early childhood educational programs increases future academic achievement scores and raises the school completion rate (Reynolds et al. 2001). Similarly, a study in the US state of Minnesota (2007) indicated that higher educational performance depends on the student's previous years' test scores.

Chinyoka (2014) examined the impact of poor nutrition on the academic performance of seventh grade students in two primary schools in Chivi, Zimbabwe. Findings revealed that malnutrition affected physical growth, cognitive development, overall health and wellbeing, and academic performance. Hungry children were less likely to attend school on a regular basis and, if they did, were less able to concentrate and learn as well as being less likely or able to participate in sporting activities at school. Food insecurity and malnutrition can have direct effects on children's performance and academic achievements in school, impacting their future employment and career opportunities. Thus, persistent food insecurity may contribute to intergenerational transmission of poverty at the local level and weak economic growth at the national level.

If malnutrition, often indicated by food insecurity, impairs proper physical development, accompanied by physiological and behavioral problems, negative and long-term effects on student academic performance are possible. One study, Alderman et al. (2006), examined the impact of malnutrition in the preschooling period on subsequent human capital formation in rural Zimbabwe. They analyzed 680 children in rural Zimbabwe in two periods: 1983–1984 and a re-survey of the same sample in 2000. Their results indicated that better-quality nutrition during preschool years fostered improved physical development of students (i.e., their height) and was associated with a larger number of completed school grades.

The trend remains the same in other geographic locations. For instance, Glewwe et al. (2001) studied a sample of 1239 Philippine children in the 1983–1987 period and in the 1994–1995 period. Their research indicated children with better nutrition perform significantly better in school due to entering school earlier and greater learning productivity per year. Themane et al. (2003) studied a sample of 1033 South African children and they found that better anthropometric measures, such as body mass index, height for age, and weight for age, were positively associated with higher test scores in mathematics and English.

This negative empirical relationship between nutrition and student performance is not a characteristic exclusive to developing countries. For instance, Florence et al. (2008) indicated that a low-quality diet depresses future achievement scores of fifth grade Canadian students. Similarly, Alaimo et al. (2001), Ames et al. (2016), and Angioloni and Ames (2015) found comparable results for the American students.

Addressing these problems in the USA, federal, state, and local level educational policies are in place to reduce the achievement gap (Roza 2010). Achievement gaps

occur when there is a significant difference in achievement across groups, such as gender, ethnicity, and income. Card and Payne (2002) studied school finance reforms across richer and poorer districts and the consequences of spending equalization for the relative test performance of students from different family backgrounds. They found that the amount of state aid available to poorer districts led to increases in the spending of these districts, narrowing the spending gap between richer and poorer districts. Moreover, using SAT scores, they found evidence that equalization of spending leads to a narrowing of test scores across family background groups. Thus, if family economic status has a negative effect on the student's performance, it may be necessary to invest a larger amount of public funds to properly deliver educational programs (Roza 2010).

The increasing pressure on public school finances makes the necessity of effective policy recommendations more urgent. An Organization for the Economic Co-operation and Development (OECD 2013) study found that the aftermath of the 2008 financial crisis has meant a significant number of countries have cut public spending on education. Despite GDP rising in most OECD countries between 2009 and 2010, public expenditure on educational institutions fell in one-third of them. In the USA, Lechman and Mai (2014) reported that States are providing less per-pupil funding for kindergarten through twelfth grade than they did 7 years ago. Similarly, in Georgia, the focus of this chapter's case study, the per-pupil spending decreased by 7% moving from $8775 in the school year 2010–2011 to $8169 in the school year 2013–2014 (Georgia Department of Education 2015).

Finally, the negative effects of poverty on student development can undermine social mobility, reducing long-term economic growth. Chetty et al. (2014) used administrative records on the incomes of more than 40 million children and their parents to describe three features of intergenerational mobility in the United States. They found that high mobility areas are associated with lower residential segregation, more income equality, better primary schools, greater social capital, and greater family stability. From a more general perspective, Clark (2016) examined and compared the family status in such diverse cases as modern Sweden and Qing Dynasty China. He demonstrated that family status is determined by ancestry and that almost all societies exhibit similar limited social mobility rates. Thus, the risk of food insecurity and accompanying lower educational achievement can generate negative repercussions over the future generations.

The National School Lunch Program (NSLP) in the USA and in Georgia Public Schools

This section considers the National School Lunch Program (NSLP) as an example of childhood nutritional programs in schools. Another example in the USA is School Breakfast Program. International examples include in South Africa, the National School Nutrition Programme and in Ireland, the School Meals Programme. The NSLP is a US government-assisted program that operates in over 100,000 public schools, residential child care institutions, and some nonprofit schools in the USA.

The goal of NSLP is to provide nutritionally balanced, low-cost, or free lunches to children each school day. Children from families with incomes at, or below, 130% of the poverty level are eligible for free meals. Those children from families with incomes between 130% and 185% of the poverty level are eligible for reduced-price meals, for which students can be charged no more than $0.40 USD.

In the 2011 academic year, the NSLP subsidized the cost of free or reduced-price lunches for over one million students in Georgia's schools (Food Research and Action Center 2013). Students in Georgia are particularly vulnerable, as the state is currently ranked seventh highest in the USA for food hardship rates. While 46% of the households with children in Georgia qualify for free lunches, an additional 21% of households qualify for reduced price lunches (Food Research and Action Center 2012). Schools where 40% or more of the students get free or reduced price lunches also qualify for Title I federal funds to pay for special programs to close students' achievement gap. Thus, the importance of the relationship between food insecurity and academic achievement has been long recognized by policy makers and analysts (Georgia Department of Education 2015).

The percentage of fourth grade student who qualified for the National School Lunch Program has been increasing over time. This is especially alarming considering US family incomes have been expected to recover from the economic downturn of the Great Recession (2008–2016). Overall, Fig. 1 shows that in the

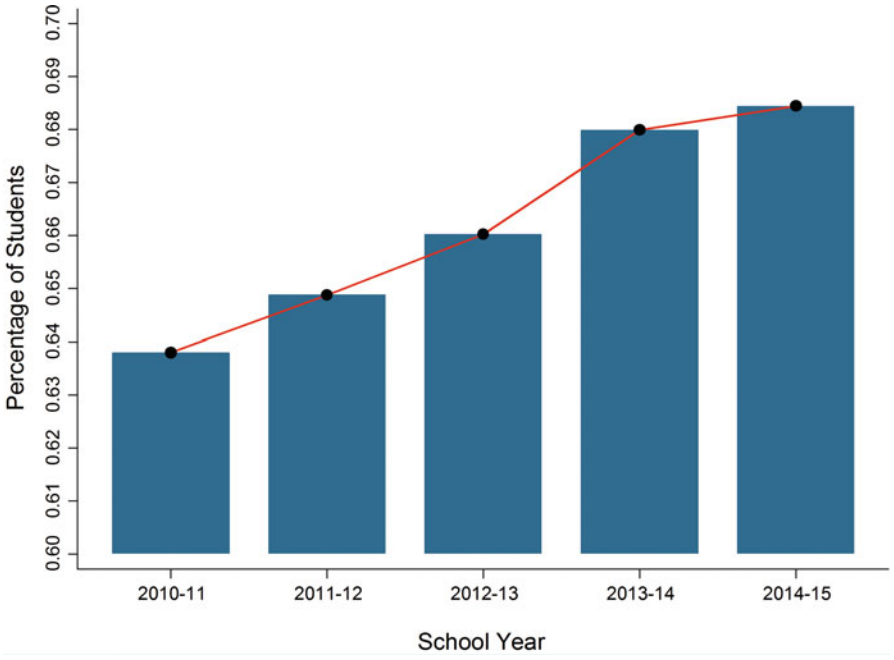

Fig. 1 Percentage of Fourth Grade Students Qualified for NSLP in Georgia's Public Schools. NSLP: National School Lunch Program. Source: National Center for Education Statistics (2016)

2011–2015 period, two-thirds of the fourth grade students qualified for free or reduced price meals in the Georgia public schools (National Center for Education Statistics 2016). Moreover, Fig. 1 shows a clear increasing trend over time of the percentage of students that took part in the NSLP. This indicates that food insecurity is not just strongly present in the Georgia public schools, but it is also gradually increasing overtime.

Achievements in Scoring Patterns

Educational achievement data were taken from the Georgia Department of Education (2016). Georgia has 159 counties, with 1283 elementary schools. The data are representative of student achievement at the school level, and they can be employed to analyze the relationship between food insecurity, as exhibited by participation in the NSLP, and achievement test scores.

With regard to the educational performance, the Pearson correlation coefficients between the percentage of students who qualified for the NSLP and those passing the fourth grade math test range from −0.64 to −0.69 in the 2011–2014 period. This is in agreement with previous studies on the inverse relationship between achievement score and food insecurity (Alaimo et al., 2001; Ames et al., 2016). Figure 2 graphically illustrates this relationship. The predicted values in Fig. 2 were estimated from a fractional probit model, as in Papke and Wooldridge (1996), to allow for the fact that the achievement score is bounded between zero and one. Figure 2 clearly indicates that an increase of the percentage of students qualified for the NSLP is associated with a lower proportion of students that reach predetermined standards on the fourth grade math test. In general, similar relationships are observed for different school exams (Angioloni and Ames 2015), grades (Ames et al. 2016), US states (Dahl and Lochner 2012; Jyoti et al. 2005), and countries (Hirsch 2007; Florence et al. 2008). This suggests that the negative relationship between food insecurity as represented by participation in the National School Lunch Program and educational performance generalizes to other locales.

The negative relationship between food insecurity and achievement score is not just limited to mathematics, but it involves every the school subject. Table 1 shows the summary statistics of the achievement scores in mathematics, reading, English Language-Arts, Science, and Social Studies for the fourth grade students in the Georgia's public schools. Moreover, Table 2 shows the correlation between these achievement scores and the percentage of fourth grade students qualified for the NSLP. As shown from Table 2, the correlation is negative and quite large in magnitude. In particular, the achievement score in Science and Social Studies showed the highest negative correlation with the percentage of students qualified for the NSLP.

With respect to the spatial distribution, Figs. 3 and 4 plot the quartile distribution of the percentage of students eligible for the NSLP and that passed the fourth grade math exam, respectively. These figures were drawn through the Voronoi tessellation, a geometric technique employed to divide space in disjointed contiguous cells, one for every observation (Turner 2014). The cells represent the closest points to a given observation. Thus, if the observations are schools as in this case, the Voronoi cell

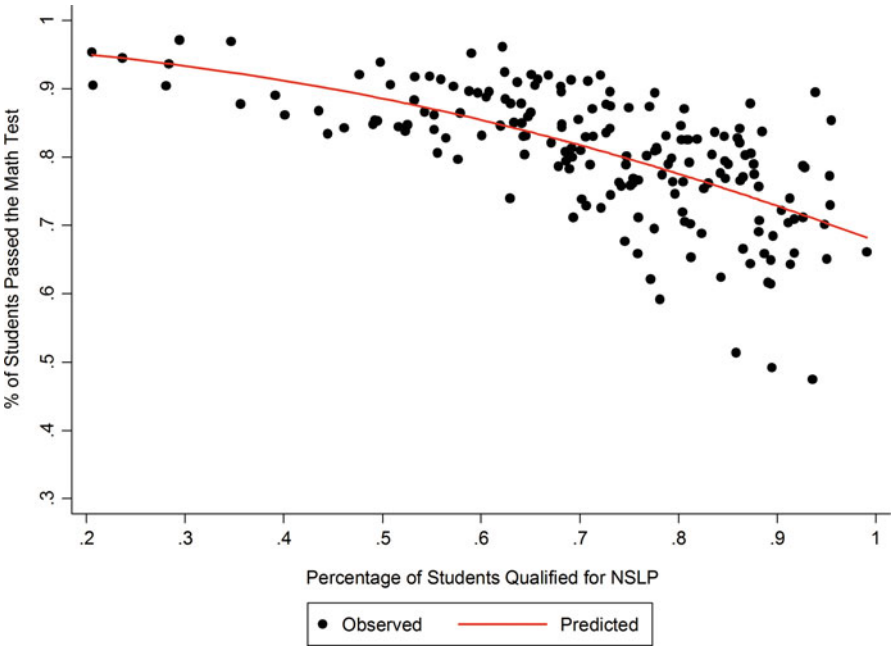

Fig. 2 Mathematics Achievement Score vs. Percentage of Students Qualified for NSLP. Math achievement score: share of fourth grade students who met or exceed the standard of the Criterion Reference Competence Test (CRCT) in mathematics (Georgia Department of Education, 2016). NSLP: National School Lunch Program (National Center for Education Statistics 2016). All the values are averaged at the school district level (180 observations)
The predicted values were estimated from a fractional probit model of the math achievement score vs. the percentage of students qualified for NSLP and a constant (Papke and Wooldridge, 1996).

Table 1 Achievement Scores and Qualification for NSLP in Georgia's Public Schools. Summary Statistic

	Average	Standard deviation	Minimum	Maximum
Mathematics Score	0.80	0.14	0.07	1.00
Reading Score	0.90	0.09	0.37	1.00
English Language-Arts Score	0.88	0.09	0.46	1.00
Science Score	0.79	0.15	0.17	1.00
Social Studies Score	0.78	0.16	0.13	1.00
% of Students Qualified for NSLP	0.66	0.26	0.00	1.00

NSLP: National School Lunch Program (National Center for Education Statistics 2016).
The achievement score of a school subject (mathematics, reading, English Language-Arts, Science, and Social Studies) corresponds to percentage of fourth grade students who met or exceed the standard of the Criterion Reference Competence Test (CRCT) in that school subject (Georgia Department of Education 2016).

represents the area around a school where students are from (Angioloni and Ames 2015; Mumm 2004). Figure 3 shows the Voronoi tessellation where each cell/school area was colored according to the quartile of the distribution of the percentage of

Table 2 Pearson Correlation Coefficients Between Achievement Scores and the Percentage of Students Qualified for NSLP in the Georgia's Public Schools

	Mathematics	Reading	English language-arts	Science	Social studies	% of Qualified for NSLP
Mathematics	1	–	–	–	–	–
Reading	0.82	1	–	–	–	–
English Language-Arts	0.85	0.86	1	–	–	–
Science	0.87	0.85	0.87	1	–	–
Social Studies	0.85	0.85	0.85	0.93	1	–
% of Qualified for NSLP	−0.66	−0.63	−0.66	−0.70	−0.69	1

NSLP: National School Lunch Program (National Center for Education Statistics 2016).
The achievement score of a school subject (mathematics, reading, English Language-Arts, Science, and Social Studies) corresponds to percentage of fourth grade students who met or exceed the standard of the Criterion Reference Competence Test (CRCT) in that school subject (Georgia Department of Education, 2016).
The Pearson correlation coefficient is defined as the ratio between covariance of two variables and the product of their standard deviations.

students qualified for the NSLP from yellow (first quartile) to dark red (fourth quartile). Similarly, Fig. 4 shows the spatial distribution of the math achievement score.

Figures 3 and 4 confirm that the negative relationship between achievement score and food insecurity is also present at the geographical level. This is particularly clear from the analysis of the Atlanta Metropolitan Area (AMA, U.S.A. Office of Management and Budget 2013). The Atlanta Metropolitan Area is the most populous metropolitan area in Georgia. The AMA is located in the Northern part of the state, and it is easily recognizable from the high density of schools and small cells. According to Fig. 3, the inner part of this region is characterized by schools with a high percentage of students qualified for the NSLP (dark red cells), while the outer part of this region, and in particular to the Northern part of the AMA, is characterized by schools with limited presence of students who qualified for the NSLP (yellow cells).

Figure 4 shows the spatial distribution of the math achievement score and it indicates a similar, but *reversed* pattern. The outer part of the AMA is characterized by high performing schools (i.e., schools with a high proportion of students that succeeded in the math test), as they are identified by the dark red cells representing the fourth quartile. In contrast, the inner part of the AMA is characterized by schools that belong to the first quartile of the distribution of the achievement score as indicated by the yellow cells.

Additionally, the spatial pattern is not limited to the Atlanta Metropolitan Area. For instance, a similar pattern is observable along the Black Belt, a region of the southern USA that crosses Georgia in the middle along the North-East, South-West

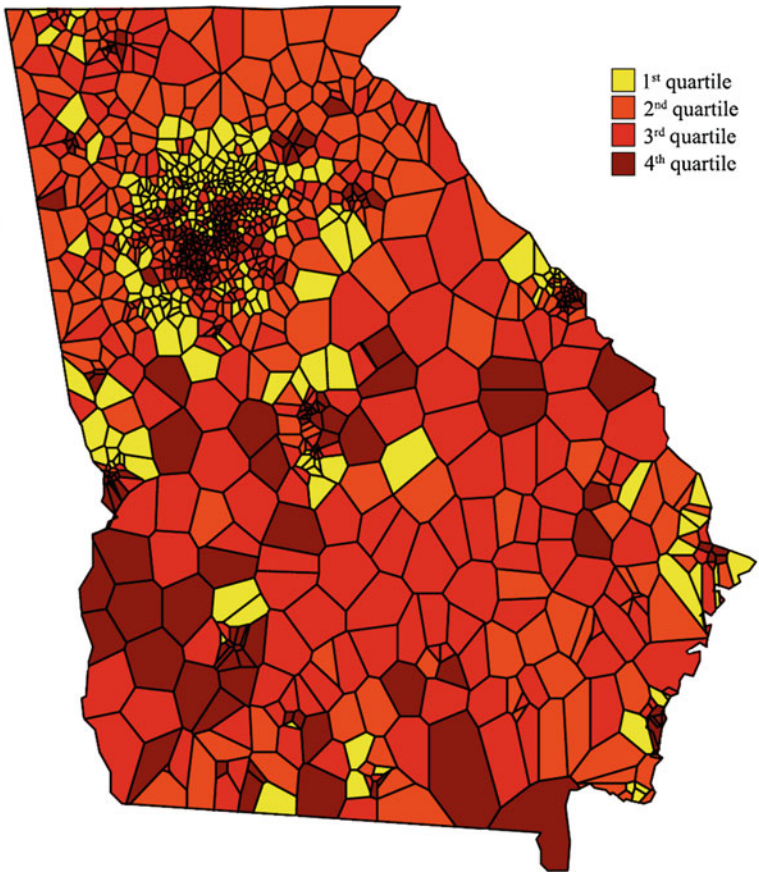

Fig. 3 Percentage of Students Qualified for NSLP in Georgia Public Schools. NSLP: National School Lunch Program (National Center for Education Statistics 2016). The figure shows the Voronoi tessellation of the public elementary schools in Georgia. Each cell corresponds to a school and was coded according to quartile of the math achievement score (Turner, 2014).The school coordinates were collected from the National Center for Education Statistics (2016)

diagonal. In Georgia, this region is mostly rural with some small towns and it is characterized by a substantial presence of African-American population (for a review of the socio-economic characteristics of the Black Belt, see Webster and Bowman, 2008). Along this region, Fig. 3 indicates that there is strong presence of food insecurity with many cells colored red (fourth quartile of the poverty rate). In contrast, Fig. 4 shows that the spatial pattern of the math achievement score is basically the opposite with areas mostly tending to be yellow in color.

Overall, Figs. 3 and 4 confirm previous studies (e.g., Angioloni and Ames 2015) and they indicate that the negative relationship between achievement score and food insecurity is present in Georgia public schools (Ames et al. 2016; Angioloni and Ames 2015). Regions characterized by high food insecurity are, on average,

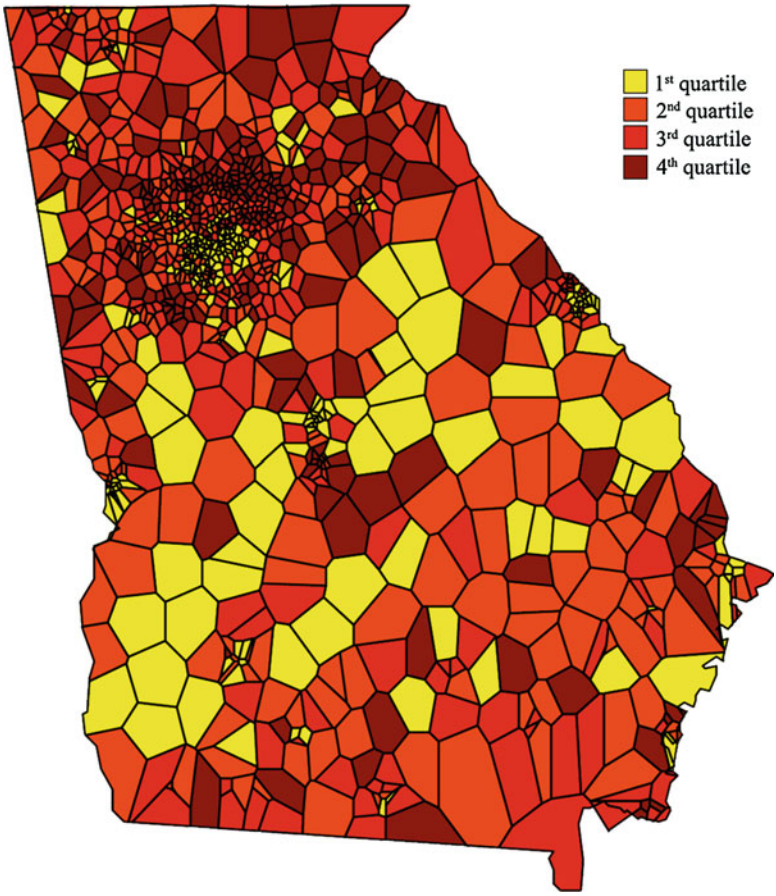

Fig. 4 Math Achievement Score in Georgia Public Schools. Math achievement score: share of fourth grade students who met or exceed the standard of the Criterion Reference Competence Test (CRCT) in mathematics (Georgia Department of Education, 2016). The figure shows the Voronoi tessellation of the public elementary schools in Georgia. Each cell corresponds to a school and was coded according to quartile of the math achievement score (Turner, 2014).The school coordinates were collected from the National Center for Education Statistics (2016)

associated with a low educational performance. Moreover, the descriptive illustrations provided in this chapter indicate that there is a clear spatial pattern with schools characterized by low poverty rates and high academic achievement and schools characterized by high poverty and limited educational achievement. This problem seems particularly worrisome from a policy prospective.

In the USA, the achievement gap between students from more affluent families and students from less affluent families increased in recent years. Reardon (2011) shows that the achievement gap between children from high- and low-income families is roughly 30 to 40 percent larger among children born in 2001 than among those born 25 years earlier. The differences in the achievement gap are also

associated with the socio-economic difference of the neighborhood. Students from high-income families are usually from wealthy neighborhoods with better jobs, infrastructure, and schools (Lareau and Goyette 2010). Thus, differences in the level of food insecurity is not just related to a better nutritional level, but it can involve a higher engagement of parents, lower level of criminality in the community and, consequently, higher chances to complete schools and find a better job (The Saguaro Seminar 2016). Thus, the inverse relationship between food insecurity and academic success is not just limited to reduce the educational performance, but it appears to be one of the causes that reinforce inequality and segregation and thus undermine the development of a more effective social mobility (Clark 2016; Chetty et al. 2014).

Policies and Protocols

This chapter has summarized previous research and studies regarding the relationship between food insecurity and educational outcome, with a particular case study focusing on the state of Georgia in the USA. In general, these studies found that academic performance is inversely related to food insecurity. Moreover, this relationship is differentiated over time. In the short term, food insecurity reduces the student's capacity to learn and it can generate cognitive and behavioral problems (Jyoti et al. 2005). In the long term, the effect of food insecurity is more articulated and potentially, stronger. In general, this may be due to the long-term effects of poverty and food insecurity being a summation of short-term effects (Hamilton 1994). Thus, the repercussions of food insecurity on the achievement score are amplified over time (Reynolds et al. 2001; Barnett 1998). In particular, the negative effects of food insecurity range from health problems as an improper development of the child's brain, lower height and weight, and reduced body mass (Themane et al. 2003). Other socio-economic consequences are that reduced educational performance over time generates higher drop-out rates from school, lower graduation rates, and substantial difficulties in finding more skilled and higher paying jobs (Reardon 2011).

The National School Lunch Program is a clear response to food insecurity in the USA and it was used to illustrate the relationship between poverty rates and achievement scores of fourth grade students in the Georgia's public schools. The descriptive analysis indicated that the negative relationship between food insecurity and limited educational performance is present also for the fourth grade students. In particular, schools in the inner part of the Atlanta Metropolitan Area are characterized by substantial poverty and limited academic success. A similar relationship is observed in rural areas, historically characterized by a substantial presence of low-income racial minority families, namely African-American.

From a policy perspective, these results indicate that food insecurity can generate negative effects on long-term academic achievement. Policies that increase the accessibility to free or reduced price meals can be optimal in schools where the students are from very low-income families (Reynolds et al. 2001). Where food

insecurity is extreme, these initiatives can be combined with tutoring and mentoring programs designed to mitigate the achievement gap where it is present, particularly in scientific subjects (Duncan et al. 2007).

These results have very broad long-term implications for local, regional, and national competitiveness. States and local administrations compete vigorously for new industries and tout the quality of the local workforce among the incentives for companies to locate or relocate to a specific locality (Georgia Department of Economic Development 2017). If an increase in the poverty rate has a negative effect on the achievement scores, then efforts to attract high tech industries to a particular county, municipality, or state will be aided or hampered by the skills of the local workforce. Since food insecurity impacts the level of educational achievement over a student's academic career, it reduces the attractiveness of the local workforce and increases the cost of training labor for new jobs in high tech industries. This clearly impacts a region's overall competitiveness in the global economy.

Future research should extend the approach highlighted in this study to analyze the relationship between food insecurity and student's performance with student-level observations and for a longer period of time, ideally from the first grade to the eighth grade. This would allow for studying the persistence effect of food insecurity over time and space on the student's academic performance.

Dictionary of Terms

- **Achievement score** – Percentage of students, per school, that pass the school test.
- **Poverty rate** – Percentage of students, per school, that are eligible for free or reduced price meals under the National School Lunch Program.
- **Food insecurity** – Limited or uncertain availability or inability to acquire nutritionally adequate, safe, and acceptable foods due to financial constraints.
- **Correlation** – The change of a variable is associated to a change of another variable.
- **Voronoi tessellation** – Given a set of points over a plane, the Voronoi tessellation divides the plane in disjointed polygons such that they are the closest ones to the given points.
- **Probit** – Statistical model that explains the behavior of a binary variable.
- **Fractional probit** – Statistical model that explains the behavior of a fractional variable.

Summary Points

- The eligibility for free or reduced price school meals is based on the family's income.
- The family's income is strongly inversely related to food insecurity.
- The eligibility for free or reduced price school meals can be employed as a proxy for food insecurity.

- Hungry children are less likely to be ready to learn and exhibit a lower academic performance.
- A higher educational performance depends on the student's previous test scores.
- Thus, an increase in food insecurity in the current school year has negative repercussions on the student's future academic performances.
- Where food insecurity is extreme, policies that increase the accessibility to free or reduced price meals may be optimal for improving the academic achievement.
- Where food insecurity is extreme, tutoring and mentoring programs can be designed to mitigate the achievement gap where it is more present.
- Specifically, 22% of Georgia's residents indicated that at times in the past 12 months, they have been without adequate resources to secure sufficient food for the family (Food Research and Action Center 2013). Furthermore, the state of Georgia has a particularly troubling number of students at risk for decreased academic performance due to food insecurity.

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Home Edible Food Waste: Nordic Aspects 16

Ole Jørgen Hanssen and Aina Stensgård

Contents

Abstract

Food waste has been of growing concern in the society over the past 10 years, and especially the focus on food waste prevention. This is the case both in Norway and the Scandinavian countries, as well as in Europe and globally. Up to now most efforts have been allocated to developing good statistics for food waste over the whole food chain, get a good definition of food waste, develop strategies and implement measures for food waste prevention at each stage in the food chain as well as between actors along the chain, and finally to develop governmental policies and regulations of food wasting and treatment of food waste. Activities to cope with increasing food waste globally have been initiated both by UNEP, FAO, OECD, and EU. EU DG Sante has established an Expert Platform for food waste prevention in 2016, with experts and representation from all 28 EU countries as well as private organizations, NGOs, etc. Main focus in 2017 will

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be on food redistribution guidelines, on guidelines for measuring food waste, and on date labeling and food waste.

Keywords
Edible food waste · Scandinavia · Household food waste · Prevention

Introduction

In Norway, the work with food waste prevention started more systematically with the ForMat project in 2010, as a collaboration between the retail sector, the wholesale sector, and the food industry (see Hanssen and Schakenda 2011), in close contact with the government (Ministry of Food and Agriculture and Ministry of Climate and Environment). All the work from 2010–2016 in the ForMat project was based in voluntary efforts from key actors in the food sector, supported by annual funding from the Government (several ministries). The project was formally closed in 2016, after having reported all the main results from 6 years work with gathering food waste statistics, carrying out network projects between actors in the food chain and implemented a number of voluntary actions and measures to promote food waste prevention (Stensgård and Hanssen 2016; Matvett 2016). During the ForMat project, a company was established by the main actors in the food chain to operate all common activities on behalf of the organizations in the food chain (Matvett Ltd). The project was then followed by an interim negotiated agreement between seven food business organizations (from primary production to hospitality sector) and five ministries. The main aim of the interim agreement was to prepare for a permanent negotiated agreement similar to other soft regulations covering different waste fractions in Norway, focusing on how to define food waste, how to measure and develop statistics for food waste, and how to develop strategies to and implement measures to prevent food waste. This agreement will probably be signed by all parties in 2017.

Similar activities have been initiated also in other Scandinavian countries. In Sweden, there has been a project organized by the Environmental Protection Agency and with representatives from the whole food chain (SaMMa project), functioning more like a network than like an organized project. Food Waste Statistics have been gathered and published for 2012 and 2014 by SMED, with financial support from the Swedish Government (Jensen et al. 2011, Naturvårdsverket 2013). Also in Denmark, there has been established a network project with the Environmental Protection Agency (Miljøstyrelsen) as the active part in initiating the network.

In Norway as well as in the rest of the Nordic region, it has been a strategy to differentiate between edible and nonedible food waste, to get a more narrow focus on those fractions of food waste that most easily can be prevented from being wasted and treated as waste. This has been a discussion in parallel with similar discussions in the rest of Europe, where the FUSIONS project proposed a definition without a clear differentiation between edible and nonedible food waste. The FUSIONS project did also define food which was used as animal feed and for bio prosperity

outside the definition of food waste. In Norway, this has not been followed up in the National definition of food waste, where both food used for feeding animals and food being used as a resource in developing new nonfood products are defined as edible food waste in the negotiated agreement. In the other Nordic countries, it has also from the beginning been differentiated between edible and nonedible food waste, but it is unclear how this will be followed up in the future after the FUSIONS definition was published, and the work in the EU Food Waste platform started in 2016.

Although all Nordic countries (except Iceland) have initiated national activities on food waste prevention and been involved in both Nordic Food Waste projects as well as EU project FUSIONS, there are some differences in the approaches that have been taken. In Norway, all food waste prevention activities were initiated by the food industry and the retail sector, and both branch organizations as well as single companies have been very active in both internal as well as common programs and activities. Governmental organizations have supported activities and also been active in promoting food waste actions by their own, but have had a more supporting than initiating and coordinating role. In Sweden and Denmark, the EPAs have been much more involved in initiating and coordinating activities, with less organized activities among companies, working more by themselves. In Denmark, Selina Juul and her organization Stop Wasting Food has taken most of the "official place" nationally, where much of the activities have been centered around her and her organization's agenda and work. There are no similar companies or organizations like Matvett Ltd. established in other Nordic countries, making the work in Norway more organized and more on the premises of the food business sector.

Edible Food Waste in Scandinavia

Food Waste Statistics in Norway 2010–2015

Through the ForMat project, edible food waste from four steps of the Norwegian food value chain was mapped from 2010 to 2015: the food industry, wholesale, retail, and households.

Food waste from primary production, the hospitality sector, parts of the food industry (fisheries, the brewery industry, and in mills and flour producers), as well as food wasted through the sewage system at the households was not mapped due to lack of data.

Edible food waste was mapped for 9 different product groups and 21 different sub-categories, where the following definition of food waste was used: "Food waste includes all edible food that could have been eaten by humans, but for some reason has been wasted or removed from the human food supply chain."

Primary data was collected from a representative sample of businesses/households within each of the four value chain steps, and scaled up to national statistics using upscaling factors based on share of total turnover (wholesale and retail), total production (industry), or number of inhabitants (household-step). For the wholesale and retail step, key figures of cost per kg edible food waste (NOK/kg) was used,

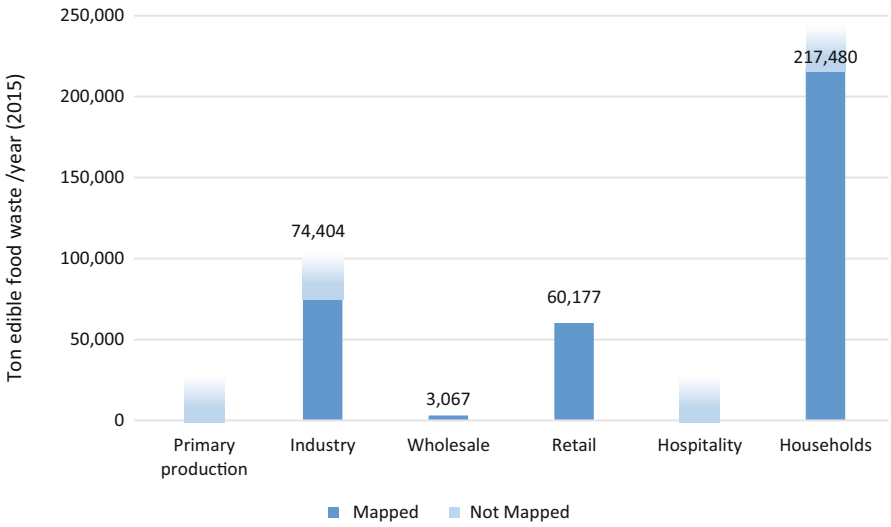

Fig. 1 Ton edible food waste in Norway and data gaps (2015)

as primary data was economic (for detailed information concerning data and methodology, see the final ForMat report, Stensgård and Hanssen 2016).

Figure 1 shows the up-scaled results of ton edible food waste per value chain step (2015) as well as markings for data gaps.

In total 355,000 tons of edible food was wasted within the four steps in 2015, equivalent to 68.75 kg/capita. The household-step was the main contributor (61%), followed by the industry (21%), retail (17%), and wholesale (1%).

The industry-, wholesale-, and retail-step provided data annually from 2009 to 2010. For the household-step, data was only retrieved in 2011 and 2015 through detailed waste-composition analysis. To fill in the missing years for the household-step (2010, 2012, 2013 and 2014) the 2011 and 2015 data was extrapolated using the trend-line function in Excel.

Figure 2 shows kg edible food waste per capita from 2010 to 2015 for each of the value-chain steps and total.

For the analyzed time period (2010–2015), edible food waste was reduced by ca. 10 kg per capita, or 12%. Food waste had decreased for three out of four steps (all except wholesale), and the reduction was most significant at the households.

At the household step, edible food waste was reduced by 4.2 kg per capita, or 11%, from 2011 to 2015 (the analyzed years). The two waste-composition analysis also showed that the development varied between the different product groups.

Figure 3 shows the development and composition of edible food waste at the household-step per product group and year.

Edible food waste at the household step was reduced from 46.3 kg/capita to 42.1 kg/capita. Bread was the product group with the most significant decline, reduced by 3.75 kg/capita or 40%, followed by "other" edible food wastes, which

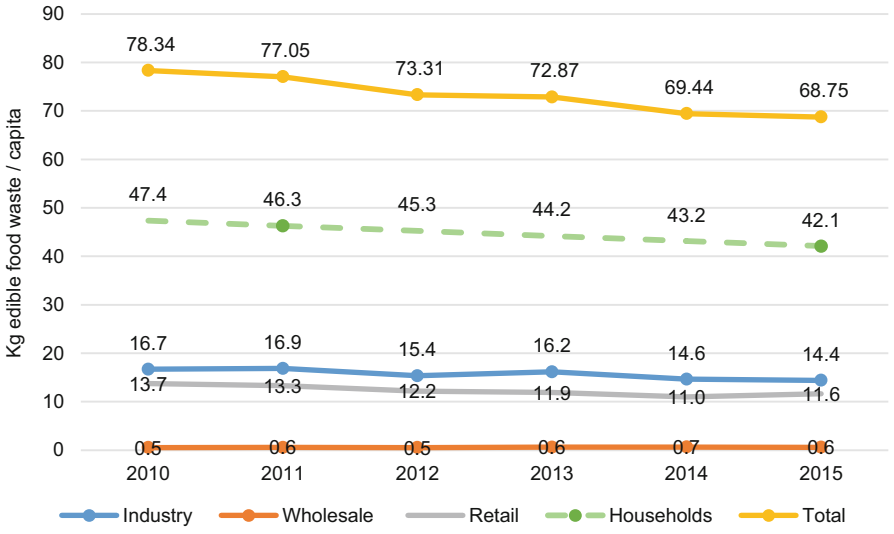

Fig. 2 Kg edible food waste in Norway per capita from 2010 to 2015, for each value-chain step and total

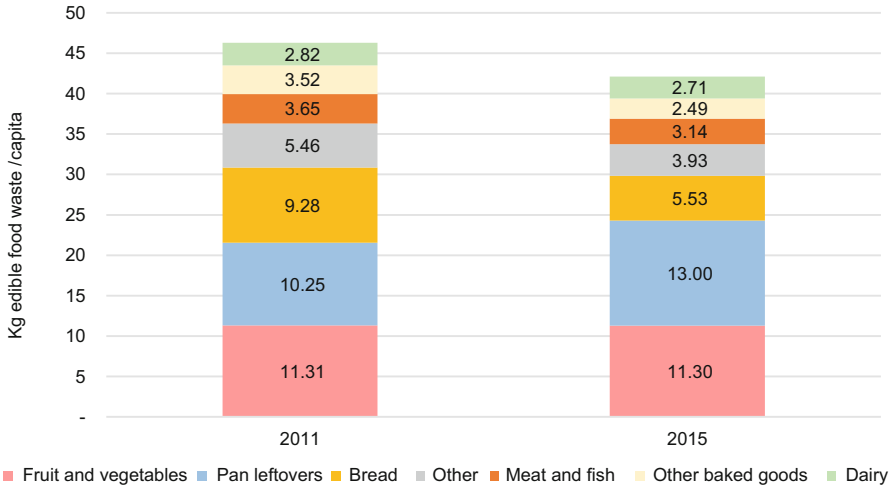

Fig. 3 Kg edible food waste per capita at the household-step, per product group and year (2011 and 2015)

were reduced by 1.53 kg/capita or 28%. Pan leftovers was the only product group that increased during the period, adding 2.75 kg/per capita.

The project also estimated costs and environmental impact (limited to greenhouse gas emissions (GHG)) related to food waste, based on the national food waste statistics. The costs were calculated by using key figures of cost per kg edible food

waste (NOK/kg) per product group as well as upscaling factors based on share of total turnover (wholesale and retail), total production (industry), or number of inhabitants (household-step). All costs were calculated in 2015 value, to ensure comparability between the years.

Environmental and Economic Effects of Food Waste at Home

The environmental impact was calculated by multiplying the amount of food waste in ton (for each product group and value chain step) by a corresponding emission factor. The emission factors were calculated through life cycle analysis methodology (LCA) in accordance to ISO 14040/44 and the European Commission JRC (2010, 2011). These factors included all GHG emissions related to production, transport, an packaging of food. Emissions related to storage at the retailers, transportation home, cooking, and waste handling was not included. All GHG emissions were converted into CO2 equivalents.

Figure 4 shows the costs and Fig. 5 shows the environmental impact related to edible food waste in Norway for each value chain step and year, as well as total (Fig. 5).

From 2010 to 2015 economic costs related to edible food waste was increased by NOK 0.3 billion, or 1.5%, and GHG emissions decreased by 25,000 tons CO2-eq., or 3%. Yet edible food waste was reduced by 7% (25,500 tons), meaning that the development of economic and environmental impact related to food waste did not align with the development of ton food wasted. The deviation between ton food wasted and economic and environmental costs is a result of offsets between different

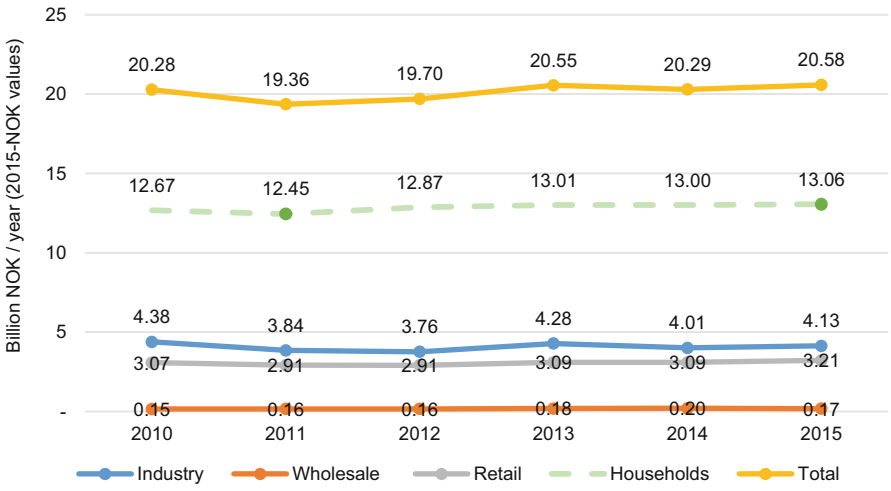

Fig. 4 Economic costs (Billion NOK) related to edible food waste in Norway per year, value chain step and total

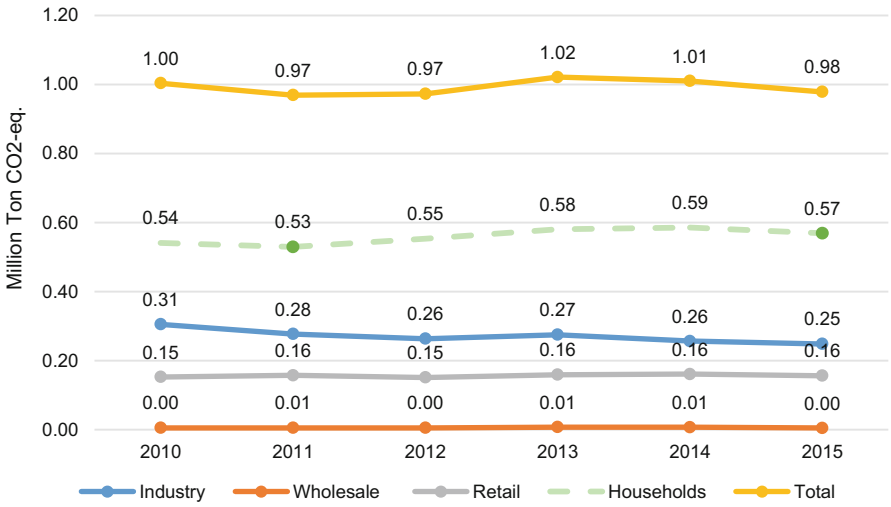

Fig. 5 Environmental impact (million ton CO2-eq.) related to edible food waste in Norway per year, value chain step and total

product groups within the food waste. Through the period 2010 to 2015, the composition of edible food waste was altered from relatively cheap and climate friendly products to relatively expensive and climate intensive products.

The household step was the main contributor to economic and environmental cots, as well as increased costs and environmental burdens per kg edible food waste. As shown in Fig. 3, the composition of edible food waste at the household step is quite rough, and the only product group that increased during the period was pan leftovers, a relatively vague product group. In the analysis, it is assumed that the cost and carbon footprint of pan leftover is relatively high compared to bread and fruits and vegetables. This is decisive for the result of GHG and costs.

Consumer Surveys Explaining Why Food Is Wasted

In the ForMat project, a number of 1000 consumers were asked about their food wasting of different food products in a given week before the study was done every year between 2010 and 2015. The main results are shown in Fig. 1, indicating percentage of consumers that said they wasted the given food product and percentage points change between 2010 and 2015. Leftovers from pots was the most frequently wasted product (26%), followed by biscuits (20%), eggs, fresh fruits (both 19%), fresh vegetables, and frozen ready-made food (both 16%). Milk/cream (14%), fresh bakery products (13%), and fresh meat (12%) did also rank high in food wasting. An interesting observation was that all food types other than fresh fruits showed reduction in frequency between 2015 and 2010 (Fig. 1), with the largest reduction by eggs (5.9%-points), leftovers of dinner meals (5.4%-points), and fresh

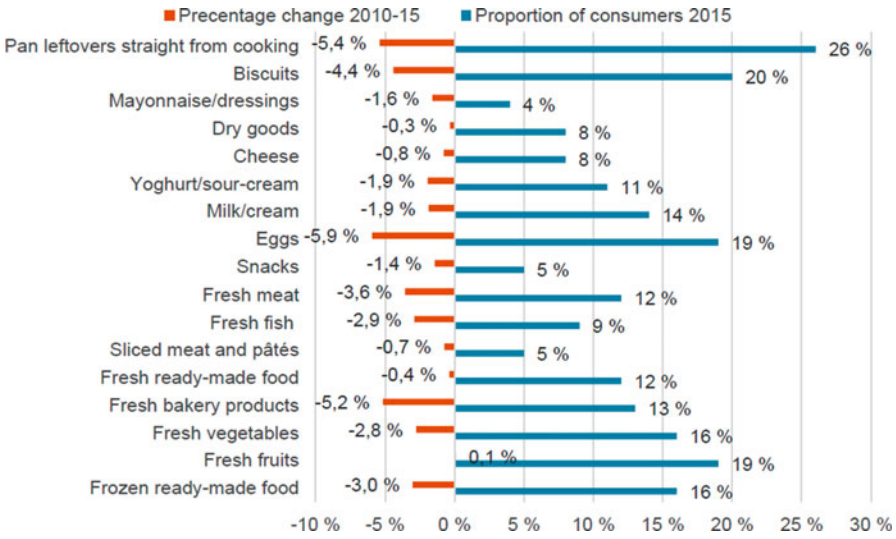

Fig. 6 Changes in share of consumers saying they are wasting different types of food waste in Norway 2010–2015 (Based in Stensgård and Hanssen 2016)

bakery products (5.2%-points). It is thus quite good agreement between the consumer study by self-reporting and the results from the waste composition analyses shown earlier in the chapter, if focusing on the type of food being wasted and the overall trends (Fig. 6).

Through the ForMat project, another cohort of consumers was asked each year from 2010 to 2015 about their behavior and attitudes with regard to important factors that could influence on food wasting from households, with main focus on shopping behavior, food preparation and eating, storing and transport of food, packaging and date labeling of food, as well as food wasting (Figs. 2 and 3). With regard to food planning, about 85% claimed to know how much they needed to buy food, whereas 64% said they normally used shopping list. Only 26% and 24%, respectively, said they either bought too much of each product or more food than they needed. All trends between 2010 and 2015 were positive with regard to food wasting behavior, as the two last factors were reduced in frequency whereas the two first increased slightly over time (Fig. 2). For eating behavior, 48% said they normally took smaller portions to prevent food waste, being more or less stable over the 5-year period. On the other hand, about 33% said they made too much food and throw away the leftovers, and 31% said they made too much food for dinner. Both frequencies were reduced over the years from 2010 to 2015 (Fig. 7).

Consumers have high thoughts about their abilities and behavior to store food properly at home (87%) and at the right temperature in cars from retail shop and markets to home (48%). Only 14% say food often are stored unproperly at home, and 9% that food is often damaged under transport to home from retail shops (Fig. 3). Forty-nine percent of the respondents said they normally store food in new and special packaging solutions at home.

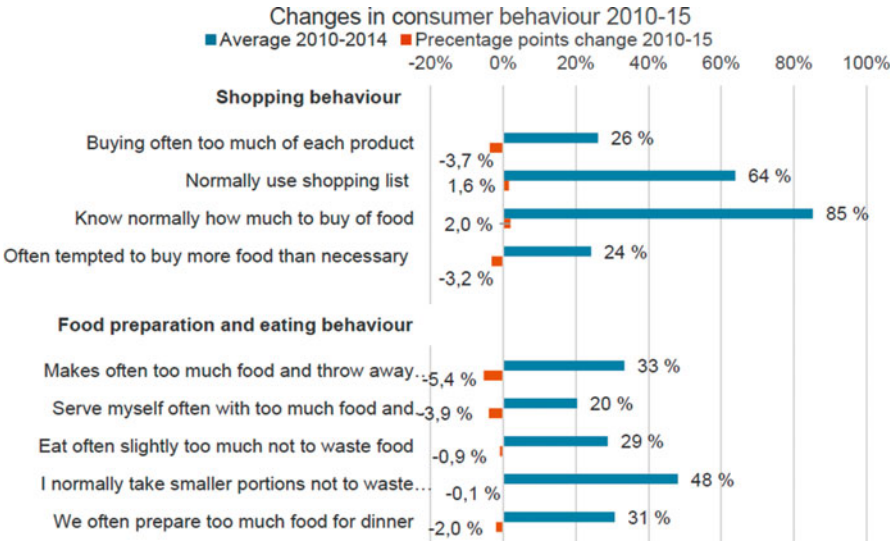

Fig. 7 Changes in behavior and attitudes regarding shopping and food preparation and eating among Norwegian consumers 2010–2015 (Based in Stensgård and Hanssen 2016)

An unexpected share of consumers said they knew well the differences between "best before day" and "used by day" (78%), increasing even slightly over time from 2010 to 2015. About 30% of consumers said they always wasted food that had passed date, and with a significant reduction (8.6%-points) over time from 2010–2015 (Fig. 3). About each third respondent said that there was too much food in each packaging unit (32%), whereas only 24% said they had to waste food due to bad packaging. This figure was also reduced with close to 5%-points over the project period from 2010 to 2015. There has been a high focus on date labeling as a reason to food waste in Norway through the ForMat project and from many different actors, which might explain the significant reduction in respondents saying they are wasting food only due to passed date labeling (Fig. 8).

Similarities and Differences Between Scandinavian Countries in Food Waste Generation and Composition

Statistics of edible food waste from households in four Nordic countries are shown in Fig. 1 and indicate that amount and to some extent composition of edible food waste differ significantly between the four countries. Denmark and Norway are both generating more than 40 kg edible food waste per capita (46.6 kg in Denmark and 42.1 kg in Norway), whereas Finland and Sweden both are generating 50% less edible food waste (Sweden 22.0 kg and Finland 23.3 kg). Also composition of edible food waste differ slightly between the countries, with fruits and vegetables as the most important food type being wasted in Sweden (37%) and Finland (32%), whereas leftovers from

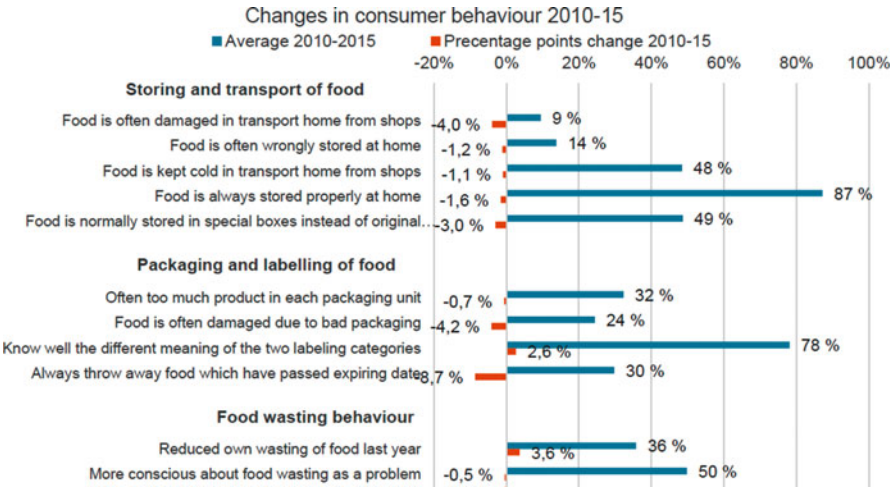

Fig. 8 Changes in behavior and attitudes regarding storing and transport of food, packaging and labeling, and food wasting among Norwegian consumers 2010–2015 (Based in Stensgård and Hanssen 2016)

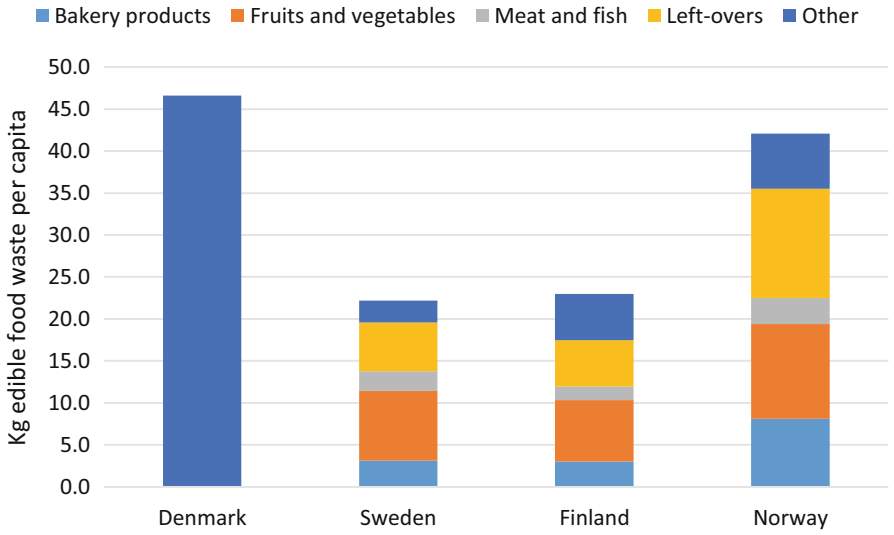

Fig. 9 Total mass of edible food waste from studies of household in Nordic countries

meals is the most important category in Norway (31%). Bread and bakery products are relatively more important in Norway (19%) than the other countries, whereas meat and fish have a higher share in Sweden (11%) than in other countries. Data are taken from Miljøstyrelsen (2014; Denmark), Katajajuuri et al. (2014; Finland), Naturvårdsverket (2016; Sweden), and Stensgård and Hanssen (2016; Norway) (Fig. 9).

As shown by Hanssen et al. (2016) total amount of food waste per capita differs less than the edible food waste per capita (Table 7). Both Finland and Sweden have lower shares of edible food waste than Norway, which coincides to some extent with the differences in data on edible food waste. The question of where to define the border between edible and nonedible food waste should thus be further discussed in follow-up studies between countries.

Differences in amounts of food waste might also be due to variations in food consumption and eating habits between countries. Norwegian consumers eat more bread-based meals than Swedish and Finnish consumers; the latter tend to consume warm lunch both in job canteens and schools. This might explain the high percentage of fresh bakery products in Norway (Fig. 9). Norwegian consumers have also different preferences for fresh bread than Swedish and Finnish consumers, with more focus on "crunchy" products which will naturally soften quickly and hence be more vulnerable to wastage. Credible statistics on food preferences in Nordic and European countries are, however not available, making such explanations more speculative than empirically based.

Policies and Protocols

Policies and Regulations of Food Waste Prevention

Treatment of food waste is strictly regulated in all 28 countries in EU, both from agriculture, food business operators, as well as from households and municipalities. Regulation of wasting of food as such is, however, so far very seldom regulated, and only from food business operators as in France, where the retail sector and wholesale sector is not permitted to waste food.

Norway

Norway has a long tradition in using soft regulations within the waste area, where negotiated agreements alone or in combination with a general regulation by law have been the most used type of policy. This is the case for packaging waste, as well as WEEE, batteries, cars, etc. Producer Responsibilities Schemes have been established by the business sectors themselves, where payment of a certain fee per unit of material used is paid by the industry. This packaging fee is paid to operate collection and recycling schemes, as well as waste prevention actions, information and motivation measures as well as development of more sophisticated and effective solutions. At the end, it is the consumers who pay the cost of the packaging waste recycling and prevention measures.

It has thus been natural to use the same approach to prevent and regulate treatment of food waste in Norway. An interim negotiated agreement between five Ministries and seven food business organizations was signed in May 2015, and will most probably be followed up by a permanent agreement which can be signed up before summer 2017. This permanent agreement will regulate food waste over the whole food chain, where the ambitions and long-term goals are defined in the common

agreement, but where the food sector will identify the best strategies and measures to prevent food waste. A common definition has been agreed upon, as well as a system for how to measure edible food waste or surplus food along the whole chain (Hanssen 2016). In parallel, the Norwegian Parliament has decided that the Government shall evaluate the need for a Law against food waste, based on the French Food Waste regulations. This can eventually be an incentive to make the negotiated agreement effective by the food sector, as regulations by Law probably will be less flexible than a negotiated agreement. The food sector in Norway has been the initiator behind food waste prevention from day one, where both the retail and wholesale sectors as well as the food industry and the hospitality sector have taken initiatives to measuring edible food waste and surplus food, as well as implementing specific measures to prevent food from being wasted. Most of the food waste is generated in households, where it is very difficult to regulate and follow up regulations of food wasting, making it questionable if a Law will be necessary in Norway if the negotiated agreement is implemented by all actors along the food chain. This is a question which will be important to answer in the next couple of years based in experiences also in other countries.

Finland

In Finland, more than a hundred (out of 200) MPs signed a bill 29/2016 of the Food Act (23/2006) proposing that edible food that is not possible to sell ordinarily, should be offered for redistribution or other ways of human food. The initiative aims to reduce amount of food waste and the effects of food waste.

In Finnish LA 29/2016, on Parliament's website is https://www.eduskunta.fi/FI/vaski/KasittelytiedotValtiopaivaasia/Sivut/LA_29+2016.aspx

Ministry of Agriculture and Forestry has commissioned a study (completed January 2017) estimating the costs related to implementation of the bill, including monitoring of food waste statistics, as well as effect of the bill to the amount of food waste and amount of food being donated.

The cost of the bill caused to the operators was estimated to be significant. Also Evira (food safety authority) evaluated the obligation to distribute (on premises) to cause more costs. Mandatory donation of surplus food alone does not lead to increase in amount of food being redistributed and reduce waste. The main problem is not lack of willingness of the actors to make food donations, it is lack of resources with the redistributing actors. Food distribution directly from the stores (shops, bakeries, food catering) is problematic, and is not supported.

Food should be eaten by humans, and not put in the trash. Opportunities to reduce food waste through legislation are sought through Prime Minister's office project "How to develop legislation to reduce food waste?" The final project report including policy brief will be completed before 31 December 2017.

Sweden

In Sweden, the Government initiated an action for food waste prevention in 2013, which was finalized with a report in March 2016. This has been followed by a new action initiated by the present Government in February 2017, where environmental

and food authorities shall identify long term measures to reduce food waste from the whole food chain in close collaboration with stakeholders and actors. The action plan shall contribute to an understanding of how Sweden shall reach the global goal of 50% reduction in food waste by consumers and retail before 2030. This is part of the program to develop policies for sustainable consumption and a circular economy. The short term action plan shall be reported in February 2018, but is not clear if this implies regulations by law or more soft regulations.

Protocols

Edible food waste is measured in a number of different ways, and there are several reports from the EU project FUSIONS that give comprehensive overview of both alternative methodologies as well as manuals with more specific descriptions of "how to measure" (Møller et al. 2013, 2014, Tostivint et al. 2016). An international protocol for measuring food waste and loss has also been developed in collaboration between United Nations Environmental Program (UNEP) and World Resources Institute (WRI), which to a large extent is based in the work from FUSIONS.

As our work with the ForMat project in Norway started well before the FUSIONS project back in 2010, we had to develop our own approach and methodologies for dealing with food waste statistics, and the methodologies are well described in the final report from the project (Stensgård and Hanssen 2016). The more detailed work with food waste from households through waste composition analyses have been described in detail by Hanssen et al. (2016).

Our work is only focused on what is defined as edible food waste, and differ in that respect from the broader definition of food waste from FUSIONS project in two directions:

- We have excluded all types of inedible food parts from peels, bones, stones, etc.
- We have included food that ends up as animal feed or to industrial nonfood products.

Our definition includes all types of food that ends up in the solid waste fractions from the food industry, wholesalers, the retail sector, the service sector, and households, including food to animal feed and new bioproducts.

Through the ForMat project, routines have been developed for voluntary reporting of edible food waste from the food industry, from wholesale companies, as well as the three big retail companies in Norway. Statistics are gathered for 21 food types within 9 main groups. The methodology is well described by Stensgård and Hanssen (2016) and by Hanssen et al. (2016) for the households. We have data covering more than 25% of the economic value of food manufacturing industry in Norway, more than 40% of the wholesale sector and 90 retail shops being representative for the whole retail sector in Norway. The food industry measures edible food waste in tones and gives data per tones of production, while the retail sector and wholesalers register food waste in economic value by scanning all

products that are wasted instead of being sold. Amount of edible food waste is then estimated based in economic value of a sample of 185 food products. All data are sent to Ostfold Research and treated confidentially and presented as aggregated figures and scaled up to national statistics based in weighted measures from economic value. Data from edible food waste from households are based in annual data on organic waste registered by all Norwegian municipalities and used to estimate amount of food waste in total and per capita based in coarse waste composition analyses from about 50 municipalities in Norway. In addition, detailed waste composition analyses were carried out in 200 households in two urban and rural municipalities in 2011 and 2015, to get specific data for the 9 main groups of food (Hanssen et al. 2016). National guidelines for how to carry out waste composition analyses to register organic waste and more detailed data on food types have been developed and published by Avfall Norge (2015).

Based in literature data from LCA studies of food production, Ostfold Research has developed indicators for GHG emissions from a number of different food products, both from Norwegian production as well as foreign production. Those data have been used to generate national statistics for GHG emissions related to food that has been produced and ended as food waste, including waste treatment processes. Economic value of the 185 sample products have been used to generate average economic value per kg of food of the 21 main food types. Further on, this has been used to estimate and upscale to gross economic value of food being wasted in Norway.

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Principles for Developing a Safe and Sustainable Valorization of Food Waste for Animal Feed: Second Generation Feedstuff

17

David San Martin, Carlos Bald, Marta Cebrian, Bruno Iñarra, Mikel Orive, Saioa Ramos, and Jaime Zufía

Contents

Abstract

Developed countries are producing and consuming more food than needed, which generates a significant amount of food waste. Within this framework, three main strategies have been proposed to control this food wastage: reduction, reuse, and recycle. In the hierarchy of valorization options for any food waste, the reuse for human food or ingredient has to be prioritized, however, due to technical requirements; this option could not be always implemented. The use of food wastes as a second generation feedstuff is an interesting option to many food sectors since it could reduce land use competition, the dependence on the current feed raw

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materials, the cost of animal feed, as well as the environmental impact associated to its production. However, the feasibility of this option depends on several legal, technical, and economical requirements as well as environmental and social sustainability conditions. In addition, this valorization alternative demands some treatments in order to stabilize and preserve effectively.

Technological cooperation, knowledge, and involvement of different actors in the demonstration of new valorization schemes, from generators to processors and end-users, are of crucial importance due to the important and frequent constraints which affect the technical and economic feasibility of their reutilization: logistic costs, processing costs, availability of raw materials, and heterogeneity of the quality and composition of the wastes. Moreover, food safety experts and regulatory bodies have to be also involved and may contribute to promote the safe and responsible use of food waste for feed.

Keywords

Food waste · Valorization · Animal feed · Second generation feedstuff · Profitability · Feasibility · Environmental and social sustainability

List of Abbreviations

ABPs	Animal by-products not intended for human consumption
CIR	Cost-to-income ratio
CRF	Capital recovery factor
EC	European Commission
EFFPA	European Former Foodstuff Processors Association
FAO	Food and Agriculture Organization of the United Nations
FUSIONS	Food Use for Social Innovation by Optimising Waste Prevention Strategies
FW	Food waste
GHG	Greenhouse gas
GIS	Geographic information systems
HORECA	Hotel, restaurant, and catering sector
IRR	Internal return rate
LCA	Life cycle analysis
LCT	Life cycle thinking
NPV	Net present values
PBT	Payback time period
SDG	Sustainable Development Goals

Introduction

One third of all food produced in the world – approximately 1.3 billion tonnes – is lost or wasted every year (Gustavsson et al. 2011) while, according to FAO estimations (2015), even as more than 800 million people are still undernourished. Major global organizations and governments agree that the food waste and losses produced along

the food supply chain is one of the biggest issues now facing humanity. Moreover, it is one of the Sustainable Development Goals published by the UNEP, "Target 12.3: By 2030, halve per capita global food waste at the retail and consumer levels and reduce food losses along production and supply chains, including post-harvest losses."

However, the precise definition of food waste or losses has generated some controversy between the stakeholders. For the European Commission (2010), food waste (FW) is all food lost or discarded in the process of manufacturing, distribution, retail, food service activities, up to the consumer level. FAO (2014) gives another perspective in their definition and considers FW a part of food loss distinguished by the causes of its generation. Thus, while "food loss refers to all food produced for human consumption but not eaten by humans," FW refers to this part that being safe and nutritious for human consumption is discarded or used for nonfood purposes and can be generated in all steps of the value chain, including primary production. Thus, FW is generated mainly due to inefficiencies in the food chain and inadequate consumption patterns.

Additionally, in 2014, Garrone et al. define the concept of surplus food, which consists of edible food products that for various reasons are not purchased or consumed by customers or people for whom they were produced, processed, distributed, served, or purchased. Instead, FW is the part of surplus food that is not recovered for human or animal consumption, for producing goods or energy. However, from the social perspective most surplus food could be considered as waste.

For the purpose of the present chapter, the most recent definition provided by FUSIONS platform (Stenmarck et al. 2016) is considered, where FW is the fraction of "food and inedible parts of food removed from the food supply chain." In this definition, feed (food going to animals) is not considered as waste. Food that is redistributed is not accounted for as FW as it does not quit the food chain.

Beyond ethical aspects that obviously underlie food wastage, it should be considered as a big failure in the efficiency of food value chain. While food demand will increase at global level but mainly in developing countries in the next years driven by population and income growth, high income countries still produce and consume more food than they need. In this context, all measures taken to increase the efficiency in the use of the resources, like avoiding food losses, is of critical significance.

According to Gustavsson et al. (2011), the causes of food losses and waste are different in low and in high income countries. While in developing countries the biggest losses, more than 40%, occur in the primary production due mainly to deficiencies in the production systems, in developed countries more than 40% of losses occur at retail and consumer level, due to inadequate planning or bad purchasing habits as well as to "high appearance" quality standards.

All actions taken against food wastage can be classified into three strategies: reduction or prevention, which means minimizing the generation of FW (as redistribution in food banks), reuse or reentering the food chain (as for animal feed), and recycle, giving FW a second life in other applications as materials or in composting or energy production.

According to the main causes of FW generation above mentioned, main actions to be taken shall be focused in first instance to prevention. Thus, in relation with

household waste generation, actions shall be focused on educational measures, increasing the awareness of the consumer or promoting social initiatives, while in relation with waste generation in the processing and distribution, on a better planning and communication between steps in the food value chain, adequate marketing or labeling strategies, redefining quality specifications and product innovation.

Regarding the reuse of food waste, following the hierarchy of waste or by-product management established by the Waste Framework Directive of the EU parliament (2008) (Fig. 1), the reuse for human food or ingredient has to be prioritized, but the use as raw material for feed is an option that interests many food sectors. One reason is the growing demand for animal products in the high income countries that will cause that livestock production will have to substantially increase proportionally to the global demand on food which is expected to increase by 60% in 2050 (FAO 2016a). As a consequence, a significant increase in the need of feed raw materials is expected.

Thus, a lot of foodstuffs which were manufactured for human consumption but have been discarded due to reasons that do not affect their safety reenter the food chain as feed raw materials. These are defined as "former foodstuffs" in the EU Catalogue of Feed Materials (Regulation (EC) No 68/2013). The European Former Foodstuff Processors Association claims that five million tonnes of former foodstuffs are nowadays used as animal feed and this can increase to seven million tonnes until 2025 (EFFPA 2016).

But there are unavoidable parts of FW that represent an important percentage of the total FW. Namely, by-products are those unavoidable fractions generated through the different food production steps that are not part of the final product. They can be recovered and recycled for different applications being upgraded as raw materials for other products, such as food, feed, or as a source of chemicals, then not considered as

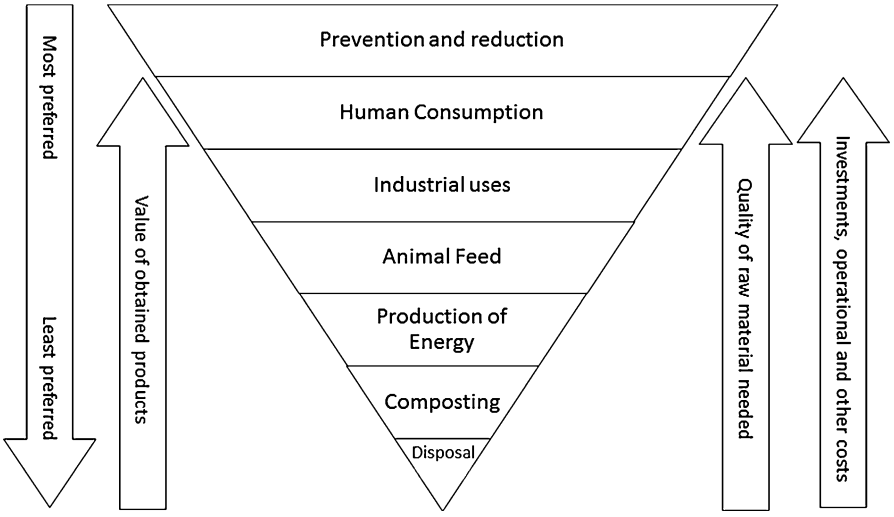

Fig. 1 Hierarchy of food waste valorization adapted from EU waste framework directive and USA-EPA

waste. However, nowadays, those by-products are sometimes managed as waste and disposed into landfills, due to economical, technical, logistic, and/or regulatory constraints.

The reduction of food loses and the recovery of food by-products into the food chain can have also an indirect impact on food security. As the livestock sector is the world's largest user of agricultural land through grazing and the use of feed crops, the use of former foodstuffs and by-products in animal feed could allow the release of land and other agricultural resources for feeding up people. The impact on the lowest income countries would be very high as agriculture accounts for more than 30% of their economic activity (FAO et al. 2012). Moreover, in these regions the expected subsequent increase in the prices of raw materials for animal feed could even exceed the price of the same crop when used for human consumption (CAST 2013).

Food loss and waste have also a high environmental impact. Organic wastes associated with food can take up space in landfills and contribute to greenhouse gas emissions during decomposition (Hanson et al. 2016). Moreover, food production consumes water, land, energy, and resources that are finally wasted when food is lost. As an example, according to a study of Kummu et al. (2012), global losses in the food supply chain account for a quarter of the crops wasted, 24% of total freshwater resources used in food crop production, 23% of total global cropland area, and 23% of total global fertilizer. In addition, livestock production uses a considerable amount of resources to produce feeds and makes a significant contribution to global carbon emissions (FAO 2016a).

Climate change can have also a negative impact on agriculture. It is estimated that depending on socioeconomic and climate change assumptions, by 2050, relative to a world with no climate change, global average crop yields will decline by 5–7%, while the area harvested will increase by around 4% (Wiebe et al. 2015). The high dependence on agriculture makes developing countries even more susceptible to the effects of climate change.

As a conclusion, all measures targeted to increase the efficiency and reduce the food waste will contribute definitively to reduce the environmental impact, increase food security alleviate poverty and favor the economic growth (FAO et al. 2015).

Food processing unavoidable waste should not be considered as waste but as a raw material that may reenter the food chain and has to be treated as such. Most food industry by-products are unstable and highly perishable, which means that they have to be stabilized and preserved adequately before being processed to obtain feed ingredients and feed products. Besides, there are still several constraints that affect the technical and the economic feasibility of their valorization such as the availability of raw materials (geographical and seasonal), the logistic costs, the processing costs, and the heterogeneity of the quality and composition of the by-products. Technological cooperation, knowledge, and involvement of different actors in the demonstration of new valorization schemes, from generators to processors and end-users are of crucial importance. Also food safety authorities and regulatory bodies have to be involved and may contribute to promote the safe and responsible use of FW for feed.

In addition, every recovery and recycling proposed option should not imply an increase in the environmental impacts in comparison with the actual treatment or

disposal option. For this purpose and in accordance to EU recommendation (COM (2005)666), life cycle analysis (LCA) appears as a useful methodology to demonstrate the environmental benefits of the alternative option.

In the present chapter, main important aspects related to the valorization of food industry by-products of different sectors for feed purposes are presented. Different approaches, technological and methodological solutions are described.

Principles for a Safe and Sustainable Valorization

Due to its characteristics and nature food by-products needs to be pretreated, often dehydrated, stabilized, and preserved to obtain safe and nutritious feed ingredients and feed products. As any raw material, feed products from FW are subjected to legal, technical, and economical requirements, as well as environmental and social sustainability conditions.

Following, the principles for a safe and sustainable valorization and the different uses of food industry by-products produced by different sectors for feed purposes are presented.

Legal, Hygienic, and Sanitary Requirements

One of the main aspects to assure the feasibility of any valorization option is to accomplish all the legal aspects that limit the hygienic and sanitary requirement for the proposed solution. Moreover, there is also some legislation that promotes the use of FW.

Regarding the EU legislation, FW is considered a biodegradable waste, or bio-waste, and has a specific regulation. On the one hand, the Landfill Directive (1999/31/EC) obliges member states to reduce the amount of biodegradable waste they landfill but without prescribing a particular treatment option. The European Commission adopted an ambitious Circular Economy Package in which the recycling strategy is the precondition using these wastes as a secondary raw material (Fig. 2).

Fig. 2 Circular economy scheme

Also the Sustainable Development Goals (UN 2015) include a target to halve per capita FW and reduce food losses along the food production and supply chains.

The Council adopted Regulation 183/2005 EC the European Parliament and of the Council laying down requirements for feed hygiene which ensures that feed safety is considered at all stages that may have an impact on feed and food safety, including primary production and operators. Main elements of the regulation are:

- The compulsory registration of all feed business operators
- To implement the application of good hygiene practices
- To introduce the Hazard Analysis Critical Control Point (HACCP) system
- To introduce compulsory requirements for feed production at farm level

Also EU legislation on undesirable substances in animal feed (Council Directive 2002/32/EC) has to be taken into account to ensure that these secondary raw materials do not represent any danger to human health, animal health, or the environment or do not adversely affect livestock production.

Regarding limiting values of contaminants for animal feed, the legislative framework differs worldwide. Currently, most comprehensive regulations are those of Europe and North America. Hence, while in the European Union, the legislation on animal feed is already harmonized, in many developing countries, particularly in Africa, around 50 countries do not still perform the statutory control of contaminants or it is still at best rudimentary (D'Mello and Macdonald 1998). However, in order to fulfill quality and safety standards for imported animal feeds, since 1999 non-EU feed manufacturers are required to have representatives based in the EU who can confirm declarations.

On the other hand, also Regulation (EC) No 1069/2009 of the European Parliament and of the Council and Commission Regulation (EU) No 142/2011 constitute the community legal framework applicable to animal by-products not intended for human consumption and products derived from them, that stablishes which by-product can be used for further purposes and the traceability, transport, and processing solutions. In brief, only category 3 materials can be destined to animal feed and are subjected to several restrictions for their use as feed for farmed animals, fur animals, pets, or aquaculture.

Technical Parameters

The feasibility of any valorization option is also conditioned by technical factors which could limit the use of food by-products as raw material for animal feed. Those factors can be found at two different levels. Hence, whereas the first level is linked to food by-products and their generation points, the second level is related to the animal feed production plant.

Regarding to the technical factors associated to food by-products, nutritional parameters determine the value of the by-products for animal feed. Preferred values are low contents of humidity and ashes and high contents of protein, fiber, or lipids. In addition, vitamins, minerals, or polyphenols are considered for animal feed. Finally, the digestibility of these parameters will determine the capacity of assimilation by the animal.

However, as well as nutritional parameter, there are also other undesirable substances known as anti-nutritional factors. These substances when present in animal feed or water reduce the availability of one or more nutrients and can negatively affect the health of animals.

Despite the nutritional quality, another strategic factor which makes by-products use feasible is their availability related to the generation rates. Only, producers with high generation rates are expected to be feasible from the logistic point of view and also in terms of the high environmental impact they cause.

With regard to the technical factors linked to the production plant, many factors can be taken into account in land studies. They should be in accordance with the required objectives, the information available, and planner's experience.

The geographic dispersion of the FW generation, as well as the ground-use area where the plant can be built or geo-natural factors, will determine the location of the processing plant and the logistics routes for centralizing all FW in the treatment plant.

Moreover, the technology used for FW processing is other technical aspect related to production plant that it is necessary to take into account because it will determine the energetic requirements, the processing capacity or the investment and the process costs.

In conclusion, a multicriteria design analysis which takes into account all technical factors, combined with geographic information systems (GIS), is advisable to ensure the feasibility of reusing FW for animal feed (GISWASTE Project 2016).

Economic and Market Factors

The profitability of reusing unavoidable FW as a raw material for animal feed depends on several economic factors.

The annual cost-to-income ratio (CIR) is one of the most important indicators of profitability and considers the main costs and incomes in a projection of 15–20 years (Perry and Green 2008).

Moreover, once quantified the CIR, it is necessary to perform a standard financial analysis to provide insight into the structure of costs and incomes and to stablish economic-profitability parameters such as:

- The net present values (NPV)
- The payback time period (PBT)
- The internal return rate (IRR)
- The capital recovery factor (CRF)

While the advantage of the NPV is its predictability and the respect of the time value money, it does not express the accurate rate of profitability. Therefore, IRR is chosen since it allows comparing very easily different proposal sizes. IRR criterion is very simple: if the project IRR is higher than the discount rate, the project is accepted, otherwise, it should be rejected. Under considered assumptions, if the IRR value is higher than discount rate the activity under study is profitable (Kwan et al. 1995).

It must be noted that the results of the financial analysis depend on unpredictable fluctuating variables. Therefore, if they are considered constant to enable the economic assessment, it is necessary to predict how the different variables may change over time and their effects over NPV through a sensitivity analysis.

Environmental and Social Aspects

Reusing FW for animal feed can be seen as environmentally and socially beneficial. However, a number of variables need to be taken into account to ensure that the new management alternative is socially faithful and environmentally responsible.

From the environmental point of view, the European Commission recommends the use of Life Cycle Thinking (LCT) when planning waste management systems (Waste Framework Directive 2008/98/EC) to reduce the environmental impacts associated with the whole life cycle of the waste management option – including the transportation, energy water and other material requirements or the emissions to the atmosphere (Fig. 3). According to several studies (Eriksson et al. 2015; Woon et al. 2016; Jin et al. 2015; Jensen et al. 2016; Franchetti 2013; Bernstad and la Cour Jansen 2012), when focusing on the environmental impact, the most relevant aspects are the following:

- Energy usage and climate change: It is necessary to take into account the energy needed to transform FW into a new feed ingredient.
- Water usage: It can also become an environmental critical point.

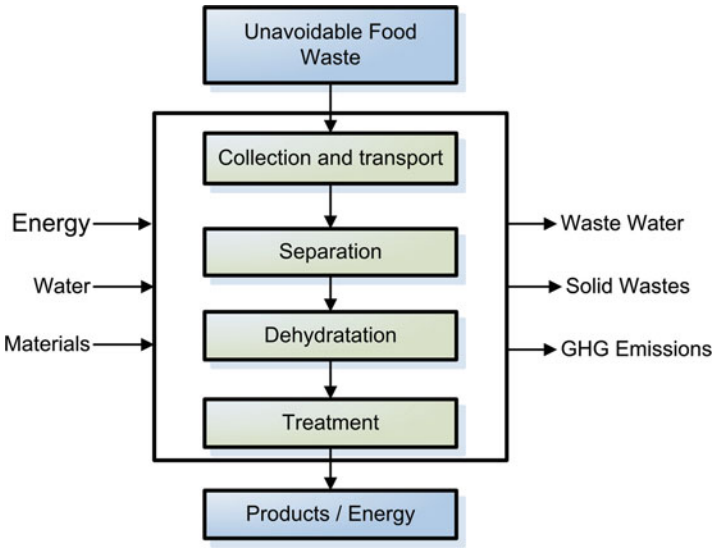

Fig. 3 Life cycle analysis scheme

- Wastewater generation: The generation of pollutant spills is also closely linked to the management of wastes and can generate a high potential for eutrophication or organic contamination of the waters where they are being discharged.
- Other aspects: Usage of toxic agents for extraction of certain compounds.

With regard to social responsibility, as discussed above, the fact of using FW as a second generation feedstuff would release the pressure exerted on the food availability in developing countries. It is estimated that about 1000 million tonnes of animal feed is produced globally every year and the 60% is produced by 10 countries, being the United States, China, and Brazil the biggest producers (FAO 2002).

Major feed ingredients have been facing market competition with human food demands of developing countries, especially for those nongrain self-sufficient countries, this is the so-called feed-food competition (CAST 2013). To cope up with this competition, feed needs to be technically treated to improve nutritional values and institutional collaborations and support is demanded in order to facilitate alternative feed utilizations.

It is also necessary to ensure that the new management model safeguards the social rights of all stakeholders in the chain. Particular attention should be paid to human rights at work such as freedom of association and collective bargaining, nonexistence of child labor, and all forms of inequality, labor conditions, or safety and health at work.

Different Uses for Food Waste

Food wastage is classified into different categories. According to FAO et al. (2015), global quantitative food losses and waste per year are roughly 30% for cereals; 40–50% for root crops, fruits, and vegetables; 20% for oil seeds, meat, and dairy plus 35% for fish. Thus, depending on the category, they could have different application for animal feed.

Following, and within this framework, different approaches, technological and methodological solutions for the main important categories of FW are described.

Vegetable and Fruit By-products

In general, vegetable and fruits by-products (Fig. 4) are characterized by their high moisture content, above 80%. The high degradation activity related to high moisture content, together with a lack of coordination between different actors in the supply chain, make that fruits and vegetables have the highest wastage rates of any food with 40–50% (FAO et al. 2015).

Only in Europe, about 200 million tonnes of fruits and vegetables are generated each year and up to 45% is wasted at different stages of the food chain (FAO 2011), in spite of having high potential to be reused as animal feed. These by-products are

Fig. 4 Vegetable by-products

an excellent source of nutrients which can contribute to supply the demand of feedstuffs for livestock, reducing the cost of feeding and giving higher profits to farmers (FAO 2013).

However, the use of these by-products as animal feed has some difficulties that can limit its feasibility. Its high degradation activity makes more difficult fulfilling hygienic and sanitary requirements, which advices subjecting them to a drying process. Furthermore, their analytical composition can vary extremely throughout the year (Westendorf 2000), which means that animal feed manufactures should have to change periodically their feed formulations depending on their composition.

Within this framework, some experiences have shown the feasibility of using vegetable wastes in animal feed. In a recent study, cauliflower and cabbage leaves or pea pods and pea vines vegetable by-products were assessed as complete feed for goat bucks (Wadhwa et al. 2006). It was concluded that they could serve as an excellent source of nutrients for ruminants and can economize the production of animals.

Moreover, the Clean Feed project (San Martin et al. 2016) showed that a vegetable flour, obtained after drying a mixture of different vegetable and fruit by-products produced by food industry and retail trade, complies with all the requirements of the animal feed market and is suitable for animal feed. In addition, Wadhwa and Bakshi (2013) assessed apple or banana by-products as livestock feed, concluding that they can act as an excellent source of nutrients.

Dairy Industry By-products

Dairy industries by-products, such as skim milk, buttermilk, and whey (Fig. 5), are valuable substances obtained during the manufacture of cream, butter, and cheese, which contain proteins, mineral salts, vitamins, and other bioactive compounds beneficial for health (Birsen Bulut and Nihat 2012).

Most of the skim and butter milk are dried and marketed as valuable ingredients for the food and feed sectors with a global production around four million tonnes/

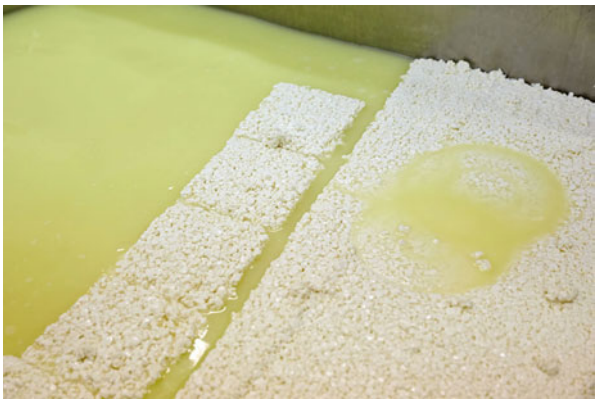

Fig. 5 Dairy by-products

year in 2013 according to OECD/FAO report (OECD and FAO 2016). Regarding to dairy whey, only about a 50% of the total generated amount (180 million tonnes/year) is used to produce whey powder (2.3 million tonnes/year) or other derived products (lactose, protein concentrates), being the rest used in a liquid form or discarded.

Dried whey and skim milk are commonly used for breeding and fattening purposes, especially as milk replacers in young pigs or calves (OECD and FAO 2016), but they are less frequently used in older animals because of possible digestive disturbances. Nevertheless, there are several recent studies indicating the benefits of the inclusion of whey in the diet of livestock.

Regarding to dried whey, results obtained in 2014 within the frame of the European Life- VALORLACT project (VALORLACT 2015) demonstrated that the addition of 6% whey in the feedstuffs produces an increase in the lying rates in hens (9%), without affecting eggs' quality, and improves the average daily rates and conversion rates in broilers (28% and 17%, respectively) as well as milk quality in sheep (increased fat and protein levels), without a reduction in production yield.

On the other hand, fresh whey feeding to animals is also a suitable and cheap alternative to avoid its disposal to the environment. It also complains the Regulation (EC) No 1069/2009 about animal by-products. However, direct supply of whey has to ensure hygienic conditions to avoid pathogenic organisms and insects proliferation. As a rule, it is desirable to provide daily fresh whey or maintain it in a refrigerated tank for a maximum period of 36 h before using. Digestive disorders such as diarrhea or bloat may also occur if the adaptation period to whey feeding is not enough (at least 1 or 2 weeks). It is estimated that ruminants can ingest from 12 to 15 liters of fresh whey per 100 kg live weight. In some cases, the intake of liquid whey can also reduce the amounts of hay or grain consumed (5–10%) (FAO 1978).

The most obvious benefit in pigs is increased feed intake, consuming on average 12% more dry matter when offered liquid rather than dry diets (Mavromichalis 2013).

According to previous cited FAO report (1978), dairy cows can drink as much as 100 liters per day, without reporting any depressive effect on milk production,

duration of lactation, or butterfat content. Experiences developed in Tunisia (Ben Salem and Fraj 2007) with lactating cows fed with 40 liters/day during 141 days showed a replacement of 2 kg of concentrate by liquid whey and an increase of 5–6% in milk fat and protein (gr/cow/day).

Fish By-products

Fish transformation industry and fish retail distribution generates a large amount of fish by-products (Fig. 6). It is estimated that 25–30% of the total fish or seafood weight ends up as a waste. These wastes, mainly considered as by-products, are typically processed to produce fish meal and oil that are used for animal feeding (FAO 2016b).

The fact that fish by-products are considered animal by-products not intended for human consumption stablishes some limitation of which by-product can be used for further purposes and the traceability, transport, and processing solutions. In brief, only category 3 materials can be destined to animal feed and are subjected to several restrictions for their use as feed for farmed animals, fur animals, pets, or aquaculture (Commission Regulation (EU) No 142/2011).

Moreover, and even though more than 85% of fish catches are used for direct human consumption, also about 10% of fish catches are used for fish meal production (FAO 2016b).

Worldwide, annual production from approximately 300 dedicated fishmeal plants has been around 5 ~ 7 million tonnes of fishmeal over the last years.

In Europe, more than 20 facilities produce approximately 500,000 metric tonnes of fishmeal and 190,000 tonnes of fish oil a year (EU Fishmeal 2016). Worldwide it is estimated that about 35% of world fishmeal production is obtained from fish residues, while in Europe this ratio is increased up to 50% (FAO 2016b). In fact, the use of fish by-products for the production of fish meal and fish oil reduces the overexploitation of the seas and the fisheries that target species for fish meal production.

Fig. 6 Fish by-products

The standard process of fish meal and fish oil obtains an average 20–25% fish meal yield and 3–5% of oil. The obtained product has an average 65–70% protein, 7–10% oil, less than 12% ashes, and less than 10% of moisture (Windsor et al. 2001).

Better quality of protein rich products can be produced through other processes such as the enzymatic hydrolyzation, increasing the amount of protein up to 80–85% and the content of ashes is considerably reduced (Chalamaiah et al. 2012).

On the other hand, these fish by-products also contain high valuable compounds such as bioactive peptides, pigments, collagen/gelatine, chitin/chitosan, chondroitin sulfate, or calcium, which can be recovered. Worldwide several industries process fish wastes to obtain such products preventing the disposal of fish waste and the use of other raw materials (Arvanitoyannis and Kassaveti 2008).

Meat Industry By-products

The majority of meat waste (Fig. 7) is produced during slaughtering and consists of the portion of a slaughtered animal that cannot be sold as meat or used in meat-products. Such waste includes bones, tendons, skin, the gastrointestinal tract content, blood, and internal organs. More than half the animal by-products are not suitable for normal consumption, due to their unusual physical and chemical characteristics. Within this framework, the vast majority of the remaining by-products after the different uses are processed to produce meat and/or bone flour in order to improve the protein levels in animal feed.

Approximately 25 million tonnes of animal by-products are rendered in North America, 15 million tonnes in the European Union, and 10 million tonnes in South

Fig. 7 Meat by-products

America and Australasia, to provide rendered products worth up to eight billion US dollars annually (FAO 2004).

Unlike other type of by-products, the recovery of meat industry by-products is bound by severe hygiene and health limitations. In the European Union, meat by-products are considered animal by-products not intended for human consumption. The Regulation (EC) No 1069/2009 stablishes the limitations for the use of these by-products regarding the traceability, transport, processing solutions, and disposal. According to this regulation, only meat by-products of category 3 can be destined to animal feed after being subjected to several restrictions for their use as feed for farmed animals, fur animals, pet-food, or aquaculture. In this category are mainly included all the meat by-products from animals with no signs of communicable disease to humans. The most dangerous disease is the Bovine spongiform encephalopathy (BSE), for which the European Union promulgated specific legislative measures to avoid that products containing BSE could end up in the meat distribution chain (Regulation 999/2001 and 853/2004). EU national authorities make official controls on these products imports from non-EU countries.

Policies and Protocols

Just one quarter of all wasted food could feed the 795 million undernourished people around the world who suffer from hunger. However, not all the food wasted could be directly used as secure human food. In this chapter, an alternative use for these wastes as animal feed is analyzed which could release enough resources to feed up to three billion people.

In this sense, farmers around the world use lots of crops that could be eaten by people. The impact on the world's developing regions may be very high as agriculture is usually their main way for subsistence. The promotion of second generation feedstuff, feedstuff produced from FWs, involves that, if farmers fed their livestock on the food that is currently wasted and on agricultural by-products, enough grain would be liberated to feed people.

Moreover, the use of FW for animal feed can reduce the competition for the use of land for some crops in circumstances when the price paid for animal feed can even exceed the price that can be paid by the human consumer in that region (CAST 2013). It could also improve social, environmental, and economic sustainability of the food chain and account as circular economy scheme, mitigating the climate change, water depletion, and other environmental impacts associated not only to the food product but also to those impacts related to the livestock production.

However, food by-products frequently need to be pretreated, stabilized, and processed adequately to obtain useful feed ingredients. Therefore, in order to ensure that the whole valorization process is economically feasible, socially just, and environmentally friendly, it is necessary to control a large amount of key parameters. Large number of technical parameters conditions the

feasibility of the proposed alternative, such as moisture, nutritional value, generation rates, or geographical dispersion. Additional variables such as market demand, prices, or energy and water requirements have to be controlled to guarantee economic and environmental sustainability of the proposed procedure. Thus, further investigation is necessary in order to explore innovative strategies for safe and sustainable production of useful second generation feedstuff.

As could be drawn from the present chapter, principal FWs from processing industries, such as fish or meat by-products, can be rather easily managed and controlled. Additionally, there are also successful approaches focused on the valorization of minor FWs, such as coffee or specific bakery processing by-products. Thus, main challenge is now focused in the valorization of highly heterogeneous FWs from distribution. These streams have also a great and valuable potential that may need further regulation for the promotion and implementation of sustainable management schemes.

Finally, it can be also concluded that innovative management systems and technologies to guarantee safety of the second generation feedstuffs could encourage EU to promote the use of FW in animal feed.

Dictionary of Terms

- **Animal by-products not intended for human consumption (ABPs)** – The whole bodies or parts of animals, products of animal origin or other products obtained from animals that are not intended for human consumption, either for health reasons or due to a decision by the operator.
- **By-products** – Those unavoidable fractions generated through the different food production steps that are not part of the final product.
- **Feed-food competition** – The market competition of major feed ingredients with human food demands of developing countries, especially for those nongrain self-sufficient countries.
- **Food waste (FW)** – Fractions of food and inedible parts of food removed from the food supply chain to be recovered or disposed, including composted, crops ploughed in/not harvested, anaerobic digestion, bioenergy production, cogeneration, incineration, disposal to sewer, landfill, or discarded to sea.
- **Second generation feedstuff** – Are the feedstuffs that are produced through the valorization of FW or any source that do not compete with human feeding.
- **Former foodstuffs** – According to the EU Catalogue of Feed Materials (Regulation (EC) No 68/2013), former foodstuffs are foodstuffs, other than catering reflux, which were manufactured for human consumption in full compliance with the EU food law but which are no longer intended for human consumption, due to practical or logistical reasons, problems of manufacturing or packaging defects or other defects, and which do not present any health risks when used as feed.

Summary Points

- Developed countries are producing and consuming more food than needed and generating a significant amount of food wasted, which is considered as a big failure in the efficiency of the food value chain.
- FW has negative consequences in the economy, the food security, and the environment, contributing as well to climate change.
- In developing countries the biggest losses occur in the primary production due mainly to deficiencies in the production systems. In developed countries most losses occur at retail and consumer level due to inadequate planning or bad purchasing habits as well as to "high appearance quality standards"
- All actions taken against food wastage can be classified into three strategies: reduction, reuse, or reentering the food chain and recycle.
- The conversion of food unavoidable waste into by-products means that this is not more considered as a waste but as a resource, a raw material that may reenter the food chain and has to be considered and treated as such.
- In the hierarchy of valorization options for any FW or by-product, the reuse for human food or ingredient has to be prioritized but the use as raw material for feed is an option that interests many food sectors.
- As any raw material for animal feed, products from FW must fulfill different principles and are subjected to legal, technical, and economical requirements as well as being environmental and socially sustainable.
- The reuse of FW in animal feed can reduce the competition for the use of land for some crops for animal feed contributing to food security.
- The reuse of food waste in animal feed implies that it has to be often pretreated, stabilized, and preserved adequately to obtain feed ingredients and feed products.
- Food wastage is classified into different categories and, depending on the category, it has different application for animal feed.
- Further research is needed to overcome some important and frequent constraints that affect the technical and the economic feasibility of their reutilization: processing costs, logistic costs, availability of raw materials, and heterogeneity of the quality and composition of the wastes.
- Technological cooperation, knowledge, and involvement of different actors in the demonstration of new valorization schemes, from generators to processors and end-users are of crucial importance.
- Innovative management systems and technologies to guarantee safety of the second generation feedstuffs could encourage UE to promote the use of FW in animal feed.

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Food Waste: Metrics, Effects, and Hunger in Hawai'i

18

Matthew K. Loke

Contents

Abstract

Food waste is often ignored in mainstream America. This is not wise as food waste inflicts various adverse effects on society. It is a major source of wasted money for consumers. It also contributes to the misallocation of resources from wasted production inputs, income losses, and higher disposal costs to businesses. It further imposes damaging effects on the environment and degradation in overall environmental quality. Finally, it decreases food security and lessens the likelihood of food donations to needy households.

While quantifying food waste is challenging, it is nevertheless measureable in three alternate ways in the food supply chain – (i) edible weight, (ii) economic

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value, and (iii) calorie equivalent. At the aggregate level, an estimated 237 thousand metric tons (523 million pounds) of edible food, valued at about $1 billion (1.54% of GDP), is wasted annually in Hawai‘i. This figure represents 26% of available edible food that is suitable for human consumption.

Opportunities exist to reduce food waste and to channel rescued food to feed hungry residents. Although the prevalence of household food insecurity in Hawai‘i is relatively low by national standards, the demand for “charity” food is still exceedingly high as suggested by local food advocates. As a tourism mecca, the ability to feed visitors and stationed military personnel ably but economic disadvantaged local residents to a lesser extent may lead to a false perception of inequitable treatment and unnecessarily aggravate social resentment.

Community activities to harness food waste and to feed the hungry serves as a practical strategy to preserve social cohesion, in addition to preserving the environment and supporting the economy. More tangible results are attainable with productivity and efficiency gains in the process to locating, rescuing, and redistributing edible food. This chapter is specific to Hawai‘i. However, its content is relevant and applicable far beyond Hawai‘i’s shores.

Keywords

Food · Waste · Loss · Supply chain · Metric · Weight · Value · Calorie · Effect · Hunger · Security · Recovery · Environment · Hawai‘i

List of Abbreviations

ERS	Economic Research Service; an agency of the U.S. Department of Agriculture (USDA). It provides information and research on agriculture and economics.
FSC	Food Supply Chain; a system referencing the processes that describe how food from farms end up on our dining tables. The processes include production, processing, distribution, consumption, and disposal.
GDP	Gross Domestic Product; a key measure of the economy and is defined as the market value of all final goods and services produced in an economy during a given time period. (Usually, 1 year or every 3 months (quarterly) in the USA.)
n/a	Not available or not applicable.

Introduction

In operations management, people are fond of saying “You can’t manage what you can’t measure.” In supply chain management, it is no different, and waste in the food supply chain (FSC) cannot be addressed without knowing what to measure. At the same time, knowing what to measure is not the only requirement for reducing food waste. Jonathan Bloom, author of the inspiring book *American Wasteland: How America Throws Away Nearly Half of Its Food*, identifies clearly our conventional mindset on food waste in our culture of abundance. He has been

widely quoted as saying, "*Food waste isn't considered problematic because, for the most part, it isn't considered at all. It's easy to ignore because it's both common and customary.*" (Bloom 2011). Reflecting this sentiment, a recent study reported that 77% of Americans expressed guilt about food waste, 42% agreed that food waste is a major source of wasted money, yet 24% acknowledged they do not have enough time to worry about it (Qi and Roe 2016). This impression is reflected fully in present-day Hawai'i, a tourism dream destination offering the vacation of a lifetime – staged in the midst of stunning, natural beauty and charming cultures, with year-round, *sun-sand-surf* activities, and lounging in world-class facilities. Samuel Clemens (Mark Twain) once described the Hawaiian Islands as "the loveliest fleet of islands that lies anchored in any ocean." (Morgan et al. 2016)

Amongst such idyllic surroundings, the issues of food waste and hunger are easy to ignore in paradise Hawai'i. In reality, situated within an isolated, fragile, and ecologically sensitive environment, Hawai'i faces the same challenges relating to food waste as are experienced elsewhere, if not more. The food waste problems facing local residents are real, ranging from the misallocation of scarce resources and the coexistence of hunger and food insecurity to obvious degradation of the environment: decaying foods contribute to foul smells, larvae infestation, greenhouse gas emissions, and leaching landfills, which in turn may contaminate groundwater aquifers, streams, rivers, and coastal watersheds, and, ultimately, may influence climate change.

The Hawaii Foodbank estimates that one in five islanders is in need of public food assistance. This figure translates into 287,000 people, or 123,000 local households, which must rely on donated food to feed themselves or go hungry. Even more sobering, this statistic includes over 46,000 elders (kupuna) and an additional 48,000 at-risk youths (Hawaii Foodbank 2016a). Sliding down the age scale, Hawai'i's children (keiki) are equally challenged. A recent University of Hawai'i report estimates that 53% of public elementary-school students (ages 6–11) received free or reduced-price lunches during the regular school year (UHCOF 2015). It is commonly recognized that a lack of adequate food and nutrition may retard the physical growth, mental acuity, and overall health of these affected school children.

This chapter examines food-waste metrics in Hawai'i; assesses the economic, social, and environmental effects of this waste; reviews the coexistence of food waste and hunger; and explores potential policy responses. In light of Hawai'i's heavy dependence on imported food – estimated at 88.4% of edible food by weight (Loke and Leung 2013) – food waste literally originates at the harbor dock during unloading. Container shipment of food from the US West Coast to Hawai'i typically takes about 5 days. Some fresh produce originates from more distant places such as Mexico, Ecuador, Chile, Australia, and New Zealand via West Coast ports, thus increasing the duration of transportation in the FSC and resulting in a higher degree of food loss. Fresh fruits, fresh vegetables, and frozen seafood are most vulnerable, particularly with exposure to sunlight and unanticipated interruptions in cold-chain logistics.

Definition and Data Sources

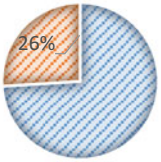
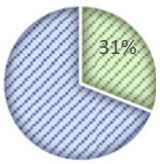
In the absence of a universal definition for food waste and/or food loss, many different forms exist in the published literature. Some authors on the subject have used the terms "food waste," "food loss," or "food loss and waste" interchangeably without any differentiation (Timmermans et al. 2014; Dou et al. 2016). An early definition refers to food loss as any change in the availability, edibility, wholesomeness, or quality of the food that prevents it from being consumed by people (FAO 1981). An alternate definition by Parfitt et al. (2010) asserts that food waste occurs at different points in the FSC and is clearly defined at the retail and consumer stages, where agricultural production outputs are unequivocally food for human consumption.

In Hawai'i, recent work efforts have followed the definition by Gustavsson et al. (2011) that food waste represents the edible portion of food, post-harvest, that is available for human consumption but is not consumed for any reason. This approach was also adopted by the ERS (Buzby et al. 2014) and facilitates the meaningful comparison of measures between the USA as a whole and Hawai'i. The most recent study also analyzed food waste in seven different food groups, including (i) dairy (fresh milk), (ii) grain (rice), (iii) seafood protein, (iv) other protein, (v) fruits (fresh), (vi) vegetables (fresh), and (vii) others (Loke and Leung 2015).

Food Waste Metrics

Food waste in the FSC can be quantified in three alternate ways – (i) edible weight, (ii) economic value, and (iii) calorie equivalent. An estimated 237 thousand metric tons (523 million pounds) of edible food, valued at about $1 billion (1.54% of GDP), is wasted annually in Hawai'i. On a per capita basis, this is equivalent to 161.5 kg (356 pounds) or $698.36 per person per year. In the supply chain, a fairly significant proportion of this food waste is generated at the consumer level (16.5%), followed by the wholesale and retail level (8.3%), and a relatively small proportion at the postharvest (farm) level (1.1%). Overall, 26% of available edible food that is suitable for human consumption is wasted in the local supply chain. The food categories that comprised the highest quantity of waste are fresh fruits (47.5%), grain rice (41.4%), seafood (39.8%), and fresh vegetables (36%). These food measures and their comparable US metrics are shown in Fig. 1.

The assessment by measure and by food group in Hawai'i yielded definitive trends. The three fresh food groups (vegetables, fruits, and dairy milk) shared a similar distribution of food waste by weight, value, and calorie equivalent in decreasing order (see Fig. 2). These lower value-to-weight and lower calorie-to-weight ratios reflected the attributes of fresh produce. In contrast, "others" displayed food waste by weight, value, and calorie equivalent in an increasing order. The *oils and fats* and *sugar and sweeteners* components in this food group represent higher added value as well as calories. The two protein groups (seafood and other) displayed higher food-waste value over weight and calories, illustrating the more expensive nature of protein products. Finally, the lower proportion of value-to-weight and

		 Hawai'i	 USA[a]
Measure of food waste: (per person per year)			
Weight:		161.5 kg (356.1 lb)	194.6 kg (429 lb)
Value:		$698.36	$521.71
Waste in food supply chain:[b]			
Post-harvest:		1.1%	n/a
Wholesale and retail:		8.3%	10%
Consumer:		16.5%	21%
Leading food waste items: (edible weight)		i) Fresh fruits (47.5%) ii) Grain rice (41.4%) iii) Seafood (39.8%) iv) Fresh vegetables (36%)	i) Seafood (39%) ii) Fresh fruits (37%) iii) Fresh vegetables (34%) iv) Grain products (31%)
Food waste (yearly):		 *26% or 237,122 metric tons (t) at a cost of $1 billion. This loss is equivalent to 1.54% of Hawai'i's GDP.*	 *31% or 60.3 million metric tons at a cost of $161.6 billion at the retail and consumer levels.*
Edible weight:		237,122 t (522.8 mil lb)	60.3 mil t (133 bil lb)
Value:		$1.025 bil	$161.6 bil
Food waste (daily): (per person per day)			
Edible weight:		i) 0.44 kg (0.97 lb)	i) 0.53 kg (1.17 lb)
Value:		ii) $1.91	ii) $1.43
Calorie equivalent:		iii) 937 kcal/person/day	iii) 1,249 kcal/person/day
Food Recovery and Hunger:		*If just 10% of the calorie food wasted in Hawai'i were recovered, it would be sufficient to feed over 53,000 needy residents for an entire year.*	

[a]Retail and consumer levels
[b]May not sum up due to rounding errors

Fig. 1 Select food-waste metrics (Sources: Loke and Leung 2015; Buzby et al. 2014)

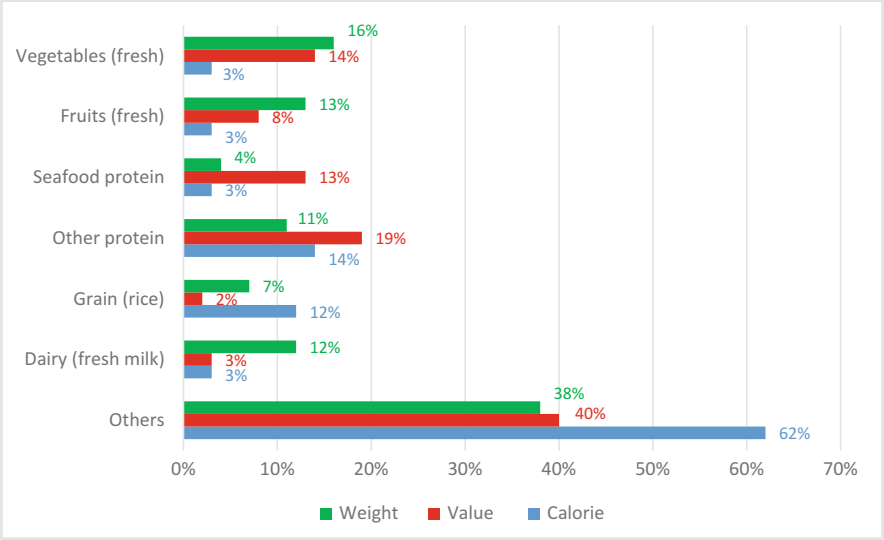


Fig. 2 Percentage of food waste by food group in Hawai'i (Source: Loke and Leung 2015)

value-to-calorie in grain rice revealed an inexpensive but high-calorie-equivalent food item, something that is highly desirable in times of emergency (natural or manmade).

Effects of Food Waste

Food waste is often viewed as an issue with multidimensional adverse effects on society and is receiving heightened attention in local media. Examples of recent coverage include Loomis (2016), Smallwood (2016), Blair (2016), and various local television networks (i.e., KITV, KGMB, KHNL). Beyond wasted money for consumers, it also contributes to the misallocation of resources, from wasted raw materials in food production to the loss of income streams and higher disposal costs paid by businesses in the FSC. It further imposes damaging effects on the environment, ranging from greenhouse gas emissions, pesticide leaching, and soil erosion to increasing disposal volumes in limited landfill areas, spillage in congested landfills, potential groundwater contamination, air pollution from fossil fuels used in waste-management equipment, and degradation in overall environmental quality.

Additionally, food waste strikes at deep social concerns about feeding the hungry. Discarded food in the supply chain decreases food security in society and reduces the likelihood of food donations to needy households. This effect weakens the social safety net and is morally wrong from an ethical standpoint. Figure 3 summarizes the positive effects of reducing food waste from a holistic viewpoint. The effects are divided into three distinct subsets – economics, environment, and social. The relative influences of these individual effects are not known, and their rankings are unlikely to be determined anytime soon.

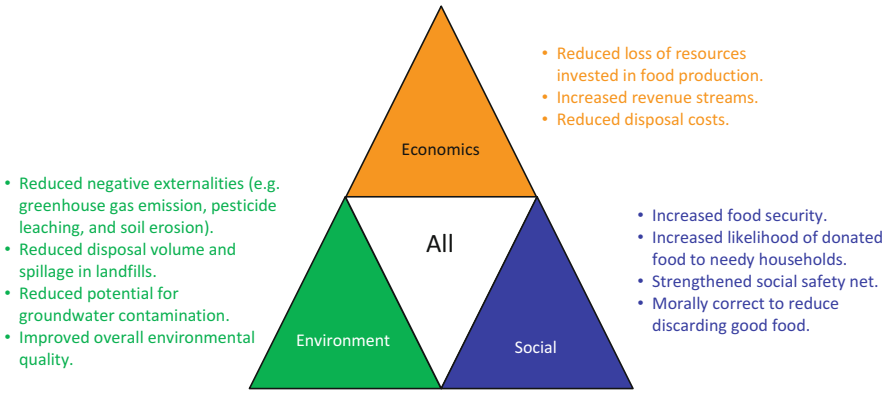


Fig. 3 Multidimensional effects of reducing food waste

Food Waste and Hunger

Feeding a growing population, particularly those in need or hungry, is an important function of society. This difficult challenge can be addressed, among other ways, by increasing the supply of food (e.g., enhancing crop yields through technology and/or productivity gains), decreasing the consumption demand of food (e.g., consuming less food per capita), or increasing efficiency in consumption (decreasing food waste). Given the current level of antagonism toward environmental degradation and biotechnology adoption in food production, and the difficulties in curbing food consumption to a sufficiency level, this leaves food-waste reduction as a practical strategy for combating hunger and food insecurity in society.

In Hawai'i, it is a common misconception to associate food availability with the concept of food self-sufficiency rather than food security. Food self-sufficiency is best described as "proportion of locally sourced production to satisfy overall food consumption." In contrast, food security is defined as "a situation that exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life" (FAO 2003). In Hawai'i, Kent (2016) articulated a definition of food security that is tailored specifically to local conditions (see Table 1).

While the "waste less to feed more" movement may address all three types of food security enumerated in Table 1, "food recovery to feed" efforts are focused narrowly at type II, so as to target the connection between food waste and hunger. Hawai'i has been fortunate to benefit from food recovery activities organized by volunteers. A University of Hawai'i at Mānoa (UHM) chapter of the Food Recovery Network (FRN) was incorporated in 2016 to fight food waste and feed people in need. A group of dietetics students led the chapter launch, in partnership with the UHM campus dining services contractor, Sodexo. They began by collecting unutilized food from campus cafeterias on a weekly basis and delivering it to the Institute of Human Services (IHS), which then distributes the food to homeless shelters in Honolulu.

Table 1 Types of food security and related characteristics in Hawai‘i

Type	Characteristic
I	Ample supplies of edible food for the general population now and into the future.
II	Attention to the special needs of low-income people, many of whom depend on federal government programs and food pantries that offer free or very low-priced food.
III	Plan for various types of public emergencies that can arise, such as shipping interruptions, crop failures, terrorism, or other force majeure events.

Source: Kent (2016)

Since then, they have increased their collection schedule to multiple times a week and distributed over half a metric ton of edible food.

Beyond campus, the nonprofit organization Aloha Harvest has been recovering quality food from participating donors (caterers, restaurants, hotels, food distributors, and others) and delivering it free of charge to other nonprofit organizations responsible for feeding the hungry in Hawai‘i. Since its inception in 1999, it has collected and distributed almost 8,165 metric tons (18 million pounds) of quality perishable and nonperishable food and beverages to feed the hungry. In 2016, it recovered over 861 metric tons (1.9 million pounds) of edible food (Aloha Harvest 2016).

This dynamic effort to reduce food waste has captured the attention of national political leaders. Hawai‘i’s Senator Brian Schatz (D) has joined his counterparts Richard Blumenthal (D-Conn.), Ron Wyden (D-Ore.), Jeff Merkley (D-Ore.), and Cory Booker (D-N.J.) in sponsoring comprehensive legislation in Congress to reduce food waste in stores and restaurants, at schools and institutions, on farms, and in American homes (Blair 2016). The legislation proposed seeks to clarify food expiration labels, increase the use of less-than-perfect-looking fruits and vegetables, and fund composting initiatives.

With an improved economy, the hunger situation in Hawai‘i appears better than before. Recent statistics published by the federal government suggest the prevalence of household food insecurity in Hawai‘i averaged 9.7% for the period 2013–2015 (see Table 2). This rate compares favorably to the 14% recorded in the immediately preceding 3-year period, 2010–2012, but it is somewhat higher than the 7.8% prevalence rate reported in 2003–2005. Between the periods 2003–2005 to 2013–2015, the rate of change in prevalence rate was 1.9%. These food-insecurity rates in Hawai‘i mirror the national trend over time, albeit at a lower rate.

Summary and Opportunities

It is important to remember that ending food waste completely is akin to chasing a rainbow, an optical phenomenon that fades upon approach and is, therefore, unattainable. Not all food waste is avoidable. Some portions of food items are not edible or are not amenable to consumer preferences. Common examples of such discarded

Table 2 Change in prevalence rate of food insecurity and very low food security, Hawai'i and USA, average, various years

Measure	Hawai'i (%)	USA (%)
Food insecurity		
Average 2013–2015	9.7	13.7
Average 2010–2012	14.0	14.7
Average 2003–2005	7.8	11.4
Change 2010–2012 to 2013–2015	−4.3	−1.0
Change 2003–2005 to 2013–2015	1.9	2.3
Very low food security		
Average 2013–2015	3.0	5.4
Average 2010–2012	5.6	5.6
Average 2003–2005	2.8	3.8
Change 2010–2012 to 2013–2015	−2.6	−0.2
Change 2003–2005 to 2013–2015	0.2	1.6

Source: Coleman-Jensen et al. (2016)

Table 3 Prevalence rate of food insecurity and very low food security, average 2013–2015

Select state	Food insecurity (low or very low food security) (%)	Very low food security (%)
Hawai'i	9.7	3.0
North Dakota[a]	8.5	2.9
Mississippi[b]	20.8	7.9
United States	13.7	5.4

Source: Coleman-Jensen et al. (2016)
[a]Top-ranking state
[b]Bottom-ranking state

portions include apple cores, bread crusts, egg yolks, meat fat, and stale bread, etc. At the wholesale and retail levels, businesses often choose to engage in surplus stocking to ensure that upswings in their customers' demand are met. Such savvy business practices build trust, loyalty, and good faith in the customer base but generate a higher level of food waste.

Additionally, along the FSC, higher food waste as one stage (say consumer) often translates into higher future sales at the preceding stage (retail) and other upstream stages (wholesale, processing, etc.). Such behavior contributes to more revenues (and profits) in the entire FSC, and consequently discourages any honest attempt at food-waste reduction. Finally, food-waste reduction is not a costless activity. Transaction, transportation, time, and other opportunity costs are often incurred by individual players in the FSC.

However, not all is lost in the fight against hunger. The household prevalence rate for very low food security in Hawai'i, averaging 3%, was markedly lower than the comparable national average of 5.4% in the period 2013–2015 (see Table 3). Likewise, the household food insecurity rate (combining rates of low and very low food security), averaging 9.7%, was also lower than the national average of 13.7%.

North Dakota was the only state with a lower prevalence rate at 8.5% but it also has a population about half of Hawai‘i’s. Nevertheless, the 9.7% food insecurity rate still represents 44,939 households in Hawai‘i (FRAC 2016). When this measure is compared to the Hawaii Foodbank’s estimate of one in five islanders (20%) in need of public food assistance, a less optimistic outlook of society’s unmet demand emerges beyond the defined food-insecurity population.

The discrepancy between the two metrics is substantial but explainable. The USDA survey-derived metric likely has a downward bias, arising from the reluctance of some residents to reveal their true food insecurity situation. Concerns over the federal survey research method have also been expressed in academic circles (Wilde 2013). Likewise, the Foodbank’s estimate may include residents who are normally able to provide for themselves but occasionally supplement with “charity” food when saddled with unanticipated personal expenses relating to healthcare, auto repairs, theft, legal fees, etc. This situation is not peculiar to normal residents living in high-cost locations.

The two metrics may also be misleading and lead to the false perception that a dream destination like Hawai‘i can competently feed its touring visitors and ably feed stationed military personnel and dependents but is lagging behind in feeding its own hungry residents. In actuality, the vast majority of visitors to Hawai‘i arrive with highly disposable budgets and members of the military establishment purchase most of their food from commissaries subsidized heavily by the US Department of Defense. Low-income residents are typically supported by various federally funded nutrition programs, administered by the state. Such programs include the Hawai‘i Food Stamp Program (SNAP), the Special Supplemental Nutrition Program for Women, Infants and Children (WIC), the School Lunch Program (NSLP), and the Senior Farmers Market Nutrition Program (SFMNP). These federal programs are complemented by a network of local charitable organizations to feed the needy. The local nonprofit organizations perform remarkable work in minimizing food waste and distributing food effectively to needy residents. For example, the Hawaii Foodbank collected about 6,214 metric tons (13.7 million pounds) of food and distributed 5,806 metric tons (12.8 million pounds) in fiscal 2016 (Hawaii Foodbank 2016b). The food-waste amount during this period is less than 6.6%, with most losses coming from rusted or leaky canned food and wilted fresh produce.

The amount of food collected is not trivial by any means. It is now known that the amount of food collected by the foodbank represents a small proportion, about 2.6% of the estimated annual food waste by weight in Hawai‘i. If all 44,939 households (or 129,874 residents); based on the 2010 Census Report of 2.89 people per average household in Hawai‘i, who are defined as food insecure are fed fully for an entire year, it would represent about 28% of the estimated food wasted in Hawai‘i. This estimate assumes food is transferable and that local residents share the same amount of food calories consumed by the average American of 2,568 kcal/capita/day (ERS 2014). Clearly, this estimate shows the potential for food recovery to feed more people exists.

Low socioeconomic status and income remain key predictors to food insecurity. The situation in Hawai‘i is exacerbated by a myriad of different factors (e.g., geographic isolation, limited land mass, desirable climate, limited housing stock, heavy regulatory framework, etc.), which have transformed the state into an elite,

high cost-of-living environment. The high cost of housing, in particular, often forces households to choose between paying for rent, food, utilities, transportation, and medicine. A recent news article from the Associated Press quoted Hawai'i as having the second-highest consumer debt in the USA, and with home mortgages accounting for 77% of the total (Bussewitz 2017). For the majority of residents, holding a higher-paying job with at least a living wage rate is all but necessary to fend off food insecurity. The phrase "living paycheck-to-paycheck" is no laughing matter in Hawai'i. Another recent report revealed that residents in Hawai'i are more likely to be living paycheck-to-paycheck as compared to residents in any other state (HNN 2016). The *GoBankingRates'* methodology is based on the percentage of a paycheck that is leftover after paying for basic necessities (housing, food, utilities, transportation, and health). California and New York ranked behind Hawai'i. Additionally, chronic under-employment is often cited as another key factor leading to food insecurity for local residents. While Hawaii's official unemployment rate was relatively low at 3% in late 2016, it is widely acknowledged that many local residents have two or three jobs. Finally, a high immigration rate of citizens from the Compact of Free Association (COFA) island nations continues to test Hawai'i's social safety net.

When additional public resources are not anticipated in the offing, community-based efforts to combat food insecurity may have to continue to rely on food-recovery programs, at least in the short-run. This simple strategy to rescue decent, edible food destined for landfills and channeling it to end hunger is a "win-win" situation for the people, environment, and economy.

Conclusions

While quantifying food waste is a daunting task, it is nevertheless measureable in three alternate ways in the FSC – (i) edible weight, (ii) economic value, and (iii) calorie equivalent. At the aggregate level, an estimated 237 thousand metric tons (523 million pounds) of edible food, valued at about $1 billion (1.54% of GDP), is wasted annually in Hawai'i. This figure represents 26% of available edible food that is suitable for human consumption.

Food waste inflicts various adverse effects on society. It is a major source of wasted money for consumers and contributes to the misallocation of resources from wasted production inputs to income losses and higher disposal costs to businesses. It further imposes damaging effects to the environment, ranging from greenhouse gas emissions, soil erosion to congested landfills, potential groundwater contamination, and degradation in overall environmental quality. Finally, it decreases food security and lessens the likelihood of food donations to needy households.

Despite the obvious challenges, there are real opportunities to reduce food waste and to channel rescued food to feed hungry residents. Although the prevalence of household food insecurity in Hawai'i is relatively low by national standards, the demand for "charity" food is still exceedingly high, as suggested by local food advocates. The ability to feed visitors and stationed military personnel ably but economically disadvantaged local residents to a lesser extent may lead to a false

perception of inequitable treatment and unnecessarily aggravate social resentment. Given Hawai‘i’s highly marketed position as a tourism dream destination, social expectations are held even higher. It is simply prudent business practice for a premier destination to competently manage its hunger issue.

With that in mind, community activities to harness food waste and to feed the hungry serve as a practical strategy to preserve social cohesion, in addition to preserving the environment and supporting the economy. More tangible results are attainable with productivity and efficiency gains in the process of locating, rescuing, and redistributing edible food. Ideally, finding a solution to Hawai‘i’s hunger problem may provide a working model for people wrestling with the same issue elsewhere in the world.

This chapter is specific to Hawai‘i. However, its content is relevant and applicable far beyond Hawai‘i’s shores. First, food waste is measurable, and knowing how and where it occurs is crucial to lessening it. Second, the adverse effects of food waste are near universal, with social, environmental, and economic impacts. Third, the coexistence of food waste and hunger is real anywhere, and opportunities to recover food to feed hunger exist. Fourth, unique strategies are applicable in specific locations to implement the “waste less to feed more” food paradigm.

Policies and Protocols

Community Policies to Strengthen Food Recovery Programs

Feeding a growing population, particularly those in need or hungry, is an important function of society. Community-based efforts to combat food insecurity can rely on food recovery programs. In moving to strengthen food recovery programs, some community policies worthy of consideration include the following:

- Provide more incentives for donation to anti-hunger entities (e.g., food banks, food redistribution outlets, and feeding centers) and recycling of produce (including composting) to wholesalers, retailers, caterers, and restaurants.
- Invest in “real-time” technologies (apps) to match food donors and recipients, manage food safety protocols, organize volunteers to collect and to deliver, rescued food between locations, and to operationalize relevant metrics.
- Increase capacity of food aggregation/distribution centers by investing in cold-chain logistics (including delivery processes), increase mechanization, and adopt conveyor belt technology to improve labor productivity of employees and volunteers.
- Dedicate resources (public and/or private) to support outreach and consumer education on reducing food waste, increasing food recovery, reuse, composting, and other tangible environmental benefits (this makes rational sense in a joint social, environment, and economic paradigm).

- Develop best practices to coordinate, motivate, and recognize the contributions of volunteers in this important community-based "food recovery to feed" effort and the broader "waste less to feed more" initiative.
- Research, prepare, and offer credible legislative proposals to raise the food tax credit limit so as to provide more relief to low-income households facing real threats of food insecurity, and therefore decelerating the growth rate of demand for "charity" food.

Essentially, various food industry associations could partner with different food recovery agencies, faith-based institutions, and media outlets to recruit, educate, and retain their members' participation in this community effort. Additionally, pro-bono work from software companies and/or academic institutions could help contribute to "real time" technologies (apps) in the food collection-delivery chain. Additional apps could support the general educational process, keep core volunteers informed and motivated, and, possibly, how to navigate the legislative process.

Dictionary of Terms

- **Cold-chain logistics** – A temperature-controlled system that provides uninterrupted transportation, storage, and distribution activities to help ensure food safety and extend shelf life of temperature-sensitive products such as fresh produce.
- **Food insecurity** – Food insecurity reports of reduced quality, variety, or desirability of diet. Little or no indication of reduced food intake (ERS 2017).
- **Food loss** – Any change in the availability, edibility, wholesomeness, or quality of the food that prevents it from being consumed by people (FAO 1981).
- **Food waste metric** – Food waste in the FSC can be quantified in three alternate ways – (i) edible weight, (ii) economic value, and (iii) calorie equivalent.
- **Food recovery** – Actions taken to harness quality food that may be discarded and channeling it to community centers that feed hungry people.
- **Food security** – A situation that exists when all people, at all times, have physical, social, and economic access to sufficient, safe, and nutritious food that meets their dietary needs and food preferences for an active and healthy life (FAO 2003).
- **Food self-sufficiency** – Food self-sufficiency is often measured by the self-sufficiency ratio and defined by FAO as the proportion of domestic production in relation to domestic food utilization, excluding stock changes.
- **Food waste (in FSC)** – Refers to food waste occurring at different points in the FSC and is clearly defined at the retail and consumer stages, where agricultural production outputs are unequivocally food for human consumption (Parfitt et al. 2010).

- **Opportunity cost** – An economics concept relating to the value of the next-best alternative foregone when a specific activity is chosen. It arises in any decision involving a tradeoff between two or more activities.
- **Real-time** – Refers to the actual time during which activities are taking place.
- **Very low food security** – Reports of multiple indications of disrupted eating patterns and reduced food intake (ERS 2017).

Summary Points

- Food waste in the food supply chain (FSC) cannot be addressed without knowing what to measure and is often ignored in our culture of abundance.
- Within an idyllic environment, the issues of food waste and hunger may not garner much attention in paradise Hawai‘i but exist with an estimated one in five islanders (20%) requiring public food assistance.
- Lacking a universal definition for food waste and/or food loss, many different forms exist in the existing literature and the terms “food waste,” “food loss,” or “food loss and waste” are often used interchangeably without any differentiation.
- Food waste in the FSC can be quantified in three alternate ways – (i) edible weight, (ii) economic value, and (iii) calorie equivalent.
- An estimated 237 thousand metric tons (523 million pounds) of edible food, valued at about $1 billion (1.54% of GDP), is wasted annually in Hawai‘i. On a per capita basis, this is equivalent to 161.5 kg (356 pounds) or $698.36 per person per year.
- Food waste inflicts various adverse effects on society. It is a major source of wasted money for consumers. It also contributes to the misallocation of resources from wasted production inputs, income losses, and higher disposal costs to businesses. It further imposes damaging effects on the environment and degrades overall environmental quality.
- In Hawai‘i, it is a common misconception to associate food availability with the concept of food self-sufficiency rather than food security. It has benefited from food recovery activities organized by volunteers to fight food waste and to feed the hungry.
- Ending food waste completely is unattainable as not all food waste is avoidable. Some portions of food items are not edible or are not amenable to consumer preferences.
- Businesses often choose to engage in surplus stocking to ensure that upswings in their customers’ demand are met, at the expense of higher food waste. Additionally, food-waste reduction is not costless. Transaction, transportation, time, and other opportunity costs are often incurred by individual players in the FSC.
- While the prevalence of household food insecurity in Hawai‘i is relatively low by national standards, the demand for “charity” food is still exceedingly high as suggested by local food advocates.

- Low income residents are typically supported by various federally funded nutrition programs, administered by the state. These programs are complemented by a network of local charitable organizations to feed the needy.
- Low socioeconomic status and income remain key predictors to food insecurity. The high cost of housing, in particular, often forces local households to choose between paying for rent, food, utilities, transportation, and medicine.
- A recent report revealed that residents in Hawai'i are more likely to be living paycheck-to-paycheck than residents in any other state.
- Community-based efforts to combat food insecurity may have to continue to rely on food recovery programs, at least in the short-run. More tangible results are attainable with productivity and efficiency gains in the process of locating, rescuing, and redistributing edible food.

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Food Wastage Prevention as a Means for Saving Food

19

Konstadinos Abeliotis, Christina Chroni, and Katia Lasaridi

Contents

Abstract

Food wastage is receiving much research and policy attention worldwide, especially during the past decade, due to the environmental, economic, and social implications that it is related to. Prevention of food wastage is a key factor in the battle against starvation on the global scale. If food wastage is halved globally by the year 2025, almost 1 billion more people could be fed, a number that corresponds to the expected global population growth between the years 2010 and 2025. Food wastage is generated in every sector of the food supply chain, namely, agriculture, processing, wholesales-retails, households, and food services. The contribution of each sector differs substantially due to a range of reasons

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dependent on the socioeconomic conditions of each country, the culture of the people, the food supply chain, the available food provisioning infrastructure, and policy. In the more developed part of the world, there is growing evidence that the contribution of the households to the food waste problem is particularly significant as the result of multiple behaviors within a household that increase the likelihood or amount of food being wasted. The aim of this chapter is to review the findings reported in peer-reviewed literature published during the past decade. Food wastage is presented in terms of: its amounts generated; its economic, social, and environmental impacts; its dependence on certain sociodemographic and behavioral factors; and finally, its placement in the waste hierarchy context. Food wastage prevention throughout the food supply chain should be among the key pillars of creating a sustainable food system.

Keywords

Food wastage · Food waste · Food loss · Food supply chain · Households · Consumer behavior

List of Abbreviations

EC	European Commission
EPA	US Environmental Protection Agency
EU	European Union
FAO	Food and Agricultural Organization of the United Nations
FSC	Food supply chain
GDP	Gross domestic product
GHG	Greenhouse gases
LCA	Life cycle assessment
LULUCF	Land use, land use change and forestry
MMT	Million metric tones
UK	United Kingdom
USA	United States of America
USDA	US Department of Agriculture

Introduction

Food wastage is receiving much research and policy attention worldwide, especially during the past decade, due to the environmental, economic, and social implications that it is related to. In the early 2010s, the Food and Agricultural Organization of the United Nations estimated that approximately one third of food produced for human consumption is either lost or wasted through the food supply chain, from agricultural production and postharvest handling and storage to processing, transportation, distribution, and consumption (Gustavsson et al. 2011). Food wastage puts a heavy burden on the global fight against starvation and famine, and measures have to be taken in order to prevent its generation.

Fig. 1 Breakdown of food wastage classification based on the life cycle stage of the food supply chain

Review of the relevant literature reveals that there is a multitude of terms describing food wastage. Several authors (Gustavsson et al. 2011; Koivupuro et al. 2012; Parfitt et al. 2010) indicate that the food materials discarded during postharvest or processing are often referred to as "food losses." Koivupuro et al. (2012) mention that food wastes are the "food products discarded in retail or consumption." In this chapter (see Fig. 1), we differentiate food wastage between "food losses" and "food waste" in line with the recommendations by Gustavsson et al. (2011) and Parfitt et al. (2010).

Food wastage is generated in every sector of the food supply chain (FSC), namely, agriculture, processing, wholesales-retails, households, and food services. In terms of the contribution of each sector in the generation of food waste, according to a study for food waste in the EU-27, households produce 42% of the total amount of food waste, the manufacturing sector 39%, and the food service – catering sector 14%, while the retail-wholesale sector produces approximately 5% (European Commission 2010).

From a global point of view, the contribution of each FSC sector to food wastage differs substantially due to a range of reasons dependent on the socioeconomic conditions of each country (European Commission 2010; FAO 2013). Moreover, the amount of food that people from different countries waste depends also on their culture, since different parts of the foods are edible in different parts of the world, while other parts are thrown away (Thyberg and Tonjes 2016). Besides cultural and societal reasons, food wastage in each part of the globe also depends on the food supply chain, the infrastructure, and the policy (Neff et al. 2015). However, despite the aforementioned specificities, generalizations are possible based on the degree of industrialization and GDP of each geographical region: Parfitt et al. (2010) stated that in developing countries food losses are higher at the immediate postharvest stages, while in industrialized and developed economies they are higher for perishable foods after they have been prepared for market or, in fact, sold to the consumer; "*for affluent economies, post-consumer food waste accounts for the greatest overall losses*." A recent analysis of the FSC sectors by geographical region showed that wastage occurring at consumption level is much more variable, with wastage in middle- and high-income regions, but much lower in low-income regions (FAO 2013).

The chapter is based on the findings reported by the authors of peer-reviewed literature, published during the past decade. The chapter is compiled as follows: after the introduction, the reported figures of the food wastage generation in different parts of the world are presented. Then the environmental, economical, and social impacts of food wastage are presented, in their respective sections. In the sections that follow, the effects of consumer behavior and sociodemographic characteristics are described. Finally, the chapter ends with the placement of food wastage management options in the EU waste hierarchy.

The Problem in Numbers: How Much Food is Wasted?

Moving on to the actual figures reported for food waste by notable international organizations, FAO of the United Nations estimated that the per capita food waste generation in Europe and North America is 95–115 kg/year (Gustavsson et al. 2011). The European Commission reports an even higher value of 179 kg/person/year as the average total amount of food waste generated in the EU-27 (European Commission 2010). On the global scale and a calorific basis, Kummu et al. (2012) report that around one quarter of the global food crops supply are lost or wasted, a number that corresponds to 1.46 10^{15} kcal/y.

However, specific data referring to countries, even the developed ones, are very scarce and not easily comparable, as there is a wide diversion in definitions and assessment methodologies (Lebersorger and Schneider 2011; Schneider 2013a). For instance, in Europe, the average amount of avoidable food waste reported for Finland was 63 kg/household or 23 kg/person (Koivupuro et al. 2012). Back in 2009, WRAP estimated that consumers in the UK throw away 31% of the food they purchase for consumption (WRAP 2009). Again in the UK, Quested et al. (2013) reported that 7.2 MMT of food and drink waste were generated in households in 2010, of which 4.4 MMT were avoidable. This avoidable waste corresponded to 160 kg per household and was equivalent to 12% of the food and drink entering the home (Quested et al. 2013). Beretta et al. (2013) reported that roughly one third of the edible calories produced for Swiss consumption are lost over the whole food supply chain. Finally, food waste generation in Greece has been quantified, by means of a diary, to 98.9 kg/person annually, of which 29.8 kg/person is avoidable (Abeliotis et al. 2015, 2016).

In the USA, Venkat (2011) reported that the total avoidable food waste at the distribution, retail, and consumer levels amounts to over 55 MMT/year, representing nearly 29% of the annual production by weight, while Thyberg and Tonjes (2016) stated that Americans dispose over 0.6 pounds (approx. 272 g) of food waste per person per day, without any distinction made between avoidable and non-avoidable. Again from the USA, Neff et al. (2015) reported that 31–40% of the postharvest food supply goes to waste; this lost nutritional value represents an estimated 1,249 calories per capita per day.

In South Africa, Nahman et al. (2012) reported that the annual food waste generated is 1.44 MMT, of which 1.16 MMT (approx. 81%) being edible, i.e., avoidable.

The Environmental Impacts of Food Waste

There is strong evidence that the most environmentally damaging form of human consumption is eating (Ehrlich 2011). Figure 2 outlines the main environmental impacts generated at each stage in the FSC life cycle. In a life cycle perspective, energy and/or material resources are added to the FSC in each one of its stages. Therefore, food wastage generated in the latter stages of the FSC (e.g. consumption) generates more environmental impacts compared to food wastage in the earlier stages (e.g. agricultural production). Overall, the environmental impacts of food wastage do not only depend on the quantity but also on the type of food, where in the FSC food is lost or wasted, and how it is recycled or disposed of (Beretta et al. 2013).

Regarding the assessment of the environmental impacts of food wastage, most of the published reports focus on GHG. Food wastage contributes to GHG emissions in two ways: (i) from the embedded emissions associated with its production, processing, transport, and retailing; and (ii) via the decomposition of the food waste after disposal in landfills (Venkat 2011). Overall, GHG emissions associated with

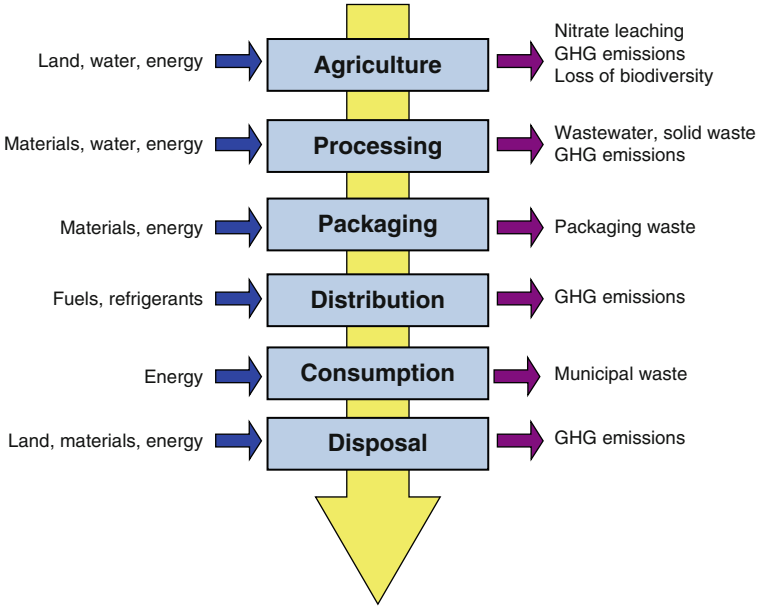

Fig. 2 Outline of the environmental impacts during the life cycle of food

food that is wasted are considered as "wasted emissions" or "emissions in vain" (FAO 2013). In terms of the GHG values reported, Venkat (2011) estimated that the production, processing, packaging, distribution, retail, and disposal of the avoidable fraction of food waste in the USA resulted in GHG emissions of at least 113 MMT CO_2e per year, which corresponded to 2% of the US national emissions. The calculated emissions were subject to an uncertainty of ±20% due to cooking assumptions. In the UK, Quested et al. (2013) reported that the 160 kg per household of avoided food waste for 2010 was estimated to contribute 17 MMT of CO_2e in GHG emissions annually and account for the total water footprint of the UK. Moreover, food waste makes up 30% of the general waste stream from UK households (Quested et al. 2013). In Greece, Abeliotis et al. (2015) estimated that a total of 5,672.5 Gg CO_2 eq. of GHG emissions per year are associated with food wasted. This figure corresponds to approx. 5.3% of the total GHG emissions (with LULUCF) in Greece for 2012.

The Economic Impacts of Food Waste

Food waste prevention is associated with economic impacts, such as business and consumer savings (Nahman et al. 2012). For example, Venkat (2011) estimated, with an uncertainty up to ±15%, that food waste costs to US businesses and consumers $190 billion per year. Consumer waste alone amounts to $124 billion, or nearly 63% of the total value, which works to about $1600 per year for a family of four. Again in the USA, Neff et al. (2015) reported that food lost from harvest to consumer in 2010 costed $161.6 billion; losses at the consumer level averaged $371 per capita or 9.2% of average food spending. In a similar report, Nahman et al. (2012) estimated that the costs of food wasted by households in South Africa are in the range of 21.2 billion rands annually. This equated to approximately 0.80% of South Africa's GDP or 9.63% of the value of retail sales by food, beverage, and tobacco in South Africa.

The Social Impacts of Food Waste

Besides the association of food waste generation with significant environmental and economic impacts, it is also related to social impacts, such as the global food and water security (Koivupuro et al. 2012; Kummu et al. 2012). FAO estimated that almost 11% of the global population is food insecure (Thyberg and Tonjes 2016). Food waste is also associated with the expanding dimensions of malnutrition, in both undernourishment (Nahman et al. 2012) and obesity terms (Parfitt et al. 2010). Therefore, food waste has a strong ethical dimension, since people in many parts of the world face the threat of food insecurity. Kummu et al. (2012) estimated that if the target of the European Parliament to halve food losses and waste by the year 2025 in the EU (see "Policies and Protocols" section) were to be applied globally, almost 1 billion more people could be fed. This number of people is equal to the

expected global population growth between 2010 and the year 2025, based on the medium variant of the UN estimation (Kummu et al. 2012).

From a sociological point of view, Evans (2012) states that food waste is an outcome of food-related behavior. This means that people who provide food for their households are more interested in the freshness and the nutritional quality of the food items (Stefan et al. 2013; Evans 2012). Therefore, in some instances, food waste generation at households is taking place because of the need of people to provide the best for their loved ones. In this case, the guilt about wasting food maybe smoothed away by collective and individual pride in reiterating norms of food safety and freshness (Waitt and Phillips 2016). As a result, food that does not (or is not perceived to) meet the aforementioned freshness criteria, including leftovers, ends up as waste.

The Effect of Consumer Behavior in the Generation of Food Waste Generation by Households

There is growing evidence that the contribution of the households to the food waste problem is particularly significant (Sharp et al. 2010; European Commission 2010). For instance, in EU-27, households are responsible for 42% of the total amount of food waste generated (European Commission 2010). Therefore, food waste generation at the consumption level is becoming an increasingly significant global issue, and household food waste mitigation is becoming a priority.

More specifically, the generation of food waste is the result of multiple behaviors within a household that increase the likelihood or amount of food being wasted (Visschers et al. 2016). These behaviors relate to many different aspects of the food's journey into and through the home: planning, shopping, storage, preparation, serving, and consumption of food (Quested et al. 2013).

Figure 3 presents the flow diagram of food reaching home. Solid lines denote the normal flow of food, while dotted lines denote food diverted to waste.

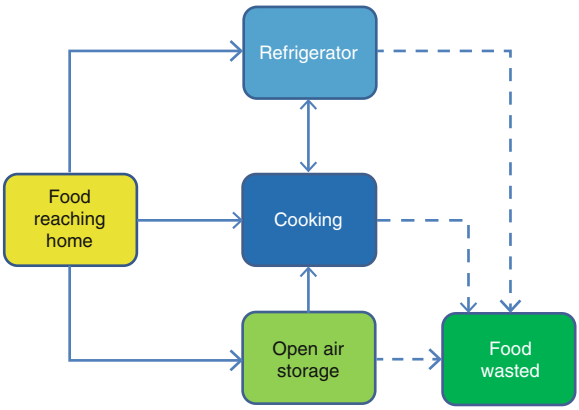

Fig. 3 Outline of possible routes of food arriving at households

More specifically, food reaching home can go directly to cooking, or to the refrigerator, or to open air storage. Food from the refrigerator can be directed to cooking; vice versa, cooked food can be directed to the refrigerator for preservation or deep freezing. Similarly, food from open air storage can be directed for cooking.

Cooked food can take the road to waste if it is served in large quantities or from other situational factors, such as the presence of young kids in the household. Food stored in open air can take the road to waste if it is rotten due to poor management. Food from the refrigerator can end up in the waste bin if it is refrigerated for a very long time, or if it past its "use before" date, or if it past its "best use before" date.

Note that both cooking and refrigeration add energy to food. Since energy results in GHGs emissions to the atmosphere and has financial cost for the household, food wasted after refrigeration and/or cooking is environmentally and economically worse compared to food wasted right after entering home.

More specifically, there is a large number of behaviors that can have a positive impact on food waste generation and, consequently, to the efforts toward food waste prevention (WRAP 2009). Such behaviors include meal planning, cupboard checking and list making before going for food shopping, proper storage of food items, use of food leftovers, cooking and serving the right amount of food, and careful use of expiration date labels (WRAP 2009; Abeliotis et al. 2014, 2016; Koivupuro et al. 2012; Stefan et al. 2013; Quested et al. 2013).

Besides human behavior, studies also indicate that technological factors of food provision such as packaging also affect the generation of food waste (Langley et al. 2011; Evans 2012; Williams et al. 2012). Effective packaging can reduce food losses, both directly and indirectly (Williams et al. 2012). Provision of the correct packaging size (e.g., for families) to the consumer has a direct negative effect on the generation of food waste. Moreover, packaging provides information to consumers regarding the "expiration date" and the "best before date." Correct comprehension of these two dates by the consumers has also an indirect negative effect on the generation of food waste by the consumers. On the other hand, packaging has a direct negative effect on food waste generation when it is used for unnecessary "multipack" promotions.

The Effect of Sociodemographic Characteristics on Food Waste Generation

At the household level, food waste generation is strongly associated with certain socioeconomic parameters (age, income, marital status). Koivupuro et al. (2012) showed that in Finnish households the amount of food waste is correlated with the size of the household, the gender of the person who is responsible for grocery shopping, the frequency of buying discounted products, and the view of the respondent to food reduction as well as to purchasing food packet sizes. Again in Scandinavia, Stancu et al. (2016) found that in Denmark lower amounts of food waste were associated with older consumers, fewer members in the household, and lower income. However, note that the amount of food waste generated per capita decreases

with increasing household size (Quested et al. 2013). People in four-person households generated approximately half the amount of food waste per capita compared to single-occupancy homes (WRAP 2009). Also, regarding the age of household occupants, Quested et al. (2013) reported that people over 65 years old in the UK generate less food waste than the rest of the population.

Regarding the effect of household income, Thyberg and Tonjes (2016) report that as incomes rise, people may be wasting food because the associated monetary losses are not a considerable fraction of their income. The concept of saving money has been found in both qualitative and quantitative research to be a powerful motivating factor toward prevention of food waste generation (Quested et al. 2013). For instance, Stancu et al. (2016) found that awareness of the economic impact of food waste has a stronger negative association on the generation of food waste compared to awareness of environmental and social consequences. Neff et al. (2015) reported that among the motivations for US consumers to reduce food waste, saving money topped the list. Jörissen et al. (2015) argue that there are active societal and economic trends that promote the generation of food waste such as the growing GDP, urbanization, and rising number of single households in addition to multiple burdens in work and family life for women. Figure 4 is an attempt to depict the complex network of food wastage implications discussed in this chapter.

Placement of Food Waste in the Context of the EU Waste Hierarchy

Food waste is normally treated alongside the rest of the organic municipal solid waste in mainstream waste treatment methods such as landfilling, centralized or home composting, incineration, and anaerobic digestion, which are known and largely accepted practices worldwide (Khoo et al. 2010; Luque and Clark 2013). Food waste, being organic in nature and therefore highly degradable, is subject to biological decomposition during the waste management chain. Among the management alternatives, landfilling is the worst in terms of environmental impacts because food waste when landfilled generates substantial amounts of methane, a strong GHG. It is estimated that two tons of CO_2 equivalents are generated per ton of food waste (European Commission 2010).

From the viewpoint of circular economy, industrial symbiosis, and green chemistry, food waste is a renewable resource of useful products (Luque and Clark 2013; Mirabella et al. 2014). In the context of these principles, food wastes are used as raw material for new products and applications focusing on the goal of "zero waste" society and economy. For example, chemicals included in food waste are sugars, phenols, starch, pectin, collagen, cellulose, chitosan, lignin, natural dyes, waxes, and hemicelluloses (Luque and Clark 2013). Waste from fruit and vegetable processing contains high concentrations of phenolic compounds, antioxidants, and fibers; waste from meat processing contains recoverable amounts of proteins, collagen, and gelatin, while waste from dairy products is a valuable source of proteins (Mirabella et al. 2014). Valorizing food waste components could lead to numerous possibilities

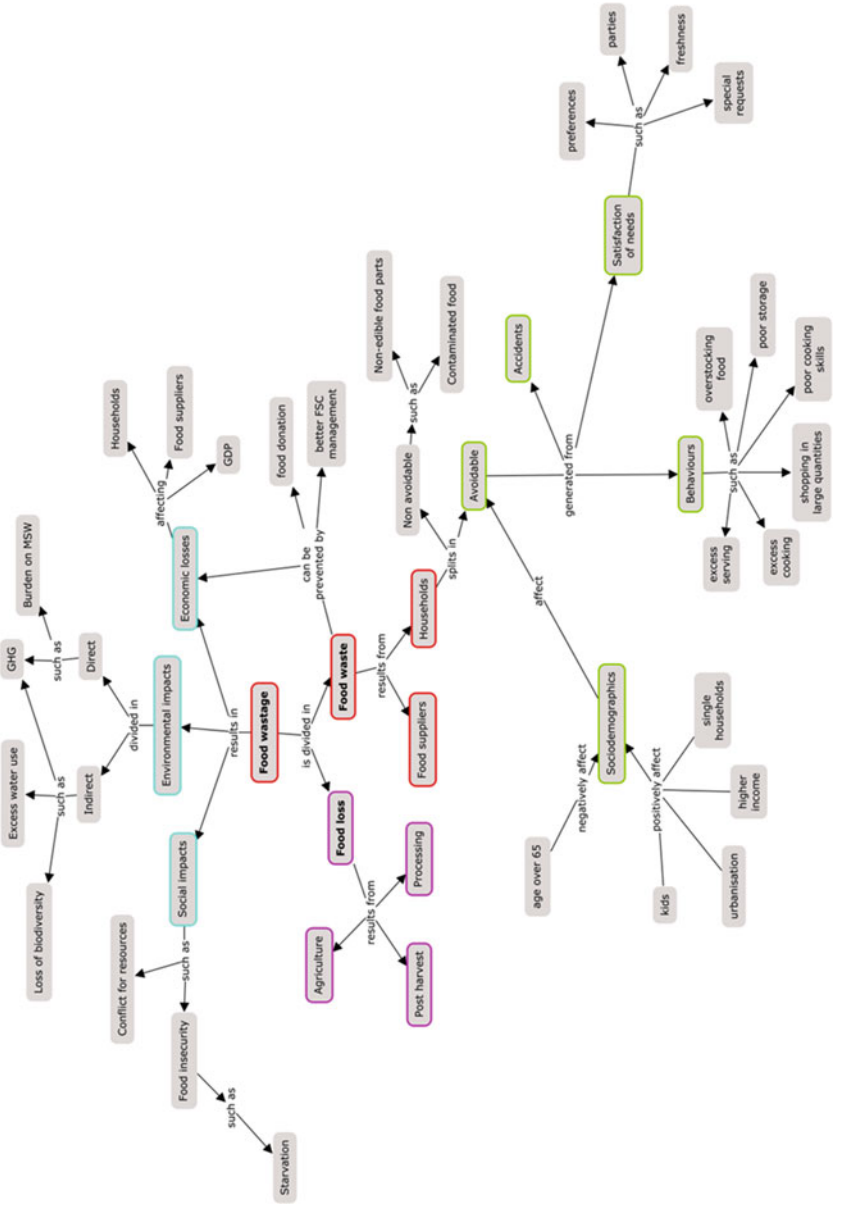

Fig. 4 The complex network of food wastage implications

for the production of chemicals, pharmaceuticals, fuels, and other valuable products (Luque and Clark 2013; Mirabella et al. 2014).

However, prevention should be the preferred management method for food waste (Salhofer et al. 2008). Waste prevention encompasses all the measures taken before a substance, material, or product has become waste, which reduce the quantity of waste, the adverse impacts of the generated waste on environmental and human health, or the content of harmful substances. Food waste prevention must incorporate practices to expand the accessibility of people on still edible foods that are older and/or less esthetically pleasing and those close to their expiration dates (Neff et al. 2015). Food waste prevention, on average, reduces GHG emissions by around eight times more than diverting the same amount of food waste from landfill to anaerobic digestion (Quested et al. 2013). Since the main purpose of food provision is human intake, among the possible food waste prevention alternatives, the donation of food to people in need is a sustainable act par excellence (Schneider 2013b) and must always be the first choice of people that have excess food to provide.

Policies and Protocols

Prevention of food waste generation is among the policy goals set by the UN, EU, and US policy agendas. More specifically:

- The 12th UN Sustainable Development Goal ("Ensure sustainable consumption and production patterns") sets the target to halve by 2030 the per capita global food waste at the retail and consumer levels and reduce food losses along production and supply chains, including postharvest losses (U.N. Sustainable Development Goals 2015).
- Food waste prevention is an integral part of the European Commission's new Circular Economy Package to stimulate Europe's transition toward a circular economy which will boost global competitiveness, foster sustainable growth, and generate new jobs. Moreover, the EU and member states are committed to meet the Sustainable Development Goals (SDG), adopted in September 2015, including a target to halve per capita food waste at the retail and consumer level by 2030, and reduce food losses along the food production and supply chains (European Commission 2017).
- The USDA and EPA have introduced the US Food Waste Challenge. This action calls on entities across the food chain – farms, agricultural processors, food manufacturers, grocery stores, restaurants, universities, schools, and local governments – to join efforts to reduce and better manage food loss and waste in the USA. Among them, the best performing participants are termed as champions. Food Loss and Waste 2030 Champions are businesses and organizations that have made a public commitment to reduce food loss and waste in their own operations in the USA by 50 percent by the year 2030. The Food Waste Challenge inventory of activities will help disseminate information about best practices and stimulate

the development of furher food waste prevention initiatives (United States Department of Agriculture 2017).

Dictionary of Terms

- **Food loss** – "A decrease in mass (dry matter) or nutritional value (quality) of food that was originally intended but is no more suitable for human consumption" (FAO 2013).
- **Food security** – The availability of and access to sufficient and healthy foods and good nutrition.
- **Food waste** – "Composed of raw or cooked food materials and includes food loss, before, during or after meal preparation in the household, as well as food discarded in the process of manufacturing, distribution, retail and food service activities"(EC 2011).
- **Avoidable losses** – Food and drink thrown away that was, at some point prior to disposal, edible (e.g., slice of bread, apples, meat).
- **Possible avoidable losses** – Food and drink that some people eat and others do not (e.g., bread crusts) or that can be eaten when a food is prepared in one way but not in another (e.g., potato skins).
- **Unavoidable losses** – Waste arising from food or drink preparation that is not, and has not been, edible under normal circumstances (e.g., meat bones, egg shells, pineapple skin, tea bags).
- **Life cycle assessment** – A methodology utilized to inform decision-making by identifying changes at every stage of a product's life cycle that can reduce its environmental impact.

Summary Points

- Food wastage is generated in every sector of the food supply chain.
- Food wastage generation is sector and country specific. However, common trends may be identified among countries of similar socioeconomic status.
- Food wastage is associated with social, economic, and environmental impacts.
- Food wastage puts a heavy burden on the global fight against starvation and famine.
- Food wastage has a strong ethical dimension since people in many parts of the world face the threat of food insecurity.
- Households are the major source of food waste in the developed world.
- There is a high level of complexity associated with the behaviors and practices relating to food waste generation at home.
- At the household level, food waste generation is strongly associated with certain socioeconomic parameters. For instance, older people generate less food waste.

- Food waste generation by households has a high variability which depends on the family structure, its eating and consumption habits, seasonality, special holidays, etc.
- The donation of excess food to people in need must always be the first choice.
- In the context of circular economy, food waste is a renewable resource of useful products such as chemicals, pharmaceuticals, and fuels.
- Food waste is a key focus for waste prevention at the household level.
- Food wastage prevention throughout the food supply chain should be among the key pillars of creating a sustainable food system.

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Preventing Food Waste and Promoting Healthier Eating among Lower-Income Families in Industrialized Nations

20

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Contents

Abstract

A wide body of research shows that lower income is highly correlated with obesity in industrialized nations. Research indicates this is largely due to lack

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of availability and affordability of healthier foods for lower-income families. In addition, lower-income families show considerable anxiety over buying healthier foods, as they are often rejected by children and potentially lead to costly food waste. Lower-income families face multiple stressors that thwart the ability to manage this tension, including economic constraints, the need to buffer stress with positive emotions, and juggling multiple responsibilities. In this chapter, we examine key issues faced by lower-income families and offer research-based solutions.

Keywords
Children · Diet · Eating behavior · Food waste · Lower-income families · Nutrition · Nutrition literacy · Obesity · Overweight · Poverty

List of Abbreviations
SNAP Supplemental nutrition assistance program

Introduction

Obesity is a major global health issue, and research supports the assertion that lower income is highly correlated with obesity in industrialized nations (e.g., Drewnowski and Specter 2004). Thus, interventions targeting lower-income families are likely to have a significant impact in addressing this epidemic. However, these families face well-researched obstacles to eating healthier diets including affordability of healthier options and access to healthier foods. Lower-income families also face a wide variety of psychological stressors that push them toward less healthy options, including economic constraints, the need to buffer stress with positive emotions (which can be temporarily accomplished with "comfort foods"), and juggling multiple responsibilities that deplete self-control resources. These factors lead to a fear of food waste in lower-income families, which drives food choices often toward unhealthy options (Daniel 2016; Connell et al. 2016).

Food waste is particularly problematic from a health perspective because fresh, healthy foods (like vegetables) are often wasted; at the same time, most individuals would benefit from increased consumption of these foods (Gunders 2012). Studies show that the average four-person American family wastes approximately $1500 of usable food yearly (Smith 2014). While researchers (and conventional wisdom) suggest that lower-income households should waste less food (see Gustavsson et al. 2011), empirical work has shown that differences in waste are minimal in lower versus higher-income households (Quested and Johnson 2009). Since wasted food equates to wasted money, it may be that the loss of food due to waste is felt more significantly in lower-income (versus higher-income) households.

In this chapter, we examine obstacles that lower-income families face regarding the tension between eating a healthier diet and preventing costly food waste, focusing particularly on obstacles that drive parental food choices for children in the household. Specifically, we examine the roles of nutrition literacy, convenience,

palatability of foods, positive and negative emotions, and potential food safety issues. We provide a review of extant research for each of these tensions, and then offer policy or parental solutions based on this research.

Nutrition Literacy

Nutrition literacy is the knowledge and motivation to incorporate nutrition information into food choices in order to promote health goals and food well-being (Block et al. 2011; Vidgen and Gallegos 2014). Lower-income and ethnic minority individuals may have more difficulty achieving adequate levels of nutrition literacy to promote healthy choices due to constraints on time and/or educational resources (Gans et al. 2009; Kumanyika 1996). Furthermore, lower-income parents must balance a tension between the priorities of good nutrition for the family and value based on economic constraints (Foley an Pollard 1998). Parents' knowledge and beliefs about food, health, and nutrition come from a variety of sources, including formal education from schooling and government agencies, education from healthcare professionals, and information from friends and family members.

Formal education sources to help promote nutrition literacy should be developed in conjunction with and customized to – the target population, such as lower-income parents (Howard-Pitney et al. 1997). Healthcare providers play an important role in promoting nutrition literacy among parents (Parra-Medina et al. 2010). Such education can help increase understanding of the connection between nutrition and downstream consequences (e.g., school performance, type 2 diabetes, etc.; Jyoti et al. 2005). Providers can help parents set concrete learning and food choice goals. Actively involving the parent in the learning and implementation process will increase the likelihood of parental adoption of recommendations by creating a positive stress experience, called eustress. This can lead to more progress toward a health goal (Mende et al. 2017). For example, helping lower-income parents to increase their nutrition literacy can help them to use caloric information to reduce consumption of sugar-sweetened beverages (Bleich et al. 2012).

Family, culture, and tradition may also influence nutrition literacy development among lower-income parents. For example, families who have lived in a food desert for generations may develop traditions around foods that are accessible in the local environment (Davis and Carpenter 2009), without having knowledge about the connection such foods have to downstream health and well-being challenges for the family.

Nutrition literacy uniquely influences parents with limited financial resources at every stage in the consumer experience including acquisition and storage, preparation and consumption, and disposal of food for the family. Lower-income parents want to purchase healthy foods for their families but perceive such foods to be unaffordable (Dammann and Smith 2009). Furthermore, parents may need to learn about how to access a variety of nutritious, inexpensive foods that can be used to prepare healthy meals for the family. At the acquisition stage, parents must be vigilant in distinguishing between promotional messages (e.g., "now with reduced

Table 1 Solutions to address the impact of lower nutrition literacy on food choice and food Waste

Problem	Recommendation
Less healthful food traditions may be learned based on being passed through families over generations	Use traditional educational sources such as healthcare providers, and new sources such as popular press to promote healthy alternatives (e.g., incorporating broccoli into a traditional family macaroni and cheese recipe to improve healthfulness without sacrificing tradition)
Parents may not be aware of what healthy options are available to them, and how to obtain them cost-effectively	Educate parents on how to access and economically prepare a wider variety of fruits and vegetables they may not have been aware of (e.g., providing information on how to obtain seasonal, less-commonly used vegetables)
Lack of food storage literacy may cause parents to consume expired or otherwise unhealthy foods in an effort to avoid waste	Promote the need for the parent to consume healthy options in order to effectively care for the children. Educate parent on food safety options

fat!") and objective nutritional information (Roe et al. 1999). A key aspect of nutrition literacy is understanding guidelines to safe and effective food storage (Connell et al. 2016; Daniel 2016). For example, understanding the nutritional content of fresh and frozen vegetables may help to save parents time and money. Although some frozen fruits and vegetables foods may have added sugar as part of their processing, frozen foods can be stored longer and can at times be convenient to prepare (Szocs and Lefebvre 2016). Families experiencing greater levels of poverty may seek shelf stable convenient foods that do not require refrigeration, which in turn limits the variety of foods that are available (Curtis and McClellan 1995).

Lower-income parents may be concerned about wasting food (Daniel 2016) and information on how to prepare and store meals in advance might help minimize the amount of usable food that is discarded. A parent might mitigate the stress of wasting money on discarded, usable food by, say, consuming items beyond listed expiration dates themselves. An understanding of the distinctions of use-by, sell-by, and best-before, and other information may influence heuristics parents use. Table 1 provides some solutions to help address the impact of lower nutrition literacy among parents.

Preparing own Meals or Buying Convenience Foods

Prepackaged or prepared healthier foods tend to be costly. For example, a Southwest grilled chicken salad costs $4.79 at McDonald's, whereas two cheeseburgers and a side of small fries costs only $3.19 (fastfoodmenuprices.com 2016). Thus, if lower-income parents wish to improve their children's diets, preparing meals themselves is the most viable option. However, many lower-income parents may lack proper cooking facilities, food storage space, and/or the knowledge necessary to cook their own meals (Carraher et al. 1998).

Lower-income families also face time constraints. Because minimum wage employment does not provide a living wage in many locations (Dube 2014), lower-income parents might need to work more than one job to make ends meet. Thus, even if lower-income parents possess adequate knowledge to prepare healthier meals, time constraints will impede their ability to plan and prepare meals. This problem is exacerbated by limited access to retailers selling healthier options such as fruits and vegetables in lower-income areas (Weatherspoon et al. 2013). Additionally, home economics education is no longer a mainstay (Lichtenstein and Ludwig 2010) and research has suggested that cooking is frequently learned from parents, particularly mothers (Carraher et al. 1999). If parents are working multiple jobs and lack time or energy to cook, then the end result is a generation that lacks the skills necessary to prepare healthy meals when they become adults.

In addition, working throughout the day is likely to tax precious regulatory resources, which already tend to be depleted as the day progresses (Baumeister 2002; Boland et al. 2013; Montoya and Scott 2013), and could increase the already difficult and potentially emotionally exhausting task of preparing a healthy meal that children may resist eating (Daniel 2016). If children refuse the meal, then parents are left with the task encouraging consumption and/or wasting food that goes unconsumed (Daniel 2016). Thus, prepared "fast" food is not only an inexpensive option, but an easier and palatable option likely to be preferred when one's regulatory resources are depleted.

A wide body of research supports the assertion that lower-income families urgently need better access to healthy, convenient, and affordable food options. Federal and local governments can provide tax incentives for grocery stores to open locations in urban areas. Because convenience stores still represent the primary food shopping destination for many lower-income families, governments could also provide convenience store owners with tax incentives to offer healthier options, like fresh produce. Governments could further develop programs lower-income families rely on to reflect today's realities. In the USA, perhaps SNAP (Supplemental Nutrition Assistance Program) could be altered so that consumers can use credits for prepared options at restaurants if they meet healthy dietary guidelines. Already, SNAP "dollars" count two-for-one at local farmer's markets, and such incentives should be pervasive.

Because many lower-income parents may lack nutrition-related knowledge, programs need to be put into place or further supported, as preparing meals at home remains an affordable option for healthy eating. As previously mentioned, home economics courses are often not required or offered in primary education (Lichtenstein and Ludwig 2010). We assert that home economics may help increase nutrition literacy, if courses are updated for modern needs. For instance, courses could emphasize understanding of nutrition labels, shopping within a budget, shopping in multiple environments, and how to prepare healthy and easy meals. Adult learning programs that teach the same skills are also necessary.

Involving children in meal preparation is also important as research has shown that when people expend effort in creating something, they develop a greater emotional attachment to the resulting product (Norton et al. 2012). Further, children

Table 2 Solutions to address the labor and convenience tradeoff

Problem	Recommendation
Inexpensive and convenient foods are calorie dense	Tax incentives to food retailers to provide healthy, palatable, and affordable options to lower-income families
Healthier prepared options are expensive	Allow consumers to use programs such as SNAP for prepared options that meet dietary guidelines
Lack of food preparation skills	Increase home economics education in schools and adult learning programs
Children have no vested interest in eating healthier options	Involve children in age-appropriate food preparation tasks

who are involved in growing their own produce and/or assist in preparing meals are more likely to enjoy consuming healthier options (Heim et al. 2009; Kim et al. 1998).Thus, if parents involve children in meal-preparation tasks that are safe and age-appropriate, some of the objections children might have to healthy food options could be attenuated. Solutions to the problems identified here are summarized in Table 2.

Consumption of Preferred Foods or Wasting less Preferred Ones

Empirical evidence supports the notion that the cost of healthful foods (e.g., fresh fruits and vegetables) is higher than the cost of alternatives that are higher in fat, sugar, and salt (e.g., packaged and processed foods; Drewnowski 2010). While research suggests that lower-income families often work to optimize their limited food budgets by selecting less expensive, nutrient poor but calorie rich options (see Drewnowski and Specter 2004), other work shows that careful planning can mitigate the monetary costs of a healthier selection of foods (Carlson and Frazao 2012). Yet lower-income families face additional risks in purchasing healthy foods for children, as children often refuse and waste less preferred, healthy foods (Connell et al. 2016; Daniel 2016). Thus, it is not merely the economic costs of food purchased that drives the decision of lower-income parents regarding what to buy, but also the risk inherent in wasting healthy foods.

Food liking is influenced by many factors. One key factor driving childhood food acceptance is palatability; children prefer sweeter (vs. less sweet) foods. The literature implicates biology (i.e., evolutionary preference) and experience (i.e., lack of exposure to healthy foods in childhood) as drivers (Mannella et al. 2016). Although people inherently prefer sweet and calorie-dense foods, most tastes are learned (Beauchamp and Mennella 2011). The finding that repeated exposure to a novel stimulus increases liking (Zajonc 1968) has been demonstrated in the domain of taste, such that individuals can develop preferences for foods that are not liked on first exposure (Birch 1999; Wardle et al. 2003a, b). Thus, experts recommend repeated exposure to less preferred foods to foster acceptance. Liking for new foods increases between five and ten exposures for two-year-olds (Birch et al. 1982), with more exposures required for older children and adults (Sullivan and Birch 1990).

This technique is effective at increasing the acceptance of a variety of foods and, in particular, increasing children's acceptance of vegetables (e.g., Ahern et al. 2014). Kalat and Rozin (1973) theorize that repeated exposures for novel foods are effective because of "learned safety"; experiencing a less palatable food multiple times without a negative physiological response increases perceptions of safety and, in turn, acceptance (Birch 1999; Wardle et al. 2003a, b).

Despite the known effectiveness of repeated exposure of novel or less preferred foods, research indicates that lower-income parents are not likely to serve children foods that they previously reject (Goodell et al. forthcoming). As discovered by Daniel (2016), a key reason underlying this is fear of food waste; while parents may be aware of the beneficial effects of multiple exposure, their food budgets simply cannot extend to provide (often) more expensive healthy foods that children refuse. Taken together with the structural factors that limit access to healthy foods in lower-income populations (e.g., food deserts with a dearth of fresh foods tend to occur in lower-income residential areas; Hendrickson et al. 2006), this makes the acquisition of such items difficult on a regular basis for lower-income families – and recall that multiple exposures of novel foods are required to establish preferences. While those of greater means may consider food waste stemming from repeated exposures as a necessary buy-in to establish healthy preferences, lower-income family may simply lack the financial flexibility to waste costly food – healthy or not.

Building on the concept of exposure leading to liking (Zajonc 1968; Pliner 1982) lower-income parents might seek out ways to provide multiple exposures of less preferred foods that minimize waste. Food storage may play a role here; foods might be prepared and frozen or canned, for instance, so that multiple exposure can be planned for later dates (as opposed to wasting remaining, perishable foods). Alternatively, less perishable forms of such foods might be purchased (e.g., frozen foods) prior to a child's acceptance so that smaller portions can be used without waste (e.g., cooking a small portion of frozen vegetables and freezing the rest until the next exposure/serving opportunity).

We also suggest that the order in which foods are provided may be intentionally altered to potentially increase acceptance. In terms of vegetables, a category which children tend not to prefer but for which consumption offers myriad benefits (CDC 2014), parents might offer children foods that align in a "risk ladder," in which sweeter foods are introduced and followed by those with increasingly bitter taste profiles (see Connell et al. 2016). This may increase acceptance by children and decrease the waste of less preferred foods. Such a strategy could work with other food categories as well; notably, similar plans are often adopted by parents in cultures where spicy foods are commonly eaten, such that children are given increasing levels of spice with repeated exposures to food to gradually increase acceptance (Rozin et al. 1982). Indeed, support would be needed to communicate the appropriate progression of vegetables (or other foods) for parents who might not possess adequate flavor knowledge to create an appropriate progression.

In addition to exposure, research also suggests that strategies based on associative conditioning may also increase liking for foods (Wadhera et al. 2015). These techniques involve associating a less preferred food with a positive or negative

consequence. For example, sweeteners can be added to items (e.g., grapefruit juice) and, with multiple exposures, children exhibit a greater preference for the item in its natural state relative to those who received multiple exposures to an unsweetened item (i.e., flavor-flavor learning; Capaldi and Privitera 2008). While studies have also looked at the provision of rewards as a basis for increasing liking, the results are mixed, making it unclear if rewards offer additional benefit over merely exposing children to foods repeatedly (see Corsini et al. 2013; Wadhera et al. 2015). However, when exposure is paired with parental modeling of food acceptance (either with or without additional rewards), children's acceptance of disliked foods increases. This supports the idea that parental modeling encourages the consumption of healthy foods and, importantly, may be a cost-efficient way of encouraging healthful eating (Holley et al. 2015).

Food presentation has also been shown to impact the consumption of healthy foods. In a series of studies, Wansink and his colleagues assigned attractive names (e.g., "X-ray Vision Carrots") to vegetable dishes in elementary school cafeterias and compared the percent of students choosing these options over generically named dishes; indeed, more students were likely to select the foods with attractive (vs. generic) names (2012a). Similar research has shown an increase in the selection of apples when they include stickers featuring child-friendly characters (Wansink et al. 2012b). These and other techniques such as serving food in "cute" shapes (e.g., Nenkov and Scott 2014) may serve to increase consumption of less preferred foods. In sum, parents may be able to utilize creativity to alter the appearance of foods as an economical means of increasing the consumption of healthy foods at home while minimizing the likelihood of waste. Solutions to the problems identified here are summarized in Table 3.

Table 3 Solutions to promote acceptance (and minimize waste) of healthy foods

Waste reduction strategy	Parent recommendation
Food storage	Freezing, canning, or otherwise preserving perishable foods
Product form	Purchasing products in less perishable forms (e.g., frozen) so that small portions can be utilized with the remainder stored for future use
Food introduction	Children might be first introduced to healthy foods that they are more likely to accept (e.g., a sweeter vegetable), followed by progressively risker options (e.g., vegetables with more bitter taste profiles)
Food associations	Pairing less liked foods (e.g., grapefruit juice) with preferred tastes (e.g., sweeteners) may generate liking over time with multiple exposures
External rewards	Children may be given non-food rewards for trying new foods or not wasting provided food
Parental modeling	The tendency for children to model their own eating after that of their parents suggests that parental consumption of healthy foods may increase the likelihood of childhood consumption
Food presentation	Foods may be given child-friendly names, associated imagery, shapes, etc. to increase liking and reduce food waste

Healthy Foods or Happy Kids (and Parents)

Indeed, food plays an important role in family life beyond its mere nutritional components that contribute to individual health (Block et al. 2011). Research demonstrates a remarkable connection between food and emotions. Studies show, for instance, that children who regularly eat less healthy foods (vs. healthy foods) are less likely to be unhappy, highlighting a tradeoff between objective (i.e., health) and subjective (i.e., emotional state) well-being (Chang and Nayga 2010). Food is often consumed as a means of consciously generating positive emotions, particularly when more negative ones are experienced, as the phrase "comfort food" suggests (Wood 2010). Foods that bring individuals "comfort" are typically unhealthy ones (e.g., potato chips, ice cream, cookies). Such foods are consumed when people seek to maintain positive emotions or repair negative emotions (Wansink and Sangerman 2000).

Given the tendency for unhealthy foods to generate positive emotions (i.e., to make kids happy), comfort foods might be used by lower-income parents to ease childhood stress that arises from food insecurity and other financial and emotional pressures associated with lower-income households (e.g., inadequate housing, taxing wage work, etc.; Leung et al. 2015; Liu et al. 2014). Unfortunately, this can lead to poor nutrition-related outcomes (e.g., obesity, high blood sugar, etc.); though, notably, experts suggest that stress may itself also lead to weight gain due to resultant hormonal changes (see Adam and Epel 2007). So, while it is important to make healthy food choices, it is also important to identify methods to reduce stress and associated negative emotions via means other than consuming unhealthy comfort foods.

In addition to emotions experienced by children, caregiver emotions can also impact food choice. Research suggests that lower-income parents associate food gratification with good parenting (Kaufman and Karpati 2007) and perceive that saying "no" to a child's request for unhealthy food is an example of failing to show love (often based on their own, recalled responses to being told "no" as a child; Herman et al. 2012). A separate study of lower-income mothers similarly identified the emotional difficulty experienced by the mother in denying children additional food after they had already consumed an adequate serving (Jain et al. 2001).

Additionally, hard-working lower-income parents may consume comfort foods to cope with stresses that spill over from work into home life (Devine et al. 2006). Rather than mitigating stressful work experiences, many lower-income working parents reduce their workload at home, often because they recognize employment instability (Devine et al. 2006). Lower-income parents also often contend with negative emotions resulting from conflicts with other adults in the household who disagree with their strategies to encourage healthy eating (Herman et al. 2012; Jain et al. 2001). Anticipated negative emotional responses from children as well as negative emotions like guilt or stress that may be induced by fellow caretakers may forestall efforts aimed at fostering healthier choices and consumption.

Additionally, family food choices can act as a means of showing affection. A poll by National Public Radio, the Robert Wood Johnson Foundation, and the Harvard School of Public Health (2013) indicates that more than 25 percent of families in the

United States equate food with love. Food and food brands represent key components of individual and family identities (e.g., food traditionally served at holidays or family dinner favorites and preferred brands; Moisio et al. 2004; Motley and Perry 2009; Trump et al. 2015; Wallendorf and Arnould 1991). These identities are based on shared memories and experiences that generate happiness. However, food-derived comfort is a learned association. That is, when a parent attempts to repair negative emotions with food, the hope is that the positive emotions experienced from consuming the food will mitigate negative emotions experienced from stress, frustration, or failure. As mentioned previously, people appear to have an innate preference for foods high in fat, sugar, and salt (Birch and Fisher 1998), which makes introducing healthier options such as fruits or whole grains a tricky proposition for parents. Such an approach would require a widespread public education effort to shift parents' use of unhealthy foods as vehicles of comfort to healthier options. This would likely require significant investments in public service announcements and use of healthy foods as a way of evoking positive emotions in public spaces such as classrooms.

Indeed, positive affect evoked from brands is known to bias people's perceptions of the advertised foods (Connell et al. 2014). Activating health goals has been shown to prompt people to reconsider their judgments and make healthier choices in some cases (Connell et al. 2014; Finkelstein and Fishbach 2010) and have the potential to strip junk foods of the pleasure people anticipate from consuming these foods (Connell and Mayor 2013). Thus, nudging health motivations via public service announcements or via signage or outdoor advertising might serve as effective means of getting people to reconsider their beliefs.

Another important consideration is understanding the multiple stressors that lower-income families face and providing them with a repertoire of behaviors to deal with these stresses (i.e., increasing resilience) that are not related to food. Research on building resilience in children focuses on seven crucial "Cs": competence (enhancing ability), confidence (having positive self-esteem), connection (strong interpersonal relationships), character (moral understanding), contribution (helping others and building community), coping (effectively dealing with stressors), and control (exercising restraint when necessary) (Ginsburg and Jablow 2011). Thus, building resilience should be a priority in our educational institutions so that parents and children have tools other than foods to deal with frequent environmental stressors. Potential solutions to issues related to experienced or anticipated emotions are summarized in Table 4.

Beyond Budget: Perceived Food Safety Impacts Waste

Another factor that likely impacts purchase of healthy foods pertains to food labeling and its impact on perceived food safety. To aid people in evaluating the freshness and safety of items to purchase as well as purchased items, the food industry utilizes a

Table 4 Solutions to reduce the tension between healthy and pleasurable eating

Problem	Recommendation
Indulgent foods are a source of affection	Public education to promote use of healthier foods with sweet flavor profiles (e.g., carrots, beets).
Positive feelings toward food brands and products create biased nutrition judgments	Use outdoor advertising, signage, and public service announcements to activate health goals, which can facilitate reconsideration of beliefs
Lower-income families face multiple stressors that create need for mood repair	Build resilience skills among children and parents via public education

number of cues to convey freshness, including information about when fresh food was packaged, the "sell-by" date indicating that food is still safe to consume, and the "use-by" or "best by" date, indicating a food is at peak quality, and expiration date. However, retailers frequently apply labels inconsistently, and they sometimes put multiple dates on packaged fruit, vegetables, protein, and dairy. Further, people tend not to receive information about what the date on their food label means (WRAP 2011). Consequently, many confuse the "sell-by" and "use-by" dates for expiration dates, and this confusion increases avoidable waste. One explanation for this confusion relates to the "anchoring and adjustment heuristic" in psychology; when people need to make a decision about food freshness, they rely on available information, such as "sell-by," "use-by," or "expiration" dates (Block et al. 2016; Tversky and Kahneman 1975). Indeed, one study indicated that 17 percent of household waste in the United States was due to food products being past their labeled dates (Van Garde and Woodburn 1987). Although "sell-by" and "use-by" dates have no bearing on a product's expiration or freshness, 50 percent of consumers still believed that eating food after their "sell-by" or "use-by" dates posed a significant health risk (Ransom 2005). Reducing the "sell-by" anchor from packaging reduced food waste by 21% in the United Kingdom (The Grocer 2016).

While these are general trends that apply to all consumers regardless of income level, conceivably, these information processing issues are exacerbated among lower-income families who tend to have low nutrition and food literacy and less time to consider whether dates on labels are impacting their eating decisions. Further, given that fresh fruits, vegetables, meats, and dairy take up a larger proportion of a lower-income family's food budget, this tension between determining whether food is fresh or should be thrown out might shift lower-income parents' preferences toward more shelf-stable foods.

When date labels are not present, people may be uncertain as to the range of a products freshness. In these cases, lower-income parents are still likely afraid of purchasing healthier items, like fruits and vegetables, because they are afraid the product will reach its expiration date before it will be consumed (Connell et al. 2016; Daniel 2016). This is consistent with research on risk-attitudes, which attests that as income decreases, concern with food safety and health risks increases (Dosman et al. 2001). Table 5 proposes strategies to overcome concerns of food safety and expiration.

Table 5 Solutions addressing concerns related to food freshness and food safety

Waste reduction strategy	Industry or governmental recommendation
Food date labeling	Remove date marks from food packaging that are unrelated to food safety and expiration (e.g., "sell-by" and "use-by" dates)
Food labeling consistency	Use one, consistent date mark on food labels
Food information	If unable to remove extraneous date marks, educate consumers about what dates on food labels actually mean (e.g., peak-freshness versus expiration)
Food packaging	Offer a range of package sizing so that consumers might pick smaller package sizes to minimize household waste

Conclusion

In this discussion, we have considered some of the unique challenges faced by lower-income parents seeking to feed their families a healthy diet while minimizing food waste – nutrition literacy, convenience, palatability of foods, positive and negative emotions, and potential food safety issues. In doing so, we seek to build on prior research aimed at generating solutions to the global issue of food waste (see Block et al. 2016; Porpino 2016). Acknowledging the complexity faced by low income families, in particular, we recognize that this is not an exhaustive list. However, it is our hope that the issues identified here may be used to generate inquiries that lead to solutions that promote the well-being of families, and may simultaneously address larger societal, economic, and environmental issues.

Policies and Protocols

In this discussion, we discuss unique obstacles faced by lower-income families in preventing food waste and promoting healthier eating, and suggest research-based policies to aid these consumers.

- Efforts to **boost nutrition literacy** by educating consumers about healthy versus unhealthy foods, where to purchase such foods, how to prepare these items, and ways to promote them within the family might promote better food decisions and less waste.
- Policies that make **healthy food more accessible and convenient** include tax incentives for retailers, increased government assistance for consumers, increased home economics education in schools, and other adult learning. Such initiatives may also lead to creative solutions for minimizing waste.

- Techniques aimed at **increasing the palatability of healthy** foods include teaching food preservation techniques (e.g., canning, freezing) and promoting the purchase of foods in less perishable forms. Moreover, children might be exposed to less risky (e.g., sweeter) foods before less palatable ones or preferred tastes might be paired with less preferred ones to increase liking. Foods might also be framed as more fun (e.g., through product naming) and strategies to promote healthy food choices for parents may lead to children modeling parental consumption.
- Given the **emotional nature of eating**, education programs might promote healthy foods, nutrition goals, and strategies for resisting food temptations.
- Polices aimed at **creating an understanding of food safety** include standardizing food dating systems and educating consumers about date meanings. Further, a variety of package sizes might work to reduce food waste.

Dictionary of Terms

- **Eustress** – A stress experience that is positive for the individual. Eustress can lead, for instance, to increased goal engagement.
- **Food desert** – A geographic area defined by a lack of healthful foods, like fresh fruits and vegetables.
- **Food insecurity** – Lacking reliable access to adequate quantities of healthy, safe, and affordable food.
- **Heuristic** – A practical shortcut for decision-making that minimizes effort and time spent in the decision-making process.
- **Nutrition literacy** – The knowledge and motivation to incorporate nutrition information into food choices.

Summary Points

- Key issues lower-income families face in providing healthy diets while avoiding food waste include those related to nutrition literacy, convenience, food palatability, positive and negative emotions, and food safety.
- Many lower-income parents lack basic nutrition literacy.
- Lower-income parents may lack time or food preparations skills to prepare healthy meals at home.
- Healthier foods tend to be less palatable for children, potentially leading to food waste.
- Food is often used as a way to reward, show love, or repair negative mood.
- Concerns over food safety of fresh foods (e.g., expiration dates) can lead to waste or avoiding these options.

- The issues identified and the research-based solutions presented here may be used to generate inquiries that lead to resolutions that promote the well-being of families, and may simultaneously address larger societal, economic, and environmental issues.

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Part IV

Biosocial and Social Aspects, Inequalities, Low Income, Refugees, and Conflict

Applying a Biosocial Perspective to Address Childhood Diarrhea-Related Morbidity and Mortality

21

Nicola Bulled, Merrill Singer, and Rebecca Dillingham

Contents

Abstract

Despite calls for greater attention to the relationship between water access and disease, diarrhea and related malnutrition remains a leading cause of morbidity in children under 5 years. Children are especially vulnerable given their inability to mount an active immune response to pathogen exposure. Social conditions,

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including the long-term effects of poverty – reduced nutrition, poor hygiene and sanitation, and diminished home environments – further exacerbate the biological limitations. This chapter offers a syndemic perspective on childhood diarrhea-related malnutrition, specifically examining the consequential interactions between pathogens, as well as pathogenic interactions with other health and social conditions that are disproportionately common among disparity populations. This perspective offers a potential for enriching and extending policy discussions and planning, addressing malnutrition-related diarrheal illness and other health challenges of impoverished populations.

Keywords
Biosocial model · Childhood diarrhea · Enteric pathogens · Malnutrition · Syndemic

List of Abbreviations

BFP	Bolsa Familia Program
DALY	Disability Adjusted Life Years
EE	Environmental Enteropathy
GAPPD	Integrated Global Action Plan for the Prevention and Control of Pneumonia and Diarrhea
GIT	Gastrointestinal Tract
iCCM	Integrated Community Case Management
IMCI	Integrated Management of Childhood Illness
ORS	Oral glucose-electrolyte Rehydration Solution
USAID	United States Agency for International Development
WASH	Water Access, Sanitation, and Hygiene
WHO	World Health Organization

Introduction

Following repeated calls for greater attention to the relationship between poor water access, hygiene and sanitation infrastructure, and malnutrition, the World Health Organization (WHO), United Nations Children's Fund (UNICEF), and the United States Agency for International Development (USAID) jointly prepared the report "Improving nutrition outcomes with better water, sanitation and hygiene," to summarize the abundance of evidence on the benefits of WASH (water access, sanitation, and hygiene) as a means to improve nutrition outcomes (WHO et al. 2015). Diarrhea, spread through contaminated drinking water or food, from person-to-person through contaminated hands, or unsafe disposal of stool, is the leading cause of malnutrition in children under 5 years.

Despite significant advancements in infrastructure and medical technology, diarrhea continues to be responsible for 9% of deaths of children under the age of five in 2015, killing over 1400 children a day, or 526,000 a year (UNICEF 2017). Most diarrhea-related deaths in children occur in those under the age of two in

marginalized communities in South Asia and sub-Saharan Africa (Keusch et al. 2006; Fischer Walker et al. 2013; UNICEF 2017). In these settings, children under age of three experience on average three episodes of moderate-to-severe diarrhea every year (WHO 2013). Diarrhea is usually a symptom of enteric bacteria, viruses, and/or parasitic infection of the intestinal tract. In 2015, rotaviral enteritis (rotavirus) was the leading cause of under-five mortality due to diarrhea globally (146,000 deaths) (Institute for Health Metrics and Evaluation 2017). Death from diarrhea usually results from severe dehydration and fluid loss. The greatest burden of disease occurs primarily in younger age groups, with 72% of deaths from diarrhea occurring in children younger than age two (Fischer Walker et al. 2013). The highest rates of severe diarrheal disease occur at age 6–11 months, as infants no longer receive passive protection from trans-placental and breast milk antibodies and begin to experience greater pathogen exposure from food, water, and their wider environment (Fischer Walker et al. 2013). Risk of diarrhea and diarrheal mortality then decreases with age. However, morbidity (i.e., stunting and cognitive impairment) related to moderate-to-severe childhood diarrhea can have profound implications throughout one's lifetime. Infants and children are more adversely affected because they are more vulnerable to both social and biological factors that facilitate infection, such as the long-term and entwined effects of poverty, malnutrition, and underdeveloped immune systems.

The tendency in existing research and intervention is to look for, and seek to resolve, individual causes of diarrheal disease or use medical treatment technologies to prevent disease or disease mortality. Simple oral glucose-electrolyte rehydration solution (ORS) can reduce diarrhea-related mortality by 93% (Munos et al. 2010), dramatically reducing acute diarrhea-related mortality from 4.6 million annual deaths globally during the mid-1980s to the current estimate of 1.6–2.1 million (Kosek et al. 2003; Keusch et al. 2006; WHO 2013a). The rotavirus vaccine, introduced as part of childhood vaccination schedules in 81 countries (as of May 2016, PATH 2016), has been shown effective at not only reducing hospitalizations due to rotavirus by 88% (95% CI 76–94), but also conferring heard immunity for older children born before the rotavirus vaccine became readily available (40–69% reduction in admission for rotavirus) (Tharmaphornpilas et al. 2017). High-doses of vitamin A supplementation helps to promote and maintain strong immune systems and can reduce all-cause mortality by 24% and cases of diarrhea by 15% (UNICEF 2017). From 2000 to 2015, the total annual number of childhood deaths from diarrhea decreased by more than 50% through these basic interventions (UNICEF 2017).

However, diarrhea is preventable and often does not occur in isolation. Worldwide, one in nine people (783 million individuals) have no source of safe drinking water, and one in three (2.4 billion) lack improved sanitation – numbers that are projected to double by 2025 (Mara 2003; WHO 2013b; The Water Project 2017). Furthermore, 946 million people practice open defecation (WHO/UNICEF 2012a; WHO/UNICEF 2017). Children who suffer from multiple bouts of diarrhea are frequently inflicted with other diseases of poverty, including malnutrition (Bennett 2009), malaria, dengue, measles, and HIV (Singer 2009). Diarrhea-related morbidity

continues to have significant long-term implications, particularly for young children (Keusch et al. 2006; Fischer Walker et al. 2012). Research suggests that diarrhea is not limited to transient illness, but that repeated episodes of diarrhea can cause physical growth impairments (Petri et al. 2008; S.P. Walker et al. 2011). A pooled analysis of nine studies showed that the odds of stunting by age 2 years increased by 1.13 (95% CI 1.07–1.19) for every five episodes of diarrhea (Checkley et al. 2008). Additional research suggests that by age 7, children suffering from multiple bouts of diarrhea can lose up to 8.2 cm in height (Moore et al. 2001). Multiple studies identify the link between repeated episodes of diarrhea and cognitive deficits, with a measured loss of up to 10 IQ points and 12 months of schooling by age 9 (Guerrant et al. 1999; Niehaus et al. 2002; Lorntz et al. 2006). Yet, the causal interactions between disease states and social conditions are understudied.

This chapter offers a syndemic perspective on childhood diarrheal disease, specifically examining the consequential interactions between pathogens, as well as pathogenic interactions with other health and social conditions that are disproportionately common among disparity populations (those suffering disproportionately from poverty, social inequality, and economic and political marginality). We present this perspective in an effort to highlight the potential for a syndemics analysis to enrich and extend policy discussions and planning, addressing malnutrition-related diarrheal illness and other health challenges of impoverished populations. Drawing from available research and current policy agendas, this chapter explores current approaches to diarrheal disease management and considers novel comprehensive disease prevention approaches, consistent with syndemic theory, that are likely to prove effective in generating sustainable and equitable disease prevention solutions. This chapter is an update to our previously published work on the topic (Bulled et al. 2014).

Current Public Health Approaches to Childhood Diarrhea

The international bodies of UNICEF and WHO have guided the global public health agenda on childhood mortality, with a primary focus on the use of biomedical technologies. The *Integrated Management of Childhood Illness* (IMCI), developed in 1995 to reduce under-five mortality, aims for an integrated approach to address the well-being of the whole child. The strategy focuses on three main components: improving case management skills of health care staff for the accurate identification of childhood illness in outpatient settings; making improvements in overall health systems to ensure the appropriate combined treatment of all major childhood illnesses; and boosting community health practices to ensure appropriate care seeking, care management, and preventive practices. The attention is on scale-up and maintenance of health care facilities.

Although the program has been introduced in more than 75 countries, clinical coverage and quality of clinical care for sick children have not reached high enough levels to achieve the expected reductions in mortality or morbidity (Chopra et al. 2012). According to a multicountry evaluation of the impact and cost-effectiveness

of the IMCI strategy conducted in Brazil, Bangladesh, Peru, Uganda, and the United Republic of Tanzania, the IMCI can reduce under-five mortality and improve nutritional status if implemented well (WHO 2016). Furthermore, it costs up to six times less per child than standard of care. However, significant reductions in under-five mortality cannot be attained unless large-scale intervention coverage is achieved (WHO 2016b). According to a study of services provided in 12 countries in sub-Saharan Africa with high diarrhea burdens, the prevalence of "good" care (defined as ORS or zinc provided, fluids given, and feeding continued) was low in all formal and informal health care setting (Carvajal-Velez et al. 2016). A median of 27% of children with diarrhea (range from 17% to 38%) received high quality of care.

Bottlenecks have been a primary impediment to access to and use of biomedical therapies (Gill et al. 2013). These include: inequitable supplies of key commodities and unequal provision within communities through public or private avenues; delayed development of national policies supporting use of certain newer strategies or integration of strategies into national plans (i.e., rotavirus vaccine); limited funding and resources; poor management and coordination of efforts; and inadequate advocacy. For example, despite evidence of effectiveness, the median coverage of ORS has decreased since the late 1980s, with just over 40% of children under age five with diarrhea receiving the recommended treatments of ORS and continued feeding worldwide (WHO/UNICEF 2012b). Use of this treatment is lowest in sub-Saharan Africa and South Asia (38% and 47%, respectively), regions with the most deaths from diarrhea (UNICEF 2017). Furthermore, even a decade after WHO/UNICEF released recommendations for treatment of diarrhea with zinc, global uptake of zinc for the management of diarrhea remains almost negligible, at 4% (Bhutta et al. 2010; UNICEF 2017).

WHO/UNICEF *Integrated Community Case Management* (iCCM) strategy for diarrhea, pneumonia, and malaria launched in 2011 offered a community-level, rather than health care facility, focus for disease prevention and treatment. The aim of this program is to enhance preventive activities such as exclusive breastfeeding for the first 6 months of life, improved vaccine coverage for rotavirus as well as *Streptococcus pneumoniae* and *Haemophilus influenzae* type b (the two most common bacterial causes of childhood pneumonia, measles, and pertussis), ORS usage, and improve water and sanitation access in health care facilities with accompanying hygiene practices.

Despite resurgence in the use of community health workers in the delivery of case management of childhood illnesses, evaluations of these community-based strategies suggest that the quality of services is poor (Strachan et al. 2012; Gill et al. 2013). Contributing factors include: poor incentives to attract or retain workers, inadequate training, no systematic approach to supportive supervision, and limited provision of appropriate treatments such as antibiotics, pre-packaged ORS, and zinc to community health workers despite their skills and access to communities.

The recently announced WHO/UNICEF *Integrated Global Action Plan for the Prevention and Control of Pneumonia and Diarrhea* (GAPPD) offers a more comprehensive approach to addressing childhood diarrhea (and pneumonia) by providing a cohesive policy framework upon which local political and civil society

leaders take the lead in the development of coordinated strategies. In so doing, the GAPPD aims to: close service delivery gaps by bringing together critical services and interventions, promote best practices and proven strategies, and establish healthier environments. The effectiveness of this integrated, multidimensional approach that attempts to speak across sectors is yet to be determined.

One concern with the GAPPD approach is the lack of direct attention to addressing social drivers of disease, including the promotion of poverty alleviation, improvement of water infrastructure, or assessing whether local communities are interested in the approaches suggested and are willing to comply. For example, an analysis of 15 highly cost-effective interventions for the reduction in childhood diarrhea (see Bhutta et al. 2013) published to coincide with the announcement of the GAPPD did not include estimations for broader-based investments in water infrastructure, focusing instead on biomedical/health care facility solutions. A qualitative analysis of the discussions of the multicountry workshop consultations, which ultimately led to the development of the GAPPD, identified limited or no country-level awareness and support for water, sanitation, and hygiene initiatives (Gill et al. 2013). This lack of national engagement in discussions of water and sanitation improvements possibly reflects the perceived magnitude of resources that are needed to develop or upgrade effective water supply and sanitation systems. This lack of conversation also highlights inequities in current geopolitical arrangements as country ministries feel beholden to the priorities of multilateral funders (Gill et al. 2013), resulting in external interests that are quickly implemented and easily monitored being favored over longer-term national priorities, possibly contributing to limited national political will and civic engagement. The result is limited attentiveness to the underlying causes of disease: poverty, inequality, and social marginalization.

Considering the Social Aspects of Disease: The Syndemic Perspective

The term *syndemic* is a portmanteau derived from the Greek work *synergos*, meaning two or more agents working together to create a greater effect than each working alone; and *demos*, or "people," commonly used in public health concept of epidemic, a disease classification that literally means "upon the people." Much like the synergistic effect of multiple drug agents working simultaneously in a system, whereby the total response is more than the predicted sum of the two individual effects, the synergistic interactions of disease conditions that are perpetuated by social disparities generates a syndemic (Fig. 1). The syndemics perspective recognizes that adverse social conditions, including social inequality, marginality, poverty, and other forms of political and economic oppression, play a critical role in facilitating the clustering of both infectious and noninfectious diseases that lead to heightened potential for deleterious disease interactions (Singer 2009).

While the development of a syndemic could indicate less overt inequalities in need of investigation, usually with infectious diseases we are arguing that conditions of poverty or other disadvantages (e.g., malnutrition, trauma, stress, environmental

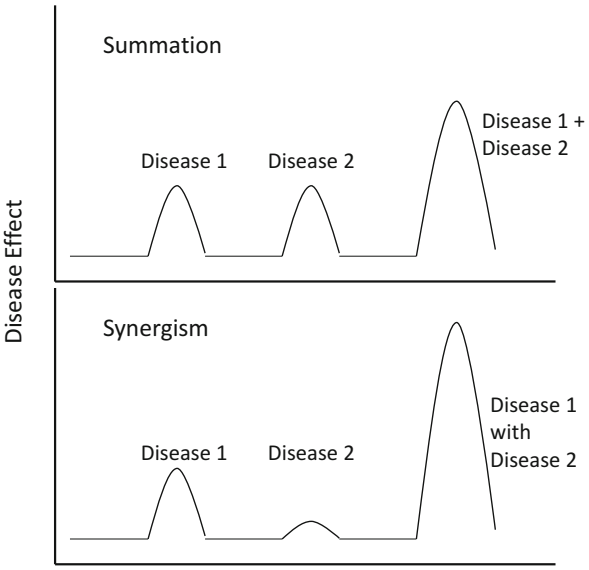

Fig. 1 Summative versus synergistic effects of co-occurring diseases. Under certain social conditions, the clustering of diseases in a specific population may be exacerbated given both micro-parasitism and macro-parasitism relationships

toxins) place bodies at heightened risk for diminished immune capacity, diminished body development and repair, and heightened exposure to pathogens and pathogen clustering interaction. Moreover, disadvantaged populations commonly face multiple infectious agents rather than a high burden of infection from a single pathogen, and these, in turn, often have the capacity to interact within human bodies to multiply their adverse effects. Syndemics theory therefore rests on recognition that health issues are never matters of biology alone but rather involve continuous bidirectional feedbacks between biological and social structure (Singer et al. 2017).

In the case of diarrhea causing enteric infections, the syndemic framework offers an understanding of the complex and dynamic relationship between *micro-parasitism*, the biological proximate causes of disease, and *macro-parasitism,* the human relations that are the ultimate origins of much disease. Globally, diarrhea is of gravest threat to populations already at comparatively high risk for a range of threats to health and social well-being. In this, diarrheal disease both exposes the vast inequities of our prevailing global social structure and the limitations of current national infrastructures to respond effectively and justly to disease.

Syndemic Interactions: Social Conditions

Latest global epidemiological estimates on childhood diarrhea project that only 2% of diarrhea episodes progress to severe disease, with a worldwide case-fatality ratio of 2% (CL Walker et al. 2013). However, diarrhea incidence and case-fatality ratios are much higher within low-income countries than in middle- and high-income countries. Asian and African world regions retain the greatest proportion of severe

diarrhea episodes at 26%. Fifteen countries (Afghanistan, Angola, Burkina Faso, China, Democratic Republic of the Congo, Ethiopia, India, Indonesia, Kenya, Mali, Niger, Nigeria, Pakistan, Tanzania, and Uganda) account for 53% of the total episodes of diarrhea globally and 56% of severe episodes. In 2011, 74% of the total burden of diarrhea mortality in children under 5 years was in these countries. The highest number of childhood deaths due to diarrhea in 2015 occurred in India (117,285) followed by Nigeria (76,980) (WHO 2015).

Infrastructural limitations and poverty conditions significantly increase exposure to diarrhea-causing pathogens. A UNICEF analysis based on 2015 estimates from the WHO and Maternal and Child Epidemiology Estimation Group and the UNICEF global databases from 2016 shows the negative relationship between improved sanitation facilities and under-five morbidity due to diarrhea (UNICEF 2017). However, infrastructural improvements are not enough; even within marginalized communities there are gradations of unhealthy environments that predispose children to increased burdens of disease. In India, for example, where 44% of the population practice open defecation, down from 75% in 1990 (WHO/UNICEF 2017), children are two standard deviations shorter than the reference mean (Spears 2013). Even children in the richest households in India are shorter than the international reference norms (Tarozzi 2008). While this may be interpreted as an inappropriate use of international normalizing standards (Panagariya 2012), Spears (2013) argues that the practice of open defecation creates a disease environment that exposes all residents to disease pathogens, although to varying degrees based on household economic capital and place of residence. In India, recurrent diarrhea and death from diarrhea are much more common among the lowest wealth index (Lahariya and Paul 2010; Avachat et al. 2011) despite somewhat universal exposure to diarrhea-causing pathogens.

Syndemic Interactions: Disease-Disease

Interactions of diarrhea pathogens with other disease states have been well documented (Singer 2010). Concurrent infections with intestinal helminth parasites have been shown to impair host immunity to enteric pathogens (Chen et al. 2005; Weng et al. 2007; Su et al. 2012). Among 30 hospitalized patients with strongyloidiasis (round worm) infections in central Kentucky state, 16 (53%) were found to have extraintestinal infections, with enteric organisms identified in 13 (81%) of these patients (Al-Hasan et al. 2007). Reasons for these relationships are not well understood, but warrant greater investigation.

Children living in diarrhea endemic areas are often at higher risk for other infectious diseases that are likely to have syndemic interactions with other pathogens, such as HIV/AIDS, pneumonia, malaria, tuberculosis, and measles. For example, a study of children under 2 years in Kenya revealed significantly greater episodes of diarrhea in children infected with HIV as compared to those without HIV (van Eijk et al. 2010). Conditions of marginalization increase the likelihood of exposure to diarrhea pathogens and contribute to HIV infection in infants. Barriers to

the prevention of mother-to-child-transmission of HIV in southern Africa overlap with factors that contribute to higher diarrhea burdens, including lower maternal education level, limited health service accessibility, and medical staff shortages (Gourlay et al. 2013).

In addition to HIV, children with measles have a greater likelihood of diarrhea. A prospective study of children in India in the late 1980s found that 25% of those with measles had associated diarrhea, and the diarrhea was significantly more dysenteric in nature (Deivanayagam et al. 1994). A more recent, multicountry study (children in the Democratic Republic of Congo, Ethiopia, India, Nigeria, and Pakistan) revealed a 12–22% reduction in diarrhea cases among children who received the measles vaccine in all countries except Ethiopia (Bawankule et al. 2017). Diarrhea associated with measles has compelled enhanced emphasis on the inclusion of measles vaccines in the GAPPD program.

Syndemic Interactions: Gut Microbiota

Extensive research exists on the dynamic relationships between malnutrition-and-diarrhea and increasingly environmental enteropathy (EE)-and-diarrhea. In isolation, malnutrition is well recognized as a widespread health problem, accounting for 11% of the total global DALYs (disability adjusted life years) (Black et al. 2008). In association with diarrhea, malnutrition is responsible for 53% of deaths in children under 5 years (Kosek et al. 2003). In an elegant study in mice, Coutinho et al. (2008) revealed the synergistic damage of the intestinal architecture caused by malnutrition and diarrheal pathogens, consequently limiting nutrient absorption. EE, the condition resulting from chronic exposure to fecal pathogens, is also marked by alterations in intestinal architecture and increased intestinal inflammation (Korpe and Petri 2012; Lin et al. 2013), and causes alterations in gastrointestinal tract (GIT) microbiota, which contributes to variation in mucosal immunity. Recent study findings indicate that rotavirus and oral polio vaccines are less effective in children who are malnourished and/or experience two or more episodes of diarrhea in their first month of life (Haque et al. 2014). Reasons for this are not well understood, although the findings indicate a complex interrelationship between diseases, immune response, and gut microbiomes, further suggesting the potential importance of syndemic interaction.

GIT microbiota are essential for the development of the gut-associated lymphoid tissue and play a vital part in shaping the immunological repertoire of the GIT (Mayer 2003; Quigley 2008), regulating the epithelial barrier function and GIT motility, and providing support for digestion and host metabolism. Microbiota prevent colonization by pathogens in large part through competition for nutrients and access to adhesive sites and receptors on the epithelial surface (Sekirov et al. 2010). Evidence suggests that GIT microbiota are 3–4 times lower following episodes of acute diarrhea (Albert et al. 1978; Tazume et al. 1990; Tazume et al. 1993). Iatrogenic suppression of GIT microbiota that occurs following antibiotic use, perhaps for repeated episodes of diarrhea or other bacterial and viral infections

including pneumonia and measles, can also disturb the normal mechanisms of GIT microbiota and possibly increase the likelihood of subsequent pathogenic infections (Cryan and O'Mahony 2011). Finally, normal colonization of the gut appears to commence at birth (Palmer et al. 2007). The microbiome of unweaned infants is simple, becoming more diverse and numerous after 1 year of age due to diet and environment (Kurokawa et al. 2007; Palmer et al. 2007). This makes infants and young children especially vulnerable to infection by multiple enteric pathogens, a process further facilitated by weaning from the protective components of breast milk. High-stress environments experienced by children living in marginalized conditions may alter the GIT microbiome sufficiently enough to increase the likelihood of repeated pathogenic infection by various microorganisms causing moderate-to-severe diarrhea in children.

Syndemic Interactions: Pathogen-Pathogen

Diarrhea-causing pathogens may also be interacting with each other in ways that increase the severity of disease symptoms, as co-infections are frequent. Recent findings of the Global Enteric Multicenter Study, a prospective case-control study of hospitalized pediatric patients with moderate-to-severe diarrhea in seven diverse sites in Africa and Asia (Kotloff et al. 2013), suggest that co-infection with multiple diarrhea-causing pathogens is common, at least among children with symptoms severe enough to interact with the formal health system. One or more pathogens were identified in 83% of diarrhea cases (compared to 72% in controls) and two or more agents in 45% of cases (compared to 31% in controls). Similarly in Brazil, 14% of previously collected rotavirus positive samples contained enteric adenovirus or norovirus (Ferreira et al. 2010). In India, diarrheal stool specimen analysis revealed that 63% of specimens had polymicrobial infections (Sinha et al. 2013).

The interactions between enteric pathogens that cause diarrhea are not yet fully understood. However, research is emerging that offers an indication of possible synergistic effects of pathogens in promoting disease. Observational studies suggest that molecular interactions may occur as a result of co-infections that exacerbate disease symptoms. For example, a prospective study of pediatric patients hospitalized for acute gastroenteritis in Rome, Italy, identified co-infection in 18% of patients (Valentini et al. 2013). Co-infected patients experienced more severe clinical presentations. A case-control study of 22 communities in Ecuador found evidence of rotavirus-Giardia and rotavirus-*E. coli* co-infections (Bhavnani et al. 2012). Researchers reported that during co-infection the pathogenic potential of each agent appeared enhanced. Collectively, these findings suggest that moderate-to-severe diarrhea in children may be the consequence of microbial interactions. Of course, these findings might also serve as markers for poor social conditions, whereby multiple pathogens are able to infect children, although not acting synergistically to worsen health outcomes. Additional investigations are needed to tease out the molecular and anatomical level interactions that may or may not be contributing to more severe clinical presentations.

As outlined, these complex relationships between disease states, pathogens, and microbiota suggest an adverse feedback loop involving high-stress environments resulting from social conditions of poverty and marginalization with diarrheal disease. These relationships are accelerated by and accelerate the development of multidisease syndemics that increase the burden of disease and negatively impact the social condition of sufferers (e.g., costs of care, long-term health impacts).

Syndemic Approaches to Childhood Diarrhea Management

Given the syndemic nature of childhood diarrhea, we believe that responses that address both disease and social interactions are likely to prove more effective in the long term. In Brazil, progressive social policies that complement the country's market-oriented reform policies provide a platform for a comprehensive syndemic approach to childhood diarrhea prevention. Policies on poverty alleviation and reduction of inequalities are in line with historical evidence that suggests that improvements in living conditions, not direct biomedical interventions or only improved supplies of water and sanitation services, have a more significant influence on reducing infectious diseases (McKeown 1978; Wegman 2001). Although also attempted in other countries in different ways, Brazil's *Bolsa Familia Program* (BFP) is the world's largest conditional cash transfer program for poverty reduction. Initiated in 2003, BFP distributed funds to over 25% of Brazil's population, or 13.4 million families in 2011, with a total budget of US$11.2 billion (Ministerio do Desenvolvimento Social e Combate a Fome 2013). Cash transfers range from US$18 to US$175 per month, depending on the income and composition of the family (Lindert et al. 2007). The conditional cash transfers require that children are sent to school where they receive at least one meal per day, vaccinations are completed per the Brazilian immunization program schedule (including rotavirus vaccine), routine health check-ups and growth monitoring are maintained per Ministry of Health guidelines, and women attend post-natal care services and receive health and nutritional education. An analysis of the effects of BFP on child survival revealed that under-five mortality decreased as program coverage increased, with the greatest positive impact occurring on poverty-related malnutrition and diarrheal disease (Rasella et al. 2013).

The BFP and other conditional cash transfer programs are not without their limitations and may not be appropriate in all situational contexts, particularly given the significant national investment required and potentials for misappropriation of funds. Nevertheless, the syndemic perspective presented here indicates that greater involvement of diverse stakeholders and strategies with equal or greater investments on improving social conditions can effectively address the biosocial dynamics of childhood diarrheal disease. The success of the BFP suggests that by operating through multiple mechanisms – improving social conditions by decreasing poverty and barriers to health care; improving food access and education; providing biomedical technology that directly addresses specific diarrheal diseases and

disease-disease interactions – as per the syndemic perspective results in significant improvements in childhood diarrhea.

Implications

Given the complex nature of childhood diarrhea including interactions between existing disease conditions, pathogens, gut microbiota, and social environments, the syndemic perspective offers a way forward for potential interventions. Interventions that focus only on technologically advanced biomedical strategies are likely to have only minimal impact or fail completely, as they do not address the social conditions of marginalized populations that promote disease clustering. However, only addressing the macro-level factors influencing childhood diarrhea, bypassing the disease completely, also has limitations. For example, in an effort to improve water distribution, particularly during times of scarcity, the Tanzanian government implemented pilot programs of a form of water "rights and fees" (World Bank 1996). A consistent year-round water supply is necessary for hygiene and sanitation practices and food productivity thereby indirectly reducing exposure to enteric pathogens and preventing malnutrition. The program requires that all commercial water users register with the government and pay a fee to obtain a "water right" which entitles them to an annual value of water resources. Evaluations of this program suggest that this form of government taxation has generated greater income and health inequalities, disproportionately burdening small-scale land owners who cannot afford to purchase the "water rights" (van Koppen et al. 2004).

Improving social conditions among marginalized communities is often considered outside the realm of health care, and consequently infrequently the focus of global public health interventions. Yet, history suggests that approaches that involve both the social and biomedical prove the most advantageous. As noted by Farmer and Becerra (2001):

> epidemic and endemic disease – are not solely biological; neither are they purely social. Yet, conventional studies typically rely on disciplinary approaches and fail to reveal the full complexity of these epidemics. Only by embracing a transdisciplinary, biosocial approach can we hope to describe fully these 'tropical' epidemics, and intervene successfully.

The GAPPD represents an emerging paradigm for global health by emphasizing diagonal financing and structuring (Ooms et al. 2008) (aiming for disease-specific results through improved health systems), with plurality across multiple sectors, collaboration between public and private partnerships, and local ownership. Programs like Brazil's BFP prove that such diverse approaches, although requiring significant national investment and coordination, can have profound effects on improving health conditions of marginalized communities. See Table 1 for an overview of current approaches to addressing childhood diarrhea.

Syndemics represent a critical element in the health profiles of disparity populations and constitutes an important contributor to addressing health inequality.

Table 1 Overview of current approaches to childhood diarrhea in comparison to an approach that considers the syndemic nature of ill health in addressing both disease agents and social factors

	Current approaches to childhood diarrhea			Syndemic approach
Program	*Integrated management of childhood illness*	*Integrated community case management*	*Integrated global action plan for the prevention and control of pneumonia and diarrhea*	*Bolsa Familia program, Brazil*
Aims	• Improving case management skills of health care staff for accurate identification of illness • Improvements in overall health system to ensure appropriate combined treatment of all major illnesses • Boosting community health practices to ensure appropriate care seeking, care management, and preventive practices	Aims to enhance preventive activities: • Exclusive breastfeeding in first 6 months of life • Improved vaccine coverage • ORS usage • Improved water and sanitation access in health care settings with accompanying hygiene practices	Cohesive policy framework aiming to: • Close service delivery gaps • Promote best practices and proven strategies • Establish healthier environments	Conditional cash transfer program requires that: • Children are sent to school • One meal per day at school • Vaccinations are complete per the Brazilian immunization program schedule • Routine health check-ups and growth monitoring are maintained • Women attend post-natal care services
Pros	Costs up to six times less per child than standard of care Improves nutritional status and reduces under-five mortality (WHO 2016)	Resurgence of community health workers in delivery of case management of childhood illness	Integrated, multidimensional approach	Decrease in under-five mortality, with greatest impact on diarrheal disease and malnutrition (Rasella et al. 2013)
Cons	"Good" care is low in high diarrhea burden countries (Carvajal-Velez et al. 2016) Inequitable supply of key commodities and unequal provision, limited funding, poor coordination, inadequate advocacy, delayed national policies (Gill et al. 2013)	Poor incentives to attract or retain community health workers, and limited provision of appropriate treatments (Strachan et al. 2012; Gill et al. 2013)	Lack of direct attention to addressing social drivers of disease, or assessing interest and willingness of local communities to comply (Gill et al. 2013)	Significant national investment and coordination, with potential for misappropriation of funds

The study of syndemics has been termed a "promising new frontier for public health action in response to the critical challenges of our time" (Leischow and Milstein 2006). A *Lancet* Series on syndemics highlights key ways in which the syndemic framework can advance public health approaches to specific diseases like childhood diarrhea (Hart and Horton 2017; Mendenhall 2017; Mendenhall et al. 2017; Singer et al. 2017; The Lancet 2017; Tsai et al. 2017). First, social, political, and ecological factors are recognized as both creating and perpetuating structural vulnerabilities that contribute to syndemic interactions. Second, an understanding of how certain individuals and groups, and not others, are vulnerable to certain syndemics is offered. Third, current strategies of prevention and care are strengthened by considering the full scope of vulnerabilities and intervening at policy and clinical levels to address the root causes of sickness and disease treatments.

Policies and Protocols

- The biosocial nature of diarrhea-related malnutrition must be acknowledged, with any intervention addressing disease combining efforts to address both the biological and social components, including a reduction in poverty and inequality, improved infrastructure and housing, and access to education and health care.
- Interventions targeting diarrhea-related malnutrition must work across government sectors, including health care, economy, housing, and infrastructure to prove effective.
- Marginalized individuals and communities must be encouraged to participate in health maintenance. Supportive social structures can include incentives, such as free meals at school and free access to preventive health care services.

Dictionary of Terms

- **Syndemic** – The adverse interaction of two (or more) diseases or health conditions within a specific population given exacerbating social factors.
- **Syndemic interaction** – The co-occurrence of social and health conditions, including social–psychological, social–biological, and psychological–biological interactions, which worsen the condition of the person or population afflicted.
- **Syndemic perspective** – The recognition that adverse social conditions, including social inequality, marginality, poverty, and other forms of political and economic oppression, play a critical role in facilitating the clustering of both infectious and noninfectious diseases that lead to heightened potential for deleterious disease interactions.
- **Syndemic approach to childhood diarrhea** – Comprehensive and multipronged approaches that address both proximal causes of disease (e.g., improved access to rotavirus vaccine, treatment antibiotics, rehydration solutions, improved sanitation and hygiene infrastructure) and macro social factors (e.g., policies on poverty alleviation, reduction of inequalities, and improvements in living conditions).

Summary Points

- This chapter draws on available research and current policy agendas, to examine current approaches to diarrheal disease management in order to consider novel comprehensive disease prevention approaches, consistent with syndemic theory, that are likely to prove effective in generating sustainable and equitable disease prevention solutions.
- Diarrhea remains the second leading cause of death in children under 5 years. Moreover, frequent bouts of diarrhea result in irreversible limitations on physical and cognitive development, particularly in marginalized communities. Children are especially vulnerable as they are unable to mount an active immune response to pathogen exposure and these biological limitations are exacerbated by social conditions.
- Syndemic theory offers a platform to explore the role of adverse biosocial interactions that increase the total disease burden of diarrhea-related malnutrition in low-resources populations and assess the limitations of recent global calls to action.
- The syndemic perspective describes situations in which adverse social conditions, including inequality, poverty, and other forms of political and economic oppression, play a critical role in facilitating disease-disease interactions.
- Given the complex micro and macro nature of childhood diarrhea-related malnutrition, including interactions between pathogens, disease conditions, and social environments, the syndemic perspective offers a new approach to disease management. While rarely the focus of health interventions, the syndemic approach suggests a coupling of advanced biomedical technologies with economic and political interventions that address the social conditions of disparity.

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Metabolic Syndrome and Social Deprivation

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Marie Blanquet, Anne Debost-Legrand, and Laurent Gerbaud

Contents

Abstract

The metabolic syndrome is a set of disorders responsible for an increased risk of all-cause and cardiovascular mortality. Social deprivation is defined as the lack of

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[one or more of the prerequisites of] security, enabling persons to assume their responsibilities into the society and to enjoy basic rights. A review of the literature published over the past 16 years (2000–2016) was performed to analyze the association between metabolic syndrome and social deprivation. Of the 652 references reviewed for title and abstract, 78 studies were selected and 55 articles were considered. The absence of uniformity in the measures used to assess metabolic syndrome and social deprivation requires some modulation of the results of our analysis. The studies were classified according to the Human Development Index (HDI) of the countries where they took place. The principal risk factors identified for metabolic syndrome are a low educational level (women especially), low income, occupation (specifically an unskilled job or absence from the labor force), and greater socioeconomic deprivation, measured by composite indices. These results are stable according to HDI level.

In conclusion, access to education, the possibility of superior education, and the acquisition of occupational credentials are three themes to prioritize in developing public health policies, irrespective of the country's level of development.

Keywords
Metabolic syndrome · Social deprivation · Geographic indices · Individual deprivation

List of Abbreviations

AHA	American Heart Association
HDI	Human Development Index
IDF	International Diabetes Foundation
NCEP-ATPIII	National Cholesterol Education Program Adult Treatment Panel III
WHO	World Health Organization

Introduction

The metabolic syndrome (MetS) is a set of disorders that includes elevated fasting glucose, high blood pressure, dyslipidemia, and abdominal obesity and is responsible for an increased risk of all-cause and cardiovascular mortality (Isomaa et al. 2001; Lakka et al. 2002; Hu et al. 2004; Eckel et al. 2005). The pathophysiologic basis of this syndrome is complex, and its first-line management consists of dietary and lifestyle changes, including a reduction in the intake of both calories and atherogenic fat as well as an increase in physical activity (Eckel et al. 2010).

Social deprivation has been defined by J. Wrezinski as "the lack of [one or more of the prerequisites of] security, such as a job, enabling individuals and families to assume occupational, family, and social responsibilities and to enjoy basic rights" (Wrezinski 1987). At the same time, Townsend defined the concept of deprivation as a "state of observable and demonstrable disadvantage relative to the local

community or the wider society to which an individual, family or group belongs." He applied this concept to conditions rather than resources and distinguished between deprivation and poverty (Townsend 1987). He also argued that deprivation is the main cause of inequalities in health and developed an index to measure deprivation over given geographic areas (Carstairs and Morris 1989).

This analysis of the association between MetS and social deprivation is based on a review of the literature published over the past 16 years (2000–2016), identified by a search on PubMed. The keywords used for this search were metabolic syndrome, psychosocial deprivation, economic status, and socioeconomic status. In all, 652 references were reviewed by title and abstract and 78 studies were selected for complete reading. Only the 55 articles with multivariate analyses were considered in this review.

The absence of uniformity in the measures used to assess MetS and social deprivation requires some modulation of the results of our analysis. We report here the different measures used to assess MetS and social deprivation as well as their association. Finally, we also analyze the association between this syndrome and social deprivation together with other factors, such as sex and level of education.

Key Principles

Definitions of Metabolic Syndrome

Its first recognized definition was established by the World Health Organization (WHO) and emphasized impaired glucose regulation in particular (Alberti and Zimmet 1998). Subsequent definitions included one by the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) and another by the International Diabetes Foundation (IDF) (Alberti et al. 2005, 2006; Grundy et al. 2004). Finally, in 2009, several international medical federations and societies reached a consensus and established a harmonized definition that enables international comparisons (Alberti et al. 2009). Definitions by the following groups were used in the selected studies, listed in order of decreasing frequency: NCEP-ATPIII, the harmonized definition, American Heart Association (AHA), IDF, and WHO (Table 1). Thirteen studies measured the prevalence of the MetS with at least two definitions.

Measures of Deprivation

Validated tools exist for measuring social deprivation. Some are ecological area measures, by geocoding; the most frequently used are those by Townsend, Carstairs, and Pampalon. The principal individual tool is the EPICES score (Pampalon and Raymond 2000; Townsend 1987; Carstairs and Morris 1989). Among the selected studies, three used the EPICES score, while the others did not use any specific measure of social deprivation. The indicators used in these studies, in order of

Table 1 Definitions of the metabolic syndrome

	WHO 1998	NCEP-ATP III 2001	AHA 2005	IDF 2006	Harmonized definition 2009
Metabolic syndrome	Glucose intolerance, IGT or T2D and/or IR + 2 or more criteria	3 or more criteria	3 or more criteria	Abdominal obesity + 2 other factors	3 or more criteria
Waist to hip ratio and/or BMI	M > 0.90; W > 0.85 >30 kg/m^2				
Waist circumference (cm)		M > 102 W > 88	M > 102 W > 88	Europe: M ≥ 94; W ≥ 80 South Asians/ Chinese M ≥ 90; W ≥ 80 Japanese: M ≥ 85; W ≥ 80	Population- and country-specific definitions
Blood pressure (mmHg)	≥160–90	≥130/85	≥130/85 or med for HBP	sBP ≥130 or dBP ≥ 85 or med for HBP	sBP ≥130 and/or dBP ≥ 85 or med for HBP
Triglycerides mmol/l (mg/dL)	≥1.7 (150)	≥1.7	≥1.7	≥1.7 or med for HTG	≥1.7 or med for HTG
HDL-C mmol/l (mg/dL)	HTG and/or M < 35; W < 39	M < 1.0; W < 1.3	M < 1.0; W < 1.3	M < 1.0 (40); W < 1.3 (50) or med for rHDL-C	M < 1.0; W < 1.3; or med for rHDL-C
Fasting glucose mmol/l (mg/dL)	IGR or T2D, IR	≥6.1 (110)	≥5.6 (100) or diagnosed T2D	≥5.6 or diagnosed T2D	≥5.6 or T2D
Others	μalb ≥20 μg/min or acr ≥ 20 mg/g				

WHO World Health Organization, *NCEP-ATP III* American National Cholesterol Education Program Adult Treatment Panel III, *AHA* American Heart Association, *IDF* International Diabetes Federation, *IGT* impaired glucose intolerance, *T2D* type 2 diabetes, *IR* insulin resistance, *BMI* body mass index, *M* men, *W* women, *sBP* systolic blood pressure, *dBP* diastolic blood pressure, *med* medication, *HBP* high blood pressure, *HTG* hypertriglyceridemia, *HDL-C* high density lipoprotein cholesterol, *rHDL-C* reduced HDL-C, *IGR* impaired glucose regulation, *μalb* microalbuminuria, *ac* albumin/creatinine ratio

decreasing frequency, were educational level, income, occupation category (including nonparticipation in the labor force), social class, and simple or composite indices of poverty.

Association Between Metabolic Syndrome and Deprivation

The studies were classified according to the Human Development Index (HDI) of the countries where they took place. The HDI scores used here come from the 2014 United Nations Development Program (Programme des Nations Unies pour le Développement 2015).

Main Conclusions

The principal risk factors identified for MetS are a low educational level, low income, occupation (specifically an unskilled job or absence from the labor force), and greater socioeconomic deprivation, measured by composite indices. The role of educational level appears to differ between men and women, with a low educational level consistently found to be a risk factor for women but not men. These results are stable according to HDI level.

In countries with a very high HDI [0.800; 1], Lidfeldt et al. included women from 50 to 60 years old at the time of the study. They found that MetS was more frequent among women with low educational level compared with women having a university degree. On the other hand, no association was found between MetS and working status in this population (Lidfeldt et al. 2003). Park et al. performed two cross-sectional studies in 1988–1991 and 1991–1994 in the general population aged 20 years old or above. Low participant's household income in women was the sole factor identified to associate with higher prevalence of MetS. Education was not associated with MetS neither in men nor in women (Park et al. 2003). Carnethon et al. used the education level to explore socioeconomic status among young adults in the Coronary Artery Risk Development in Young Adults (CARDIA) study and found that low educational level was associated with MetS in women only and irrespective of the women ethnic groups (Carnethon et al. 2004). Dallongeville et al. found that MetS decreased with high educational level both in men and women. High household income (estimated by family income tax) and high occupational categories were at low risk of MetS in women only. Finally, tenants were at higher risk of MetS than owners, irrespective of the gender (Dallongeville et al. 2005). Langenberg et al. who included persons 53 years old in 1999 found that least educated people were at higher risk of MetS in men only. No evidence was found for social class irrespective of the sex (Langenberg et al. 2006). Loucks et al. focused their study on civilian non-institutionalized population aged at least 25 years old and showed that low education is related to MetS both in men and women. The research team used the poverty income ratio (PIR), which is the ratio of the midpoint of

observed family income category to the official poverty threshold, and found no significant result (Loucks et al. 2007). Perel et al. included people aged from 35 to 55 years old based on office staff. Education, employment grades, annual personal income, annual household income, and amounts of money the respondent would have if all household assets were cashed and debts all paid were assessed. In men, low employment grades, low annual personal income, low annual household income, and low amount of money were associated with MetS, whereas for women, this association was found for low education only (Perel et al. 2006). La Rosa et al. used the EPICES score to measure deprivation among patients of health examination centers aged more than 16 years old and found that deprived patients were at higher risk of MetS (La Rosa et al. 2008). Lucove et al. included 25- to 50-year-old community-based persons; evaluated education, home ownership, employment status, and occupation; and found that high education level was at lower risk of MetS (Lucove et al. 2007). Park et al. included people age of more than 20 years old in general population and showed that women with high education and upper monthly income were at low risk of MetS, whereas no significant result was found for men (Park et al. 2007). Prescott et al. targeted people aged of at least 60 years old and evaluated duration of schooling, education, longest held occupation, and household income. They demonstrated that high education level was associated with low risk of MetS (Prescott et al. 2007). Salsberry et al. used also the PIR to measure deprivation status and that low PIR was related to MetS in women only (Salsberry et al. 2007). Chichlowska et al. studied family income and neighborhood Z-score in general population aged from 45 to 64 years old, stratifying on gender and ethnicity. For women, both low family income and low neighborhood Z-score were associated with high risk of MetS either in Black or White. For men, in Black only medium neighborhood Z-score was related to MetS and in White only intermediate family income (Chichlowska et al. 2008). Ramsay et al. assessed social class in a cohort at two times and found that manual class was at higher risk of MetS (Ramsay et al. 2008). Santos et al. evaluated MetS in non-institutionalized inhabitants of more than 40 years old in Porto and revealed that low education, housewives, and low social classes were associated with MetS in women only (Ramsay et al. 2008). Scuteri et al. included people aged from 42 to 52 years old and demonstrated that low education level was related to MetS, whereas no significant result was found for yearly income (Scuteri et al. 2008). Manuck et al. assessed deprivation with subjective socioeconomic status by Mac Arthur scale and objective socioeconomic status by education, annual family income, and computed measure by averaging standardized values of the two index variables for each individual. They demonstrated that both subjective and objective socioeconomic status and MetS were associated inversely (Manuck et al. 2010). Phillips et al. performed a study on men who served in Vietnam from 1965 to 1971. They used monthly income, household income in midlife, education, and index of occupational prestige to analyze socioeconomic status. They showed that men in the lower two groups of combined measures were at risk of MetS (Phillips et al. 2010). Riediger et al. explored education and income adequacy in the general population aged at least 18 years old. They revealed that people with high education and the highest income adequacy experienced lower risk of MetS

(Riediger and Clara 2011). Sygnowska et al. performed a study in general population aged from 20 to 74 years old, using education and per capita income with two definitions of MetS. They showed that in women, for the both definitions, higher education level was a protective factor (Sygnowska et al. 2012). Navarro et al. assessed MetS in postmenopausal Caucasian women by calculation poverty index based on annual income of the family. They found no significant results (Navarro et al. 2013). Al-Daghri considered patients of a primary health-care center aged from 18 to 70 years old regarding total monthly household income, occupation, and education. They showed that low income was the protective factor of MetS and that retired men and unemployed women experienced higher risk of MetS. They revealed also that education in women was inversely associated with MetS (Al-Daghri et al. 2014). Keita et al. focused their study on residents in the lower 48 states of the USA aged more than 45 years old and explored socioeconomic status by measuring neighborhood deprivation, education, and total annual household income. In White adults, the most the neighborhood deprivation is, the higher the MetS risk is (Keita et al. 2014). Yang et al. carried a study on person aged more than 20 years old on education, current occupation, equalized gross household income per month, and combined adult socioeconomic status. They revealed that in women, low educational level, manual and inactive, low equalized gross household income, and low combined adult socioeconomic status were associated with higher risk of MetS. On the other side, in men, low education level and manual and inactive were at lower risk of MetS (Yang et al. 2014).

Blanquet et al. used EPICES score on patients consulting in health examination centers aged at least 16 years old. The most deprived persons experienced higher risk of MetS (Blanquet et al. 2016). Gannar et al. used a combined measure based on income, crowding, and education on persons aged 18–75 years old in the general population. They revealed that higher combined measure was a protective factor of MetS in the Spanish population. Opposite results were found for Tunisian men (Gannar et al. 2015). Lim et al. studied education and economic status in patients aged more than 20 years old in a health center and did not found significant results. (Lim and So 2015). Ortiz et al. used the ENRICHD social support inventory and a combined measure of education and income (socioeconomic position) in adult of a former cohort (MESA Latino cohort). They showed that high socioeconomic position was a protective factor of MetS (Ortiz et al. 2015). Martin et al. performed a study on 25–54-year-old permanent residents of the Illawarra region, and they did not found any significant results (Martin et al. 2016). Klijs et al., who evaluated education and household equivalent income in a Dutch cohort aged more than 30 years old, revealed that low education was associated with higher risk of MetS (Klijs et al. 2016). The results are summarized in Table 2.

In countries with a high HDI [0.700; 0.800], Schooling et al. carried a study on persons aged at least of 50 years old and measured education and longest held occupation. They found that high education was a protective factor in women only (Schooling et al. 2008). Erem et al. explored in general population aged 20 years old or above education, occupation, and household income. They showed that education was inversely related to MetS and that housewives were at higher risk of MetS

Table 2 Studies performed in countries with a very high human development index

Reference	Population	Socioeconomic status	Results of the multivariate analysis (ORa)
Lidfeldt et al. 2003 (Sweden)	Women's birth date between 02/12/1935 and 01/12/1945	**Education** (comprehensive school; upper secondary school; university degree)	**(ref university degree)**: comprehensive school 1.62 [1.41; 1.87]; upper secondary school 1.40 [1.24; 1.57]
		Working status (full time; part time; unemployed/ long-term sick list/with a disability pension)	*ns*
Park et al. 2003 (USA)	≥20 years old in the general population	**Education** (years) (<8; 8–12; >12; unknown)	**Women and men**: *ns*
		Participant's household income for the previous year (≤$15,000; $15,001–25,000; >$25,000; unknown)	**Women**: (ref > $25,000): ≤15,000$ 1.5 [1.0; 2.3] **Men**: *ns*
Carnethon et al. 2004 (USA)	18–30 years old in general population	**Education** (high school or less; more than high school)	(ref more than high school): **Overall**: high school or less 1.52 [1.24; 1.87] **Black women**: high school or less 1.84 [1.20; 2.82] **White women**: high school or less 1.63 [1.05; 2.52] **Men**: *education ns*
Dallongeville et al. 2005 (France)	35–64 years old	**Education** (primary; secondary/technical; university)	**Men**: (ref primary): university 0.52 [0.37; 0.73] **Women**: (ref primary): intermediate 0.61 [0.44; 0.84]; university 0.33 [0.22; 0.51]
		Accommodation status (owners; tenants)	**Men**: (ref owners): tenants 1.54 [1.16; 2.03] **Women**: (ref owners): tenants 1.54 [1.12; 2.10]
		Household income estimated by family income tax (no income tax; <€760; €760–2300; >€2300)	**Men**: ns **Women**: (ref no income tax): <€760 0.66 0.44; 0.99]; €760–2300 0.52 [0.36; 0.76]; >€2300 0.38 [0.25; 0.57]
		Occupational categories (skilled manual workers; company clerks; middle executives; senior executives)	**Men**: ns **Women**: (ref unskilled manual): middle executive

			0.52 [0.34; 0.81] senior executive 0.36 [0.18; 0.73]
Langenberg et al. 2006 (UK)	53 years old in 1999	**Education** (higher education; advanced secondary education; ordinary secondary education; below ordinary secondary qualifications)	**Men**: (ref most educated): least educated 2.5 [1.3; 4.5] **Women**: *ns*
		Social class (professional; intermediate; skilled nonmanual; skilled manual; semiskilled and unskilled)	**Women**: *ns*
		Cohort members' occupation at the age of 53 years or 43 years	**Women and men**: *ns*
Loucks et al. 2007 (USA)	≥25 years old civilian noninstitutionalized population	**Education** (years) (<12; 12; >12)	**Women**: (ref > 12): <12 1.77 [1.39; 2.24]; 12 1.46 [1.16; 1.84] **Men**: (ref > 12): 12 1.32 [1.04; 1.68]
		Poverty income ratio (PIR)	–
Perel et al. 2006 (UK)	35–55 years old in London-based office staff	**Education** (higher education; advanced secondary qualifications; ordinary secondary qualifications; no academic qualifications)	**Women and men**: *ns*
		Employment grades (unified grades 1–7 = high employment grades; executive officers = middle grades; clerical and support staff = lower grades)	**Women**: (ref administrative): clerical/office support 1.91 [1.17; 3.12] **Men**: (ref administrative): professional/executive 1.32 [1.11; 1.56]; clerical/office support 1.64 [1.20; 2.24]
		Annual personal income (≥£50 000; £25 000–49 999; £ 15 000–24 999; <£15,000)	**Men**: (ref ≥ £50 000): <£15 000 1.41 [1.05; 1.90] **Women**: ns
		Annual household income (≥£60 000; £40 000–59 999; £20 000–39 999; <£20 000)	**Men**: (ref ≥ £60 000): <£20 000 1.54 [1.11; 2.14] **Women**: (ref ≥ £60 000): £40 000–59 999 2.41 [1.04; 5.60]; £20 000–39 999 2.51 [1.15; 5.49]; <£20 000 3.43 [1.54; 7.62]
		Amount of money the respondent would have if s/he cashed in all household assets and paid off all	**Men**: (ref ≥ £500,000): £40 000–99 999 1.61 [1.06; 2.47]; <£40 000 2.26 [1.43; 3.57]

(*continued*)

Table 2 (continued)

Reference	Population	Socioeconomic status	Results of the multivariate analysis (ORa)
		debts (≥£500,000; £100,000–499,999; £40 000–99 999; <£40 000)	**Women**: (ref ≥ £500,000): £40 000–99 999 2.71 [1.04; 7.07]; <£40 000 4.12 [1.57; 10.8]
La Rosa et al. 2008 (France)	16–91 years old visited health examination center	**EPICES score**: deprived ≥ 40	(ref not deprived): 1.22
Lucove et al. 2007 (USA)	25–50 years old, community based	**Education** (≥high school; <high school),	(ref < high school): 0.70 [0.51; 0.96]
		Home ownership (not home owner; home owner)	*ns*
		Employment status (unemployed; employed)	*ns*
		Occupation (unskilled; skilled)	*ns*
Park et al. 2007 (Korea)	>20 years old in general population	**Education (years)** (<7; 7–9; 10–12; >13).	**Women**: (ref <7): 10–12 0.55 [0.44; 0.70]; >12 0.31 [0.22; 0.43] **Men**: *ns*
		Monthly income (lower <€1400; middle €1400–2500; upper > €2500)	**Women**: (ref lower): upper 0.80 [0.66; 0.97] **Men**: *ns*
Prescott et al. 2007 (Denmark)	≥60 years old	**Duration of schooling** (3 categories)	–
		Education (1=low =none; short; skilled worker; more advanced; 5= high=complete college)	(ref 1=low): level 3 0.68 [0.57; 0.81]; level 4 0.60 [0.47; 0.76]; level 5 0.40 [0.30; 0.53]
		Longest held occupation (6 categories)	–
		Household income (6 levels)	–
Salsberry et al. 2007 (USA)	≥21 years old	**Poverty income ratio** (PIR) (low; middle; high)	**Women**: (ref high): middle 1.58 [1.09; 2.29]; low 1.89 [1.28; 2.81] **Men**: *ns*

Chichlowska et al. 2008 (USA)	45–64 years old	**Family income** (<$24,000; $24,000–$49,000; ≥$ 50,000)	**Black women**: (ref ≥$ 50.000): $24.000–$49.000 1.56 [1.03; 2.34]; <$24.000: 1.92 [1.41; 2.61] **White women**: (ref ≥$ 50.000): $24 000–$49 000 1.20 [1.05; 1.37]; <$24.000: 1.36 [1.18; 1.58] **Black men**: *ns* **White men**: (ref ≥$ 50 000): $24 000–$49 000 1.14 [1.03; 1.26]
		Neighborhood deprivation (tertiles of Z-score high; medium; low)	**Black women**: (ref high): medium 1.25 [1.03; 1.53]; low 1.20 [1.04; 1.40] **White women**: (ref high): medium 1.14 [1.00; 1.30]; low 1.17 [1.00; 1.37] **Black men**: (ref high): medium 1.28 [1.01; 1.62] **White men** : *ns*
Ramsay et al. 2008 (UK)	1st part men 40–59 years old; 2nd part men 1998–2000 all surviving subjects aged 60–79 years old	**Social class** (I professional; II managerial; III nonmanual; IV partly skilled; V unskilled)	*ns*
		Social class (manual III, IV, V; nonmanual I, II)	(ref nonmanual): manual 1.21 [1.2; 1.43]
Santos et al. 2008 (Portugal)	≥40 years old, noninstitutionalized inhabitants Porto	**Education** (years) (0–4; 5–11; ≥12)	**Women**: (ref ≥ 12): (0–4 years) 2.28 [1.48; 3.52] **Men**: *ns*
		Occupation (active; retired; unemployed; housewives)	**Women**: (ref active): housewives 1.77 [1.16; 2.70] **Men**: *ns*
		Social classes (I = upper class to V lower class)	**Women**: (ref I): IV 2.56 [1.45; 5.72]; not employed 2.59 [1.32; 4.79] **Men**: *ns*
Scuteri et al. 2008 (USA)	42–52 years old	**Education** (<high school; high school graduate/ equivalent; some college; college graduate; post-college education)	(ref post-college education): high school or less 1.38 [1.02; 1.85]
		Yearly income (<20,000$; $20,000–34,999; $35,000–49,999; $50,000–74,999; >$75,000)	–

(*continued*)

Table 2 (continued)

Reference	Population	Socioeconomic status	Results of the multivariate analysis (ORa)
Manuck et al. 2010 (USA)	30–54 years old in Community Volunteers	**Subjective SES**: MacArthur scale of subjective social status	*ORa 0,79 [0,67; 0,93]*
		Objective SES: **education; annual family income** (<$ 25,000; 25–34,999; 35–49,999; 50–64,999; 65–80,000; >80,000)	*ORa 0,79 [0,66; 0,93]*
		Computed measure (Z-score)	–
Phillips et al. 2010 (USA)	Men who served in Vietnam from 1965–1971	**Monthly income** (≤$144/month; >$144/month)	**5 multivariate models (A B C D E)**: –
		Household income in midlife (≤$20,000/year; >$20,000/year)	**5 multivariate models (A B C D E)**: (ref high): low (A) 1.27 [1.05; 1.54]; (B) 1.41 [1.15; 1.72]; (C) 1.23 [1.01; 1.50]; (D) ns; (E) 1.23 [1.01; 1.49]
		Education (≤12 years; >12 years)	**5 multivariate models (A B C D E)**: (ref high): low (A) 1.30 [1.09; 1.55]/(B) 1.31 [1.10; 1.56]; (C) 1.26 [1.05; 1.51); (D) ns; (E) 1.28 [1.07; 1.53]
		Index of occupational prestige (≤50th percentile; >50th percentile)	**5 multivariate models (A B C D E)**: (ref high): low (A) 1.35 [1.13; 1.61]; (B) 1.35 [1.15; 1.65]; (C) 1.30 [1.08; 1.56]; (D) 1.21 [1.00; 1.47]; (E) 1.32 [1.11; 1.58]
Riediger et al. 2011 (Canada)	≥18 years old in general population	**Education** (<secondary school graduation; secondary school graduation; some post-secondary education; postsecondary education)	(ref secondary school graduation): some post-secondary 0.3 [0.13; 0.7]; post-secondary graduation 0.45 [0.25; 0.81]
		Income adequacy (lowest income group; lower-middle income group; upper-middle income group; highest income group)	(ref lower middle income): upper middle income 0.49 [0.26; 0.93]; highest income 0.4 [0.21; 0.78]
Sygnowska et al. 2012 (Poland)	20–74 years old in general population	**Education** (<secondary; secondary; higher)	**AHA**: **Women**: (ref <secondary): higher 0.72 [0.54; 0.98] **IDF**: **Women**: (ref <secondary): higher 0.72 [0.55;

			0.95] **AHA and IDF**: **Men**: *ns*
		Per-capita income	**AHA**: **Women**: *ns* **IDF**: **Women**: *ns* **AHA and IDF**: **Men**: *ns*
Navarro et al. 2013 (Spain and Tunisia)	Postmenopausal Caucasian women	**Poverty** = annual income <€6346.8/family member	*ns*
Al-Daghri et al. 2014 (Saudi Arabia)	18–70 years old and visited primary health care center	**Total monthly household income** (upper income class; middle; low)	**Men**: (ref low income): middle class 2.2 [1.5; 3.5]; upper class 2.3 [1.5; 3.5] **Women**: (ref low income): middle class 0.65 [0.48; 0.89]
		Occupation (government; private; retired; unemployed)	**Men**: (ref government): retired 1.4 [1.0; 1.9] **Women**: (ref government): unemployed 1.6 [1.2; 2.2]
		Education (uneducated; precollege; college/high education)	**Men**: *ns* **Women**: (ref uneducated): higher education 0.38 [0.26; 0.56]
Keita et al. 2014 (USA)	>45 years old resident in the lower 48 states of the United States	**Neighborhood deprivation** (Quintiles of Z-score Q1 most deprived to Q5 least deprived)	**White adults**: (ref: Quintile 5): Q1 1.78 [1.50; 2.12]; Q2 1.62 [1.39; 1.88]; Q3 1.56 [1.36; 1.79]; Q4 1.38 [1.21; 1.57] **Black adults**: *ns*
		Education (<high school; high school; some college; college)	*Model adjusted for education*
		Total annual household income (<$20K. $20K–34K; $35K–74K; ≥$75K)	*Model adjusted for income*
Yang et al. 2014 (Korea)	>20 years old	**Education** (high; middle; elementary school/low)	**Women**: (ref university): high school graduate 1.78 [1.38; 2.28]; middle school graduate 2.18 [1.63; 2.90]; elementary school or lower 3.29 [2.45; 4.42]

(*continued*)

Table 2 (continued)

Reference	Population	Socioeconomic status	Results of the multivariate analysis (ORa)
			Men: (ref university): elementary school/low 0.76 [0.60; 0.96]
		Current occupation (Nonmanual; manual; inactive)	**Women**: (ref nonmanual): manual 1.34 [1.04; 1.73]; inactive 1.40 [1.09; 1.81] **Men**: (ref nonmanual): manual 0.82 [0.69; 0.98]; inactive 0.79 [0.64; 0.99]
		Equalized gross household income per month (high; middle; low)	**Women**: (ref high): middle 1.16 [1.00; 1.35]; low 1.60 [1.32; 1.94] **Men**: *ns*
		Combined adult socioeconomic status (higher scores= lower SES)	**Women**: (ref highest quartile): quartile 3 1.81 [1.33; 2.46]; quartile 4 (lowest) 2.69 [1.92; 3.78] **Men**: *ns*
Blanquet et al. 2016 (France)	≥16 years old consulting in health examination centers	**EPICES score**: Binary (deprived ≥ 30.17; nondeprived < 30.17) By quintile (from Q1 least deprived to Q5 most deprived) Continuous variable	**Low waist circumference**: (ref Q1): Q2 1.18 [1.06 ; 1.31]; Q3 1.35 [1.21 ; 1.51]; Q4 1.72 [1.54 ; 1.92]; Q5 2.47 [2.21 ; 2.77] **High waist circumference**: (ref Q1): Q2 1.16 [1.03 ; 1.31]; Q3 1.38 [1.21 ; 1.56]; Q4 1.71 [1.51 ; 1.94]; Q5 2.69 [2.38 ; 3.05]
Gannar et al. 2015 (Spain and Tunisia)	18–75 years old in the general population	**ICE (higher social class with higher value) = income** (family income in quintiles); **crowding** (household crowding index); **education**	**Spain**: **Men**: (ref ICE1): ICE 5 0.67 [0.51; 0.90] **Spain**: **Women**: (ref ICE1): ICE2 0.72 [0.58; 0.90]; ICE4 0.65 [0.50; 0.85]; ICE5 0.39 [0.29; 0.53] **Tunisia**: **Men**: (ref ICE1): ICE4 3.88 [1.05; 14.27] **Tunisia**: **Women**: *ICE ns*

Lim and So 2015 (Korea)	>20 years old who visited a health center in Seoul	**Education** (elementary school or lower; middle school; high school; college or higher)	*ns*
		Economic status (very poor; poor; rich; very rich)	*ns*
Ortiz et al. 2015 (USA)	Adults of the MESA Latino sample	**Social support** was assessed with the ENRICHD Social Support Inventory (ESSI) (high= high support)	*ns*
		Socioeconomic position (SEP) = Education + Income	0.91 [0.85; 0.99]
Martin et al. 2016 (Australia)	25–54 years old, permanent residents of the Illawarra region	**Education**	*ns*
		Average household income	*ns*
Klijs et al. 2016 (Nertherlands)	≥30 years old in the Dutch Life Lines Cohort Study	**Education** (tertiary; upper secondary; lower secondary; elementary)	(ref tertiary): upper secondary 1.54 [1.25; 1.90]; lower secondary 1.55 [1.18; 2.04]
		Household equivalent income (<€1100; €1100–1499; €1500–1899; ≥€1900/month)	*ns*

ORa adjusted odds ratio, *ref.* reference, *ns* nonsignificant

(Erem et al. 2008). Marquezine et al. measured socioeconomic classes through a population aged 25–64 years old in residents of the municipality of Vitoria. They revealed that the most deprived women experienced higher risk of MetS (Marquezine et al. 2008). Zuo et al. measured education and economic status among people aged 35–74 years old and found that low education level was associated with higher risk of MetS (Zuo et al. 2009). Silveira explored education and family income in persons born in 1982 considering two definitions of MetS. In women, low education was associated with high risk of MetS regardless of the definition, whereas upper family income was a protective factor (da Silveira et al. 2010). Ferguson et al. performed a study in 25–74-year-old people by measuring education and total monthly household income. For men, high education level and also high total monthly household income were associated with higher risk of MetS (Ferguson et al. 2010). Romaguera et al. considered education level of 36–82-year-old women for two MetS definitions and found that high education level was a protective factor when the IDF definition was used (Romaguera et al. 2010). Sidorenkov et al. included outpatient clinic in northwest Russia aged 18 years old or above and measured education and income without any significant results (Sidorenkov et al. 2010). Belfki et al. focused their study on 35–74-year-old persons in the general population and explored education and occupation. They showed that high education level was associated with higher risk of MetS, whereas intermediate occupation was a protective factor in men (Belfki et al. 2013). Gronner et al. measured education and family income in 30–79-year-old urban population and demonstrated that low education was at risk of MetS (Gronner et al. 2011). Tan et al. in 25–64-year-old citizens measured education, monthly household income, location of residence, length of typical work day, and activity involved by work and used IDF and NCEP-ATP III definitions of MetS. They showed that education was inversely associated with MetS, whereas income was positively related to MetS in the pooled sample, irrespective of the definition used (Tan et al. 2011). Ni et al. explored level of occupation and education in the general population aged 18 years old and above. Women with high socioeconomic status have low risk of metabolic syndrome (Ni et al. 2013). Zhan et al. evaluated years of education, personal monthly income, and household monthly income in the general population of adulthood. Women with high education level and high personal monthly income were at low risk of metabolic syndrome (Zhan et al. 2012). Podang et al. carried out a study based on the evaluation of education, monthly income, and types of job in a single enterprise among 25–54-year-old employees. In men, low level of education and low social class were risk factors of MetS. On the other hand, in women low monthly income was the sole factor identified to be at risk of MetS (Podang et al. 2013). Zhao et al. performed in a rural population (18–80 years old) a study measuring education and family economic level. The higher the education level was, the better the protective factor of MetS was. On the contrary, high family economic level was at high risk of MetS (Zhao et al. 2014). Lao et al. compared urban versus rural area in the general population and did not find any difference between these two areas (Lao et al. 2014). Enkh-Oyun et al. analyzed education, monthly income, and social class in Mongolian people aged 40 years old or above and found no significant results (Enkh-Oyun

et al. 2015). Ebrahimi et al. explored, in Iranian population aged 40–64 years old, education and home assets and revealed that education was a protective factor (Ebrahimi et al. 2016). Soysal et al. evaluated education and self-perceived economic status and measured MetS by using two definitions, IDF and NCEP-ATP III. They showed that low education and worst self-perceived economic status were associated with high risk of MetS irrespective of the definition (Soysal et al. 2016).

In countries with a medium HDI [0.550; 0.700] or low HDI (<0.550), Son et al. highlighted that homemakers and retired workers were at higher risk of MetS than unskilled workers in the general population (Son et al. 2005). Khanam et al. performed a study in a rural district of Bangladesh among 60-year-old people. They found that poverty, measured by household assets and housing characteristics, was a risk factor of MetS (Khanam et al. 2011). Kaduka et al. reported, in urban dwellers of Nairobi province aged from at least 18 years old, that high socioeconomic status was associated with higher risk of MetS both for men and women. In men only, higher degree of education was also associated with MetS (Kaduka et al. 2012). Adedoyin et al. measured salary level, annual income, occupation, and education for socioeconomic stratum in the resident of the Ilora community (30–70 years old) and found no significant results (Adedoyin et al. 2013). Binh et al. performed a study in the general population regarding education, occupation, and income and did not revealed any significant results (Binh et al. 2014). Finally, Villamor et al. recruited school-aged children and their family and found that women with high level of education were at low risk of MetS (Villamor et al. 2016) (Tables 3 and 4).

Policies and Protocols

Policies

Regardless of the country's level of development, access to education, the possibility of superior education, and the acquisition of occupational credentials are three themes to prioritize in developing public health policies.

It is also important that these policies do not focus on extreme poverty, which is different from social deprivation, a distinction clearly defined by Wrezinski and Townsend (Townsend 1987; Wrezinski 1987). Socially deprived individuals must be able to receive the appropriate management that they lack, policies of prevention and health promotion specifically designed for them, and the development of their capabilities in the health domain, especially through health literacy (Sørensen et al. 2012).

Protocols

Future research should use the validated composite deprivation indices, geographically coded (Carstairs, Townsend, and Pampalon), which exist or that are based on

Table 3 Studies performed in countries with a high human development index

Reference	Population	Socioeconomic status	Results of the multivariate analysis (ORa)
Schooling et al. 2008 (China)	≥50 years old in the members of a community social and welfare association	**Education** (≤primary school=low; junior middle school=medium; ≥senior middle school=high)	**Women**: (ref low): medium 0.77 [0.66; 0.90]; high 0.59 [0.45; 0.68] **Men**: *ns*
		Longest-held occupation (manual; non-manual)	**Women**: *ns* **Men**: *ns*
Erem et al. 2008 (Turkey)	≥20 years old in the general population	**Education** (illiterate; primary; secondary; high school; university)	(ref illiterate): primary 0.79 [0.64; 0.97]; secondary 0.74 [0.54; 0.99]; high school 0.59 [0.44; 0.79]; university 0.49 [0.34; 0.73]
		Occupation (worker; agricultural worker; tradesman; unemployed; housewife official)	(ref worker): housewife 1.56 [1.13; 2.16]
		Household income (1–250; 250–500; 500–750; >750)	*ns*
Marquezine et al. 2008 (Brazil)	25–64 years old residents of the municipality of Vitoria	**Socioeconomic classes** based on education level of the head of the family and the type and quantity of domestic equipment in the home (A, higher income, to D, lower income)	**Women**: 1.64 ($p < 0.0001$) **Men**: *ns*
Zuo et al. 2009 (China)	35–74 years old in area of Jiangsu Province	**Education** (<middle school; ≥middle school)	(ref < middle school): ≥middle school 0.85 [0.72; 1.00]
		Economic status (<6000 low; 6000–15,000= middle; ≥15,000)	(ref low): middle 1.17 [1.01; 1.36]
Da Silveira et al. 2010 (Brazil)	Persons born in 1982	**Education (years)** (<4; 5–8; 9–11; ≥12)	**NCEP-ATP III**: **Women**: (ref ≥ 12): 5–8 years 2.53 [1.03; 6.21] **NCEP-ATP III**: **Men**: *ns* **IDF**: **Women**: (ref ≥ 12): 0–4 years 3.60 [1.29; 10.07]; 5–8 years 3.78 [1.55; 9.21]; 9–11 years 2.98 [1.27; 7.00] **IDF**: **Men**: *ns*
		Family income = total income of the family members in the month before the interview (lower; upper)	**NCEP-ATP III**: **Women**: (ref lower): upper 0.52 [0.28; 0.96] **NCEP-ATP III**: **Men**: *ns* **IDF**: **Women**: (ref lower): upper 0.53 [0.31; 0.90] **IDF**: **Men**: *ns*

Ferguson et al. 2010 (Jamaica)	25–74 years old	**Education** (primary or lower; secondary; post-secondary or tertiary)	**Men**: (ref primary): secondary 3.12 [1.54; 6.34]; post-secondary 2.61 [1.33; 5.11] **Women**: *ns*
		Total monthly household income (≤J$1000; J$ 1001–6000; >J$6000; not reported)	**Men**: (ref low income): high income 6.0 [2.22; 16.19] **Women**: *ns*
Romaguera et al. 2010 (Caribbean Island)	36–82 years old women	**Education** (≤bachelor's degree; ≥master's degree)	**IDF**: **(ref ≤ bachelor's degree)**: ≥master's degree 0.45 [0.23; 0.92] **NCEP-ATPIII**: *education ns*
Sidorenkov et al. 2010 (Russia)	≥18 years old in outpatient clinic in Northwest Russia	**Education** (low; average; high)	*ns*
		Income based on self-related occupational status (very low; low; medium; high; unknown)	*ns*
Belfki et al. 2013 (Tunisia)	35–74 years old in general population	**Education** (years) (illiterate; low level ≤6; intermediate level 7–13; higher education ≥14)	**Men**: (ref illiterates): low 1.65 [1.13; 2.41]; intermediate 2.18 [1.43; 3.32]; higher 2.24 [1.29; 3.89] **Women**: *ns*
		Occupation (not working/retired; employee worker; intermediate grade; upper grade)	**Men**: (ref upper): intermediate 0.55 [0.33; 0.91] **Women**: *ns*
Gronner et al. 2011 (Brazil)	30–79 years old in urban population	**Education** (fundamental= elementary school; middle school=high school; and higher=university)	(ref higher): 2.41 [1.47; 3.96]
		Family income (minimal national wages)	*ns*
Tan AKG et al. 2011 (Malaysia)	25–64 years old in adult citizens	**Education** (years)	**IDF**: **Pooled sample**: education 0.97 [0.94: 0.99] **IDF**: **Women**: education 0.95 [0.92; 0.98] **IDF**: **Men**: *ns* **NCEP-ATPIII**: **Pooled sample**: education 0.97 [0.94: 0.99] **NCEP-ATPIII**: **Women**: education (years) 0.96 [0.93; 0.99] **NCEP-ATPIII**: **Men**: *ns*
		Monthly household income	**IDF**: **Pooled sample**: income 1.11 [1.01; 1.21] **IDF**: **Women**: *ns* **IDF**: **Men**: income 1.19 [1.01; 1.36] **NCEP-ATPIII**: **Pooled sample**: income 1.15 [1.06;

(*continued*)

Table 3 (continued)

Reference	Population	Socioeconomic status	Results of the multivariate analysis (ORa)
			1.25] **NCEP-ATPIII**: **Women**: *ns* **NCEP-ATPIII**: **Men**: income 1.20 [1.03; 1.36]
		Location of residence (urban; rural)	**IDF**: **Pooled sample**: *ns* **NCEP-ATPIII**: **Pooled sample**: *ns*
		Length of typical work day	**IDF**: **Pooled sample**: *ns* **NCEP-ATPIII**: **Pooled sample**: *ns*
		Activity involved by work	**IDF**: **Pooled sample**: *ns* **NCEP-ATPIII**: **Pooled sample**: *ns*
Ni et al. 2013 (Taiwan)	≥18 years old in the general population	**Two-factor index of social position based on occupation and education** (1 lowest to 5 highest)	**Women**: 0.86 [0.75; 0.98] **Men**: *ns*
Zhan et al. 2012 (China)	≥18 years old in the general population	**Education** (years) (<7; 7–12; >12)	**Women**: (ref < 7): 7–12 0.87 [0.75; 0.99]; >12 0.83 [0.62; 0.91] **Men**: *ns*
		Personal monthly income (lower; middle; higher)	**Women**: (ref lower): middle 0.94 [0.86; 0.97]; higher 0.72 [0.65; 0.88] **Men**: *ns*
		Household monthly income (lower; middle; higher)	**Women**: *ns* **Men**: *ns*
Podang et al. 2013 (Thailand)	25–54 years old among employees of the Electric Generating Authority of Thailand	**Education** (<bachelor degree; bachelor degree; >bachelor degree)	**Women**: *ns* **Men**: (ref higher than bachelor degree): lower than bachelor degree 2.28 [1.32; 3.93]; bachelor degree 1.87 [1.20; 2.91]
		Monthly income < baht 50,000; baht 50,000–99,999; >baht 99,999)	**Women**: (ref > baht 99,999): baht 50,000–99,999 3.01 [1.28; 7.06] **Men**: *ns*
		Types of job (lower class; middle class; upper class)	**Women**: *ns* **Men**: (ref high class): middle class 1.44 [1.01; 2.05]

Zhao et al. 2014 (China)	18–80 years old in a rural population	**Education** (illiterate; elementary; middle school; high school and above)	(ref illiterate): elementary 0.73 [0.51; 0.98]; middle school 0.71 [0.52; 0.97]; high school and above 0.57 [0.60; 0.87]
		Family economic level (low; moderate; high)	(ref moderate): high 1.23 [1.01; 1.50]
Lao et al. 2014 (China)	≥20 years old in the general population	**Urban vs. rural area**	*ns*
Enkh-Oyun et al. 2015 (Mongolia)	≥40 years old in the general population	**Education** (years) (<7; 7–9; 10–12; 13–14; >14) **Monthly income** (lower; middle; upper) **Social class** (I professional; II managerial; III semiskilled nonmanual; IV partly skilled; V unskilled; VI nonemployed)	*ns*
Ebrahimi et al. 2016 (Iran)	40–64 years old	**Education** (years)	0.98 [0.96; 0.99]
		Home assets (high; middle; and low)	*ns*
Soysal et al. 2016 (Turkey)	>30 years old in residents of the Balçova district	**Education** (without degree; with degree)	**IDF definition**: (ref: with degree): without a degree 1.26 [1.09; 1.45] **NCEP-ATPIII definition**: (ref: with degree): without a degree 1.28 [1.10 ; 1.47]
		Self-perceived economic status (worst/bad; fair; very good/good)	**IDF definition**: (ref very good/good): fair 1.16 [1.001; 1.34] and worst/bad 1.24 [1.02; 1.51] **NCEP-ATPIII definition**: (ref very good/good): fair 1.19 [1.01; 1.79] and worst/bad 1.46 [1.18; 1.80]

ORa adjusted odds ratio, *ref.* reference, *ns* nonsignificant

Table 4 Studies performed in countries with a medium, low, or high human development index

References	Population	Socioeconomic status	Results of the multivariate analysis (ORa)
Son LNTD et al. 2005 (Vietnam)	≥20 years old in the general population	**Occupation** (unskilled workers; office workers; homemakers; and retired workers)	(ref: unskilled workers): homemakers 1.89 [1.01; 3.57] and retired workers 1.96 [1.01; 3.83]
		Household income (low, medium, high)	*ns*
Khanam et al. 2011 (Bangladesh)	≥60 years old in rural districts of Bangladesh	**Literacy** (illiterate, literate)	*ns*
		Asset index (poverty tertile)	(ref: lowest tertile): middle tertile 2.95 [1.41–6.15] and highest tertile 2.78 [1.31; 5.89]
Kaduka et al. 2012 (Kenya)	≥18 years old in urban dwellers of Nairobi Province	**Education** (years) (none; primary 1–8; secondary 9–14; university >14)	**Women**: *ns* **Men**: **(ref none)**: university 9.8 [1.1; 86.2];
		Occupation (formal, self-employed; petty trade)	**Women**: *ns* **Men**: *ns*
		Monthly income	**Women**: *ns* **Men**: *ns*
		Wealth index (by quintile: lowest, second, middle, fourth, highest)	**Women**: (ref lowest wealth quintile): 4th wealth quintile 3.7 [1.4; 9.8] **Men**: (ref lowest wealth quintile): middle wealth quintile 6.3 [1.6; 25.1]; highest wealth quintile 14.9 [3.5; 62.5]
Adedoyin et al. 2013 (Nigeria)	30–70 years old resident of the Ilora community	**Salary level, annual income occupation, education for socioeconomic stratum** (low, middle, and upper)	*ns*
Binh et al. 2014 (Vietnam)	40–64 years old in the general population	**Education** (elementary; intermediate; secondary; post-secondary)	*ns*
		Occupation (heavy occupation yes/no)	*ns*
		Income (<25th percentile; 25–<50 percentile; 50–< 75 percentile; ≥75 percentile)	*ns*
Villamor et al. 2016 (Guatemala, Honduras, Salvador, Dominican Republic, Nicaragua, Panama, Costa Rica, Chiapas, Belize)	School-age children and their families	**Education** (years) (1–5, 6, 7–11, 12, ≥13)	**Women**: (ref ≥ 1–5 years): 12 years 0.44 [0.23; 0.85] and ≥13 years 0.60 [0.36; 1.00] **Men**: *ns*
		Household assets (sum of affirmative responses)	**Women**: *ns* **Men**: *ns*
		Home ownership (yes/no)	**Women**: *ns* **Men**: *ns*

ORa adjusted odds ratio, *ref.* reference, *ns* nonsignificant

individual measure (the EPICES score) to enable the global comparison of results and to check the nature of the concepts measured – individual or geocoded measures of deprivation that have equivalent predictivity in terms of morbidity and mortality (Pampalon and Raymond 2000; Carstairs and Morris 1989; Townsend 1987; Labbe et al. 2015). This research should also use the harmonized definition so that the identification of risk factors of developing metabolic syndrome is not based on a waist measurement inappropriate to the population studied. This would also make it possible to conduct international comparisons.

As the results appear to differ by sex, it would be appropriate for studies to stratify their results routinely for this variable.

Dictionary of Terms

- **Metabolic syndrome** – a set of disorders including elevated fasting glucose, high blood pressure, dyslipidemia, and abdominal obesity (Eckel et al. 2010).
- **Social deprivation** – social disadvantage related to local conditions for people lacking socioeconomic security but not in extreme poverty.
- **Human Development Index (HDI)** – composite indicator based on three criteria: gross domestic product (GDP) per inhabitant, life expectancy at birth, and educational level.
- **Multivariate analysis** – analysis that simultaneously introduces several explanatory variables in a statistical analysis.
- **Capabilities** – diverse combinations of functioning (states and actions) that a person can accomplish; consequently capability is a set of vectors of functioning that indicate that the individual is free to lead a particular type of life (Sen 1999).
- **Health literacy** – "Health literacy is linked to literacy and entails people's knowledge, motivation, and competence to access, understand, appraise, and apply health information in order to make judgments and decisions in everyday life about health care, disease prevention and health promotion to maintain or improve quality of life during the life course" (Sørensen et al. 2012).

Summary Points

- Most of the studies have been performed in developed countries.
- In countries with very high HDI, 15 studies showed that high education was a protective factor, 9 studies showed that socioeconomic deprivation measured by composite index was a risk factor, and 8 studies revealed that low income was a risk factor too.
- In countries with high HDI, 12 studies identified that low education was a risk factor, and 6 studies found conflicted results regarding income.

- In countries with medium or low HDI, three studies and two studies found discordant results concerning association between education and MetS and poverty index, respectively.
- High level of education was the most frequent determinant identified in women population.
- Income was the most frequent factor identified in men but with conflicting results.
- Regardless of the country, public health policies must be adapted to the deprived and must have as their principal objective of the development of the capabilities of these individuals.

Future research must use as tools specifically validated measures of social deprivation.

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Soup Kitchens: Homeless Adults and Gaps in Meeting Their Nutritional Needs

23

Lisa G. Sisson and Deborah A. Lown

Contents

Abstract

Homeless persons have multiple risk factors for malnutrition and have a greater prevalence of chronic diseases. Soup kitchens, where free meals are available to people in need, are the most common source of food for homeless persons and play a crucial role in meeting their nutritional needs. Homeless persons have special nutrient needs. Due to a high prevalence of excessive alcohol intake, smoking, and high body weight from high caloric alcohol intake they

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are at risk for protein, vitamin, and mineral deficiencies. The nutritional content of soup kitchen meals does not meet their special nutrient needs. This food tends to be high in fat and sodium and lacking in essential micronutrients, instead exacerbating their chronic diseases. Dietary intake assessments of homeless persons indicate a low intake of fruits, vegetables, and dairy products and consumption of high amounts of fats, saturated fats, and sodium and low amounts of fiber.

These problems cannot be resolved by ad hoc charitable organizations; a systemic policy response to the nutritional status of the homeless is essential to address these issues. Increased allocation of funds for soup kitchens, mandatory nutrition standards, coordination among soup kitchens, educated staff, removal of barriers to enrolling in entitlement programs, and assisting the homeless with applying for benefits are all necessary steps to meet the nutrient needs of homeless persons.

Keywords

Emergency Food System · Food insecurity · Homeless · Homeless persons · Homeless women · Hunger obesity paradox · Malnutrition · Soup kitchen

List of Abbreviations

BMI	Body mass index
NHANES	National Health and Nutrition Examination Survey
RDI	Recommended Daily Intake
SNAP	Supplemental Nutrition Assistance Program
TEFAP	The Emergency Food Assistance Program
USDA	United States Department of Agriculture

Introduction

Homelessness, described as a state of not having a regular residence and either sleeping outside, or in temporary housing, is an issue all too common in developed industrialized nations (Shelter 2014). Consistent findings from studies of homeless persons suggest an accurate depiction of their health; they have multiple risk factors for malnutrition, including poverty, food insecurity, a high prevalence of substance abuse and smoking, and limited access to health care (Sisson and Lown 2011). Homeless people also have a greater risk for chronic diseases with a reported greater prevalence of hypertension, hypercholesterolemia, and diabetes, which are often under diagnosed and poorly treated resulting in premature death (Cheung and Hwang 2004).

Soup kitchens are locations where free meals are available for on-site consumption to people who do not live at the site, often located within organizations providing other services such as food pantries or shelters (Ohls et al. 2002). They are the most common source of food for homeless persons and play a crucial role in meeting their nutritional needs (Darmon et al. 2001; Johnson and McCool 2003;

Sprake et al. 2014). Homeless persons have a high prevalence of chronic diseases, substance abuse, and poor access to a healthy diet. In this review chapter focused on developed nations, we describe the common characteristics of homeless persons and review the characteristics of typical soup kitchens and the nutrients they provide. The nutrition, health status, and special dietary needs of homeless people are also summarized. The chapter concludes with suggested methods to improve the health and nutrition of homeless persons through improved food quality at soup kitchens and systemic policy changes.

Common Characteristics of Homeless Persons

In developed nations, the prevalence of homelessness is approximately 1% of the urban population (Turnbull et al. 2007). Homelessness affects a wide variety of people of which the majority are minority, single men (Hwang 2001). Single women, mainly older women, make up one quarter to one third of homeless persons. Homeless youth (15–25 years of age) constitute the remainder of homeless people (Hwang 2001). One third of homeless people are chronically homeless, defined as homeless for 1 year or four episodes of homelessness in their lifetime (McQuistion et al. 2014). Compared to first time homeless persons, chronically homeless persons are more likely to receive disability benefits, have chronic physical problems, higher rates of arrest, and higher prevalence of substance abuse without treatment (McQuistion et al. 2014). They are also more likely to be older as compared to first time homeless persons (Goering et al. 2002). A three-nation study found homelessness can be partially attributed to a lack of affordable housing and services (Crane et al. 2005). Differing policies across nations can result in varying economic climates contributing to homelessness. For example, health problems as a contributing factor to homelessness are lower in England with a national health care system, whereas gambling is a greater contributor in Australia due to legal gaming machines creating a greater risk for gambling debts (Crane et al. 2005).

As shown in Table 1 the median age of homeless persons is increasing; presently 30–50% of homeless people are 50 years of age or older (Crane et al. 2005; National Coalition for the Homeless 2009). The aging baby boomer cohort is one factor for the increase in the median age of homeless persons (Crane et al. 2005). Half of older homeless persons (>50 years of age) experience homelessness for the first time after the age of 50. Among older individuals, compounding risk factors for homelessness include chronic health problems preventing employment, financial crises, forced unemployment, mental health issues, substance abuse, lack of family and social support, widowhood, divorce, death of a parent, and prior incarceration (Crane et al. 2005). Older homeless persons in Boston, USA, London, UK, and Melbourne, Australia, were found to be mainly single men. Seventy-seven percent of these individuals experienced their first onset of homelessness between the ages of 54 and 60; 60% were previously employed, and 50% previously lived alone in a rented home (Crane et al. 2005).

Table 1 Demographic characteristics of homeless people in emergency shelters in the United States, 2009[a,b]

Characteristics	Percentage
Gender	
Male	67.5
Female	32.5
Race/ethnicity	
White non-Hispanic	38
Black non-Hispanic	42
Hispanic	20
Native American	4
Asian	2
Age (years)	
> 51	30.6
Other	
Veteran status	40
Dependence on alcohol	38
Drug abuse	26
Mental illness status	25
LGBTQ youth[c]	20

[a]This statistic does not capture those living on the streets, in transitional housing, living with relatives or friends
[b]National Coalition for the Homeless (2009)
[c]LGBTQ stands for lesbian, gay, bisexual, transgender, and queer/questioning

The Emergency Food System and Characteristics of Soup Kitchens

Food insecurity, a prevalent problem throughout the world, exists whenever the availability of nutritionally adequate and safe foods or the ability to acquire acceptable foods in socially acceptable ways is limited or uncertain (Anderson 1990). Food insecurity may lead to periods of starvation interspersed with periods of binge eating when food is available (Hampton 2007). Hunger and malnutrition may or may not be present with food insecurity; however, it is positively associated with many adverse physical and mental health outcomes (Vozoris and Tarasuk 2013).

The emergency food system provides food for food insecure individuals and households who cannot meet their nutrient needs. It consists of private and public entities and partnerships, the majority being nonprofit faith-based organizations (Pettes et al. 2016; Ohls et al. 2002). The main types of emergency food providers are soup kitchens, food pantries, food banks, food rescue, or gleaning organizations and emergency food organizations (Ohls et al. 2002). Soup kitchens typically serve a variety of food insecure individuals and families, including immigrants, formerly incarcerated, sex workers, and individuals living with mental illness. However,

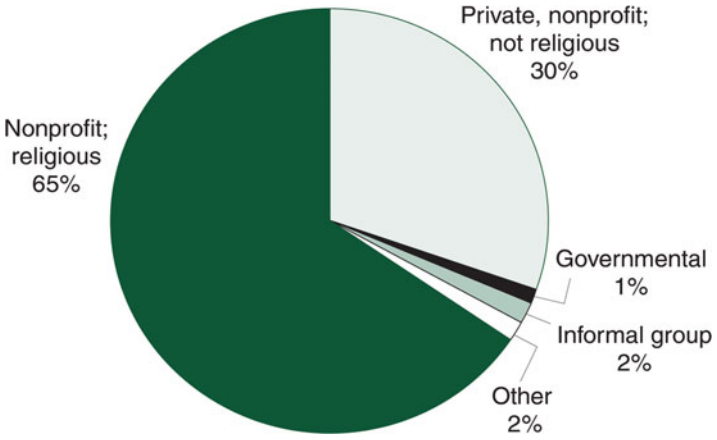

Fig. 1 Types of organizations operating soup kitchens (Ohls et al. 2002)

homeless persons make up the majority of those who utilize soup kitchens (Pettes et al. 2016).

Individual soup kitchens develop organically, while sharing an overarching goal of meeting the immediate food needs of those who utilize their services (Dachner et al. 2009). Soup kitchens are mainly charitable organizations and have limited budgets for food purchases. Most of their food consists of shelf-stable, refined, processed foods (Ohls et al. 2002) sourced through community donations, leftovers from places that serve food, farmers or growers, donations from manufacturers or wholesalers, and food banks. An additional source of food in the USA is The Emergency Food Assistance Program (TEFAP) of the United States Department of Agriculture (USDA), which provides agricultural commodities to soup kitchens (Pelham-Burn et al. 2014) (Figs. 1 and 2).

Soup kitchens are mainly staffed by volunteers with little to no specialized food or nutrition education resulting in meals of poor nutritional quality (Coppenrath 2001). Compounding this problem are the lack of policies relating to nutrition standards. Canadian kitchens often use nutrition standards to guide meal preparation (Pettes et al. 2016), while UK and US kitchens do not (Silliman and Wood 2011; Pelham-Burn et al. 2014) (Fig. 3).

Most soup kitchens are located in urban communities and provide food to an average of 100 individuals per meal. A small percentage of soup kitchens (15%) serve more than 200 people at a single meal (Ohls et al. 2002). Few soup kitchens are open for three meals a day, 7 days a week; most are open only for lunch during the work-week (Dachner et al. 2009; Ohls et al. 2002). According to Pettes et al. (2016) fewer than 65% of the surveyed soup kitchens were open year around and fewer than 50% served meals 5 or more days per week (Pettes et al. 2016). Soup kitchen directors have indicated there is an unmet need for food (Dachner et al. 2009) (Fig. 4).

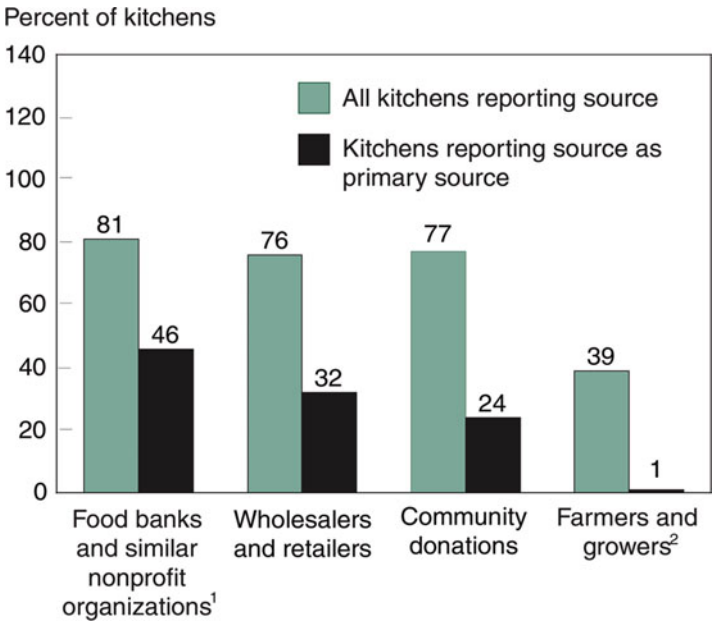

Fig. 2 Sources of food for soup kitchens (Ohls et al. 2002)

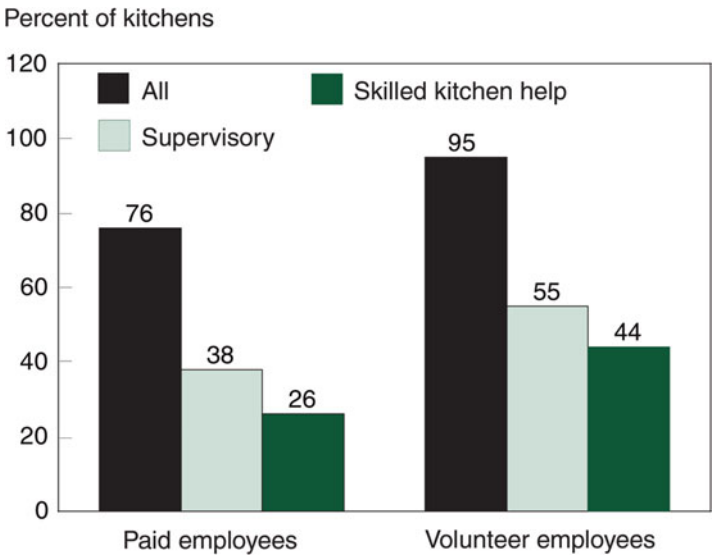

Fig. 3 Uses of paid and volunteer staff by soup kitchens (Ohls et al. 2002)

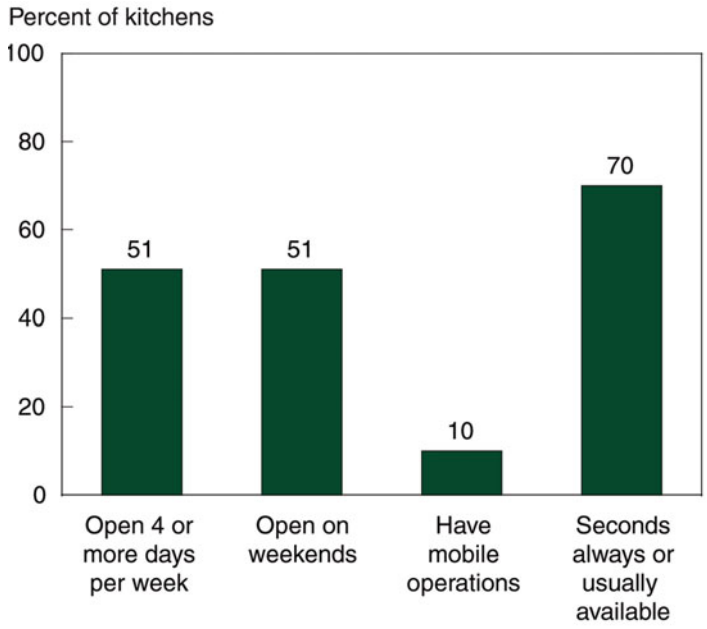

Fig. 4 Selected meal service characteristics of soup kitchens (Ohls et al. 2002)

Common Sources of Food for Homeless Populations

Statistics indicate that homeless individuals reliant on soup kitchens for their meals likely have access to only one meal 5 or fewer days per week. This is consistant with the average reported 1.4–2 meals consumed per day by homeless persons (Malmauret et al. 2002; Ohls et al. 2002). Forty percent of homeless persons, as compared to 3% of low-income households, report not eating for one entire day because they could not afford to buy food (Burt et al. 1999). Twenty-eight percent of American homeless adults as compared to 12% of low-income American adults said they sometimes or often do not get enough to eat (Burt et al. 1999).

Most homeless people obtain the majority of their food from soup kitchens (Darmon et al. 2001; Ohls et al. 2002; Sprake et al. 2014; Popma-Metsaars 2014). Other sources of food for homeless persons include fast food restaurants, convenience stores, community centers, and food purchased with money from panhandling or other income sources. However, these sources of food are seldom used because money is required. Additional food resources include dumpster diving, theft, friends and family, food pantries, and handouts from food sharing organizations (Mahadevan and Fischer 2010). In one study, 93% of homeless women reported eating regularly from dumpsters (Mahadevan and Fischer 2010).

Food assistance programs such as the Supplemental Nutrition Assistance Program (SNAP) in the USA are a potential source of funding for purchasing food; however, their usage is limited among homeless persons. Reports of the use of food assistance by homeless persons is highly variable with Mahadeven (2010) reporting 0% (Mahadevan and Fischer 2010); Obrien (2004), 19% (O'Brien et al. 2004); Martins (2015), 55% (Martins et al. 2015); and Popma-Metsaars (2014), greater than 80%. (Popma-Metsaars 2014). To enroll in food assistance programs, individuals require documentation, access to transportation, and a permanent address, which homeless persons often lack. Distrust of government oversight, bureaucracy, lack of knowledge regarding the programs, and ineligibility are additional reasons homeless persons may not partake in food assistance benefits (Nwakeze et al. 2003; Freedman and Bartoli 2013). Lack of participation results in food insufficiency, placing homeless persons at a high-risk for malnutrition, chronic diseases, and poor health outcomes (Vozoris and Tarasuk 2013).

Nutritional Content of Soup Kitchen Meals

Meals at soup kitchens are derived largely from donated foods which tend to be inexpensive items high in carbohydrates such as pastas, breads, and bakery products. These foods are calorie dense, high in total fat (Sisson and Lown 2011; Pelham-Burn et al. 2014; Silliman and Wood 2011) and saturated fat (Pelham-Burn et al. 2014; Sisson and Lown 2011; Davis et al. 2008), adequate in protein but lacking in fiber (Sisson and Lown 2011; Silliman and Wood 2011; Davis et al. 2008) and many micronutrients. The energy content of soup kitchen meals varies. Some findings indicate that adequate to excess calories are served (Sisson and Lown 2011), possibly contributing to obesity, while others indicate that inadequate calories are provided (Pelham-Burn et al. 2014). Studies found dairy, fruits, vegetables, and fish are unavailable or served in minimal amounts in soup kitchen meals (Ohls et al. 2002; Tarasuk et al. 2015; Davis et al. 2008).

The content of soup kitchen meals contributes to the malnutrition and exacerbates the chronic diseases of homeless persons. These meals commonly do not meet the requirements for vitamin A (Pelham-Burn et al. 2014; Tarasuk et al. 2015), vitamin C (Pelham-Burn et al. 2014; Sisson and Lown 2011), calcium (Silliman and Wood 2011; Tarasuk et al. 2015), zinc (Pelham-Burn et al. 2014; Sisson and Lown 2011; Silliman and Wood 2011; Tarasuk et al. 2015), and magnesium (Sisson and Lown 2011; Pelham-Burn et al. 2014; Silliman and Wood 2011; Tarasuk et al. 2015). Other nutrients found to be inadequate were B vitamins, selenium, and potassium (Pelham-Burn et al. 2014). Sodium was often found in amounts greater than recommended (Sisson and Lown 2011), contributing to hypertension and cardiovascular diseases. Fiber was found to be inadequate in all studies reviewed (Sisson and Lown 2011; Pelham-Burn et al. 2014).

Freedman and Bartoli (2013) found tray waste at soup kitchens indicated a high consumption of satiating protein and carbohydrates with low intake of fruit and vegetable offerings, resulting in inadequate vitamin and mineral intake (Freedman and Bartoli 2013). When surveyed why they consumed low amounts of fruits and

vegetables, the respondents indicated the desire to "fill-up," or they were unfamiliar with the vegetable served or did not like how the vegetable was prepared. Most studies have found that homeless people have a highly variable intake from day to day, often uncertain of when they might eat again, contributing to binge eating when food is available (Sprake et al. 2014).

The Nutritional Status of Homeless Persons

Malnutrition in the presence of obesity is known as the hunger obesity paradox and is commonly found in homeless persons (Sisson and Lown 2011; Koh et al. 2012; Martins et al. 2015). This may be due to a combination of the low nutrient dense, high fat, protein, and carbohydrate food served by soup kitchens and eating behaviors which favor consumption of high satiating foods. Dietary intake assessments indicate a low intake of fruits, vegetables, and dairy products (Martins et al. 2015; Langnäse and Müller 2001; Mahadevan and Fischer 2010) and consumption of high amounts of fats, saturated fats and sodium, and low amounts of fiber. Homeless persons consume inadequate amounts of fat-soluble vitamins A, D, and E (Johnson and McCool 2003; Sprake et al. 2014; Coppenrath 2001; Malmauret et al. 2002). Thiamin, niacin, and riboflavin intake is low even in nations where flour is fortified with B vitamins (Sprake et al. 2014; Darmon et al. 2001; Johnson and McCool 2003). Homeless persons also have inadequate intake of minerals (iron, calcium, and magnesium) and trace elements (zinc, copper, manganese) (Sprake et al. 2014; Coppenrath 2001; Darmon et al. 2001; Malmauret et al. 2002).

The reported calorie intake in homeless people is highly variable, perhaps due the prevalence of a single rather than the usual 3-day 24-h recall method of assessment (Sprake et al. 2014; Malmauret et al. 2002; Darmon et al. 2001). Darmon et al. found 7.3% of French homeless men were underweight compared to 1.7% of nonhomeless French men (Darmon et al. 2001). Whereas Koh (2012) found only 1.6% of homeless persons in Boston were underweight, while 65.7% were overweight and 32.3% were obese (Koh et al. 2012). This reflects the prevalence of overweight and obesity in nonhomeless persons. Langnäse and Müller concluded that the body mass index (BMI) of homeless persons was not solely related to their diet, but also to their chronic illnesses and drug or alcohol use (Langnäse and Müller 2001) (Table 2).

Nutritional Status of Homeless Women

Johnson (2003) found that elderly homeless women had low body weight and that their nutrient intake was less than the recommended amount for all nutrients except iron and phosphorus. These women had adequate protein intake at 52 g per day. But consistent with meals provided by soup kitchens, "less than one-half of the recommended amounts of dietary fiber, vitamins A, D, E and K, biotin, folate, pantothenic acid, calcium and magnesium were consumed. Both zinc and vitamin C were consumed at just slightly more than one-half of amounts recommended"

Table 2 Comparison of weight status by sex in the homeless persons[a]

Weight category %	Males (N = 4535)	Females (N = 1097)	*P* value
Underweight (BMI < 18.5 kg/m^2)	1.3	2.9	<0.001
Normal weight (BMI 18.5–24.9 kg/m^2)	33.4	29.4	0.01
Overweight (BMI ≥ 25 kg/m^2)	65.2	67.7	0.12
Obese (BMI ≥ 30 kg/m^2)	29.7	42.8	<0.001
Grade 1 (BMI 30–34.9 kg/m^2)	18.1	19.2	0.40
Grade 2 (BMI 35–39.9 kg/m^2)	7.3	12.3	<0.001
Grade 3 (BMI ≥ 40 kg/m^2)	4.3	11.2	<0.001

[a]Koh et al. (2012)

(Johnson and McCool 2003, 12). Average daily sodium intake was high at 2700 mg. Most of the women exhibited low body weight, muscle wasting, and/or obesity (Johnson and McCool 2003). Popma-Metsaars (2014) found 31% of homeless women were overweight and 37% suffered from Class III obesity with a BMI > 40 kg/m^2. The median daily fruit and vegetable intake was low, sodium intake was almost twice the recommended amount, and 38.9% of calories were from fat (12.2% from saturated fats) (Popma-Metsaars 2014). This is consistent with the US National Health and Nutrition Examination Study (NHANES) showing greater obesity in females (42.8%) as compared to males (29.7%) (Koh et al. 2012). In HIV, drug abusing homeless women's cultural beliefs and nutritional misconceptions also contributed to their inability to consume a healthy diet (Mahadevan and Fischer 2010).

Homeless pregnant women perceive excessive barriers to prenatal care which are associated with poor infant outcomes. Contributing factors to poor infant outcomes are stressful high risk life-styles (alcohol and drug abuse, smoking) and sexually transmitted diseases (Bloom et al. 2004). Homeless women have a greater number of preterm delivery and small for gestational age babies (Stein et al. 2000).

Special Nutrient Needs of Homeless Adults

Adult homeless persons experience a greater prevalence than housed adults of certain behaviors, physical and clinical problems that can alter their nutrient requirements and nutrient intake putting them at a greater risk for malnutrition. These underlying conditions include smoking, drug and alcohol abuse (Shelton et al. 2009; McQuistion et al. 2014; Magura et al. 2002), obesity (Martins et al. 2015; Koh et al. 2012; Chant et al. 2014), chronic diseases (Gozdzik et al. 2015; Seligman et al. 2010; Shelton et al. 2009), infectious diseases (hepatitis C, HIV, tuberculosis) (Chant et al. 2014; Gelberg et al. 2012; McAdam et al. 2009; Magura et al. 2002), and poor oral hygiene (Figueiredo et al. 2013).

Adult homeless persons have a high prevalence of excessive alcohol intake. In the UK, 35% of homeless persons consume alcohol greater than twice a week (Thorley et al. 2015). Homeless persons who consume large quantities of alcohol are often overweight due to the high calorie intake (Shelton and Knott 2014), but at risk for

protein, vitamin, and mineral deficiencies with low nutrient intake (Ross et al. 2014). Malnutrition also may be due to poor nutrient absorption with toxic damage of alcohol to the gastrointestinal tract (Bujanda 2000). Alcohol metabolism increases the requirements for folate, thiamin, and niacin (Thorley et al. 2015). Blood tests in housed individuals recovering from alcohol and drug abuse indicated 20% were deficient in either iron or vitamin A or both (Ross et al. 2014); this finding is likely similar in homeless persons with substance abuse. As alcohol intake/drug use is associated with smoking, which increases vitamin C requirements, 8% of these individuals have been found to have subclinical scurvy (Ross et al. 2014; Leblanc et al. 2002). Hepatitis which can result from long-term alcohol intake (Bedogni et al. 2007), obesity (Lomonaco et al. 2012), and risky behaviors resulting in virus transmission (Levorato et al. 2017) can lead to fat, fat soluble vitamins (Williams and Sidorov 1971), and calcium malabsorption (Slater et al. 2004).

Homeless persons are at high risk for nutritional anemias through poor intake and decreased absorption of iron, B_{12}, and folic acid. Homeless persons experience a high prevalence of obesity and infectious disease which decrease iron absorption. These inflammatory conditions decrease absorption of iron (Tussing-Humphreys et al. 2012). Older homeless persons, similar to older housed persons, are at risk for B_{12} deficiencies due to atrophic gastritis (Baik and Russell 1999). Homeless persons are at even greater risk due to gastric problems with alcohol abuse, and poor meat intake due to its expense and frequent poor dentition (Sahyoun et al. 2013) (Table 3).

Table 3 Nutrient deficiencies and reasons due to common conditions in homeless adults

Condition	Nutrients	Reasons
Excessive alcohol intake	Protein, vitamins, and minerals especially thiamine, folate, niacin, B_{12}, vitamin A, C, and iron[a]	Poor intake, malabsorption, urinary loss, and increased requirements with alcohol metabolism
Smoking	Vitamin C[b]	Increase requirements with free radicals
Hepatitis	Fat, fat soluble vitamins,[c] and calcium[d]	Decrease in bile production
Obesity/ inflammation/ infection	Iron[e]	Decrease in absorption due to increase in hepcidin production
Atrophic gastritis	B_{12}[f,g]	Low acid-pepsin secretion by the gastric mucosa, which in turn results in a reduced release of free vitamin B12 from food proteins Poor meat intake due to expense and poor dentition

[a]Thorley et al. (2015)
[b]Leblanc et al. (2002)
[c]Williams and Sidorov (1971)
[d]Slater et al. (2004)
[e]Tussing-Humphreys et al. (2012)
[f]Baik and Russell (1999)
[g]Sahyoun et al. (2013)

Special Nutrient Requirements of Women of Childbearing Years

As in all adult homeless persons, alcohol/drug dependence was significantly higher in homeless women as compared to housed women (Caton et al. 2000). Additionally, homeless women have a greater rate (75%) of unplanned pregnancies as compared to housed women (Gelberg et al. 2008). These pregnancies increase their nutritional requirements, especially if they are teens who are still growing. Malnutrition prior to and during pregnancy, particularly in the first trimester, impacts the short-term outcomes of low-birth weight, preterm delivery, and future outcomes of increased rates of obesity, cardiovascular disease, diabetes, and hypertension (Fowles et al. 2011).

Policies and Protocols

Policy Recommendations to Alleviate Malnutrition in Homeless Persons

Improving the health and well-being of homeless persons will require a joint effort between agencies that provide food for them and local and federal governments. Soup kitchens alone cannot bear the burden of meeting the nutritional needs of homeless persons. However, there are steps they can take to improve the quality of their food offerings. Soup kitchens that provide one meal a day, or less, should strive to meet the micro- and macro-nutrient requirements of an adult in every meal (Tarasuk et al. 2015). They can achieve this through increasing the amount of nutrient rich foods served, especially fruits, vegetables, and dairy products, without significantly increasing the calorie content (Tarasuk et al. 2015). The use of healthy meal guidelines by soup kitchens and setting nutrition standards for programs that receive government funding has potential to improve the quality of meals served. The city of New York has such guidelines (New York City Health 2014); however, implementation on the national level, as with child care providers and senior meal programs, has not yet occurred. Moreover, implementing standards without improving funding and food sourcing options for soup kitchens may instead have a negative impact by forcing underfunded operations to serve fewer meals.

Mandatory nutrition education standards for staff and volunteers could at a minimum eliminate inaccurate nutrition beliefs held by those preparing food. The use of dietitians/nutritionists for meal planning is suggested by some (Silliman and Wood 2011); however, this is costly and unless the ingredients necessary for healthy meals are available, will have little impact. The use of donated food and foods sourced from leftovers seriously limits the nutritional status of meals that can be prepared. Therefore, increased governmental funding of soup kitchens is indicated to allow for purchasing of hard to resource foods, such as fresh fruits, vegetables, and dairy products.

In the USA and other developed nations, food manufacturers, retailers, and some growers are allowed special tax deductions for donating food to those who serve homeless persons. Changing these deductions in a manner that encourages the donation of healthy foods such as fresh fruits and vegetables and eliminating deductions for unhealthy items such as soft drinks, highly sugared cereals, and candy could positively change the foods that are available to soup kitchens (Fitzgibbon et al. 1996).

Other policies that could enhance the quality of food sourced include developing partnerships between growers, gleaners, and soup kitchens to increase the availability of fresh fruits and vegetables. When charitable meal programs adopt and implement healthy food policies, they see an overall improvement in the nutritional quality of the foods donated which in turn improves the quality of the meals served. These policies can consist of providing lists of nutrient dense foods that are desired, as well as those less acceptable or that are not accepted due to their poor nutritional value. The policies can go further by requiring that donors may only provide foods that meet specific nutrient guidelines or a nutritional balance of donated foods (Shimada et al. 2013; Davis et al. 2008). Targeted food drives for specific healthy foods and provision of educational pieces to potential donors, explaining the needs of homeless people and the goals of the organization providing meals, can change the paradigm of food available.

Coordination among local soup kitchens of meal times and days that allow for daily adequate intake by homeless persons should be undertaken. Additional suggestions to meet the nutritional needs of homeless people include providing specifically designed fortified food products (Darmon et al. 2001), providing nutrition supplements, such as multivitamins with minerals (Coppenrath 2001; Kinder 2004), and take-away snacks (Kinder 2004).

Removing barriers to enrolling in entitlement programs, increasing the amount of money allocated for emergency food programs, and assisting homeless people with applying for benefits such as SNAP is strongly advocated by many (Nwakeze et al. 2003). Others advocate for allowing purchase of prepared foods with SNAP benefits and providing community kitchens and food storage facilities, enabling homeless persons to utilize food from pantries and grocery stores (Davis et al. 2008).

Charitable meal programs have developed out of a need to reduce hunger, but the efforts are uncoordinated and tackle the problem from the position of an emergency need to reduce hunger, rather than a longer-term goal of meeting nutritional needs and reducing or alleviating chronic diseases (Dachner et al. 2009). Unlike homelessness that has been approached as a problem to be resolved by large scale government intervention, hunger of homeless persons has been left to under-resourced charities to solve. Lack of coordination between organizations and lack of adequate funding, nutrition education, and regular trained staff all contribute to the issue of meals that do not meet the specialized needs of homeless persons.

We conclude that these problems cannot be resolved by ad hoc charitable organizations. A systemic policy response to the nutritional status of

homeless persons is essential to address this issue (Dachner et al. 2009; Tarasuk et al. 2015) and will require increased cooperation, funding, and governmental oversight.

Dictionary of Terms

- **Food insecurity** – Lacking access to adequate amounts of safe foods.
- **Emergency food system** – Provision of food to those in need, mainly by nonprofit, faith based institutions.
- **Homeless person** – Someone who does not have a regular place to sleep indoors.
- **Soup kitchen** – location that provides free meals to people in need.
- **Hunger-obesity paradox** – Malnutrition in the presence of obesity.
- **Anemia** – A condition marked by a deficiency of red blood cells or of hemoglobin in the blood.
- **Atrophic gastritis** – A process of chronic inflammation of the stomach mucosa, leading to loss of gastric glandular cells, which impairs the stomach's secretion of essential substances such as hydrochloric acid, pepsin, and intrinsic factor.

Summary Points

- The majority of homeless persons are minority, single males.
- Soup kitchens are the main source of food for homeless persons.
- Homeless persons consume an average of 2 or fewer meals per day.
- Most meals at soup kitchens are calorie dense, high in total fat and saturated fat, adequate in protein but lacking in fiber and many micronutrients.
- Dairy, fruits, vegetables, and fish are served in minimal amounts in soup kitchens.
- The content of soup kitchen meals contributes to malnutrition and exacerbates the chronic diseases of homeless persons.
- Homeless persons consume inadequate amounts of vitamins A, D, and E, thiamin, niacin and riboflavin, minerals (iron, calcium, and magnesium), and trace elements (zinc, copper, manganese).
- Homeless women have a greater number of unplanned pregnancies, preterm delivery, and small for gestational age babies.
- Homeless persons have increased rates of smoking, drug and alcohol abuse, chronic diseases, infectious diseases, and poor oral hygiene that alter their nutrient requirements.
- Soup kitchens should increase the amount of nutrient rich foods served, especially fruits, vegetables, and dairy products.
- A systemic policy response to the nutritional status of homeless persons is essential to meet their nutrition needs and will require increased cooperation, funding, and governmental oversight.

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Nutrition Status of Those Receiving Unprepared Food from Food Banks: Overview of Food Bank Users in High-Income Countries and Their Diet

24

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Contents

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Abstract

Across all countries, the number of food bank users in high-income countries has increased considerably over the years. More and more people appear to depend on the distribution of food donations. Besides being considered economically disadvantaged, the group of food bank users can be described as diverse in terms of educational background, age, gender, and ethnicity. However, mental or physical disabilities, job loss or working poor, living in a large household, an increase in housing costs, living in a nonmetropolitan area, and/or food insecurity are all main reasons for using food banks. While the types of foods offered by food banks vary substantially across countries, the energy of the provided food seems to meet or exceed nutritional guidelines. Nevertheless, often the provided foods lack vitamins and minerals and despite the small number of existing studies, it seems that food bank users do not consume milk and milk products, fruits, and vegetables in sufficient amounts. In the future, the dietary needs of people in need should be considered without jeopardizing the amount of donated food.

Keywords

Food banks · Food pantries · Food bank users · Diet quality · Dietary status · Micronutrient intake · Macronutrient intake · Economically disadvantaged populations · Donations · Food groups · Food types

List of Abbreviations

FEBA	European Federation of Food Banks
GFBN	Global Food Banking Network
U.S.	United States
USA	United States of America
UK	United Kingdom
WHO	World Health Organization

Introduction

This chapter will focus on food banks and its users in high-income countries because features and arrangements of food banks differ substantially between low-, middle-, and high-income countries. Many food banks in low-income countries go beyond the donation and distribution of foods and support people in farming or harvesting activities. For instance, food banks in Uganda directly provide seeds and practical training in farming and agribusiness (Watuleke 2015), whereas food banks in high-income countries only sometimes cooperate with local farmers and farmers' cooperatives to expand food contributions (Weinfield et al. 2014).

How donated food reaches its recipients varies considerably. People can collect unprepared food from food banks and food pantries or they receive prepared meals in soup kitchens or homeless shelters. This chapter will cover the former, while (Chap. 23, "▶ Soup Kitchens: Homeless Adults and Gaps in Meeting Their Nutritional Needs" by Lisa Sisson) will discuss the later. Therefore, the term "food bank" here will be used to describe programs that directly distribute foods to the people in

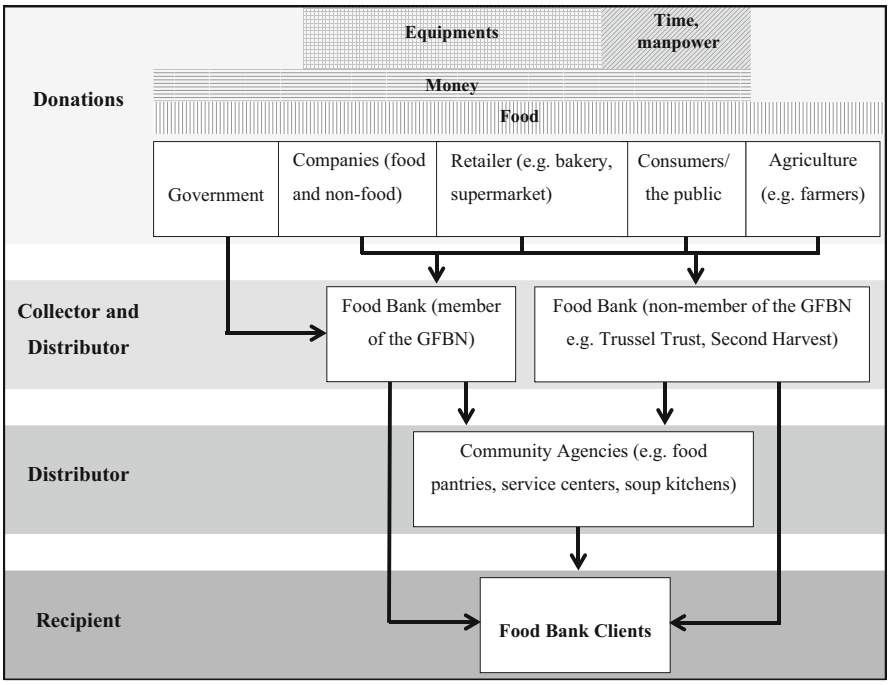

Fig. 1 Food distribution pathway across the different food bank distribution systems

need. However, the terms "food banks," "food pantries," "food parcel programs," and "food aid" are often used interchangeably. In the USA, the term "food pantry" is primarily used for programs that collect food including perishable groceries themselves and directly serve those as parcels to take home for needy clients while food banks go beyond these distribution activities as described in Box 1. In many other countries, no distinction is made and only the terms "food bank" or "local and central food bank" are used. Thus, central food banks usually receive large quantities of foods from the industry, manufactures, and federal or supranational sources such as the European Union food aid and distribute these foods to smaller charitable agencies including soup kitchens and local food banks (or food pantries in the USA). Traditional local food bank programs supply eligible households with predetermined bags of nonprepared food items donated by retailers, manufactures, industries, producers, churches, community members, and other donors. The complex scheme of food banks is described in Fig. 1.

Food Banks and Their Networks

The concept of the first food bank was born in Arizona, USA, in 1966. John Van Hengel, a retired businessman collected foods from a soup kitchen that would otherwise have been wasted and distributed them to people having not enough to

eat (Cotugna and Beebe 2002). The idea of minimizing food waste by giving it to people in need spreads around the world.

The Global Food Banking Network (GFBN)

In 2006, the GFBN was established with the aim to "fight world hunger by creating, supporting, and strengthening food banks around the world" (GFBN 2016a). Currently over 32 countries from all continents, for example, the United Kingdom, Hong Kong, India, or Egypt, are members. The member countries are represented by organizations such as the Feeding America Network, the Arab Food Banking Regional Network, or the European Federation of Food Banks (GFBN 2016b). While the majority of existing food banks are part of the network, not all food banks have so far joined forces, for instance, the Trussel Trust in the UK (The Trussel Trust 2016a), Second Harvest in Canada (Second Harvest 2016), or the Tafel (translated: tables) in Germany (FEBA 2016). More than 6.8 million people were impacted by the network in 2015 (GFBN 2016a). Food banks and their networks generally distribute food, maintain public relations, and share their experiences across organizations, but they also offer other resources such as education programs for people in need as well as volunteers. Nontraditional programs such as rehabilitation programs or community garden projects are also often initiated or supported by food banks.

Box 1 Definition of Food Banks by the GFBN (2016b)
The GFBN defines **food banks** as nonprofit organizations, that "**acquire donated food, much of which would otherwise be wasted, from farms, manufacturers, distributors, retail stores, consumers, and other sources, and make it available to those in need through a network of community agencies.** These agencies include school feeding programs, food pantries, soup kitchens, AIDS and tuberculosis hospices, substance abuse clinics, after-school programs, and other non-profit programs that provide food to the hungry."

USA

In the USA, the food bank and pantry system is highly institutionalized and widespread. Throughout the country, there are over 200 central food banks which distribute large quantities of food to over 46,000 local agencies. More than 67% of these partner agencies represent grocery programs including food pantries which directly provide a predetermined bag of unprepared food to people in need, whereas the others include meal programs such as soup kitchens and other grocery programs such as school pantries (Weinfield et al. 2014).

Local food banks play a significant role in the food environment of low-income population groups in the USA. In 2015, 5.2% of US households reported to have used a food pantry at least once in the previous 12 months (Coleman-Jensen et al. 2016).

Most food banks and food pantries have joined Feeding America; the nationwide umbrella organization that secures donations from national sources such as food manufactures and government agencies, assists food banks in providing education programs and informs the general public about hunger in the USA (Weinfield et al. 2014). The majority of the grocery products distributed through the network are non-perishable foods such as canned products, pasta or rice. Most of the food is donated, but in contrast to most food banks in Europe, U.S. food banks are also allowed to use donated money to buy additional food (González-Torre and Coque 2016).

Canada

In 2013, over 800,000 people in need were provided with groceries through food banks in Canada (Food Banks Canada 2016a). Their umbrella organization is called Food Banks Canada and is comparable to the American network with support of 3000 agencies that help distribute the collected foods. The food and funds donated stem mainly from private companies or other foundations (Tarasuk et al. 2014a). At the Canadian food banks, 38% of provided food is fresh, including milk, eggs, fresh or frozen fruits and vegetables, and bread, whereas 62% of distributed food consists of nonperishable items such as pasta, breakfast cereal, rice, and canned fruits and vegetables (Food Banks Canada 2016b).

Canadian food agencies operate similar to the US food pantries; however, in addition to the provision of prepacked bags containing a certain number of days' worth of food, many smaller food banks also have a grocery store distribution method where clients can select a certain amount of food at no cost or for a nominal cost (Tarasuk et al. 2014a).

Europe

The European Federation of Food Banks (FEBA) with 23 member countries and 4 project partners incorporates around 265 food bank organizations and is estimated to serve 5.7 million people in 2015 (FEBA 2016). Contrary to the food banking system in the USA, every food bank organization works independently and they are not linked among each other. They can vary in structure and distribution system. Some examples are described below:

Half a million Britain have used food banks in the UK (FEBA 2016). One British food bank organization is the Trussel Trust. Although not a member of FEBA, it distributes food through 400 food banks across the country. These food banks are set up as local food banks providing three-day emergency food supply of nonperishable food (Fig. 2) to people in need who were issued a foodbank voucher by care

Fig. 2 Content of a standard bag for one person distributed by The Trussell Trust (With permission from Alexandra Smart, The Trussel Trust)

professionals such as doctors or social workers. Trussel Trust food banks distributed over 500,000 three-day emergency food supplies over the last six months (April to September 2016) of which almost 200,000 emergency food supplies went to children (The Trussel Trust 2016a).

In Germany, the national federation of food banks is the main organization of German food banks (Tafeln), and around 1.5 million people in need were served in 2015 (Bundesverband Deutsche Tafel e. V. 2015). It emphasizes the provision of food as a supplement to the usual food bought in commercial food stores and the existing federal welfare (Bundesverband Deutsche Tafel e.V. 2012). In contrast to most other countries, it has always provided mainly fresh fruit and vegetables (Fig. 3), whereas nonperishable food items are rarely provided. Many Tafeln, in particular those setup as food redistribution centers, are located in parochial facilities (Fig. 4), whereas others, in particular those in a supermarket-style, are operated by other charitable organizations such as the German Red Cross.

Those setups as redistribution centers distribute the foods in predetermined quantity based on household size for a small fee or at no cost, while others use the supermarket like concept with a small shop where eligible individuals can purchase each food product. German food banks are financed mainly by food donations and monetary contributions of companies and the public.

In the Netherlands, currently, 160 locally based food banks and 510 distribution points are part of the umbrella organisation of the "Vereniging van Nederlandse Voedselbanken" which is the Dutch Food Bank Foundation and around 94.000 people were using food banks in the Netherlands in 2014 (Galli et al. 2016). In contrast to most other food bank organizations, clients of a Dutch food bank have to be in contact with social workers to address their food poverty as well as other existing poverty-related problems.

Fig. 3 Typical food distributed by the German food bank system (With permission from Dietmar Gust, Berliner Tafel e.V)

Fig. 4 German local food bank providing fresh produce (Berliner Tafel e.V., Gust D. Pressefotos [press photos] 2017. http://www.berliner-tafel.de/berlinertafel/presse/pressefotos/. Accessed 20 May 2017)

Across all countries, the number of food bank users has increased substantially over the years. For instance, the number of 3-day emergency food supplies given by Trussell Trust food banks in the United Kingdom is currently (2015–2016) almost 20 times higher than in the year 2011 (The Trussel Trust 2016b). In the USA, food pantry user rates have almost doubled from 1999 to 2014 (Baracio and Shaefer

2016). In Germany, while 220 food banks around Germany in 1999 existed, 919 food banks were open in 2014 (Bundesverband Deutsche Tafel e.V. 2016).

Campus Food Banks

One more food bank system should be mentioned. In recent years, campus food banks have been emerging since food insecurity has increasingly become an issue on college and university campuses (Hughes et al. 2011). In the USA, the College and University Food Bank Alliance was founded in 2011. Today, it includes 347 campus food banks. Campus food banks also exist in Canada with 51 campus-based food banks nationwide (Jessri et al. 2014). However, in contrast to people relying on community food banks, the situation of clients of food banks located at universities often differs insofar as students may have better future prospects and the time students need food bank assistance is limited to their student status.

Food Bank Users

Generally, users of food banks can be described as groups of economically disadvantaged populations. These vulnerable populations have certain characteristics in common, such as low- or no income, unemployment or low paid jobs, lack of money (Lightman et al. 2008; Kicinski 2012) and their reliance on welfare assistance. A recent UK study revealed that almost 28% of food bank users indicated benefit delays as primary reason for food bank use, while over 23% indicated a low income as primary reason (Loopstra et al. 2015). Independently of the type of welfare system, there seems to be a strong relationship between receiving federal welfare benefits and food bank usage (Tarasuk et al. 2014b; Daponte and Bade 2006).

However, this is where the commonalities stop. While food bank users are usually adults with or without children, their age range varies across countries, and food banks. Some studies report the highest percentage of users between the ages of 35–49 years (Algert et al. 2006), others report the largest percentage between the ages of 40–50 years (Lyman and Seo 2015; Depa et al. 2015) or 40–59 years (Robaina and Martin 2013). Nevertheless, the mean age in most studies is between 40 and 50 years of age.

Even more diverse is the educational background, and research shows that food bank users report a wide range of educational background starting from no high school education to a completed university degree. Most commonly, with over half of the users, a high school degree or more ranging from 41% to 91% is reported (Lightman et al. 2008). Thus, a low educational level is not predictive of food bank use nor is a university degree protective of becoming unemployed or poor and in need of social assistance. Furthermore, many users are reported to suffer from chronic illness and disabilities (Lightman et al. 2008). For instance, Depa et al. (2015) reported that 64.5% of their food bank participants indicated to have at least one chronic illness. High blood pressure, diabetes, and obesity seem to be the most

common medical conditions (Castetbon et al. 2011; Neter et al. 2014; Martin et al. 2013). Many food bank users also smoke (Depa et al. 2015; Starkey et al. 1998). Despite the observed low energy intake among food bank users summarized below, the large percentage of overweight and obese food bank users, in particular women, is of concern and raises the question of dietary quality of the food consumed by food bank users. Primary reasons for long-term food bank use can be found in Box 2.

Particularly in the United States, minority groups are more likely to use food banks compared to non-Hispanic Whites (Weinfield et al. 2014; Algert et al. 2006) and in Europe, food bank users appear to be more often not born in the country of study (Neter et al. 2014; Castetbon et al. 2011). While in some studies, more single households seem to visit food banks (Martin et al. 2013; Lightman et al. 2008), others report more visits by 2–4 person households (Neter et al. 2014). Households with children, seniors, and particularly single parent households appear to be increasingly using food banks across all countries (Nord et al. 2009; Tinnemann et al. 2012, Nichols-Casebolt and Morris 2002; Bartfeld 2003).

Box 2 Primary Reasons for Long-Term Food Bank Use
Mental or physical disabilities, working poor, living in a large household, an increase in housing costs, living in a nonmetropolitan area, and/or food insecurity (Kicinski 2012; Bhattarai and Duffy, 2003; Biggerstaff et al. 2002; Daponte 2000).

There is one more aspect that the majority of food bank users have in common across various countries; they have been using a food bank not as an emergency short-term service which was the initial intention by food bank founders, but they rely on food banks for support for one year and more (Depa et al. 2015) with many of them visiting a food bank more than once a week (Martin et al. 2013). In general, food insecurity is highly prevalent among food bank users even more so than in other low income households (Coleman-Jensen et al. 2016; Tarasuk et al. 2014b; Castetbon et al. 2011). For example, around 85% of food bank users in the USA are estimated to suffer from food insecurity (Weinfield et al. 2014).

Types of Food Offered

Since the primary source of food as well as of financial capital of most food banks is represented by donations, food banks are highly depend on food surplus and the goodwill of their suppliers. Another important fact regarding the offered food types is the difference in the way food banks collect, provide, and distribute foods between and sometimes even within countries.

Therefore, the dependence on donations makes the amount and types of food to be distributed unpredictable for both food banks and its users. It is, therefore, not surprising that a recent systematic literature review including nine studies from

Canada, the USA, and Australia found large variations in the nutritional quality of the food provided between and within studied food banks (Simmet et al. 2016a). Since most food banks provided bags of diverse sizes depending on the number of individuals in recipient's households, the nutritional value of the supplied food per person also varied between bags of different sizes, even when provided by the same food bank.

Provision of Energy

As reported by the systematic review from Simmet et al. (2016a), the mean provision of energy per day met or exceeded recommendations of the Food and Agriculture Organization of the United Nations for energy intake (given a moderate activity level of 1.75 and an assumed body weight of 60 and 80 kg for women and men respectively (WHO et al. 2004)) in four of six studies. The recent Dutch study also found that the provided amounts of energy were higher than the nutrition guidelines (Neter et al. 2014). However, reviewed studies also revealed that between 33% (Teron and Tarasuk 1999) and 99% of bags (Irwin et al. 2007) provided an amount of energy less than recommended.

Provision of Food Groups

Dairy products were one of the food groups most often provided in inadequate amounts (Table 1), as nearly all studies considered by the review reported an insufficient supply at least for some bag sizes (Simmet et al. 2016a). In contrast, the provision of grains generally was within recommendations, although some food banks provided fewer amounts of whole grains (Nanney et al. 2016).

More heterogeneous were the results for the provision of fruit and vegetables as well as for the provision of meat and alternatives. While the mean provision was found to be adequate for some of the studied food banks, the supply was far away from the national recommended intake for other food banks (Simmet et al. 2016a). The recent Dutch study also found that the mean supplies of fruits and of fish were too low (Neter et al. 2014).

Even if the mean supply was adequate in terms of the number of provided servings, fruit and vegetables were often represented by tomato sauce, canned fruit or vegetables and juice (Starkey 1994; Willows and Au 2006) and only some food banks provide perishable foods such as fresh produce and yoghurt when available (Friedman 1991; Jessri et al. 2014).

Provision of Macronutrients and Micronutrients

As revealed by the systematic review, the supply of macronutrients as represented by the percentage of energy generally met nutrition recommendations, but some food

Table 1 Comparison of mean group intake of food bank users and mean bag content by food groups with national recommendations (Unpublished, adapted from Simmet et al. 2016a,b)

mean bag content	food group	mean group intake
	grains	
	fruit and vegetables	
	vegetables	
	fruits	
	milk products	
	meats and alternatives	

Number of icons represents the number of studies; green icon: mean group intake/bag content was within recommendations; red icon: mean group intake/bag content was below recommendations; green-red color mix: mean group intake/bag content was partly within recommendations (e.g. for one sex or for some bags)

banks provided relatively low amounts of fat (Simmet et al. 2016a), whereas others provided too much saturated fat and protein (Neter et al. 2014).

Among micronutrients, vitamins A and C as well as calcium were the micronutrients which were provided in particularly low amounts (Simmet et al. 2016a). In addition, the provision of iron and zinc was reported to be inadequate at least for some bag sizes. Iron was mostly provided by nonheme beans. While the mean provision of the other micronutrients was mostly within recommendations, large variations were found between different bag sizes, days, and food banks.

Differences in Food Types Offered Across Countries

In most countries, food banks claim to provide a certain number of days' worth of food per person, usually 2 to 4 days (Tarasuk et al. 2014a), but the supply of fresh produce tends to be low (Simmet et al. 2016a). In countries such as the USA, Canada, France, and Australia, donated foods used to come from the food industry's production and distribution channels and included waste products such as damaged containers or mislabeled tins (Campbell et al. 2015). Historically as well as up to now, donated foods therefore have mainly included nonperishable food items.

Recognizing the need of a more comprehensive nutritional evaluation of foods distributed through the food bank systems, some food banks and researchers, for instance in the USA and France, have started to apply a nutrition-profiling system to measure the distributed foods in terms of MyPyramid days (Hoisington et al. 2011) or to use linear programming to identify food items required to mitigate nutritional deficiencies in the food donations (Rambeloson et al. 2008).

Since the food industry has become more efficient, leading to less food waste in the food production and distribution and subsequently to reductions in donations, many food banks sought for new donors and, in particular, food banks in Canada and the USA have started to distribute more fresh fruits and vegetables (Campbell et al. 2015; Handforth et al. 2013). To increase its supply, they have begun to work with local farmers and farmers' cooperatives (Webb et al. 2012; Weinfield et al. 2014), whereas others adopted a "No Soda and No Candy" donation policy (Campbell et al. 2009).

While some food bank managers show concerns in regards to restricting the types of food distributed (Handforth et al. 2013), the provision of more fresh produce and also the implemented policies have improved the nutritional quality of the supplied food (Jessri et al. 2014; Campbell et al. 2009). It requires, however, immense investments in trucks, fuel, refrigerator systems, and manpower to distribute fresh produce which often is close to its best-before-date. Moreover, the provision of fresh fruits and vegetables depends on availability and is usually highly variable.

Diet of Food Bank Users

Energy Intake and Overall Dietary Quality

Users of food banks and food pantries are not only heterogeneous in terms of their demographic characteristics, in particular their educational background, they also differ in their nutrition status.

Another recent systematic literature review by Simmet et al. (2016b) included 11 studies and observed large variations in the nutrition status between users of different programs, countries, and even within user populations.

Despite variations, mean energy intake tended to be below the recommendations of the Food and Agriculture Organization of the United Nations in both sexes and nearly all investigated age-groups (Simmet et al. 2016b). For instance, by using the Healthy Eating Index, one US study assessed the overall diet quality among 48 female food bank users and revealed that 71% of the women consumed a diet that is not health-promoting (indicated by a Healthy Eating Index score below 50 points) (Duffy et al. 2009). The average score was only around 43 points and the majority of women reported no consumption of fruits, whole grains, and dark green or orange vegetables or legumes.

Food Groups

Among different food groups, mean intake of fruits and vegetables and of milk products was below the national recommended intake in nearly all investigated age groups and both sexes (Table 1) (Simmet et al. 2016b). Considering individual intake, many of the studied food bank populations seem to consume fruit,

vegetables, and milk products in amounts lower than recommended. In a recent German study among 267 food bank users, around 50% of them consumed fruit and around 66% of them vegetables less than daily (Depa et al. 2015). Daily fruit consumption was even lower in this sample than in the general German population of low socioeconomic status. Similarly, a French (Castetbon et al. 2011) and an US (Bell et al. 1998) study found food bank users meeting less frequently the national intake recommendation than the general population. Only one Canadian study reported that the percentage of people with an adequate fruit and vegetable intake was higher among food bank users than in the general population (Jacobs Starkey and Kuhnlein 2000). All three studies comparing milk intake between food bank users and the general population found the prevalence of adequate intake lower among food bank users than among the general population (Simmet et al. 2016b).

In contrast, mean intake of meat and alternatives was adequate in all five studies comparing mean intake with national recommendations (Canada, Australia, and the USA) (Table 1) (Simmet et al. 2016b) and a small US study revealed that food bank users consumed more high-fat meat than the general population (Bell et al. 1998).

More heterogeneous were the results for the intake of grains. In two Canadian (Jacobs Starkey and Kuhnlein 2000; Rush et al. 2007) and one Australian study (O'Reilly et al. 2012), mean group intake was within the recommendations, whereas another Canadian (Tarasuk 2001) and one US study (Duffy et al. 2009) found the mean intake of grains inadequate among food bank users. Confirming the heterogeneity, large variations were also observed in the prevalence of inadequate intake, as between 13% and 61% of food bank users had an intake of grains below the national recommended number of servings (Simmet et al. 2016b). In particular, the intake of whole grains was observed to be low (Duffy et al. 2009; Yao et al. 2013).

Macronutrients and Micronutrients

While food bank users' intake of macronutrients expressed as percentage of total energy intake met recommendations, intake of some micronutrients tended to be less than recommended at least in subpopulations of food bank users (Simmet et al. 2016b). Reflecting the low consumption of milk products, calcium seemed to be among the micronutrients which were consumed in particularly low amounts. One Canadian and one US study reported that the mean intake was below recommendations among female food bank users (Bell et al. 1998; Jacobs Starkey et al. 1999). While the mean intake of other micronutrients tended to meet recommendations, one older US study also found that the mean intake of vitamin D, pyridoxine, iron, magnesium, selenium, and zinc was inadequate in women (Bell et al. 1998). Even when the mean intake was adequate, subpopulations of food bank users were reported to consume amounts of micronutrients lower than recommended (Lenhart and Read 1989; Tarasuk and Beaton 1999).

Correlates of the Dietary Quality Among Food Bank Users

Food insecurity seems to be related to the tendency of a less healthy diet while controlling for other variables such as household income and education level (Holben 2012; Robaina and Martin 2013; Tarasuk and Beaton 1999). Given the high prevalence of food insecurity among food bank users, this finding is of particular concern. Moreover, female food bank users appeared to have a higher risk of having an inadequate intake of micronutrients than men (Simmet et al. 2016b). Women's roles in managing family feedings might make them more vulnerable to negative nutritional consequences of food insecurity by neglecting their diet in exchange for a better diet of their children.

Besides food insecurity and the female sex, other characteristics identified to be negatively related to a healthy diet seem to be the frequency of food bank use, smoking, and household size, whereas a higher educational level, born outside the study country, and age seem to be positively related to a healthy diet among food bank users (Jacobs Starkey et al. 1999; Martin et al. 2013; Tinnemann et al. 2012).

Due to the small number of studies, the generally small study samples, and different instruments and methods of data collection, it is hardly possible to reliably compare the nutritional status of food bank users between different countries. It is, however, important to note that across all studied countries, food bank users reported a low intake of milk products, of fruits and vegetables and of calcium.

Given that many food bank users rely on public welfare (Depa et al. 2015; Loopstra et al. 2015; Tarasuk et al. 2014b), it is likely that besides the food provided from food banks and individual correlates, the type and extent of public welfare benefits also impacts the nutritional status of food bank users, but the available data do not allow to verify this assumption.

Intervention Measures to Improve the Nutrition Status Among Those Using Food Banks

Potential intervention measures to improve the nutrition status among food bank users are manifold and should be adapted to the needs of the target population.

Many food bank users desire to receive more meat (Campbell et al. 2011; Greger et al. 2002; Verpy et al. 2003) and perceive their meat intake as less than acceptable (Hoisington et al. 2002) although it actually may be within or even above the recommended level of intake (see Table 1). The introduction of nutrition education tools may assist food bank users to evaluate their own diets. Nutrition education could also serve as a way to improve food bank users' nutrition status since many food bank users do not know how to prepare all the food they receive from the food bank (Greger et al. 2002). More precisely, the provision of cooking classes (Caspi et al. 2016; Flynn et al. 2013) and recipes and forcing self-efficacy (Yao et al. 2013) seem to help these people to improve their dietary quality. As described above, some food bank associations provide valuable platforms to exchange information and

intervention tools to improve the nutrition status and health of food bank users (for instance http://healthyfoodbankhub.feedingamerica.org/).

Food bank interventions that address the complex origins of malnutrition and food insecurity have been conducted so far only in pilot studies (see a good-practice example in Box 3). Another example is the use of local food banks as nontraditional settings for diabetes support given the high prevalence of diabetes among food bank users (Seligman et al. 2015).

Box 3 Good Practice Example of an Innovative Food Bank Model

In 2010, Freshplace was founded in Hartford, USA, by three community agencies to address potential roots of food insecurity and malnutrition. The main differences between the Freshplace model and traditional local food banks include:

- **Client-based choice model**: Clients are allowed to choice the foods from a variety of fresh produce and healthy basic foods to meet the health and cultural needs of their families
- **Individualized case managers**: Clients meet trained case managers monthly who offer individualized case management using motivational interviewing to help clients set and monitor goals for becoming food secure and self-sufficient
- **Additional programs and services:** Cooking Matters classes, exposure to nutrition education, resumé building, and job assistance are provided.

Results: Over one year, Freshplace members were less than half as likely to experience very low food security, increased self-sufficiency, and increased fruit and vegetable consumption by one serving per day compared to the control group of clients of a traditional food bank (Martin et al. 2013).

Practical Implications for Researchers

The observed large variations in the dietary quality along with the high prevalence of overweight and obesity among food bank users highlight the need of future research to gain a deeper understanding of forces and barriers for a health-promoting diet among food bank users.

Very few studies have investigated the impact of food banks on individual dietary intake at long term (Martin et al. 2013). By applying well-planned longitudinal study designs, future studies should also investigate the impact of the assistance of food banks on individual health and body weight.

Unfortunately, very little is also known about the impact of different food bank schemes on users' nutrition status. Whether the clients are allowed to choose foods based on needs and preferences or whether they receive a predetermined bag likely has a substantial impact on the dietary quality and potentially on the

self-efficacy of users, but there is so far only one randomized controlled trial comparing the impact of a traditional and a novel food bank including a choice model as outlined in Box 3.

Since also few studies investigated correlates of the dietary intake among food bank users, future studies are further recommended to identify characteristics associated with the nutrition status. This would allow the identification of subgroups at a particular high risk of unhealthy diets among the diverse populations of food bank users. Moreover, it may assist the development of effective intervention measures to improve food bank user's nutrition status.

Policies and Protocols

Policies to improve the nutrition status of food bank users have to be developed carefully and need to consider the charitable character of food bank organizations, including the dependency on donors.

They should cover three main areas:

- Identification
- Implementation
- Monitoring

Identification Although many food banks in the USA, Canada, and UK claim to provide a certain minimum of days' nutritionally balanced food, they usually lack nutritional standards for a defined food bag provided to food bank users. Given that half of the US food banks still reported to use "common sense" in classifying the nutritional quality of their food inventory (Webb et al. 2012), more food banks are recommended to monitor the nutritional quality of food distributed.

For some food banks, it may be worthwhile to consult researchers how to improve the reliability and validity of their routine data in order to get a more comprehensive picture of their activities, the number of people served, as well as the nutritional quality of the food provided. Moreover, the identification of knowledge gaps regarding nutritional quality by food bank personnel should be made and closed by nutrition experts cooperating with food banks.

Implementation The most obvious short-term solution to improve the nutrition status among those using food banks may lie in the distribution of greater amounts of fruit and vegetables, milk products, and whole grains, although this requires substantial personnel and financial capacities. For smaller food banks, this may, however, be a logistic challenge to distribute perishable foods which are often close to its best-before-date. The initiation and maintenance of local networks likely make it easier, in particular, for rural food banks to gain and distribute more fresh produce.

In the light of the shifting needs of food bank users (e.g., long-term use rather than emergency use), food banks should not hesitate to adopt bans on energy-dense foods of low nutritional quality such as soda, sugar-sweetened beverages, and sweets. Food banks that have already implemented such policies may assist other food banks in doing so and with it, reduce the potential worries of losing much needed donations in any shape and form.

Given the charitable character of most food bank programs, they are hardly able to provide the full range of measures to improve dietary quality. Due to the institutionalization of food banks and their valuable experiences gained over decades, they have, however, an immense potential to play an important role in addressing nutrition-related topics in a population group which otherwise is hard to reach. Moreover, they provide an important setting for other health-related interventions. Food banks and other community resources including health centers, social workers, and physicians as well as research institutions are, therefore, recommended to join forces to address the roots of food insecurity, health risks, and malnutrition.

Monitoring Easy applicable monitoring tools should be developed to control changes in the nutritional quality of food provided as well as the impacts of implemented interventions. Establishing a database of good-practice examples of effective food bank intervention measures may further enhance information exchange and provide a valuable resource for other food banks to learn from.

Outlook

Besides these potential policy concepts, food banks will have to face many challenges in the future. Given the rise of people at risk of poverty and the raise in food banks observed across high income countries, the demand is also increasing. However, the supply seems to decrease while the food industries and particularly the grocery stores have become more efficient and waste conscious. The system in place in the Netherlands that fights food poverty also by offering additional nonfood-related help might become a necessary step to support people in need. For Europe in particular, the current increase in refuges will likely impact food banks substantially further limiting their ability to be effective in relieving people in need.

Dictionary of Terms

- **Central food bank** – A central food bank works as a central warehouse and collects large quantities of food from the industry, (supra-)national sources, and retail and distributes the food to other charitable organizations such as local food banks, soup kitchens, and homeless shelters.

- **Dietary quality** – Dietary quality describes the overall diet and dietary patterns based on current nutrition knowledge with the aim to define people's diet and to provide estimates of national nutritional status.
- **Food bank** – A food bank is a nonprofit and charitable organization that collects food from various sources including but not limited to manufacturers, distributors, retailers, or growers and distributes the donated food to other organizations and people in need.
- **Food insecurity** – Individuals suffer from food insecurity when they are not able to acquire a sufficient amount of food on a daily basis and therefore do not consume enough food each day. As a result, people might suffer from chronic hunger, poor nutrition, and malnutrition.
- **Food pantry** – The term "food pantry" is primarily used in the USA for charitable organizations that operate as local food banks (and collect food from central food banks, retailers, manufactures, and growers and provide the donated food to people in need).
- **Local food bank** – A local food bank is a charitable organization that collects food from central food banks, retailers, manufactures, and growers and provides the donated food to people in need.

Summary Points

- In this chapter, the wide range of food bank programs and the nutrition status among those using these programs was illustrated.
- Food bank programs, their distribution systems, and their funding mechanisms vary widely across and sometimes within countries.
- Food bank users have been described as a vulnerable population groups at high risk of food insecurity, diverse health problems, and a low dietary quality.
- In particular, a low intake of fruits and vegetables, milk products, and calcium can be observed among food bank users.
- Good practice examples show the potential to improve the dietary quality of vulnerable population groups using food banks.
- It is important to develop and implement strategies to improve the dietary quality of people using food banks.

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Healthcare, Inequality, and Epidemiologic Transition: Example of China

25

Nan Zou Bakkeli

Contents

Abstract

China has achieved great improvements in population and public health, such as a dramatically lower mortality rate, increased life expectancy, and extensive immunization coverage. However, new health challenges emerge in China today. Noncommunicable diseases such as cardiovascular diseases have become the leading cause of deaths, while there still are serious malnutrition issues among poor segments of the population. New health problems and increased income inequality have both emerged during the period of economic reform and market transition of

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healthcare systems. This chapter aims to give an overview of three possible factors that influence population health in transitional China. This includes (1) the healthcare system, (2) income inequality, and (3) epidemiological transition.

Keywords
Health · China · Welfare system · Healthcare reform · Income inequality · Health inequality · Malnutrition · Market transition · Epidemiologic transition · Non-communicable diseases

List of Abbreviations
NCDs Noncommunicable diseases

Introduction

In the period since the economic reforms started in the early 1980s, China has experienced tremendous growth, and its society has continuously been in a state of rapid transition. The structural changes have emphasized economic development, in terms of the introduction of market-oriented social policies, and the development of infrastructure.

The market reforms have had an ambiguous impact on Chinese society. On the one hand, millions have been lifted out of poverty, people's living standards have been dramatically improved, household and individual income has increased greatly, and the average annual GDP growth has been around 10% for over three decades. On the other hand, income inequality has increased, creating a more divided society. The Gini coefficient is an indicator measuring the degree of inequality in a society. It was around 0.30 in 1980 in China, but had nearly doubled to 0.55 by 2012 (Xie and Zhou 2014). This places China as one of the most unequal countries in the world.

China has achieved great improvements in population health since its establishment in 1949, but is now experiencing several emerging public health problems (Chan et al. 2008). With millions of cigarette smokers, China has become the largest tobacco consuming country in the world; obesity has increased in the population; about 10% of the population is infected with Hepatitis B; the country has experienced a progressing HIV epidemic; tuberculosis (which was previously under control) has broken out again; and in some underdeveloped areas, cholera and other epidemic diseases have been spreading (DRC 2005).

How has the current health situation in China evolved in the reform period, and what contributes to the health challenges? This chapter aims to go through important factors for health situations in China, by asking three questions:

1. What characterizes developments in health and healthcare systems?
2. Is growing economic inequality affecting the health situation in China?
3. How can one understand the interplay between social and health situation, with emphasis on the epidemiologic transition?

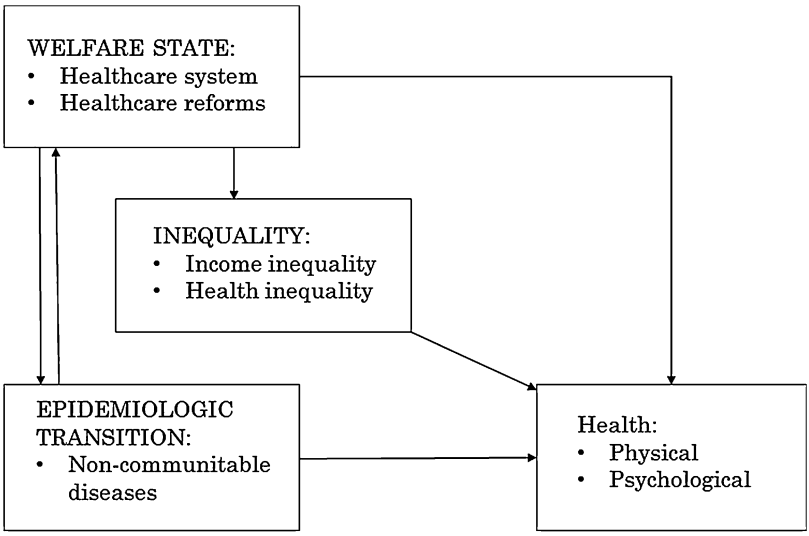

Fig. 1 Relation between welfare state, inequality, epidemiologic transition and health

Healthcare reforms, inequality and epidemiologic transition are three factors that are central to understand population health in China (see Fig. 1). High inequality may lead to negative factors such as lower social cohesion and more mental stress and therefore cause worse health (Wilkinson 1996, 1999). At the same time, market reforms have in many ways given profit-making a high priority. For example, publicly funded mental health rehabilitation facilities were closed down, merged, or down-sized to smaller-scale psychiatric hospitals. For example, only in Shanghai, the number of mental health rehabilitation facilities had decreased by 62% by June 2004, compared to before the 1990s (Liu et al. 2011). This has had a negative impact on Chinese mental health. One study showed that nearly one in five adults had suffered from at least one mental disorder in the past month, that is, roughly 173 million Chinese people (Phillips et al. 2009).

The pattern of type of diseases and leading causes of deaths is also changing. According to the World Health Organization, about 230 million Chinese people (20%) have a cardiovascular disease. The percentages of deaths associated with cardiovascular diseases in urban and rural areas are 20.9% and 17.9% of China's total numbers of deaths per year respectively (WHO 2015). There are also serious malnutrition issues among the poor: the stunting rate among rural children was 22% in 1998, and in some poor provinces, it was as high as 46% (see Park and Wang 2001). The prevalence of underweight among children under 5 years old was 1.8% in cities in 2005, but 8.6% in rural areas (Chang et al. 2006). A well-functional welfare state is not only important to narrow down income and health inequalities, but it is also a central infrastructure to solve health challenges caused by the epidemiologic transition.

Welfare State and Healthcare

The Market-Oriented Healthcare System

The process of marketization and privatization has not only reformed the labor market, but has also affected other social spheres. One example is the changing structure of welfare regime and the social security system.

In the prereform era, there was relatively wide provision of basic healthcare services in China. The state and work units were primarily responsible for offering health care services in cities, and the healthcare services for rural citizens were organized as part of the integrated rural collective commune system. Although rural health benefits were less institutionalized than those in urban areas (Wagstaff and Lindelow 2008), the gap was relatively small, and the general level of social welfare was considered high (Gao 2008; Leueng 2005). Contrary to what many believe in the West, this model of healthcare was widely accepted as successful and it was understood to contribute to better national health (Djukanovic et al. 1975; Sidel 1993).

Overall, the market-oriented economic reform that took place from the early 1980s led to a dramatic reduction in healthcare insurance coverage. In urban areas, healthcare provision was based on which sector people were employed in. Many lost their coverage when they lost their jobs in state enterprises, and only employees with close family members employed in the public sector and state-owned enterprises remained insured (Leueng 2005). By 2003, about 65% of urban residents had to pay for healthcare themselves, and self-financed medical spending had increased 13 fold compared to 1990 (Mok et al. 2010). In rural areas, the de-collectivization of agriculture communities led to reduction of public services, and households became responsible for themselves. As a result, the healthcare system collapsed. There was a fall in health service coverage from about 90% of the rural population in 1980 to 5% in 1985 (Liu and Cao 1992).

One of the major challenges facing the Chinese healthcare system after the economic reforms is that the health services have become heavily hospital-oriented, while primary care has been given low priority (Chan et al. 2008). Before the economic reforms, healthcare delivery was based on a three-tier healthcare system. In urban areas, primary healthcare was provided by the community health units and district hospitals, while municipal and provincial hospitals focused on tertiary medical services. In rural areas, village clinics were responsible for the primary tier, township public hospitals served as the second tier, and county hospitals provided the highest tier for inpatients.

However, as a result of the localization and privatization of health services during the economic reforms, local governments now only provide limited subsidies for health services. Primary care has almost ceased to function. In urban areas, 80% of people visit hospitals for even minor illnesses. In rural China, clinics do not provide preventive care without reimbursement (Chan et al. 2008).

The process of decentralization of welfare provision was combined with a reduction in health expenditure and privatization. Total national health expenditure dropped from 28% in 1978 to 14% in 1993, and health expenditure in rural areas fell

from 20% to 2% (Bloom and Gu 1997). In 2002, the central government required each township to provide only one government-run hospital, while the rest was contracted out or privatized. Healthcare providers such as hospitals and clinics were expected to become "independent accounting units of profit-making entities with independent management," and a revenue-based bonus system was used to increase productivity (DRC 2005).

As a result, healthcare services have been transformed from being institutions responsible for addressing public healthcare needs, to "revenue-maximizing organizations" (Tang and Meng 2004). In order to increase revenue, many hospitals have invested in expensive medical equipment to charge more; they are also recommending unnecessary treatments and selling more expensive medicines.

The government has attempted to regulate drug and healthcare cost, but they have also set prices for new, high-end services above the cost regulation and have allowed a 15% profit margin on drugs. This has prompted hospitals to encourage doctors to prescribe more and costlier medicines, increase expensive high-tech services, and avoid using cheaper medicines covered under regulation (Chan et al. 2008). Among patients who caught a cold, 75% were prescribed antibiotics (Yip and Hsiao 2008).

Aware of these growing challenges, from the beginning of the 2000s the Chinese central government launched several new social programs to extend medical insurance. In urban areas, medical insurance was aimed at covering all urban workers and intended to function as a unified system across different work units. In rural areas, a new medical scheme, launched in 2003, was implemented to cover the whole rural population. From 2005 to 2006, coverage was increased from 600 to 1,433 counties, and by 2008 the program had been implemented in around 95% of all rural counties in China (Zhang et al. 2010).

New Age: Privatizing Public Hospitals in 2010s

Public hospitals in China are the most important healthcare providers and deliver more than 90% of the country's health services. One problem with Chinese healthcare is the lack of primary healthcare services, and the expansion and overuse of secondary and tertiary services. It has led to increased total costs and greater inequality. In 2010, the Ministry of Health started a pilot project to reform the public hospitals. The pilot project was started in 17 cities. The central goals of the reform are to increase equity, affordability, and accessibility (MoH 2010). No practical effort was made to reintroduce primary healthcare. Instead, the important components were to allow for private investment in healthcare, as well as to encourage and support the private ownership and operation of health services.

The 12th Five-Year-Plan for Health was announced in 2012, emphasizing non-public health service providers and an extension of the pilot project to reform public hospitals in other counties and cities (MoF 2014; State Council 2012, 2014b). At least one city in each province was chosen to participate in the public hospital reform during 2014, and over half of the counties will be included in reforming their county-level public hospitals, according to the plans (State Council 2014a).

The main goals have been the same across the cities, but local goals and practices also vary. It seems that most of the governments chose to adapt the market mechanisms and private actors as healthcare solutions. Only four of these cities mentioned goals of providing access to affordable basic healthcare through public and nonprofit hospitals. Some other cities, to varying degrees, shifted their strategies towards market competition and private ownership of public hospitals (Yip and Hsiao 2014). In Hubei Province, the number of new approved private health care institutes increased from 32 in 2012 to 130 in 2013. In Beijing, in just the first two quarters in 2013, 163 new private hospitals and clinics were approved (Chinanews 2014).

If the public hospital reform in China reaches its goal of equal and affordable access to health services, it would lead to efficient and quick treatment of patients, independent of their background. However, to date, most entrants in China have been motivated by profit, including the private hospital chains, pharmaceutical sector, medical equipment producers, and real estate developers (Yip and Hsiao 2014). The opening of the market for private health providers may also reinforce the use of high-tech medical tests and expensive medicines in order to signify high quality of services to patients. This may create an even higher barrier for the poor and for people with poor health, and these developments may be followed by greater income inequality.

Inequality and Health

Inequality in Healthcare System

Notwithstanding these attempts to improve the welfare system, in the middle of the 2000s, the quality of public health care actually worsened (Chan et al. 2008). Medical costs became even more expensive, and reimbursements were low. Many poorer people were thus excluded from access to basic healthcare. About 13% of Chinese households had to pay more than 40% of their disposable income on health, and half of inpatient costs were paid out-of-pocket by an average patient (Ouyang 2013). The share of households' out-of-pocket expenditure in health reached a top of 60% in 2001, with a reduction in the following years. However, health still takes a major part of the health cost. In 2010, households' used 35% of their out-of-pocket expenditure on health accounts (see Fig. 2).

The system was fairly unequal, with rural residents in the lowest income quintile spending the highest share of their income on healthcare (Yip and Hsiao 2009). Further, real income per capita in 2003 was almost double that of 1993, but health expenditure per admission/visit had quadrupled in the same period. On average, the cost of one inpatient hospitalization was 70–80% of an individual's annual income in 1993. In 2003, this had increased to be, on average, twice as high as an individual's annual income.

As a result, illness has become a major contributor to poverty: more than 20% of those living under the poverty line were in this situation because of disease or injury (Liu et al. 2003). Income has become a dominant factor in healthcare utilization: in

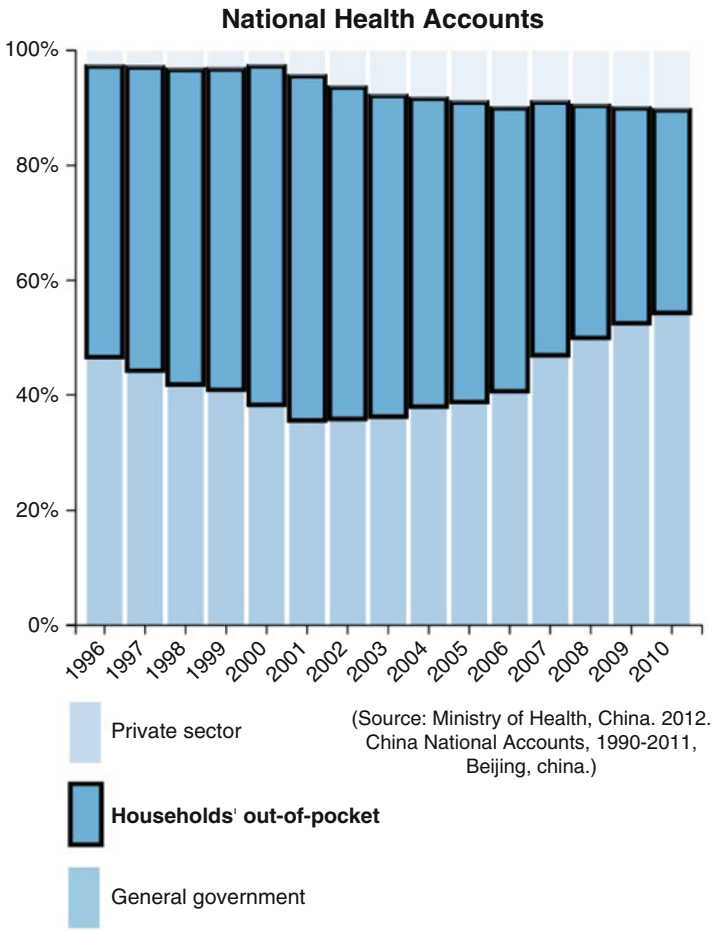

Fig. 2 Share of people in need of hospitalisation that were not hospitalised

rural and urban China, respectively, 24% and 16% of the population refused to be hospitalized due to financial burdens in 2003. Among those hospitalized, 32% of them discharged themselves against medical advice because they could not afford to stay (Yip 2010).

Overall, health inequalities have widened. In 2005, life expectancy was 6 years longer for urban citizens compared to rural citizens and 15 years longer for people living in eastern China compared to those in western China (Chen 2012). The rural to urban ratio of infant mortality rate has increased from 1.5 in 1981 to 2.1 in 1995 (Zhang and Kanbur 2005). Rural mortality was 30% higher than urban mortality among older people (Zimmer et al. 2007), and the maternal and under-five mortality rates in rural areas were about three times greater than those in urban areas (WHO and DRC 2005). In some rural areas, infant mortality even rose in the 1990s (Liu et al. 1998).

At the same time, primary healthcare provision failed in its function to provide preventative care. On average 120,000 new cases of resistant tuberculosis have been reported each year (Shan 2013). In 2012, there were 7 million cases of infectious disease that caused 17,000 deaths (DPES 2005). Some of the previously nearly controlled infectious diseases, such as schistosomiasis, began to spread again (Blumenthal and Hsiao 2005).

The Malnourished

Earlier research has shown that the impact of income inequality on health is strongest for the poorest groups (Fang and Rizzo 2011; Yang and Kanavos 2012). According to the China Family Development Report 2015, the household incomes of the top 20% of households in China are 19 times higher than that of the bottom 20% (NHFPC 2015a). One direct way of measuring the impact of poverty on people's health is to look at malnourishment. During 2005 and 2007, 130.4 million Chinese people were undernourished, accounting for 10% of the total population (FAO 2010). The prevalence of anemia among children aged 6–12 months living in rural China was 35% in 2005, and the corresponding number was as high as 28% when urban children of the same age were included (MoH 2012). Some studies reported prevalence rates of anemia for children living in rural areas in China ranging from 20% to 60%, implying more than 10 million children were affected (Miller et al. 2012). A report from UNICEF referred to an estimation of 12.7 million stunted children in China – that is the same size as the population of Tokyo. The authors found that in poor rural areas in central and western China, one out of 10 children under 5 years old were stunted. In Qinghai Province, the prevalence of anemia among children aged 6 months to 2 years old was above 70% (Liang 2013).

As recently as 2015, malnutrition among adults and children/adolescents in China was estimated to be 6% and 9%, respectively (NHFPC 2015b). Childhood anemia may also impair cognitive development, school performance, and work outcomes and also cause lower socioeconomic status throughout the life course (Stoltzfus 2001).

Inequality and Health in China

Following the process of market transition, many Chinese face greater challenges and pressure. The labor market has become more competitive, stressful, and demanding; job situations are more unsecure since the formerly lifelong employment in the state sectors has been reduced; family obligations lie more heavily on individuals because of the lack of welfare support. The tremendous changes inevitably influence people's daily life, psychological condition, lifestyle, and health-related behaviors and are further embodied in their physical health outcomes (WHO and DRC 2005). Health-related behavior and lifestyle changes such as nutrition, physical activities, smoking, and alcohol have been recognized as important reasons

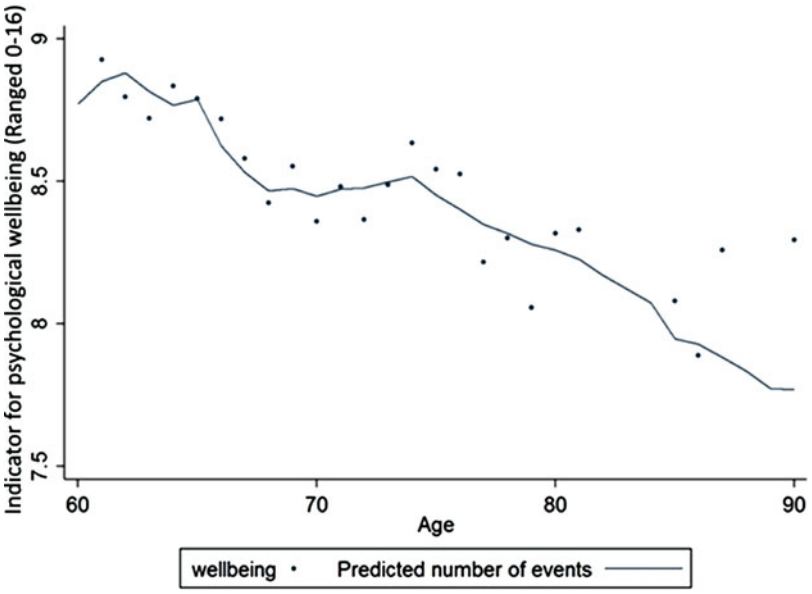

Fig. 3 Correlation between age and psychological wellbeing (Data source: Chinese Health and Nutrition Survey, 2006–11)

for increased health problems (DRC 2005; WHO 1999). By using CHNS 2006 data, Yang and Kanavos (2012) concluded that poorer people are less physically active and less likely to report their health as good or excellent. Using four waves of the same data set from 1991 to 2000, Li and Zhu (2006) found that self-rated health is positively associated with per capita income, but high inequality in a community increases the probability of harmful health-related behavior such as smoking and drinking.

Another vulnerable group is the elderly in China. Subjective wellbeing, as an indicator of psychological health, reduces rapidly with age for people over 60 (Fig. 3). Chinese people born in and before the 1950s have spent more than 25 years of their lives under socialism and experienced a time with rapid social changes, including increased inequality and reduced welfare support.

When considering psychological health, Bakkeli (2016b) found that higher income inequality is associated with lower subjective wellbeing, when studying older adults living in China. This relation is illustrated in Fig. 4. Furthermore, in places with higher degrees of income inequality, poorer individuals may suffer even more from health problems. Chen and Meltzer (2008) found that in rural China, obesity and hypertension are positively associated with increased income inequality in a county, but also with the county's average income. Fang and Rizzo (2011) confirmed that income inequality was negatively correlated with self-reported health, and the effect of inequality on individual health was stronger for individuals from lower income households, compared to those from richer families. Empirical

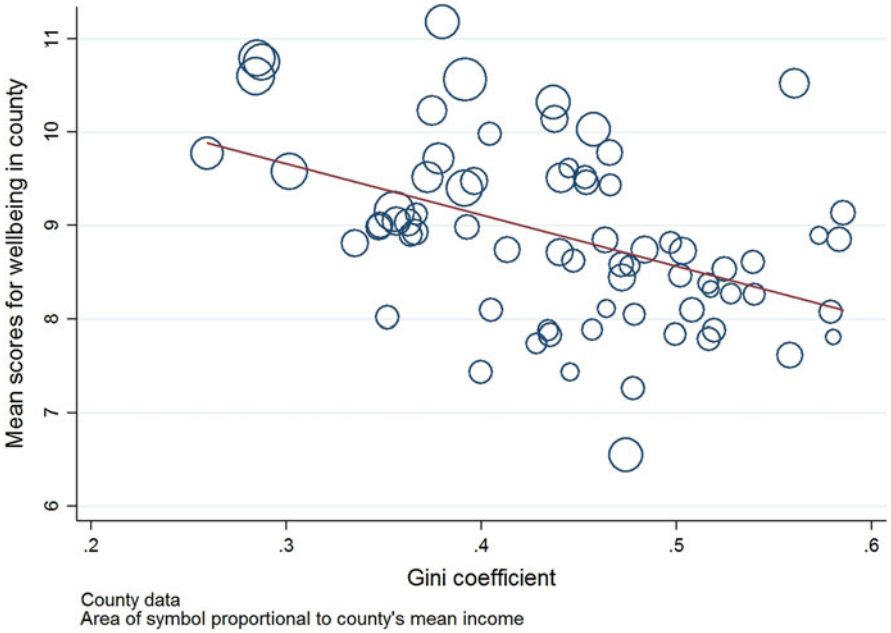

Fig. 4 Correlation between income inequality and subjective wellbeing in China, by counties (Data source: Chinese Health and Nutrition Survey, 2006–11)

studies of the income inequality hypothesis from China are relatively few, and the findings are rather mixed. For example, Pei and Rodriguez (2006) claimed that after adjusting for individual and household variables, the effect of inequality on health disappears. When analyzing longitudinal data sets and defining health using different physical measures, no statistically significant connection was detected between inequality and health outcomes in China (Bakkeli 2016a).

Health Situation and Epidemiological Transition

Recent Health Situation in China

The numbers of Chinese people diagnosed with cardiovascular diseases, hypertension, and obesity increased during the reform period. The Chinese Centre for Disease Control and Prevention estimated that 200 million Chinese people were diagnosed with hypertension in 2010, about one in five adults (CGMH 2011). Accordingly, above 40% of all deaths each year were caused by cardiovascular and cerebrovascular diseases; half of them were caused by high blood pressure. Some official sources have shown that about 24% of those aged 15 or older in China had hypertension. Among youth and adolescents, about 15% of them were

diagnosed with high blood pressure, and approximately 30% of them were diagnosed with obesity (The Central People's Government of the PRC 2013).

Previous studies have recognized occupational and psychological stress, which is a complex biopsychosocial situation, as a major health hazard worldwide; it contributes to different diseases, such as depression, cardiovascular disease, abnormal blood pressure, and even obesity. Similarly, one of the major factors contributing to cardiovascular diseases in China was recognized to be lifestyle and work pressure (Gong et al. 2006). The rising degree of competition, increased income inequality, and labor market stratification in China may also have an impact on people's stress levels, and may cause health problems.

Diabetes has also become an important public health challenge in China. The prevalence of Type 2 diabetes and impaired glucose tolerance was reported to be about 1% in a survey carried out in 1986 for urban citizens aged 25–74 in northeast China (Li et al. 1996). The numbers for the above-mentioned diseases increased to 2.5% and 3.2% in 1994, respectively (Pan et al. 1997). Some other reports found the prevalence of diabetes in the adult population to be much higher than the previously reported results. According to the International Collaborative Study of Cardiovascular Disease in Asia, in 2000–2001, the prevalence of diabetes for Chinese men and women aged 35–74 was as high as 5.2% and 5.8%, respectively (Gu et al. 2003). Furthermore, the rate in northern China was higher than in southern China (7.4% compared to 5.4%) and higher in rural areas than in urban areas (7.8% compared to 5.1%).

Some have argued that when considering noncommunicable diseases (NCDs), familial factors and inherited genetic variants may be more relevant in Chinese people than in Europeans (Ma et al. 2014). Although genetic predispositions may be an important factor in the development of cardiovascular diseases, environmental factors are also of major importance. Epidemiological studies have identified several risk factors associated with the development of cardiovascular diseases, such as overweight and lifestyle, air pollution, environmental noise, and the social-cultural context.

NCDs such as type II diabetes are highly preventable. However, the management rates for such diseases in China are very low: less than half of those who were diagnosed by hypertension and diabetes in China were treated by health care providers in 2008. Because of a lack of prevention information, about 30% of those who had hypertension were not aware of their condition before diagnosis, and 54% had never received blood pressure tests by health providers (Meng and Tang 2013). This may be a reflection of the Chinese healthcare system: despite the fact that the central government in China has started to focus more on wellbeing and human development, local governments have still prioritized economic development.

Phases of Transition

China may be facing a new epidemiological transition today. Epidemiologic transition is the changing pattern of diseases and mortality in a society, and the theory of

epidemiological transition, developed by Omran (2005), focuses on the complex change in patterns of health and disease, and how these patterns are connected to the demographic, economic and sociological determinants. The decline in mortality is closely linked to the process of modernization. While socioeconomic development was recognized as the most important factors for improving living standards and reducing mortality in the western model, medical technologies and public health were considered more important for the accelerated and contemporary model.

Cook and Dummer (2004) attempted to apply the model of epidemiologic transition to China. They documented that before 1949, infectious disease and natural catastrophes such as flooding and drought were the major causes of mortality in China, corresponding to Omran's "age of pestilence and famine." From the 1990s, China experienced an important overall decline in infectious diseases of poverty and underdevelopment, such as cholera, dysentery, hepatitis, and typhoid. In the last decade, China has experience increases in death rates from chronic diseases, including cancers and heart disease. These shifts reflected Omran's model quite well. However, Cook and Dummer emphasized that epidemiologic transition in China is segmented, with large variations between rich and poor, and between urban and rural areas, as well as an emerging "medical poverty trap" that reinforces the widening inequalities of access to healthcare services.

One may argue that income inequality is a component in and unintended consequence of the economic transition and that it further mirrors the ongoing epidemiological transition. However, Wilkinson demonstrated the relation between income inequality and life expectancy in wealthier industrialized countries that have passed through the epidemiological transition and where the cause of mortality has changed from infectious diseases to chronic diseases. Observing that life-expectancies had increased in countries with lower income inequality, while countries with higher inequality had fallen behind in terms of life-expectancies, Wilkinson argued that whereas material deprivation leads to poverty and infectious disease, social disadvantage provokes stress and chronic disease (Wilkinson 1996).

Similarly, Gaylin and Kates (1997) criticized epidemiological transition theory for being overly optimistic about the demise of infectious diseases and argued that it ignored the importance of social inequalities. They suggested that socioeconomic inequality should be brought to the center of the analysis and that epidemiologic differences between population subgroups should be taken seriously.

Epidemiological Transition in China?

This new phase of epidemiologic transition is defined by a shift in the burden of disease to NCDs. Because of economic growth and poverty reduction, the transition is being accelerated by a shift from malnutrition to overnutrition. The population faces a new set of health problems, including diseases of affluence, the impact of smoking and drinking, hypertension, environmental pollution-related health risks, and the rise of infectious diseases (Yang et al. 2013; Zhou et al. 2016).

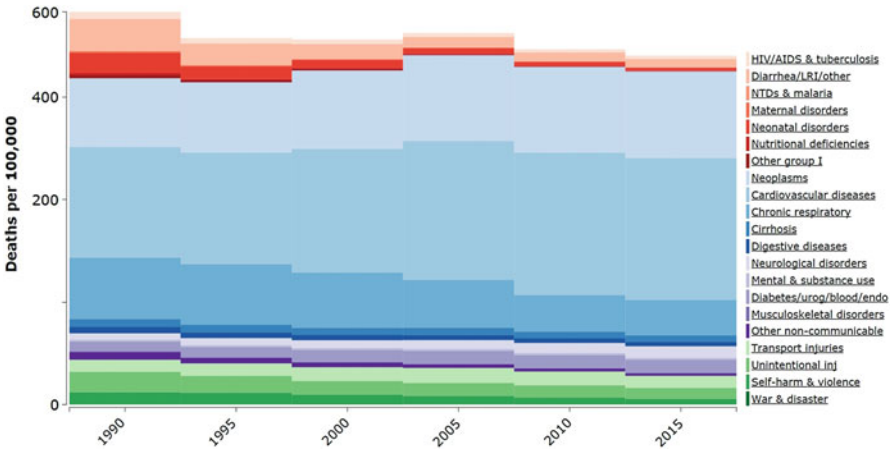

Fig. 5 Leading death causes in China, 1990–2015 (Data source: Institute for Health Metrics and Evaluation (IHME))

See Figs. 5 and 6 below for changes in diseases. Of the 8.3 million deaths in China per year, 7 million are due to NCDs. The leading risk factors for disability-adjusted-life-years in 2010 in China were cardiovascular diseases, cancers, low back pain, and depression. Other risk factors included dietary risks, high blood pressure, tobacco, and air pollution (Yang et al. 2013).

By investigating how patterns of cause of death had changed from 1990 to 2003 in 33 provinces in China, Zhou et al. (2016) show that during this period, life expectancy had improved, the death rate had fallen by almost a third, and that the major causes of mortality had changed. In 1990, the most important causes of death in most provinces were lower respiratory infections and preterm birth complications. In 2013, the leading causes of death were stroke, ischemic heart disease, chronic obstructive pulmonary disease, and lung cancer. Mortality due to infectious diseases, diarrheal disease, and lower respiratory infections had fallen substantially. Throughout the country, an increased proportion of deaths were caused by road traffic injuries throughout the country.

At the same time, challenges related to urbanization and widening social and regional disparities persist. The epidemiological transition is not unrelated to social contexts. For example, Lei et al. (2010) found that hypertension affects all socio-economic classes independently. But people with a better education living in urban China are more aware of their condition, and there is therefore a higher treatment percentage and better control among this group. Further, public healthcare services fail to inform patients of their hypertension status. Yang et al. (2013) interpreted the rapid rise of NCDs as driven by urbanization, rising incomes, and aging. Richard Horton, the present editor-in-chief of *The Lancet*, commented on China's transition and suggested that China needs to place a greater emphasis on building a primary healthcare system, addressing environmental threats, and reducing inequalities (Horton 2015).

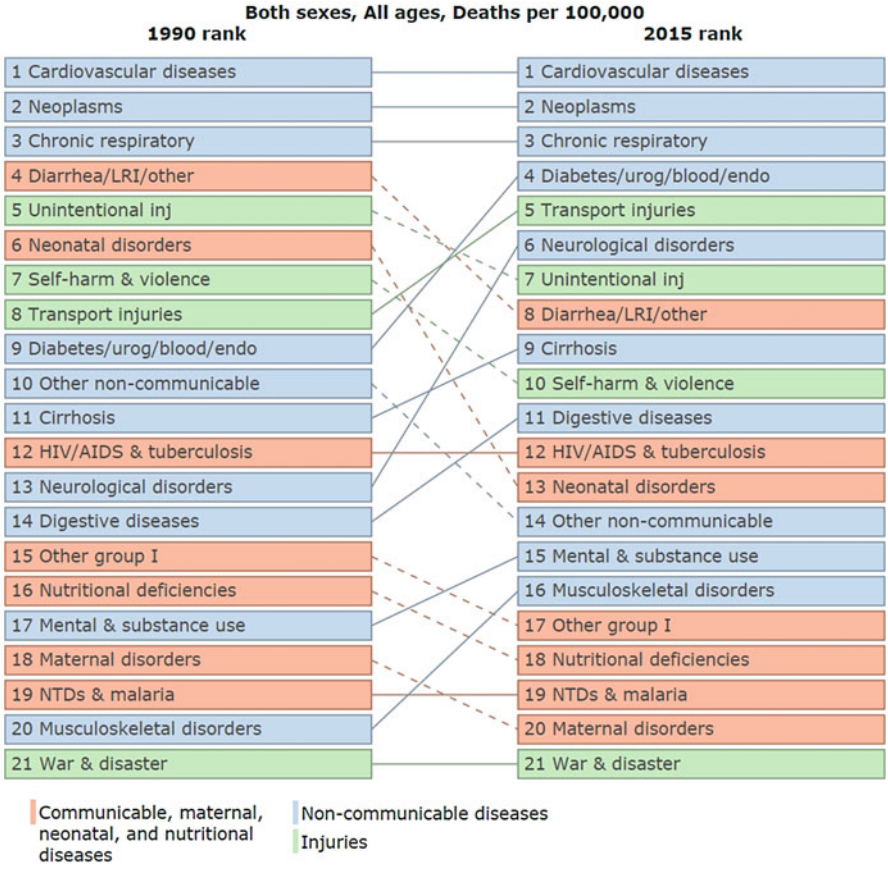

Fig. 6 Ranking of leading death causes in China, 1990 and 2015 (Data source: Institute for Health Metrics and Evaluation (IHME))

Conclusion

There is an increased awareness of the need to not only keep an eye on factors like income inequality or income in itself, but also to have a more nuanced discussion that includes the ongoing epidemiologic transition in China. There is also an urgency to place more emphasis on developing China's healthcare structures, as well as general discussions concerning welfare support. This faces challenges due to China's demographic and epidemiologic transitions. The population is aging rapidly. The increasingly elderly population, one-child families, dual-employed parenthood, and large-scale rural-to-urban migration led to a more fragile family base. At the same time, China has experienced an epidemiologic transition in a much shorter time than many other countries – there has been a shift

in leading mortality causes and behavioral changes such as smoking and lack of physical activity. With 177 million adults already diagnosed with hypertension, 303 million smoking adults, and the emergence of NCDs and chronic diseases, China's healthcare reforms and healthcare institutions face particular challenges.

The challenges include the integration of primary care services with secondary and tertiary healthcare, prevention strategies and treatment for chronic diseases, and public policy support. Further, such policies are integrated with welfare policies for employment, income maintenance, housing, education, etc.

Reducing NCDs is one of the key interventions for a reduction of poverty and social and health inequalities. Therefore, it is important that welfare institutions can also adapt to new challenges caused by the transition.

Policies and Protocols

In this chapter, we have discussed three major factors that have been important for health in China. These are healthcare system and reforms, inequality, and epidemiologic transition. We have discussed the impact of market-oriented healthcare reforms and its possible impact on health inequality and population health. Developing a well-functioning healthcare structure and social policies are important for both users of welfare state, as well as new challenges connected to social, demographic and epidemiological transitions. Following are some of the policy suggestions.

- China needs a broader public policy support with a focus on different social strata. More attention to prevention strategies and treatment for chronic diseases is essential.
- An important challenge for future healthcare reforms is to integrate primary care services with secondary and tertiary healthcare.
- Besides further development of infrastructure, it is urgent to reduce overall inequality in China.
- Reducing noncommunicable diseases is one of the key interventions for poverty reduction and increased equity and equality.

Dictionary of Terms

- **Noncommunicable diseases** – Refers to a diverse set of chronic diseases, including heart disease, stroke, cancer, diabetes, and chronic lung disease.
- **The epidemiologic transition** – Describes changing patterns of different demographic trends. This includes age distributions in a society, mortality, fertility, life expectancy, type of diseases, and causes of death. In this chapter, we focus on the epidemiologic transition in which dominant causes of death has changed from

infectious diseases, malnutrition and war, to noncommunicable diseases, accidents, pollution, etc.

- **The Gini-coefficient** – The Gini-coefficient is a statistical measure of income inequality in a society. It is ranged from 0 to 1, from total equality to maximal inequality. A value placed between these extremes indicates how unequal the income distribution is. As the value rises, the degree of income inequality is higher.
- **Malnutrition** – Primarily refers to both undernutrition and overnutrition. This chapter focuses more on the undernutrition aspect of malnutrition, i.e., individuals who do not consume enough food.
- **Privatization** – Primarily refers to state-owned enterprises passing under private ownership, in addition to the growth of the private sector and private shareholders. Furthermore, the general withdrawal of state or public services in certain areas is a form of privatization, since it reduces state responsibilities and increases the reliance on private service providers.

Summary Points

- This chapter reviews three factors that contribute to health problems in China: healthcare system, inequality, and epidemiologic transition.
- The market-oriented healthcare reforms in China have led to more profit-oriented medical services, while to some extent ignoring the healthcare system's primary function of infectious disease prevention and securing population health.
- The healthcare reforms have led to uneven distribution of healthcare and increased economic burdens for the poor.
- China is facing challenges of increasing health and income inequalities. A higher degree of inequality may also lead to lower psychological and physical wellbeing.
- The ongoing epidemiologic transition requires more policy attention. Reduction, treatment, and prevention of noncommunicable diseases should be given higher priority.

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Drinking-Water Access and Health in Refugee Camps

26

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Abstract

Drinking-water deprivation in terms of access, quantity, and quality poses serious health hazards both in the short and long term. This is especially critical in refugee chronic situations that last for decades, such as the Saharawi refugee camps (>40 years). Water access and water quantity improves health, as water is not only needed for direct consumption or cooking but also for hygiene, directly related to health. On the other hand, water quality avoids not only gastrointestinal diseases in the short term, but also other health issues that appear under a long-term exposure. This chapter reviews the history of drinking water and health at the Saharawi refugee camps, showing how water access has improved over the years but still does not guarantee the minimum quantity of 20 l/person/day established by the United Nations High Commissioner for the Refugees (UNHCR). It also shows how water treatment using chlorination has reduced dramatically the diseases associated to microbiological contamination, and how the poor raw water quality that a percentage of the population is still consuming has produced long-term health effects, such as the prevalence of fluorosis and goiter.

Keywords

Refugee camps · Protracted · Drinking water · Access · Quantity · Quality · Health risk · Microbiology · Diarrhea · Nitrate · Blue-baby syndrome · Fluoride · Dental fluorosis · Iodine · Goiter

List of Abbreviations

NGO	Nongovernmental organization
SPHERE	Sphere Humanitarian Charter and Minimum Standards in Disaster Response
UNHCR	United Nations High Commissioner for the Refugees
UNRWA	United Nations Relief and Works Agency
WFP	World Food Programme
WHO	World Health Organization

Introduction: Water and Health

Why drinking water when talking on starvation or nutrient deprivation? Although discussing on nutrition issues is often directly related to food income, water should be always considered as one of the main sources of nutrients in any nutritional study. As the United Nations High Commissioner for the Refugees (UNHCR) states, "Water is one of the main nutrients, along with fat, protein, carbohydrates, and micronutrients, that the human body needs on daily basis" (UNHCR 2016a). Water is needed for food preparation; therefore, safe drinking water is essential as otherwise it can cause diarrhea, producing loss of nutrients that results in malnutrition. This is especially dangerous for children under five as malnutrition contributes to almost 60% of deaths of this fragile population (UNHCR 1992).

But not only microbial safety is one of the main required characteristics of drinking water, there are also physicochemical requirements to guarantee water safety. Water quality includes also a series of micronutrients that can affect health, by absence or excess, and both are equally risky. Some examples are nitrate presence, fluoride, or iodine, which will be explained further in the next sections. And while microbiological contamination produces short-term health effects, causing diarrhea almost immediately, most of physicochemical components will only show their effects after several years of exposure (long-term effect), which is especially risky in some refugee situations that last for long periods of time (decades).

Finally, the other main challenge is to provide sufficient water quantity to the population, which is not only related to the amount of safe water available for drinking and/or cooking, but to other uses such as hygiene (personal washing, household cleaning) and sanitation, which are directly associated to health benefits. How to decide how much water is needed? In the past, organizations and relief agencies used to have different criteria till the development of the SPHERE Project (The Sphere Project 2011), which defines the minimum requirements for water quality and quantity, along with water access, for emergencies situations. In this line, UNHCR adapted these standards for refugee situations (UNHCR 2017), considering that refugees might stay longer in a camp that an "emergency situation" (Table 1). A minimum of 20 l/person/day of water is established, along with no microbiological contamination and a minimum of 0.2 mg/l of free residual chlorine to ensure water safety.

Guaranteeing drinking-water access at refugee camps is actually a worldwide challenge, especially in situations where the resources are scarce, and also in cases of

Table 1 Water guidelines for refugee situations. Guidelines on water minimum standards for refugee populations defined by the United Nations High Commissioner for the Refugees (UNHCR) (and comparison with the Sphere Humanitarian Charter and Minimum Standards in Disaster Response (SPHERE) Project for emergencies), including water quantity, quality, and access

Parameters	Standard	UNHCR (refugee camps)	SPHERE (emergencies)
Water quantity – basic needs for health and well-being	Average quantity of water available per person/day	>20 l	>15 l
	Water containers per household (average of 5 members)	1×20 l, 2 × 10 l, 2 × 5 l	2 of 10–20 l
Water quality – prevention of health risk	Number of fecal coliform organisms at distribution point (microbiological content)	0 per 100 ml of treated water	0 per 100 ml of treated water
	Free chlorine residual concentration in disinfected water	0.2–0.5 mg/l	0.5 mg/l
Water access	Distance from farthest dwelling to water point	<200 m	<500 m
	Number of persons in each water point	80 to 100/tap 200 to 300/hand pump/well	250/tap500/hand pump 400/well

chronic crisis, the "protracted refugee" situations (Loescher et al. 2008; UNHCR 2009). According to UNHCR, "6.7 million refugees were in a protracted situation by the end of 2015," plus 5.2 million Palestinian registered with the UNRWA, about 12 million in total (UNHCR 2016b). Among the longest refugee camps, there are the Palestinian population in Gaza, Jordan, Lebanon, and the West Bank (1948, >65 years), the Sudanese in Ethiopia (since 1950), the Burundians in Tanzania (1971) and the Saharawi camps in Algeria (1975, >40 years). In these long-term cases, the effects on health from an inadequate nutritional intake, including an appropriate drinking-water source, are always more serious, not only because of the long-term effects of malnutrition, but also because the population keeps depending on international external assistance for fulfilling their basic needs, for an indefinite period of time, staying in a "limbo" situation.

This chapter reviews the case of the Saharawi refugee camps in Algeria, describing their challenges for a clean drinking water source, and the health effects that the deprivation of safe drinking water has produced in the refugee population since the early days of the camps.

Drinking-Water Access at the Saharawi Refugee Camps

Saharawi refugee population from Western Sahara is one of the "forgotten" international conflicts that remain in a protracted situation. After 40 years, there are still about 165,000 refugees – according to local authorities and the estimations of the Government of Algeria – that live under the harsh conditions of the Sahara desert, with very limited access to basic needs, including those related to health, energy, and water. Meanwhile, there has not been any significant advance in the peaceful political conflict resolution and most of the refugee population still live in very precarious conditions.

The refugee camps were established in 1975 in the initial stages of the international conflict in Western Sahara. At present, they are composed by one institutional center (Rabouni) that comprises the administrations, and five refugee camps located near Tindouf (Algeria). These refugee camps are divided into five "wilayas" (Fig. 1): El Aiun, Awserd, Smara, Dahkla, and Boujador (Boujador is near Rabouni, not shown in the Figure). Each wilaya is organized in smaller communities called "dairas." Most of the population live in tents ("jaimas") or rudimentary houses made of adobe, made with a mixture of sand and water ("mud-houses"), with no direct access to water neither electricity (Fig. 2).

Drinking-water access has been one of the main concerns since early days of the refugee situation, starting with "survival" emergency shallow handmade wells, then building deep groundwater wells, and finally incorporating water treatment using reverse osmosis and/or chlorination; 100% water supply in terms of quantity and quality has not yet been achieved. This evolution corresponds to the natural change from an initial "temporary" settlement (emergency) to a more permanent one (prolonged crisis). Three stages on the water supply access can be differentiated (Fig. 3):

Fig. 1 Situation of the Saharawi refugee camps. Map of Western Sahara (UNHCR 2006) and detail of the Saharawi camps area, showing the different wilayas. Data are from UNHCR (2006), with permission from Publishers

Fig. 2 Overview of Saharawi refugee camps. Overview of the Saharawi refugee camps. Tents and adobe houses constitute the dwellings of most of the population. Picture is from the authors

Stage I (1975–1993) – Shallow Groundwater Wells

In 1975, the drinking-water supply was set up as an "emergency" solution to the Saharawi population that fled the Western Sahara region to Algeria. It consisted of traditional handmade shallow groundwater wells and superficial ponds dug at El Aiun and Dakhla. They supply 75% of the population, up to 20 l/person/day, which is above the minimum standard given by the Sphere Project (15 l/person/day) (The Sphere Project 2011) or the WHO (WHO 2011a) for emergency situations. Water quality was originally good, although contamination was a main risk and water quantity was seriously limited.

Stage II (1994–1999) – Deeper Groundwater Wells

In 1994, after already almost 20 years of settlement without any significant evolution in the water supply access, unusual strong rains caused devastating floods that contaminated the shallow wells mainly due to the lack of a sanitation system in the camps, originating a cholera epidemic at El Aiun. This led to a considerable change in the water supply as the shallow wells were no longer a safe solution.

Deeper groundwater wells of about 100 m with automatic pumping were created followed by a chlorination stage (only microbiological treatment). Water supply was centralized in these new wells rather than in the immediate vicinity of the households as for the previous traditional wells, and then water distribution was operated by tanker trucks to the different camps. Another important aspect was the introduction of the first water microbiological analysis from that moment onwards (*E.coli* and total coliforms analysis). These improvements guaranteed safer drinking-water access, but the available water quantity per person decreased dramatically to

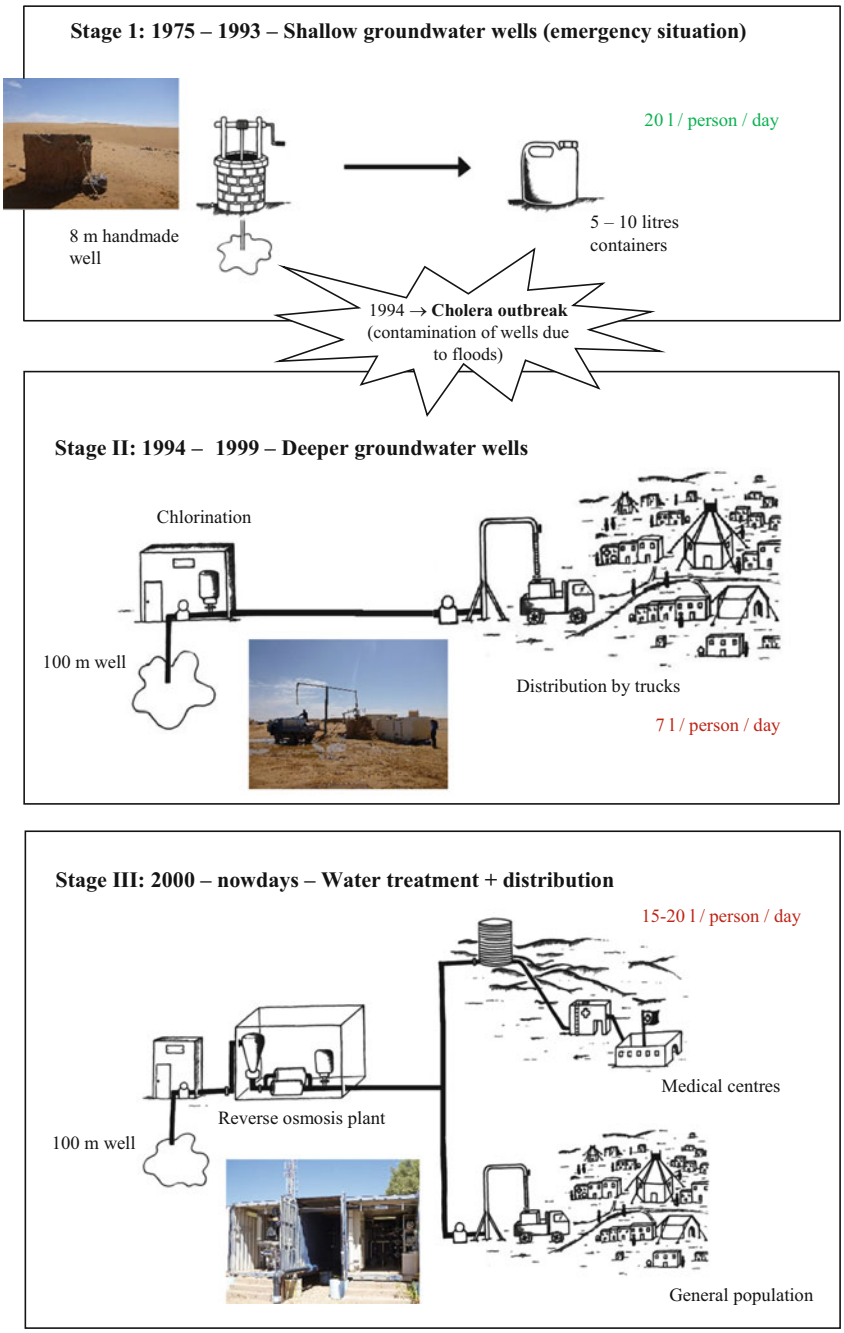

Fig. 3 Water access at the Saharawi camps over time (1975–2016). Different stages and the water supply access characteristics of each of them: a) Stage I (1975–1993): shallow 5–8 m deep hand-made wells, b) Stage II: 1994–1999: deeper groundwater wells (~100 m) plus chlorination and

7 l/person/day, below the minimum standards of 15 l/person/day given by the Sphere Project. Simultaneously, the associated costs increased notably.

Stage III (2000–Currently) – Water Treatment and Distribution

After a more than 25 years in the camps, and with no significant advances in the resolution of the political conflict, the Saharawi authorities and UNHCR decided to further improve the current water supply facilities. The main improvements included: (a) raw water treatment using reverse osmosis, (b) water quality control (physicochemical and microbiological analyses), and (c) expansion of water distribution networks. Now, general drinking-water supply at the camps is organized in three zones that include one or several wilayas:

- *Zone 1: El Aiun, Awserd*. Water supply consists of groundwater wells and a reverse osmosis plant for both wilayas. The treated water quantity in the plant is not currently sufficient to supply continuously the population in these two wilayas so each wilaya receives treated water in turns of 20 days.
- *Zone 2*: *Smara, Rabouni, Boujador*. Groundwater wells and two reverse osmosis plants working alternately provide continuous treated water to the zone.
- *Zone 3*: *Dakhla.* This wilaya is located about 140 km far from the rest of the refugee camps, so it has a dedicated water supply which consists of a groundwater well followed only by a chlorination stage due to the good quality of the raw water.

Water Quantity and Health

The different uses of safe drinking water are directly linked to health effects on the population. First, the main use of water is for human consumption, which includes drinking and cooking. As stated by WHO and Kleiner (Kleiner 1999; WHO 2003a), "Water is a basic nutrient of the human body and is critical to human life. It supports the digestion of food, adsorption, transportation and use of nutrients, and the elimination of toxins and wastes from the body." The immediate consequence of not having access to an adequate quantity of safe drinking water is dehydration, which can cause diarrheas. As an indication established by WHO, the volumes of water required for maintaining hydration are of 2.2–2.9 l/day for adults and 1.0 l/day for children.

Regarding cooking, water is needed for the preparation of foodstuffs, and quantities can vary significantly depending on the diet, although a minimum water quantity must be always included to be able to prepare the basic food basket of a

Fig. 3 (continued) water distribution by trucks, c) Stage III: 2000 - nowadays: groundwater wells followed by reverse osmosis, wider water distribution including also medical centers. Data, drawings and pictures are from the authors

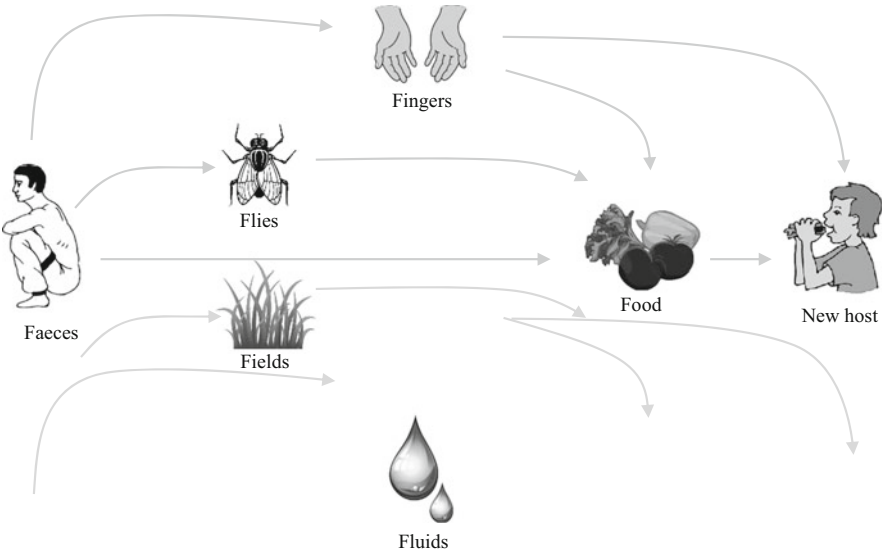

Fig. 4 Fecal-oral route for transmission of diseases, showing the importance of personal hygiene (requiring a sufficient quantity of safe water). Scheme from the authors

family. Additionally, it is important if the milk is provided as powder or not, as this would increase the required quantities of water.

The other important use of water that is related directly to the health status of a population is hygiene, including personal washing and domestic cleaning. Within personal washing, one critical aspect is handwashing, as an inadequate habit of handwashing can accelerate cases of diseases transmitted through the fecal-oral route (it is necessary to hand wash before eating and cooking and after defecation), such as diarrhea and skin and eye diseases (Fig. 4). In many developing regions, the main reason behind an inadequate personal hygiene is the lack of an appropriate quantity of water (WHO 2003a).

As defined by UNHCR, the minimum water quantity in a refugee situation should be at least 20 l/person/day. Recent data on the quantity of water supplied to the Saharawi people report that it is currently of about 15–17 l/person/day in all the settlements (below the UNHCR standard) excepting Dakhla that reaches 20–25 l/person/day (Vivar et al. 2016), although other sources (AECID 2015) report even lower quantities: 15–20 l/person/day in Dakhla, Smara, and Boujador and only 9–11 l/person/day in El Aiun and Awserd camps. UNHCR also reported in 2015 an average quantity of potable water distribution for the Saharawi refugees around 18 l/person/day (UNHCR 2015). In conclusion, the quantity of water provided to the population is currently insufficient and improvements on the service should be implemented to guarantee the minimum amount of water established by UNHCR.

Another important consideration regarding water quantity is the reliability of the service, or in other words, the water supply continuity. One of the problems of the

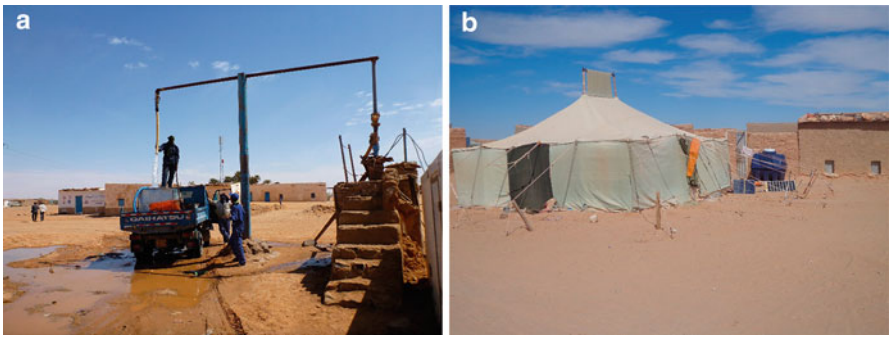

Fig. 5 Water distribution at the Saharawi camps . Example of water distribution network at the Saharawi camps: a) main water supply point in Dakhla using trucks for distribution to households; b) typical tent in the camps, with a water tank in the vicinity (in this case it is adequate for human consumption, vs. other tanks made of metal or other non-food grade materials). Photos from the authors

Saharawi water distribution system is that is based on trucks that transport the water to the different wilayas (Fig. 5). According to the latest reports, the condition of the fleet is not adequate and the trucks are in very poor condition, which often results in the fact that they are not able to adhere to the distribution calendar, producing shortcomings of water in the population (WFP and UNHCR 2013). Although in some cases the amount of water might reach the minimum requirements, often these quantities are not provided continuously, with periods of time with insufficient water to cover the basic needs.

Finally, the other important issue in the Saharawi camps is the variations of demand of water over the year. WFP and UNHCR conducted a survey in 2012 and found that the quantity of the water distributed during the winter (December–February) was considered sufficient to meet the needs of households, while in the summer months (June–August) the demands for water increased due to the high temperatures in the desert (>50 °C) and it was not sufficient (WFP and UNCHR 2013).

Water Quality and Health

Water quality is essential to guarantee a healthy status of any human being. An inadequate water quality has not only immediate effects on health (usually associated to gastrointestinal diseases), but the continuous consumption of water with inappropriate chemical content can origin other diseases in the long-term.

Short-Term Health Risks: Microbiological Content and Nitrates

In general, the effects of short-term ingestion of contaminated water are more widely known, as they produce rapid and acute effects on health such as watery diarrheas

that often lead to death in developing countries (mainly due to the lack of safe water to prevent dehydration during diarrhea), especially in children under five. These gastrointestinal diseases transmitted by water might have their origin in the microbiological content of water: microorganisms that are pathogen for the human, such as bacteria, viruses, and protozoa. These microorganisms have their optimum growth temperature about 37 °C, which is the average normal human body temperature, so when they are in the human organism they can proliferate rapidly.

The most common bacteria related to fecal microbiological contamination of water (Fig. 4) are *Escherichia coli*, *Intestinal enterococci*, and *Clostridium perfringens*, which are used as indicators of water contamination. These bacteria are present in the normal intestinal flora of humans and animals, where they can live with no harm for the host. The problem arises when they enter in other parts of the body via ingestion of water where they can cause infections.

Another group of bacteria that is used as an indicator of water contamination after it has been disinfected is total coliform bacteria, which should be absent immediately after disinfection, so their presence indicates inadequate treatment or contamination problems in the distribution network.

Finally, one of the most common groups of bacteria in epidemics in developing countries associated with water contamination is *Vibrio*, with pathogenic species such as *V. cholerae*, producing cholera, with symptoms that include fulminating and severe watery diarrhea. To guarantee microbiological safety of water, WHO standards (WHO 2011a) indicate that the content in water of any of the *microbial indicators* must be *0 in 100 ml of water*.

There are also a number of chemical components that at high concentrations can produce immediate effects on the human body posing serious hazards. Despite the general understanding that inadequate physicochemical properties of water show their effects in the long term, there are chemicals such as nitrates (NO_3^-) that are especially dangerous for the infant population, for those being fed with artificial milk ("bottle-fed").The hazard appears when water from ground wells containing high concentration of nitrates is used for the preparation of milk. When the water is ingested by the infant, large quantities of nitrates are converted into nitrites (NO_2^-) that react with hemoglobin in the red blood cells and forms methaemoglobin, blocking the oxygen transport. It is commonly called the "blue baby syndrome" because of the blue-gray skin color. The disease can evolve rapidly causing coma and death if not treated adequately (WHO 2011b).

As today, the standard limit given by WHO is 50 mg/l of nitrates in drinking water.

Long-Term Health Risks: Fluoride and Iodine

Other chemical compounds that might be present in the water will show their health effects only after several years of exposure. These are long-term health risks and are especially relevant for the case of chronic humanitarian crisis. For the case of the Saharawi camps, the main chemical contaminants naturally present in water are fluoride and iodine:

- Fluoride

Fluoride (F^-) in high concentrations can appear in groundwater, usually in areas with naturally fluoride salts. While at low concentrations fluoride is used as protection against dental caries and it is added to toothpastes, gels, or even drinking-water, higher concentrations than >1.5 mg/l if ingested during years can lead to important diseases as dental fluorosis and skeletal fluorosis. Fluoride is absorbed by the bones, and with time it can increase the possibilities of bone fractures and produce chronic pain. Fluorosis in teeth in children usually produces a characteristic "mottled enamel" aspect that in some cases can cause physical damage to the teeth (WHO 2003b).

WHO standards for fluoride in water is 1.5 mg/l.

- Iodine

Although WHO does not establish a direct relationship between iodine in drinking water and associated health effects due to either deficiency or excess of iodine, it does give an indication of the total dietary requirement for adult humans, ranging from 80 to 150 μg/day. This number includes all the nutritional sources that compose the dietary intake, not only the drinking water, but in many parts of the world, the water is an important source of deficiency or excess of iodine, and it affects directly the total iodine daily ingestion. Iodine is an essential element for the synthesis of thyroid hormones and, in inadequate quantities, can lead to severe adverse effects on health, including hypo and hyperthyroidism, goiter, and neurological development.

Only the Chinese legislation establishes standard values to evaluate the iodine concentration in water: adequate (10–150 μg/l), inadequate (high iodine zone >150 μg/l), and iodine excess goiter zone (>300 μg/l) (Pichel and Vivar 2017).

Water Quality and Health at the Saharawi Camps (1991–2016)

The relationship between water quality and health at the Saharawi camps can only be understood following the evolution of water quality along the years and the water supply infrastructure improvements (section Drinking-Water Access at the Saharawi Refugee Camps). This is especially important when studying diseases with origin in long-term exposures to certain substances.

- Microbiological content

The main epidemic related to microbiological contamination of drinking water at the Saharawi camps is the cholera outbreak in 1994 after unexpected floods contaminated all the shallow groundwater wells. These wells were closed and water from deeper groundwater wells followed by a chlorination step was used from then onwards. Although there has not been any other report on problems associated to gastrointestinal diseases from unsafe water, recent data from 2016 (Fig. 6) show that

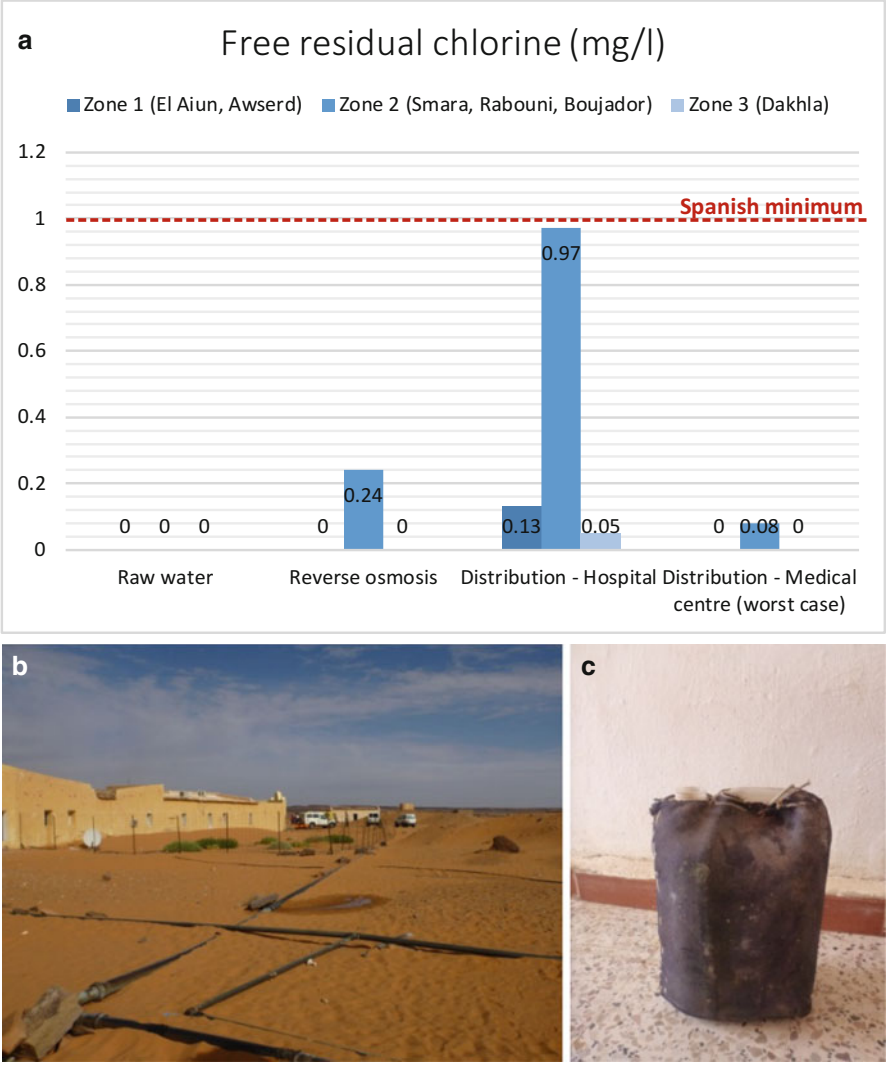

Fig. 6 Sources of microbiological contamination. (a) Free residual chlorine (mg/l) from sampling at different water supply and distribution points at the Saharawi refugee camps in 2015, (b) points of potential contamination due to failures in the distribution network; and (c) 10 l containers showing total coliforms contamination. Data and photos from the authors

the free residual chlorine levels in distribution points (tanks and taps sampled) do not reach the minimum values established by the Spanish legislation (Spanish RD140/2003 2003) neither by WHO guidelines, detecting total coliforms, especially in 10 l containers. Some distribution points also presented turbidities above 5 NTU, probably due to contamination in the distribution network, indicating potential microbiological contamination (Vivar et al. 2016).

- Nitrates

As of today, no published study has reported cases of blue-baby syndrome within the population of the Saharawi camps, but the reality is that nitrate levels have been high in the past, posing a health risk until the water started to be treated. On the other hand, maternal breastfeeding has been always promoted as the main lactation method for infants, thus reducing the use of water to prepare baby milk.

High levels of nitrates in water at the camps were first reported 2001 by Docampo (2006), and 3 years later, in 2004, another Spanish team (Docampo 2006) reported once again the same high levels of nitrates affecting the drinking water quality at El Aiun and Smara.

In 2007 two reverse osmosis plants were built in Smara, and several years later, in 2010, another reverse osmosis plant was commissioned to provide water to El Aiun and Awserd. As the raw water quality in Dakhla was adequate, only a chlorination stage was included as water treatment. These reverse osmosis plants reduced the high levels of nitrates. As of 2015, current nitrate levels (Fig. 7) in raw water in Smara were of 125.5 mg/l, decreasing to 15.9 mg/l after the osmosis. Smara water supply is the best regarding quality in distribution within the refugee camps, as it is always treated water from one of the two available reverse osmosis plants, vs. El

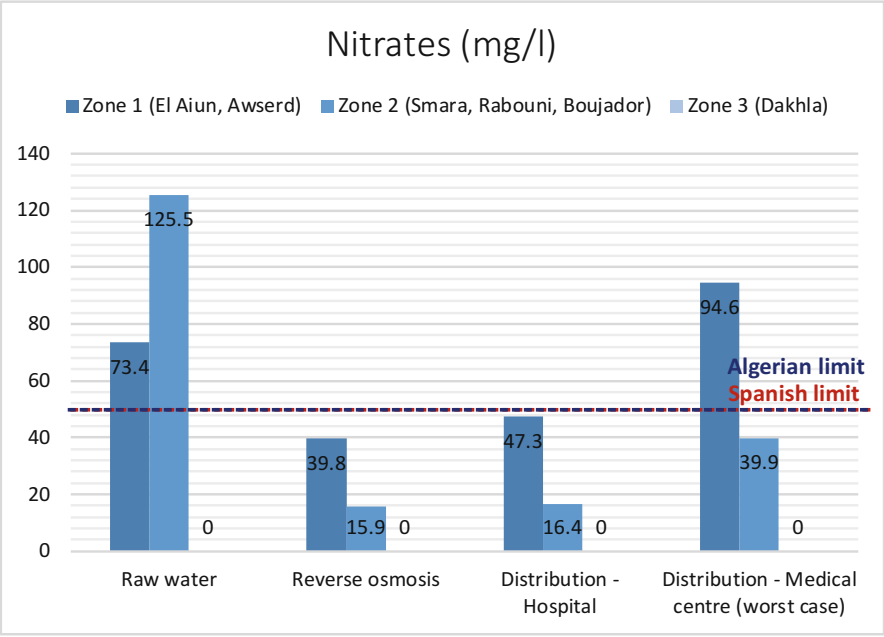

Fig. 7 Current levels of nitrates in Saharawi drinking water. Nitrate levels in water (mg/l) from sampling at different water supply and distribution points at the Saharawi refugee camps in 2015. Spanish and Algerian (Algeria 11–125 (2011)) legislation limits are shown as reference. Data are from the authors

Aiun and Awserd, which receive treated water only in turns of 20 days, receiving treated water and raw water directly alternatively. In this case, latest levels of nitrates in raw water are of 73.4 mg/l, well above the recommended limit of 50 mg/l given by WHO, posing a health risk for the infant population if bottle feeding is used. Finally, levels of nitrates in Dakhla are low, 8.88 mg/l, so there is no health risk associated (Vivar et al. 2016).

- Fluoride

Dental fluorosis can be directly observed nowadays in the population of El Aiun and Awserd in their brown spotted enamel, especially in those who have already been born in the refugee camps (young population) and have been exposed to fluoride since birth. The most detailed study on the dental status of the refugee children at the Saharawi camps is the conducted by Almerich-Silla et al. in 2008 (Almerich-Silla et al. 2008). They already associated the unbalanced diet, poor oral hygiene habits, and high concentration of fluoride in drinking water (~2 mg/l) to the current dental status. They worked with the refugee camps of Smara, El Aiun, and Awserd, and they observed that among the 6- to 7-year-old children, 36.9% were free of fluorosis, 15.6% presented moderate fluorosis, and 7.8% presented severe fluorosis. Among the 11- to 13-year-old children, only 4.2% were free of fluorosis, 30.2% exhibited moderate fluorosis, and 27.4% presented severe fluorosis. They also studied decayed permanent teeth and caries prevalence, and they concluded that these were higher on children affected with severe fluorosis, suggesting that it could increase the susceptibility to dental caries (Fig. 8).

Regarding fluoride content in water at the refugee camps over time, in 1991, Díaz-Cadórniga et al. (2003) reported already values of fluoride in water exceeding the recommended standard limit (1.5 mg/l) of 1.6 mg/l, which were reported again in 2000, ranging from 0.7 to 1.5 mg/l in the camps. In 2001, "Aguas de Sevilla" (Docampo 2006) conducted a more detailed water analysis at the camps, obtaining also high fluoride values at El Aiun and Smara (1.4 and 1.8 mg/l, respectively). Three years later, in 2003, another Spanish team (Docampo 2006) analyzed the water, reporting once again the same high values for fluorides in water at El Aiun and Smara.

Another study from the Norwegian College (Akershus University College 2008) in 2008 conducted a series of water analysis after the reverse osmosis at Smara was designed and set-up and their results reflected this improvement. While El Aiun continued to present high fluorides values, Smara water supply dramatically reduced them, from 1.8 mg/l to 0.16 mg/l.

Latest available data correspond to 2015, with the reverse osmosis plant of El Aiun and Awserd already in function. In this case, raw water quality still presents high values of fluorides (1.95 mg/l) that although are reduced to 0.23 mg/l after osmosis, still critical because the population uses this source of raw water as drinking water in turns of 20 days. On the other hand, despite the high values of raw water in Smara (1.8 mg/l), all the population in this camp receives treated water (0.12 mg/l), so no posing any health risk. Once again, Dakhla presents low values of fluorides (0.6 mg/l), not requiring any water treatment in this regard (Vivar et al. 2016).

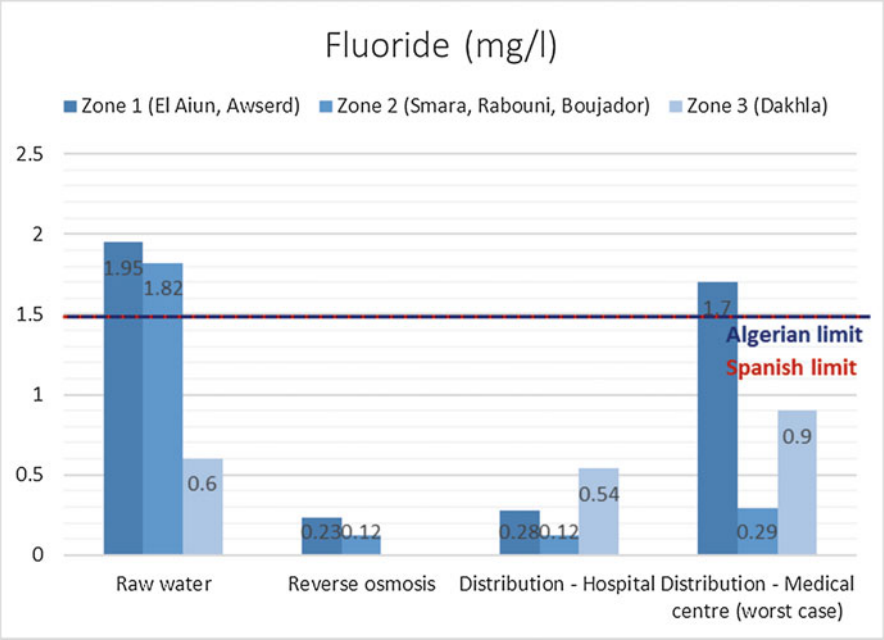

Fig. 8 Current levels of fluorides in Saharawi drinking water. Fluoride levels (mg/l) from sampling at different water supply and distribution points at the Saharawi refugee camps in 2015. Data are from the authors

- Iodine

The Saharawi refugee camps have been considered as an endemic goiter region (more than 5% of the individuals presenting goiter) since the first studies in the 1990s reported significative percentages of the population presenting goiter. For example, Prezzino et al. (1998) results showed 28% of a school children population with palpable goiter prevalence, and in the year 2000, Díaz-Cardóniga et al. (2003) reported even a higher value for goiter prevalence in the same age range population, 58%. Both studies associated prevalence of goiter with excess iodine in drinking water, with values up to 934 μg/l in El Aiun.

Another group of studies in 2007 by Henjum et al. (2010, 2011) and Barikmo et al. (2011) showed again high prevalence of goiter among school children (6- to 14-year-old) and women (15- to 45-year-old), with values of 11% and 18%, respectively. These studies also attributed excess iodine in the daily intake to drinking water, but also showed that iodine content in milk was very high (70–13,071 μg/l), so not only drinking water could be the cause of the excess iodine in the diet. Later studies by the same research group in 2007 and 2010 (Aakre et al. 2015a, b) on iodine and goiter at the camps reported also high prevalence of goiter

and continued to associate it to drinking water, although iodine concentrations in water decreased to values of 80–254 μg/l on average in 2007, vs. iodine content in animal milk of 1175–2049 μg/l, much higher, up to 20 times. Water iodine concentration continued to decrease in 2010, with an average of only 58 μg/l, but the thyroid dysfunction diseases continued to be as high as 34.1% in the surveyed population.

Overall, the iodine concentration in drinking water was strongly reduced from the high average values of 638 μg/l in 1998 (corresponding to an endemic goiter region according to the Chinese legislation, >300 μg/l), to adequate levels of iodine in water in 2010, with values of only 58 μg/l.

Latest analysis conducted by Pichel and Vivar in 2015 and 2016 (Pichel and Vivar 2017) show only high iodine concentrations in raw water before the reverse osmosis plants at El Aiun and Smara, with concentrations of 193 μg/l and 357 μg/l, down to 81 μg/l and 95 μg/l after the water treatment. On the other hand, iodine content in drinking water in Dakhla was of 108 μg/l. Average iodine in drinking water in the camps ranged from 81 μg/l to 139 μg/l, considering that the values are higher for El Aiun and Awserd that receive treated water each 20 days. According to Chinese legislation, water iodine concentration below 150 μg/l would be adequate for human consumption. As a reference, this study also shows the iodine concentration in Madrid (Spain), in an area of adequate levels, reporting values of 66 μg/l for mineral water and 57 μg/l for tap water. Finally, the study concludes that although iodine excess seems to be the cause of prevalence of goiter in the Saharawi refugee camps and drinking water has been associated directly to this goiter since the first studies on the matter, it would be convenient to analyze the entire dietary intake of the population to detect other potential sources of iodine, especially after iodine content in water has been dramatically reduced in the last years and after animal milk has shown high values of iodine in the latter literature studies (Fig. 9).

Policies and Protocols

Water Guidelines on Access, Quality, and Quantity for Refugee Situations

In this chapter, the importance of water in refugee situations has been described along with the effect of unsafe water in the Saharawi population, both under short- and long-term exposure. Guidelines on water access have followed the recommendations given by UNHCR, guidelines and policies on water quantity (>20 l/person/day) have also followed UNHCR and WHO standards, and water quality in terms of microbiological (0 CFU/100 ml), nitrates (<50 mg/l), fluorides (<1.5 mg/l), and iodine content (<300 μg/l) have followed the WHO guidelines for drinking water as well as the Algerian and Spanish legislation. For the case of iodine, the Chinese legislation has been also considered.

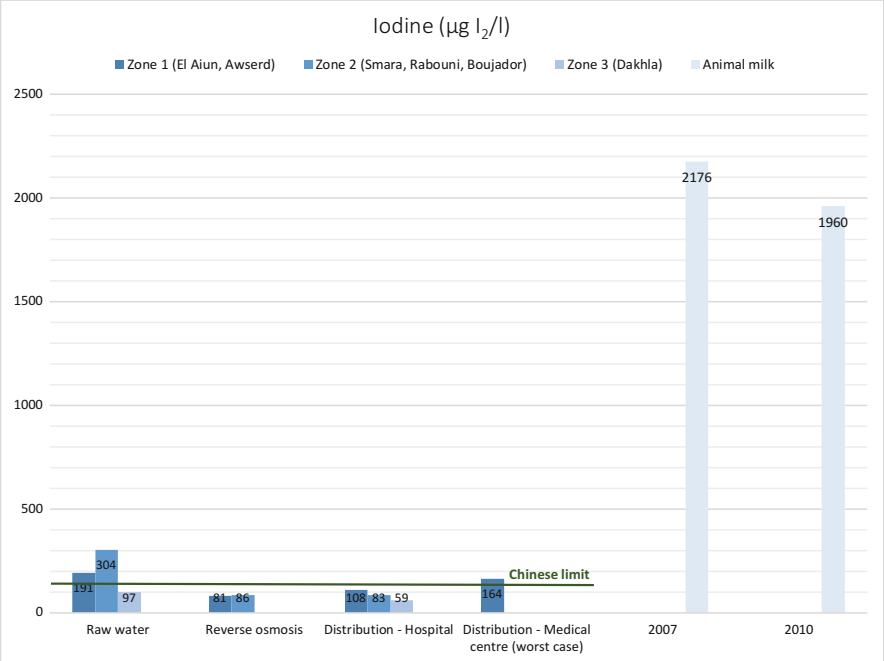

Fig. 9 Current levels of iodine in Saharawi drinking water and animal milk. Iodine levels (mg/l) from sampling at different water supply and distribution points at the Saharawi refugee camps in 2015. Iodine concentration in animal milk from the studies of 2007 and 2010 is shown as comparison and potential source of excess iodine. Data on water are from the authors, data on milk from referenced studies

Water Quality Analysis

Field-measured parameters included temperature and dissolved oxygen, pH, conductivity, and turbidity. Residual-free chlorine, total iodine, and fluoride were measured daily after sampling. Microbiological analyses were conducted using 3M Petrifilm plates with chromogenic *E.coli* and total coliforms culture medium, incubated 24 h at 37 °C. Quantification limit was of 1 CFU/ml.

At central laboratories in Spain, water samples were analyzed for different parameters according to the "Standard Methods for the Examination of Water and Wastewater" (APHA 2005): pH, conductivity and redox potential, alkalinity, total phosphorous, total nitrogen, organic content, major ions including chloride, nitrate, nitrite, phosphate, sulfate, fluoride, sodium, ammonium and potassium; and single elements such as iodine, chrome, manganese, iron, nickel, copper, arsenic, cadmium, lead, mercury, antimony, boron, aluminum, and selenium using ICP-MS (inductively coupled plasma mass spectrometer).

Dictionary of Terms

- **Blue-baby syndrome** – Disease that is common in babies under 3 months that have been ingesting water with high levels of nitrates, which react with the hemoglobin in the red blood cells and blocks the oxygen transport. It is called "blue baby syndrome" because of the blue-gray skin color.
- **Daira** – A smaller urban administrative area similar to a district or suburb. A wilaya can be composed of several dairas.
- **Fluorosis** – Fluoride that is present in drinking water is absorbed by the bones, and with time it can increase the possibilities of bone fractures and produce chronic pain.
- **Goiter** – Disease caused by a thyroid dysfunction, which causes the enlargement of the thyroid gland.
- **Protracted refugee situation** – Populations of at least 25,000 refugees who have lived in exile for at least 5 years in developing countries.
- **Wilaya** – An administrative region within the Saharawi urban organization, similar to a town.

Summary Points

- This chapter focuses on the relationship between deprivation of drinking water and health issues at the Saharawi refugee camps.
- The Saharawi refugee camps were established in 1975 and are one of the oldest chronic refugee crisis.
- Drinking-water deprivation in terms of access, quantity, and quality poses serious health hazards both in the short and long term.
- Water access at the camps has improved from the first emergency handmade wells to deep groundwater wells combined with water treatment via reverse osmosis.
- Water quantity is still insufficient in the camps, 18 l/person/day, below the minimum standard of 20 l/person/day established by the United Nations High Commissioner for the Refugees (UHNCR).
- Raw water quality has been an issue since early days at El Aiun, Awserd, and Smara, with high values of nitrates, fluorides, and iodine that pose a health risk.
- Currently, water treatment via reverse osmosis has reduced the hazards of raw drinking water, although El Aiun and Awserd still consume raw drinking-water in 20 days turns.
- These parameters have affected the population that after a long exposures how dental and skeletal fluorosis and goiter problems.
- Microbiological water content has been reduced since the cholera outbreak in 1994 when handmade wells were closed and chlorination water treatment was introduced.

- But the levels of residual-free chlorine at the distribution point and maintenance problems of the distribution networks still pose a health risk.
- It is necessary to extend the distribution of treated water with adequate quality for all the population and to increase the available water quantity in order to improve the refugees' health status.

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Impact of Transnational Migration: Underweight and Obesity in Contemporary Europe

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Sylvia Kirchengast

Contents

Abstract

Periods of famine and starvation have been major push factors of migration since prehistory. From Paleolithic times onward, a lack of available food forced *Homo sapiens* and their ancestors to move to new habitats. This behavior continued until now. At the beginning of twenty-first century, however, transcontinental migration is only seldom the result of long periods of starvation. Nevertheless migration is often associated with profound changes in dietary habits. In the present review, the weight status of Indian and Turkish immigrant women in Austria is focused on. As typical of immigrants in Europe, immigrant women in Austria suffer seldom from undernourishment. In contrast the main problem of transnational and transcontinental immigrants in European countries is the high prevalence of obesity and associated disorders. In Austria, female immigrants from India but also from Turkey show

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extraordinary high rates of obesity. This is especially true of women and children. As the main reason, the rapid transition to Western lifestyle is discussed.

Keywords
Starvation · Transnational migration · Turkish immigrants · Indian immigrants · Austria · Obesity · Overweight underweight · Thrifty phenotype · Modernization

List of Abbreviations

BMI	Body mass index
FAO	Food and Agriculture Organization of the United Nations
Perz	Percentile
WHO	World Health Organization

Introduction

Migration to new habitats is widely found in nature. Long-distance movements, which are typical of birds, fishes, and mammals, are mainly triggered by climate factors, mating behaviors, seasonality, and changes in the availability of food and water resources (Mascie-Taylor and Lasker 1988). Among humans and their ancestors, migration can be assumed since the emergence of hominids. Transcontinental migration starts with the dispersal of *Homo erectus* out of Africa to Eurasia about 1.8 million years ago. Our own species *Homo sapiens* evolved in Africa about 180,000 years ago and moved out of Africa 70,000 years ago. 30,000 years later, *Homo sapiens* had spread across Australia, Asia, and Europe. 20,000 to 15,000 years ago, *Homo sapiens* reached America, and by 2,000 years ago, most of the Pacific Islands were colonized (Henke and Hardt 2011). Consequently *Homo sapiens* has a long history of migration, and migration is clearly not only a recent phenomenon. Today rural urban migrations, transnational migration, and even transcontinental migration are widely found and triggered by push and pull factors which force people to leave their homelands and move to new areas. First of all war but also economic, political, and environmental push factors influence a person's decision to migrate. Especially important push factors are harsh environmental conditions, such as drought and famine which cause starvation and hunger; these factors however induce only seldom transcontinental migrations. In contrast affected people try to move to better areas nearby – a behavior which was typical for more than 99% of our history when the subsistence of *Homo sapiens* was based on hunting and gathering. It can be assumed that hunter-gatherer suffered repeatedly from food shortages of few days (Pentice 2005); longer periods of starvation however have occurred seldom. This is mainly due to the fact that hunter-gatherer societies consisted of few members and followed a mobile lifestyle. Their reaction of an acute food shortage was to move to new hunting and gathering grounds (Pentice 2005). This kind of lifestyle was typical of our ancestors until the so-called Neolithic transition, which started about 10,000 years BC. Even today the few remaining hunter-gatherer societies suffer very seldom from periods of starvation. Consequently from the

viewpoint of evolutionary anthropology, we can assume that to move away and to migrate are adapted strategies of *Homo sapiens* to avoid starvation. Since the Neolithic transition, however, the frequency of famines and periods of starvation increased drastically. The domestication of animals and plants and the adoption of agriculture changed the lifestyle of our ancestors. They became able to produce a surplus of staple foods and to create contingency stores to buffer future food shortages. As a consequence populations started to grow, and they gave up their mobile lifestyle. Permanently settled villages and later towns emerged. This new lifestyle patterns however created new vulnerabilities because this new system relies more or less on constant and predicable climatic conditions but also on the absence of intra- and interpopulation conflicts (Larsen 1995). Consequently droughts and flood waters, wars, but also periods of epidemic diseases caused catastrophic famines often accompanied with mass mortality. Mainly environmentally induced famines are documented for nearly every ancient culture (Keys et al. 1950; McCance 1975) but also for medieval, premodern, and modern Europe (Lucas 1930). The Little Ice Age during the seventeenth and early eighteenth century caused starvation in many European regions and forced waves of migration (Engler et al. 2013). The most prominent example of starvation-induced mass emigration is the Irish Potato Famine also called "The Great Famine" starting in September 1845. English winds had carried an airborne fungus called *Phytophthora infestans* to Ireland, wiping out the potato crop and causing a horrible period of starvation. Many people died or emigrated, and the Irish population decreased by 1.6 million people or about 17% of the total population. Without their main food crop to rely on, a large number of Irish citizens emigrated from their country to escape starvation (Daniels 2002). The large population of Americans and Canadians of Irish descent can trace its ancestry to this period (Daniels 2002). During the twentieth century, both world wars, but first of all World War II, caused periods of famine and severe starvation in Europe such as the Siege of Leningrad (1941–1944) and Dutch famine (1944–1945) (Young 1943; McCance 1975). After World War II especially during the time of the so-called *Wirtschaftswunder*, famine and periods of mass starvation disappeared in Europe as in all first world countries. Nevertheless, famine and starvation are present in many parts of the world and force migration mainly to Europe but also other first world areas. Migration is a worldwide phenomenon in 2017, and it has a profound impact on nutritional habits but also on the prevalence of underweight, overweight, and obesity of immigrants.

The aim of this paper is to review the impact of transnational and transcontinental migration on weight status of immigrants. The main focus lays on the situation of Turkish and Indian immigrant women in Austria.

Starvation and Malnutrition Worldwide

The FAO defines starvation as a severe deficiency in caloric energy needed to maintain an organism's life. Starvation represents the most extreme form of malnutrition (FAO 2015). According to the Oxford English Dictionary (1971), the term

hunger has three meanings: the uneasy or painful sensation caused by want of food, the want or scarcity of food in a country, and a strong desire. A lack of sufficient food supply results undernourishment. Although often used as synonyms, malnutrition and undernourishment are not the same. While malnutrition refers to all deviations from adequate and optimal nutritional status, including undernourishment but also overnutrition and therefore obesity, undernourishment describes a generally poor nutritional status mainly characterized by underweight (Shetty 2006). The term underweight describes a weight status too low to be considered as healthy, usually defined as a body mass index below 18.50 kg/m^2. Undernourishment and underweight are primarily caused by an inadequate intake of dietary energy (Shetty 2006). Underweight and undernourishment are caused by exogenous factors such as an inadequate availability of food as a result of famine, war, conflict, or poverty but also endogenous factors such voluntary reduction of food intake, i.e., anorexia nervosa, intestinal disorders, parasitic infestations, organ failure, neoplasm, or infections (Shetty 2006). The consequences of undernourishment are weight loss, loss of body fat, wasting of muscle mass, and the atrophy of visceral organs (Shetty 2006). Inadequate food supply or starvation, especially during intrauterine development and childhood, causes fetal growth restriction, stunting, poor health, first of all an increased prevalence of infectious diseases, but also general weakness, low-energy levels, and reductions in mental functioning (Victoria et al. 2008). In this way starvation can lead to even greater poverty by reducing people's ability to work and learn, thus leading to even greater hunger (Victoria et al. 2008). During the last 25 years, the prevalence of undernourishment was reduced markedly worldwide. According to the United Nations Food and Agriculture Organization (FAO), about 795 million people (10.9% of the world population) were suffering from chronic undernutrition in 2014–2016. Nearly all the chronically starving people, 780 million, live in developing countries, i.e., 12.9% of the population of developing counties. About 11 million people are undernourished in developed countries (FAO 2015; Rosen et al. 2016). In developed countries undernourishment is mainly caused by endogenous factors such as psychic or somatic diseases but only rarely by poverty. Famines affecting food supply do not exist in developed countries.

As to be seen in Table 1 even in developing countries, the prevalence of undernutrition decreased drastically from about 23% in 1990 to 12.9% in 2014. This was especially true of Southeast Asia, but also in sub-Saharan Africa, the prevalence of undernourishment is felt from 33.2% in 1990 to 23.2% in 2014.

Obesity Worldwide

Malnutrition characterized by obesity is mainly seen as a very recent phenomenon, because it can be assumed that at population level obesity was largely unknown up to the 1950s (Brown 1991). In 2008, however, for the first time in the long history of *Homo sapiens*, the number of obese people on earth exceeded the number of people suffering from starvation and undernourishment (FAO 2008). In 2014, 39% men and 40% of women aged 18+ were overweight (BMI $\geq$ 25 kg/m^2) and 11% of men and

Table 1 Prevalence of undernourishment 1990/1992 and 2012/2014 (Source: FAO 2015)

Prevalence	1990/1992	2012/2014
Worldwide	18.6%	10.9%
Developed countries	<5.0%	<5.0%
Developing countries	23.3%	12.9%
Africa		
Sub-Saharan Africa	33.2%	23.2%
Asia		
Eastern Asia	23.2%	9.6%
Southeast Asia	30.6%	9.6%
Southern Asia	23.9%	15.7%
Latin America	14.7%	5.5%
Oceania	15.7%	14.2%

Table 2 Prevalence of overweight in 2014 according to WHO regions (Source: WHO 2016)

	Overweight (BMI > 25.00 kg/m^2)	
	Male	Female
African region	22.9%	38.6%
Americas	62.8%	59.8%
Eastern Mediterranean region	43.8%	50.1%
European region	62.6%	54.9%
Southeast Asia	19.3%	25.3%
Western Pacific region	34.7%	31.2%

15% of women were obese (BMI ≥30 kg/m^2).This means that more than 1.9 billion adults, 18 years and older, are overweight. Of these over 600 million correspond to the definition of obesity (WHO 2016). Prevalence rates of overweight among adult people (>18 years) are presented in Table 2.

This high prevalence of obesity is a matter of concern because obesity is associated with premature mortality, morbidity, and a markedly reduced health-related quality of life (Ford and Mokdad 2008; Wang et al. 2011). Once considered a condition typical of affluent societies of developed countries, overweight and obesity rates are now fast growing in poor neighborhoods of developed countries but also in many developing countries, too (Hossain et al. 2007; Pijl 2011). The main reason of this trend is the globalization of increasingly obesogenic environments characterized by rapid urbanization, mechanization of jobs, improvement of transportation services, the availability of food of high-energy density, and reduced physical activity (Popkin 2001; Popkin and Gordon-Larsen 2004; Poston and Foreyt 1999; Ulijaszek and Lofink 2006; Ulijaszek 2007). Many developing countries have achieved a remarkable improvement in nutrition status in the past decades, and consequently obesity rates increased. Nevertheless the prevalence of undernourishment is still a serious problem. The double burden of obesity and underweight-induced malnutrition exists in almost all developing countries (Abdullah 2015).

In societies of economic and nutritional transition, the paradox situation can be observed that malnutrition and obesity rise parallel (Chatterjee 2002). One example is India which has a population of over one billion. Seventeen years ago, India was considered as one of the poorest countries in the world mainly suffering from undernourishment (Krishnaswami 2000; World Bank 2001). Since that time India has undergone a rapid economic development and is increasingly faced by a rising epidemic of obesity (Singh et al. 1995; Krishnaswami 2000; Garg et al. 2009; Florentino 2002; Bhadra et al. 2005; Yoon et al. 2006; Ackerson et al. 2008; Khokhar et al. 2010; Masoodi et al. 2009). Obesity in India is associated with modernization, rapid urbanization, and a transition in lifestyle and nutritional habits. Besides a rapid rise in rates of overweight and obesity, a consequent rise in noncommunicable diseases such as type 2 diabetes is found. This trend is observable among several societies of transition. A very fast variant of transition takes place as a result of transnational and especially transcontinental migration. The transition from a lifestyle typical of developing country to a life in a Western society is accompanied by modernization and sometimes acculturation. Both affect nutritional habits, physical activity patterns, and consequently weight status (Green et al. 2003). The present investigation focused on the weight status of two different immigrant populations in Austria. In detail the weight status of Indian women originating from Punjab and Turkish women in the Austrian capital Vienna was analyzed.

Weight Status of Refugees and Immigrants Worldwide

Recently, starvation-driven migration is mainly found in sub-Saharan Africa, where political conflicts and climatic change-related drought result in famine (Grijalva-Eternod et al. 2012). Only a few hungry refugees had enough energy to migrate long distances through the Mediterranean Sea to Europe; most of them reach refugee camps in neighboring areas only. As to be expected, in these camps, first of all in Ethiopia but also in Western Africa, high prevalence rates of underweight, stunting, and wasting are found among young children, adolescents, and women (Jemal and Haidar 2014; Kelati et al. 2015), although overweight and even obesity have been documented among women of reproductive age from Western Sahara in Algerian refugee camps (Grijalva-Eternod et al. 2012). A recent study focusing on refugee children mainly from Somalia, Iraq, and Burma in the United States showed that nearly one-half of these children had at least one form of malnutrition (44.9%). Refugee children were affected by wasting (17.3%), stunting (20.1%), overweight (7.6%), and obesity (5.9%) (Dawson-Hahn et al. 2016).

In contrast to refugees, immigrants in developed countries suffer only very seldom from undernourishment. Contrary, numerous investigators have demonstrated that migrant status increases the risk of overweight or obesity dramatically. This is true of Hispanic immigrants in the United States (Kaplan et al. 2004; Park et al. 2009), as well as of immigrants originating from Mediterranean countries or the Middle East in Central and Northern Europe (Brussard et al. 2001; Hoppichler and Lechleitner 2001; Uitewaal et al. 2004a, b; Fredriks et al. 2005; Kirchengast and

Fig. 1 Migration increases obesity rates. Transnational and transcontinental migration is often associated with a change from a traditional society into an obesogenic environment, characterized by a surplus of energy dense food and a lack of physical activity. This process of modernization and acculturation often results in increasing obesity rates among immigrants

Schober 2005; Misra and Ganda 2007; Dijkshoorn et al. 2008; Wolin et al. 2008). A high prevalence of overweight and obesity (3.6–49.4%) is also found among immigrants from Northern Africa in Europe. The highest frequency of physically inactive or lightly active people among immigrants was observed in first-generation Sudanese and Moroccans in Amsterdam (males, 57.1%; females, 74.2%), with increasing rates in second-generation females (Toselli et al. 2014). Especially high rates of overweight and obesity are found among children of immigrants (Kirchengast and Schober 2005). This observation seems to be mainly due to the fact that most immigrants hope for better lives for themselves and their children and interpret energy dense food, characterized by a high amount of sugar and fat but also physical inactivity as a luxury lifestyle (Green et al. 2003; Hosper et al. 2007; Lawrence et al. 2007). Immigrants adapted very fast to an obesogenic environment in Europe or the United States. They and their children are confronted with advertisements and opportunities to purchase food that is relatively cheap and of low nutritional quality. Therefore, immigrant obesity is interpreted as the result of an unhealthy assimilation of immigrants (Van Hook and Balistreri 2007; Faskunger et al. 2009; Park et al. 2009; Ujicic-Voortman et al. 2009) (see Fig. 1).

Immigrants in Austria

Up to the period shortly after the end of World War II, Europe was mainly a continent of emigration and not of immigration. After World War II, many parts of Europe experienced a period of enormous economic growth but also political stability and increasing food security. Consequently Europe turned from a continent of emigration to a continent of immigration. Since the 1960s, an increasing number of people originating from Southern European countries but also from Turkey and Northern Africa moved to Central and Northern European countries such as Austria. Austria, a small country in the center of Europe, has about 8.5 million inhabitants; 21% of them have a background of migration, which means that at least one of their parents was born outside Austria (Statistik Austria 2016). The majority of these people settled in

Vienna, the capital of Austria. Today Turkish people and people from Former Yugoslavia, who have been actively recruited as migrant workers, represent the largest groups of immigrants in Austria. The social status of many immigrants, especially those originating from Turkey, is significantly lower than that of the Austrian majority. Furthermore the health status of many immigrants is quite poor. People with a background of migration show higher rates in morbidity and mortality in comparison with their Austrian counterparts, although the public health system in Austria is highly developed and enables Austrians as well as immigrants access to health service centers and medical treatments free of charge (Hoppichler and Lechleitner 2001; Statistik Austria 2016). During the last 10 years, even before the great waves of immigration took place in 2015 and 2016, increasingly people from the Middle East, Asia, Africa, and Latin America migrated to Austria. The main push factors of these migration waves to Austria were mainly conflicts, political factors, and economic problems in the countries of origin but very seldom famine and starvation.

Weight Status of Indian Immigrants in Austria

The Indian community is a quite small one in Vienna. According to Statistik Austria (2016), about 3,600 immigrants from India live in Vienna. For comparison, about 700,000 people with a background of migration, mainly originating from former Yugoslavia and Turkey, live in Vienna in 2016. The Indian population in Vienna was chosen for this analysis because – as pointed out above – a marked nutritional transition leading to increasing rates of obesity has taken place in India during the last 20 years. Indian immigrants in Europe and in the United States, however, also experience an extremely fast transition in lifestyle and nutritional habits resulting in increasing rates of obesity (Bhatnagar et al. 1995; Landman and Cruickshank 2001; Abate and Chandalia 2007).

In the present study, weight status and nutritional habits of 50 Indian women aging between 19 and 58 years ($x = 38.2 \pm 10.8$) living in Vienna have been analyzed. All women originated from Punjab area in India. Punjab is a state in the northwest of India. According to the "India State Hunger Index 2008," Punjab has the lowest level of poverty in India. But due to educational differences, there is still a large disagreement in living conditions, following women rights and health conditions between the people living in rural areas and in cities. For comparison 65 women aging between 17 and 80 years ($x = 39.1$; ± 16.8) living in rural and urban areas of the district of Jalandhar in Punjab were enrolled in the study.

Weight status was determined by using the body mass index (BMI) kg/m^2. To define overweight and obesity, the BMI cutoffs for South Asians were used (WHOEC 2004) (BMI < 18.50, underweight; BMI 18.50–22.99, normal weight; BMI 23.00–24.99, overweight; BMI > 25.00 obese). As presented in Table 3, underweight among Punjabi women was much more prevalent in India than in Vienna, while in contrast overweight and obesity were significantly ($p < 0.01$) more prevalent among Punjab women in Vienna.

Table 3 Weight status of Indian women in Vienna and in Punjab (Source Kirchengast and Singh 2011)

Weight status	Indian women in Punjab	Indian women in Vienna
Underweight BMI < 18.50 kg/m^2	18.5%	6.0%
Normal weight BMI 18.50–22.99 kg/m^2	47.7%	14.0%
Overweight BMI 23.00–24.99 kg/m^2	9.2%	26.0%
Obese BMI > 25.00 kg/m^2	24.6%	54.0%

While more than 18% of Punjabi women in India were classified as underweight, this was only true of 6% among Indian women in Vienna. Furthermore a high prevalence of overweight and obesity (nearly 35%) was documented for Punjabi women in India; among Indian women in Vienna, 80% were classified as overweight and obese (Kirchengast and Singh 2011). This observation is in accordance with the results of numerous studies which showed high rates of obesity among immigrant women in Europe (Misra and Ganda 2007; Kilaf and Kirchengast 2011; Park et al. 2009). The high prevalence of overweight and obesity among Punjabi women in India, however, seems extraordinarily high for a developing country, although the National Family Health Survey III in India (2010) revealed that nearly 30% of Indian women living in urban areas and nearly 9% of women living in rural areas are obese. This is especially true of Punjab region (Garg et al. 2009; Ackerson et al. 2008; Khokhar et al. 2010). Therefore the results of this Viennese study are in accordance with the general trend of weight status among Indian women. There is no doubt that this high obesity prevalence in India is due to an extremely rapid economic development accompanied by a dramatic transition in lifestyle and dietary patterns (Ramachandran 2004). As a consequence the prevalence of obesity among Indian women increased from 10.6% in 1999 to 12.6% in 2006. Despite this trend, about 35–40% of the Indian population still lives below the poverty line, and more than 30% of Indian women of all age groups are underweight and suffer from malnutrition, anemia, and deficiencies in micronutrients and vitamins. Underweight is mainly found among women of low socioeconomic status and low education level, while overweight and obesity are most frequently found among women of high socioeconomic status living in urban areas (Ackerson et al. 2008).

Turkish Immigrants in Austria

The second immigrant sample considered in this review represents the second largest immigrant group in Austria. According to National census, about 250,000 Turkish immigrants live in Austria (Statistik Austria 2016). As presented in Table 4 from early childhood onward, Turkish girls exhibit a high prevalence of overweight and obesity, while the prevalence of underweight was extremely low (Kirchengast and Schober 2005).

Extraordinary high prevalence rates in overweight and obesity were also found among adult Turkish women aging between 18 and 56 years living in Vienna (Kilaf

Table 4 Weight status according to age group among Turkish immigrant girls and adult Turkish women living in Vienna (Source: unpublished data from Kirchengast, Kilaf & Schober)

Weight status	Turkish girls 6 years	Turkish girls 10 years	Turkish girls 15 years	Turkish women 18–50 years
Underweight (<10 Perz)	3.3%	5.1%	2.9%	2.3%
Normal weight (10–90 Perz)	71.8%	64.8%	70.3%	42.4%
Overweight (90–97 Perz)	12.7%	16.7%	14.1%	32.3%
Obese (>97 Perz)	12.2%	13.4%	12.7%	23.0%

and Kirchengast 2011). As demonstrated in Table 4, the prevalence of underweight was extraordinary low in this sample (2.1%), while more than 55% of the women were classified as overweight or obese according to the BMI categories of the WHO (1995).

In European countries immigrants originating from Mediterranean region and from Near East and Middle East show an extremely high prevalence of overweight and obesity (Papandreou et al. 2008). This is especially true of children and adolescents as well as women (Ömer et al. 2004; Dijkshorn et al. 2008; Dinc et al. 2006; Tanyolac et al. 2008). The results of the present study corroborate these reports. 23.4–30.2% of Turkish children in the Netherlands were classified as overweight or obese (Fredriks et al. 2005). Dijkshorn et al. (2008) found a prevalence of overweight/obesity of more than 80% among Turkish immigrants in the Netherlands. Obesity is also a major health concern in Turkey (Erem et al. 2001, 2004; Gültekin et al. 2009). According to Iseri and Arslan (2008), 56% of the adult Turkish population is overweight or obese. Oguz et al. (2008) reported a prevalence of overweight of 36.0% and a prevalence of obesity of 30.4% for the adult Turkish population. Nearly all authors reported higher obesity rates for women than for men. The prevalence of obesity has significantly increased among Turkish women over the past 20 years from 28.5% in 1990 to 44% in 2010 (Erem 2015). This trend is mainly due to a transition in lifestyle, changing nutritional habits and increased physical inactivity, which is found in Turkey as well as in host countries (Hosper et al. 2007; Lawrence et al. 2007; Papandreou et al. 2008). Underweight and undernourishment represent only a marginal problem among young girls and adult Turkish women in Turkey but also in European countries.

Weight Status Among Immigrant from the Viewpoint of Evolutionary Anthropology

What are the reasons for these extremely low rates of underweight and high rates of overweight or obesity among immigrants in Austria and general in Europe? From the viewpoint of evolutionary anthropology in the high prevalence of obesity and overweight associated with migration to new cultural settings, acculturation and modernization may be interpreted as a result of a maladaptation of recent *Homo*

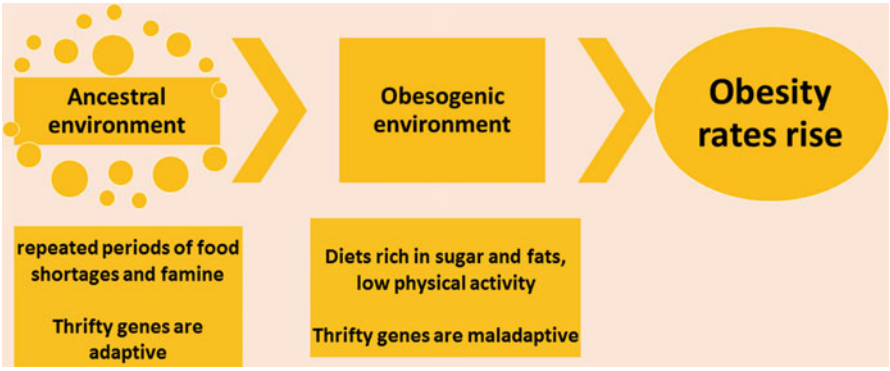

Fig. 2 Thrifty gene hypothesis. Our ancestors are adapted to an environment characterized by frequent food shortages, a diet poor in sugar and fat, and a high degree of physical activity. Thrifty genes are adaptive in this environment. In contemporary societies this thrifty genes are maladaptive because they prepare the body for starvation periods which never occur. Therefore obesity and type 2 diabetes rates increase

sapiens to postmodern environments. As early as 1962 James Neel proposed the thrifty gene hypothesis to partially explain the rise in type 2 diabetes and obesity in the twentieth century (Neel 1962). The central premise of his theory is that natural selection has favored the ability to store and utilize energy efficiently because our ancestors went through a cycle of feast and famine. Individuals whose energy storage or utilization was increased were more likely to survive and reproduce during periods of starvation. Consequently efficient energy storage was a beneficial adaptation. In our recent obesogenic environment, however, efficient energy storage turned to be maladaptive resulting in obesity and all associated problems (see Fig. 2). During the 1980s and the early 1990s, an alternative hypothesis to explain the high rates of obesity and type 2 diabetes was introduced. The British epidemiologist David Barker developed a theory of prenatal programming of later life diseases based on in utero nutritional deficiencies Barker 1999). According to Barker, life course plasticity was the key to explain obesity epidemic. In particular low birth weight newborns respond to their low level of nutritional intake in early life through alterations in growth and metabolism, which increase the risk of obesity and type 2 diabetes in later life (Barker and Clark 1997) (see Fig. 3). Barker postulates that low birth weight newborns have metabolically thrifty mechanisms for fat storage and glucose sparing with reduced rates of glucose oxidation in insulin-sensitive target tissues (Barker 1999). Furthermore poor nutrition in early childhood is suggested as an important driving factor behind the rising obesity rate in most developing countries. Indian and Turkish women were adapted to periods of food shortage up to recent times. Economic change but also the migration to new environments has improved the food supply and decreased physical work load, and consequently obesity rates are rising. As a result underweight rates drop down and obesity rates rise dramatically with the process of modernization and westernization, especially among immigrants.

Fig. 3 Thrifty phenotype hypothesis. Poor intrauterine environmental conditions result in intrauterine growth restriction, low birth weight, and a thrifty phenotype. The organism is prepared for poor food supply and energy stress. Improved environmental conditions during later life increase obesity rates

Policies and Protocols

In this chapter the impact of transnational and transcontinental migration on weight status was focused on. Research limitations: The studies reviewed have some obvious limitations. The number of immigrant women enrolled in the studies is quite small because the recruitment of participants is difficult among minority groups. The Viennese immigrant study population has been recruited in cooperation with the Viennese Fond of Integration, the Viennese school authority, and members of the Indian community in Vienna. Policy should cover three main areas: intensive cooperation between government and NGOs, improvement of education of immigrant women, and the inclusion of school authority, teacher, and parents to avoid excessive weight gain among immigrant children.

The assessment of weight status is problematic. BMI categories defined by the WHO are widely used; however, these weight status categories have met a lot of criticism. Although body composition analyses would yield more precise data, in the field it is often not possible to collect reliable body composition data. Therefore the BMI is still the most practicable indicator of weight status. For South Asian populations, separate categories have been defined. In the present studies, weight status categories defined by the WHO were used for the adult Turkish sample; South Asian weight status categories were used for Indian women. Weight status of children and adolescents have been classified by BMI percentiles according to sex and age group.

Dictionary of Terms

- **Migration** – means the movement of people from one place to another with the intentions of settling, permanently in the new location.
- **Malnutrition** – refers to all deviations from adequate and optimal nutritional status, including undernourishment but also overnutrition.

- **Obesogenic environments** – means living conditions characterized by rapid urbanization, mechanization of jobs, permanent availability of food of high-energy density, and reduced physical activity.
- **Modernization** – means the progressive transition from a traditional to a "modern" society.
- **Acculturation** – means the process of cultural change that results following meeting between cultures.
- **Thrifty gene hypothesis** – is an attempt by James V. Neel to explain in an evolutionary manner why certain populations today are prone to obesity and diabetes type.
- **Thrifty phenotype hypothesis** – suggests that early-life metabolic adaptations help the organism to survive by selecting an appropriate trajectory of growth in response to environmental cues.

Summary Points

- Starvation and famine have been major push factors of migration since prehistory.
- In 2008 the number of overweight or obese people surpassed the number of undernourished one for the first time in history.
- The prevalence of periods of starvation and consequently undernourishment is rapidly decreasing through the process of modernization.
- The double burden of obesity and underweight-induced malnutrition exists in almost all developing countries.
- The prevalence of overweight and obesity is high among immigrants in First World countries.
- Indian women and Turkish women in Austria show extraordinary high rates of obesity.
- Underweight is extremely seldom among immigrant children and women in Austria.
- Thrifty gene and thrifty phenotype hypotheses try to explain the high rates of obesity from the viewpoint of evolutionary anthropology.

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Adult Undernutrition in Rural Post-conflict Northern Uganda

28

Stine Schramm and Morten Sodemann

Contents

Abstract

This chapter outlines the prevalence and high-risk groups for adult undernutrition and discusses the social, behavioral, and structural mechanisms that can lead to food insecurity and undernutrition in a post-conflict setting like northern Uganda. In summary, adult undernutrition is higher in the post-conflict area of Uganda compared to areas that did not experience the war. Undernutrition varies by gender and age groups, higher among men than women, with young men and elderly being most likely to be underweight. Social and behavioral risk factors for undernutrition specific to post-conflict areas, such as substance use, and disruption of traditional values and norms will be discussed. The high prevalence of undernutrition among men may be a result of a "syndemic" interaction between

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mental illness, HIV, substance abuse, and undernutrition itself, which further interacts with the social and structural conditions such as gender inequality, stigma, stress, feelings of disempowerment and shame, poverty, and limited access to health care. Focusing on conventional target groups in health interventions and failure to adapt to the local context may have contributed to disruption of existing family and social structures and created other risk groups for undernutrition that are not targeted.

Keywords

Post-conflict · Adult undernutrition · Anthropometry · Gender · Health inequality · Northern Uganda · Mental illness · HIV · Syndemic · Demographic health surveillance

List of Abbreviations

AIDS	Acquired immune deficiency syndrome
ANC	Antenatal care
BMI	Body mass index
CMR	Crude mortality rate
DHS	Demographic and health survey
GAM	Global acute malnutrition
GoU	Government of Uganda
HDSS	Health and Demographic Surveillance Site
HIV	Human immunodeficiency virus
IDP	Internally displaced person
LRA	Lord's Resistance Army
MoH	Ministry of Health
NGO	Nongovernmental organization
PTSD	Post-traumatic stress disorder
STD	Sexually transmitted diseases
TB	Tuberculosis
UBOS	Uganda Bureau of Statistics
UNHCR	United Nations High Commissioner for Refugees
UPDF	Uganda People's Defence Force
USA	United States of America
U5MR	Under-five mortality rate
WFP	World Food Programme
WHO	World Health Organization

Introduction

The health of populations in post-conflict settings is determined by both direct and indirect effects from war, including trauma and injuries during the war, long-term poverty, and collapse of basic infrastructure. All aspects of human security, including food security, are further affected by disruption of families and social networks, land

conflicts, and continued ethnic clashes. When a peace agreement is signed, most humanitarian organizations pull out, while the governmental systems often do not yet have the capacity to take over. As a consequence, some public health indicators may be worse in post-conflict period than during the conflict. Hence, the real and tough battles to sustain peace and rebuilt society begin after the war. Nutritional status is a good indicator for the general health status of the affected populations. Based on published data from both epidemiological and ethnographic studies from northern Uganda, this chapter outlines the prevalence and high-risk groups for adult undernutrition and discusses the social, behavioral, and structural mechanisms that can lead to adult undernutrition and poor health in a post-conflict setting like northern Uganda.

Historical Background of Northern Uganda: Internal Displacement and Distributions of Food Rations

The population of northern Uganda experienced two decades (1986–2006) of armed conflict between the rebel movement Lord's Resistance Army (LRA) and the Government of Uganda's (GoU) military, the Uganda People's Defence Forces (UPDF) (Fig. 1). The population of the North was subject to torture, sexual violence, murder, and mutilations. Children and youth were abducted and forced to become soldiers, laborers, and brides (Branch 2008; Finnström 2008). In response to the insecurity, protected camps or internally displaced persons' (IDP) camps were created by the government and guarded by the UPDF. Most camps were established around preexisting villages or military detaches. They were created to protect civilians, but also to achieve military victory over LRA by separating civilians from rebels. While some families moved to the IDP camps voluntarily, many were forced by the UPDF to leave their homes and livelihoods and move into IDP camps (Branch 2008; Finnström 2008; Whyte et al. 2014). At the peak of the conflict, over 1.8 million people (approximately 90% of the population affected) lived in IDP camps (IDMC 2010). The camp size range from 5,000 to 65,000 people that lived in mud brick huts with thatched roofs (Whyte et al. 2014) (Fig. 2). Although the camps were "protected" and guarded by the UPDF, the IDPs were subject to traumatic experiences and human rights violence in the camps from both rebels and UPDF soldiers (Roberts et al. 2008; Nibbe 2010; Whyte et al. 2012). Camp residents did not have access to their own farmlands and livelihoods, due to insecurity, travel distances, and restrictions by the UPDF to leave the camps (Roberts et al. 2009; Nibbe 2010). The United Nations World Food Programme (WFP) provided general food assistance to IDPs from 1997 to 2008. At that time, it was considered to be one of the largest and longest food aid interventions in the world. The distributions were reported to be insufficient both in quality and quantity (Olwedo et al. 2008; Roberts et al. 2009; Nibbe 2010; WFP Uganda 2012). The rations were shipped from the United States of America (USA) and contained maize or sorghum, beans or pulses, cooking oil (fortified with vitamin A and D), and at times corn soya blend fortified with vitamin A (though only a short period). They were distributed monthly in collaboration with the Norwegian

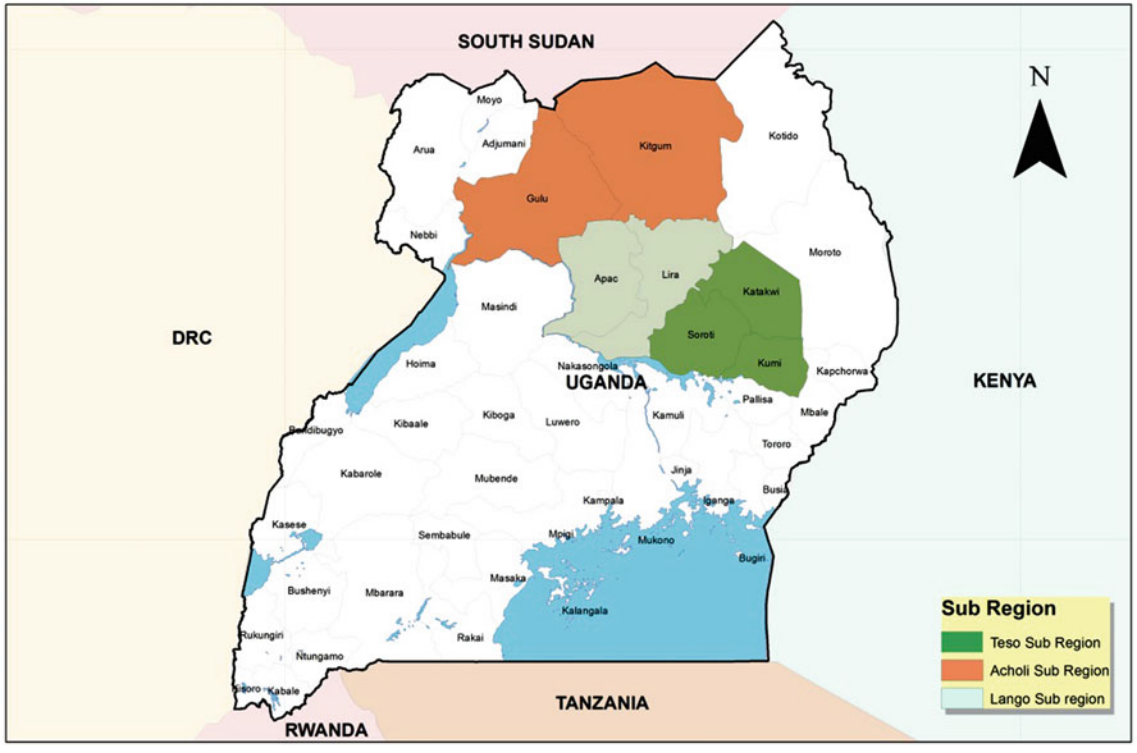

Fig. 1 Map of Uganda. The subregions of Acholi, Lango, and Teso were most affected by the armed conflict in 1986–2006. The map was developed by and used with permission from Ceaser Okumu Law, Gulu HDSS, Faculty of Medicine, Gulu University. Key: *HDSS* Health and Demographic Surveillance Site

Fig. 2 Part of an internally displaced persons' (IDP) camp in northern Uganda. The picture shows a part of an IDP camp in northern Uganda during the war. The IDP camp size range from 5,000 to 65,000 people that lived in closely build mud brick huts with thatched roofs. The picture is taken by Professor Susan Reynolds Whyte, Department of Anthropology at the University of Copenhagen, and is presented with permission from Professor Whyte

Refugee Council, camp leaders, and local leaders. Only vulnerable groups (elderly and disabled) received rations covering 100% the standard daily requirement throughout the relief period, while the general population received between 40 and 100% dependent on the supply from the USA and security situations (Nibbe 2010; WFP Uganda 2012). A standard food ration for general food relief from WFP contains 2,100 kcal per person per day, which represents the nutritional requirements for an average healthy adult (WFP). Not until 2003, supplementary and therapeutic feeding for pregnant women and malnourished children were implemented.

The IDP camps were characterized by environmental, social, and physical dangers including overcrowding, food insecurity, poor housing, collapse of health services, inadequate safe water supply, and poor sanitation, all resulting in high prevalence of acute malnutrition among children, physical illnesses, mental disorders, and high mortality rates (Salama et al. 2004; MoH Uganda 2006; WHO 2007, Spiegel et al. 2010). Accorsi et al. (2005) documented how the conflict "shaped the disease profile" of Gulu district using hospital discharge records between 1992 and 2002 from Lacor Hospital in Gulu. In 1997 and 2002, there was a sharp peak in malnutrition when the conflict escalated. The number of admissions due to measles and pneumonia also peaked in 1996 when there was large migration to IDP camps (Accorsi et al. 2005). A study in Omoro camp in 2006 reported that 6% of the 672 children below 5 years of age suffered from global acute malnutrition (GAM) and 52% were stunted, reflecting the long-standing food shortage and irregular food supply (Olwedo et al. 2008). No studies were conducted on nutritional status among adults. It has long been recognized that both moderate and severe malnutrition have large effects on childhood mortality in low-income countries. Especially, the synergy between malnutrition and infections has an impact on childhood mortality (Pelletier

et al. 1995). Correspondingly, childhood malnutrition measured as low weight-for-age has been shown to predict childhood mortality in Uganda (Vella et al. 1992a, b). In 2005, the Ministry of Health (MoH) of Uganda and the World Health Organization (WHO) conducted a large health and mortality survey among IDPs in the Acholi region (MoH Uganda 2005): The crude mortality rate (CMR), defined as the number of deaths per 10,000 persons per day (see Dictionary of Terms), was 1.54 and above the emergency level of 1 death per 10,000 persons per day. Children below 5 years accounted for 40% of these deaths, with an under-five mortality rate (U5MR) at 3.18 deaths per 10,000 per day (U5MR noncrises, <1.14; emergency threshold, 2.0) (MoH Uganda 2005).

The characteristics of IDP camps were also very similar to the recognized risk factors associated with noncommunicable diseases in developing countries: unplanned rapid urbanization, aging population, increased alcohol and tobacco consumption, decreased physical activity, and diet diversity (Kett 2005; Maher and Sekajugo 2011). Especially, the high level of mental illness contributed to the harmful health behaviors, such as hazardous drinking and increased smoking (Roberts et al. 2011). Roberts et al. (2011) showed that the absence of basic social goods and services was positively associated with mental health disorders, where *lack of food* was the most frequent reported reason for not feeling safe in the camp (93%), and was positively associated with symptoms for depression (Roberts et al. 2008). The forced displacement and loss of livelihood also increased risky behavior for human immunodeficiency virus (HIV) infection (Rujumba and Kwiringira 2010). The coping strategies to meet the basic necessities of life included sex for food, money and other material gifts, marrying off young girls and marital breakdown in favor of those who had money, and sexual violence. Additionally, the Acholi cultural practices like polygamy, widow inheritance, and early marriage (which excited before the war) added to the vulnerability of HIV and other sexual transmitted diseases (STDs) (Westerhaus et al. 2008; Rujumba and Kwiringira 2010). The above-mentioned health and mortality report by WHO found that acquired immunodeficiency syndrome (AIDS) was the second most frequently reported cause of death accounting for 13.5% of all deaths and 71.8% of deaths among 25–50 years old adults in IDP camps in 2005 (MoH Uganda 2005) (Fig. 2).

Adult Undernutrition in Rural Post-conflict Northern Uganda

Northern Uganda has been in a post-conflict transition since signing of the cessation of the hostilities agreement in 2006. In 2008–2010, local government officials put pressure on IDPs to return home, resulting in another population movement from urban camp setups to rural homes of origin. Satellite or transit camps were established to gradually decongest the IDP camps toward their villages. The closing of IDP camps and related challenged have been described in detail by Whyte and colleagues (Whyte et al. 2012; Whyte et al. 2014). The WFP's general food

distribution stopped in 2008 and distributions to vulnerable groups in 2010, and in 2012, all therapeutic feeding programs in health centers closed in the Acholi region. In 2009, the MoH Uganda reported a marked decrease in food security. Main reasons given were poor infrastructure, lack of assets (61% poverty), sudden withdrawal of WFP, and increase in the prevalence of HIV/AIDS (MoH Uganda 2010).

Adult undernutrition in post-conflict areas is a public health concern. Optimal health and nutritional status in post-conflict populations is highly needed for work ability, providing care for families and enhancing independence to rebuild society. Underweight weakens the immune response, reduces physical and cognitive function, and in turn affects morbidity and mortality (Pierce et al. 2010; Roh et al. 2014). Additionally, adult malnutrition has long-term health effects in terms of poor reproductive outcome and poor childhood growth of the next generation (Black et al. 2013). Lastly, poor health status and well-being in a post-conflict vacuum is a risk to human security that can trigger violence and instability.

A population-based study, conducted at the Gulu Health and Demographic Surveillance Site (HDSS) in 2011–2013, found a high prevalence of undernutrition among adults. Undernutrition was classified by body mass index (BMI) (see "Dictionary of Terms" for definition).The prevalence of undernutrition was 22.3% for men and 16.0% for women, whereas the prevalence of overweight was 1.5% for men and 7.6% for women (Schramm et al. 2016). The study included 2,062 men and 2,924 women aged 15 years and above, representing the adult population from a rural subcounty in Gulu district, northern Uganda. This was a cross-sectional community-based study that included anthropometric measurements (weight and height) and survey interviews with information on sociodemographic characteristics, food security, alcohol and smoking consumption, and medical history (Schramm et al. 2016). No other studies have so far been conducted to determine adult nutritional status in northern Uganda. The prevalence of underweight found in the post-conflict area was higher compared to the national prevalence in rural areas reported by the DHS Uganda in 2011, (19.2% and 12.9%, for men and women, respectively), whereas the prevalence of overweight was about half of the national rural prevalence (2.6% and 14.3%) (UBOS and ICF International Inc. 2012). Four community-based studies in the southern regions of Uganda have assessed nutritional status, where the prevalence of underweight ranged from 8.1 to 29.8% among men and 5.9 to 16.5% among women (Maher et al. 2011; Mayega et al. 2012; Kavishe et al. 2015; Kirunda et al. 2015). An overview of the community-based studies examining adult nutritional status in Uganda is presented in Table 1. It should be noted that the studies are not directly comparable; age of study population, underweight classification of young adults, and urban/rural residence should be considered when comparing studies. The highest prevalence of underweight was found by Maher et al. (2011) in southwestern Uganda (Maher et al. 2011). However, the study included participants from 13 years of age and 38.1% of the study population was below 20 years of age. As young adults are at higher risk of being underweight, this may overestimate the prevalence. Additionally, age- and sex-specific BMI cutoffs were not applied for participants below 19 years of age by Maher et al. (2011) and UBOS and ICF

Table 1 Overview of community-based studies examining adult nutritional status in Uganda. Underweight is defined by body mass index (BMI) <18.5 for adults >18 years. Age- and sex-specific BMI cutoff values should be applied to young adults <18 years (see full definition in Dictionary of Terms). Maher et al. (2011) used the BMI <18.5 for all age groups, whereas Schramm et al. 2016 applied age- and sex-specific cutoff values

Author	Study location	Rural/ urban	Year of data collection	N	Age of study participants	Underweight prevalence		Overweight prevalence	
						Men	Women	Men	Women
Maher et al. (2011)	Rakai, southwestern Uganda	Rural	2008–2009	6,678	≥13 years	29.8%	16.5%	4.1%	18.4%
Mayega et al. (2012)[a]	Iganga and Mayuge district, southeast Uganda	Rural and peri-urban	2011	1,656	35–60 years	17.6%	14.6%	9.7%	25.1%
Kavishe et al. (2015)[b]	Rural areas outside of the capital Kampala and Entebbe	Rural	2012–2013	432	≥18 years	16.0%	10.0%	9.2%	41.7%
Kirunda et al. (2015)[a]	Iganga and Mayuge district, southeast Uganda	Rural and peri-urban	2013	1,210	≥18 years	8.1%	5.9%	14.4%	35.7%
Schramm et al. (2016)	Gulu district, northern Uganda	Rural	2011–2013	4,986	≥15 years	22.3%	16.0%	1.5%	7.6%
UBOS and ICF International Inc (2012)[b]	National sample of rural areas	Rural	2011	3,524	Men: 15–54 years Women: 15–49 years	19.2%	12.9%	2.6%	14.3%

Key: *BMI* body mass index, *UBOS* Uganda Bureau of Statistics

[a]Kirunda et al. (2015) included participants both from rural and peri-urban areas ($n = 912$ [75%] from rural areas). Presented estimates are combined rural and peri-urban populations, as data was not stratified by gender in rural areas. The overall (combined gender) prevalence of undernutrition in rural areas was 8.1% vs. 4.0% in peri-urban; the overall prevalence of overweight was 15.8% in rural vs. 23.8% in peri-urban areas. Estimates from Mayaga were not stratified by rural/ peri-urban populations ($n = 1352$ [84%] from rural areas)

[b]Only data from rural populations are included in the table. Kavishe et al. (2015) and UBOS and ICF International Inc (2012) also included participants from urban areas ($n = 484$ and $n = 929$, respectively), not shown in the table

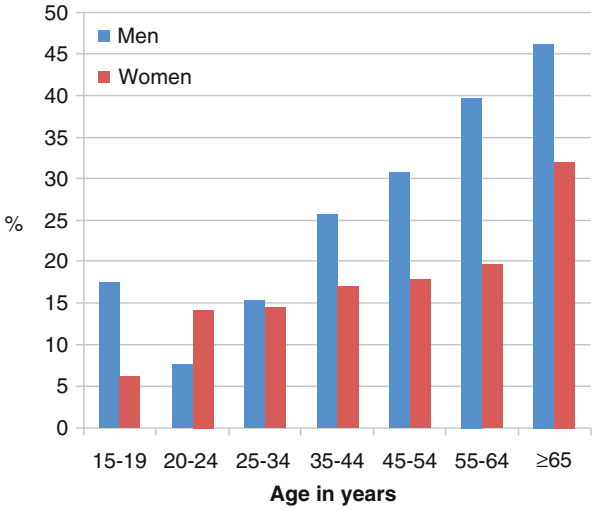

Fig. 3 Prevalence of undernutrition in northern Uganda by gender and age. The prevalence of adult undernutrition is presented by age categories and gender with men in the blue column (*left*) (Figure produced by results from Schramm et al. (2016))

International Inc. (2012) which further can overestimate the prevalence (Maher et al. 2011; UBOS and ICF International Inc 2012). Nevertheless, all studies report the same trend with underweight being more prevalent among men than women and overweight being more prevalent among women than men.

Besides gender differences, Schramm et al. (2016) found that undernutrition varied substantially across age groups, where young men and elderly were most likely to be underweight (Fig. 3) (Schramm et al. 2016). Risk factors for undernutrition were also different between men and women. Among adult men, being divorced/separated compared to being married, smoking daily vs. not smoking, and frequent alcohol consumption vs. not drinking were risk factors for being underweight. However, the association between alcohol consumption and undernutrition was not statistically significant in adjusted analysis. Although 17% of the adult women were widows, being a widow was not associated with being underweight. Smoking was more prevalent among men (32.0%) and virtually nonexisting among women (0.5%). The frequency of alcohol consumption was also higher among men than women. Lastly, among women, but not men, seasonality was associated with undernutrition. Women who were measured during the "hungry-gap rainy season" (May–July) were more likely to be underweight (Schramm et al. 2016). Hence, social and behavioral factors were associated with undernutrition among men but not among women (Table 2). Risk groups or factors associated with underweight were not examined in any of the other studies presented in Table 1 as this was not the main outcome of interest in these studies.

The following section will discuss mechanisms that can contribute to the observed gender difference in the prevalence and risk factors for adult undernutrition (Fig. 3).

Table 2 Individual-level risk factors associated with undernutrition among adults in post-conflict northern Uganda. Summary of findings from a logistic regression analysis using generalized estimation equation from the study by Schramm et al. 2016, examining risk factors for undernutrition among 4,986 adults in post-conflict Uganda (Reproduced and presented with permission from authors (Schramm 2016))

	Male	Female
Age	Young (<20 years) and older (>45 years) age was associated with increased odds of being underweight	Increasing age (from 20 years and above) was associated with increasing odds for being underweight
Marital status	Being divorced/separated was associated with ~2.0 higher odds of being underweight	No association. Note: Being divorced/separated or widowed was *not* associated with underweight
Smoking daily	Smoking daily was associated with 2.5 times higher odds of being underweight	No association
Alcohol consumption frequency	Drinking more than once per week was associated with being underweight (though not statistically significant)	No association
Measured during hungry-gap rainy season	No association	Being measured during hungry-gap rainy season was associated with ~30% higher likelihood of being underweight

Disruption of Traditional Gender Roles and Gender Differences in Undernutrition

When referring to differences between men and women or males and females, the term "gender" is used to describe a social construct, whereas "sex" is used when referring to a biological construct (Krieger 2003). Gender differences can occur from social, cultural, or structural conditions that shape the norms, values, experiences, and behavior of men and women. Poverty and trauma can interact with gender and contribute to excess disease burden in one group. Differences between genders may also be a result of inequality, that is, systematic empowerment of one group over another group (see Dictionary of Terms).

Before the civil war in northern Uganda, the traditional Acholi culture of northern Uganda had clear and defined gender roles and responsibilities between men and women. The man was the income provider, responsible for managing livestock and agricultural activities that require hard physical labor. The man had authority over the women and children and was also responsible for protecting and providing for them. A wife's responsibility was to take care of the children and the household, primarily engaged in the kitchen and subsistence farming activities that are less labor intensive (Adams et al. 2013; Sengupta and Calo 2016).

Disruption of social structures and altered gender roles during the conflict affected men more than women in northern Uganda. During the displacement to

camps, limited access to land and distribution of food relief resulted disempowered and "jobless" men. Women's roles around reproducibility and domestic work did not change to the same extent as men's roles. Men were no longer able to provide and protect their family. Boredom in camp, trauma, disempowerment, and loss of social masculine identity led to deep shame, humiliation, anger, and frustration, which further resulted in high consumption of alcohol and substance use, as well as other unhealthy behaviors among men. Women were producing alcohol and sold it to men. Women also became in power of food within the household as food rations by WFP was by policy given to women of the household at one point (Branch 2008; Kizza et al. 2012; Dolan 2013). Dolan has described this with the concept "The proliferation of small men in Northern Uganda," where women gained power within households and young men could not fulfill social expectations (including finding a wife and providing and protecting the family) and instead they engaged in alcohol abuse, violence, crime, and suicide (Dolan 2002; Dolan 2013). A qualitative study by Schlect et al. (2013) provides in-depth details on the changes and disruptions of marital traditions in northern Uganda (Schlecht et al. 2013).

Structural Mechanisms that Can Lead to Gender Differential Prevalence in Undernutrition

Differential exposure and vulnerability to disease, disability, and injuries are somewhat well known. For example, men are more prone to injury during war, and women are more prone to infectious disease due to closer contact with children. Moreover, the way that men and women are valued in society, including the health-care system, can affect the health behavior of individuals. Policies, health interventions, health systems, and health research are rarely neutral and can contribute to both equity and inequity between population groups. When targeting specific groups for intervention or care, health systems (including international and local health organizations) can be exclusionary and inequitable (Mackintosh 2001; Accorsi et al. 2007). International donors and agencies often see the post-conflict period as an opportunity to undertake reforms of public health institution that include gender policies and women's empowerment interventions (Percival et al. 2014). A study by Percival et al. (2014) suggests that donors in conflict and post-conflict settings, including northern Uganda, have prioritized reproductive and maternal health outcomes to a large extent (Percival et al. 2014). The focus on women (especially pregnant women) and children in health-care development programs and research is not new and reflects a long-term focus of international organizations and donors that are also presented in non-conflict settings.

Taking away "men's 'job'" by prolonged displacement and targeting food aid rations to the women of the household undermines the cultural gender roles and affected men's behavior. A qualitative study by Sengupta and Calo (2016) reported that local leaders perceived the "breakdown of traditions" to be due to non-governmental organizations' (NGOs) focus on gender rights and numerous women's empowerment programs. Sengupta and Calo (2016) further found that NGO

programs were perceived as *women's programs* by the male respondents of the study and that few NGOs target male involvement in women's rights program (Sengupta and Calo 2016). However, the MoH Uganda introduced the policy "male involvement strategy" for antenatal care (ANC) in November 2014 (WHO Africa 2014). The male involvement strategy was intended to encourage men to accompany their spouses during ANC visits. However, the interpretation of the policy was left to health-care providers who interpreted male partner attendance as compulsory and with compulsory HIV testing at the first ANC visit (Rudrum et al. 2015). Rudrum et al. (2015) reported that women are often sent away and miss essential health care if they come without their male partner. The policy of male involvement further contributed to the shift in power between genders; making women responsible for bringing their husbands to the health center shifts the responsibility from the health care system to the women. Leaving women to negotiate with men can also have consequences for the marital relationship and domestic violence (Rudrum et al. 2015).

Gender Differential Exposure to Trauma, Mental Illness, and Substance Use

When reviewing any public health aspect in a post-conflict setting, it is crucial to consider the role of trauma exposure and mental illness. Numerous studies have been conducted on mental health disorders in northern Uganda, and some of them will be presented here. The most commonly reported mental health disorders from northern Uganda are post-traumatic stress disorders (PTSDs), followed by depression and anxiety. Other published domains include suicide ideation or attempts, alcohol abuse, partner violence, child abuse, and feelings of guilt and revenge (Dokkedahl et al. 2015). A review by Dokkedahl et al. (2015) summarized published studies from 2004 to 2014 on mental health disorders in northern Uganda (Dokkedahl et al. 2015), but several studies have been published since. While there are no studies that have investigated the relationship between mental health and nutritional status directly, parallels can be drawn between study results.

Schramm et al. (2016) found that especially young adult men were at higher risk of being underweight (Schramm et al. 2016). Child soldier recruitment was predominantly among 11–16 years, who represent individuals aged 15–30 years at the time of the study. Exposure to abductions and other severe traumatic experiences was more common among boys and men than girls and women (Ovuga et al. 2008; Ertl et al. 2016). This differential exposure itself can put men at higher risk of developing mental illness and poor nutritional status compared to women.

The postwar environment, including community and family contextual factors, can interact with individual-level factors (sex, age, and previous trauma exposure) and either increase or decrease the burden of mental illness in post-conflict populations. For example, Amone-P'Olak et al. (2013) reported an indication that marriage (family context) was a protected factor for poor functioning of formerly

abducted young adults; individuals that were divorced/separated had a higher poor functioning score compared to individuals that were married (Amone-P'Olak et al. 2013). Likewise, Schramm et al. (2016) found that adult men that were divorced/separated had increased odds of being underweight compared to men that were married (Schramm et al. 2016). No gender difference was reported by Amone-P'Olak et al. (2013). On the community level, poor access to mental health-care services plays an enormous role in the burden of mental illness. Only 17% of former abducted youth had seen a mental health-care worker despite ~70% acknowledged that they experienced emotional and behavioral problems and wished to receive help. More women than men had sought help from mental health services. Reported barriers were stigma/discrimination, fear of family breakup, and lack of health-care providers (Amone-P'Olak et al. 2013).

As a consequence of extremely limited mental health care, stigma, and disruption of tradition gender roles, some people cope with psychological stress and symptoms themselves. Alcohol and substance use can be used as a self-medicating coping strategy. Ertl et al. (2016) found a strong association between war-related trauma and alcohol use disorder, as well as emotional abuse in the family and alcohol use disorder (Ertl et al. 2016). Similar findings were found by Roberts et al. (2011) during the insurgency period (Roberts et al. 2011).

Alcohol and substance abuse has been linked to undernutrition in other populations (Falck-Ytter and McCullough 2000). Further, Schramm et al. (2016) found that smoking and alcohol were risk factors for adult undernutrition, but only among men (Schramm et al. 2016). Likewise, Roberts et al. (2011) and Ertl et al. (2016) found alcohol consumption among Acholi women to be rare, which stems from the Acholi cultural tradition where it is not well accepted for girls and women to consume alcohol (Roberts et al. 2011; Ertl et al. 2016).

HIV

It is well known that being HIV-infected can be a cause of undernutrition (Koethe and Heimburger 2010). Additionally, being underweight with HIV further weakens the immune response which can comprise the effects of antiretroviral therapy, increase the risk of opportunistic infections such as tuberculosis (TB) (Holmes et al. 2003), and increase the risk of mortality (Van der Sande et al. 2004; Koethe and Heimburger 2010). The estimated prevalence of HIV infection in northern Uganda in the post-conflict period varies greatly by study, but is consistently higher among women than men. The estimates by the MoH Uganda (2011) were 10.1% among women and 6.3% among men aged 15–49 years, which was higher than the average national estimate of 8.2% and 6.1%, for men and women, respectively (MoH Uganda 2012). In a cohort study with 2,388 people aged 13–49 years from three different districts of northern Uganda, the prevalence of HIV infection was 14.6% among women and 8.5% among men (Malamba et al. 2016). Another study among only young adults in transit camps found the HIV prevalence to be 15.6% and 9.9%, among women and men, respectively (Patel et al. 2014). Data collection methods, selection, and inclusion criteria of participants should be considered when comparing the studies. The prevalence of opportunistic infections has also been found to be

higher among women than men and more prevalent with increasing age (>30 years) in northern Uganda (Rubaihayo et al. 2016).

When the civil war in northern Uganda ended, many of the social, structural, and environmental risk factors to HIV infections persisted. For example, food insecurity is strongly associated with increased risky behavior for HIV transmission (Westerhaus et al. 2008; Rujumba and Kwiringira 2010). A qualitative study by Rumumba and Kwiringira (2010) found that sexual immorality was linked to the perceived severity and relevance of different threats or problems. While participants were aware about the risks and consequences of HIV infection, people were more worried about food, water, and other basic needs than contracting HIV. HIV was described as "something for the future" and not something you die of now like hunger. Hence, unmet basic necessities had overshadowed the concern for HIV/AIDS (Rujumba and Kwiringira 2010).

The nutrition surveys presented in Table 1 were not able to explore if HIV could explain the prevalence of undernutrition. The consistent gender difference in nutritional status (higher prevalence of undernutrition among men than women) does not follow the same pattern with the gender difference in the prevalence of HIV infections. If HIV infection is well treated and managed, the risk of weight loss is lower. Thus, the different patterns may be explained by access to treatment, adherence, and stigma of HIV in northern Uganda. In general, women are more in contact with health care systems due to reproduction, responsibility of caring for children, and factors mentioned above. Increasing evidence suggest that disproportionately less men than women in Africa are accessing HIV treatment, men begin treatment with a more advanced disease stage, and men are more likely to interrupt treatment than women (Cornell et al. 2011). Yet, the rapid scale-up of ART programs has largely focused on women (Cornell et al. 2011). Ethnographic accounts from studies of ANC in northern Uganda give indication that HIV stigma is more present among men than women: "a man's status is greatly reduced by being known to have HIV" (Rudrum et al. 2015, p. 3), [men] fear that they have the virus and would "rather not know" (Rudrum et al. 2015, p. 4), and common cases of women bringing a taxi driver instead of their husband for HIV testing or the husband would show up drunk so he would not be able to have the test taken (Rudrum et al. 2015).

Lastly, associations between HIV and mental illness have also been established in northern Uganda. Mugisha et al. (2015) found HIV to be a risk factor for common mental disorders (Mugisha et al. 2015). Likewise, Malamba et al. (2016) found that participants living with HIV were more likely to report traumatic events, have probable PTSD and depression, and report suicidal ideation (Malamba et al. 2016). Palermo et al. (2013) found that among people living with HIV/AIDS, living in severe food insecure households had a significantly lower mental health score than people living with HIV/AIDS living in food secure households (Palermo et al. 2013).

Elderly

Older age was one of the high-risk groups for undernutrition in the study by Schramm et al. (2016). This is consistent with other studies in sub-Saharan Africa (Kimokoti and Hamer 2008; Cheserek et al. 2012). This is also in line with general

physiologically decline in both weight and height with older age, with a greater decline in weight. Consequences of underweight in elderly include alteration in immune function, decline in functional and cognitive ability, decreased ability to contribute to household and economic activities, poor quality of life, and mortality (Assantachai 2012). In post-conflict areas with migration and withdrawal of food assistance, the ability of older people to meet their basic needs can be challenging (Burton and Breen 2002). Elderly are increasingly being recognized as a vulnerable group in complex emergencies; however, they are still rarely included in nutrition assessments (Young et al. 2004). Elderly are also not a priority in development programs as they are viewed as contributing little to the society (Kimokoti and Hamer 2008). Most health studies and surveys, including the DHS, do not collect data on elderly. As a result, this group is systematically excluded from health research, public health planning, and policy, including fund allocations. In sub-Saharan Africa, the older population (>60 years) are projected to increase fourfold between 2005 and 2050 (UN DESA 2001). Elderly have a higher disease burden and will therefore have large contributions to the cost of health care in the future.

Policies and Protocols

Focusing on conventional target groups in health interventions and not adapting to the local context may have contributed to disruption of existing family and social structures and thereby create other risk groups that are not targeted. These nutritional consequences may be maintained after the conflict. Moreover, issues of gender inequality and social disruption may mirror a more general pattern in low-income countries that only becomes more pronounced in this post-conflict setting (Wyrod 2008). Health policy and interventions must consider gender relations within the historical and traditional aspects when implementing human rights and women empowerment strategies. As described above, post-conflict stressors are experienced and responded to differently between men and women. Health research and health data collected by organizations involved in conflict and post-conflict settings should systematically analyze data disaggregated by gender to be able to adjust health interventions to the local context and avoid unintended consequences.

Moreover, new approaches to better understand the complex interaction between behavioral, social, and structural determinants of health and disease in post-conflict settings are warranted. Social factors such as poverty, stigmatization, and structural violence may have great importance to the nature of morbidity and mortality. The term “syndemic” was first introduced by Merrill Singer in the 1990s and refers to two or more epidemics or diseases that interact with each other and with social and structural conditions which generate excess morbidity and mortality due to a synergetic effect (Singer et al. 2017). Social stigmatization of disease can have great importance for syndemic interactions. For example, stigmatization of HIV can result in the experience of enhanced stress; stress can further lead to adverse coping strategies such as substance abuse and social harmful behaviors which can lead to increased risk of mental illness and malnutrition; psychological distress and

malnutrition can further lead to other diseases. Syndemics may occur more easily in vulnerable groups such as post-conflict populations that are already affected by adverse social conditions. The high prevalence undernutrition among men may be a result of interactions between high levels of mental illness, HIV (or other diseases), substance abuse, and undernutrition itself, which further interacts with the social and structural conditions such as gender inequality, stigma, stress, feelings of disempowerment and shame, poverty, and limited access to health care. Additionally, early life trauma may be a significant social condition that increases the likelihood of syndemics within conflict and post-conflict populations. Recognizing and understanding these clusters and interactions of diseases and conditions are crucial for improving public health in post-conflict settings. This includes investigating the effect of co-occurring conditions and social conditions when assessing specific health outcomes. Because excess disease burden may be the result of social and structural (political) factors, interventions may be within and outside of the official health care system. A detailed description and recommendation for further research and practices have been published in the Lancet Series of Syndemics (Lancet 2017).

Dictionary of Terms

- **Internally displaced persons** – Internally displaced persons (IDPs) are defined as people who have been forced or obliged to flee their home to protected areas, particularly due to armed conflict, situations of general violence, violence of human rights, or natural or human-made disasters. IDPs flee or are forced to move to protected areas within their country and do not cross recognized international borders. They remain under the protection of their own government, not protected by international law or eligible to receive the same type of assistance as refugees (Salama et al. 2001). However, nonbinding legal principles for internal displacement have been developed to address shortfalls in protecting IDPs (Deng 1999). Refugees are people who may leave for the same reason as IDPs but flee to another country and cross international borders. They are protected under the United Nations 1951 Refugee Convention, where the United Nations High Commissioner for Refugees (UNCHR) has an international mandate to protect the rights of refugees and provide basic services (UNHCR 1967).
- **Post-conflict** – There is no universally accepted definition of a post-conflict setting. However, there is consensus that a country or an area is considered "post-conflict" when hostilities and violence have ceased and a peace agreement has been signed (Canavan et al. 2008). The transition can be very complex and context-determined. Often, several conflicts co-exit where some end and others continue. Some conflicts become chronic and displaced persons remain in camps or are without settlement for decades. A formal end to a conflict can also be replaced by high level of violence and instability, sometimes result in renewal of war (Haar and Rubenstein 2012). The post-conflict recovery and reconstruction can be understood as a process that includes efforts to improve restoration of law and order, political governance, economic rehabilitation, and development and

social conditions, including health. Hence, the real and tough battles to sustain peace and rebuilt society begin after the war. International organizations and donors often see the post-conflict period as a window of opportunity to implement a wide range of public sector reforms, including improving health care and ensuring equity and human rights, with the political will to "bring better back" (Percival et al. 2014).

- **Adult undernutrition** – This chapter focuses on undernutrition in terms of underweight, classified by body mass index (BMI [weight (kg)/height (m) ^{2}]). Adult individuals above 19 years of age are classified as underweight when their BMI is below 18.5. For young adults aged 15–19, underweight is classified by age- and sex-specific z-scores compared to the World Health Organization (WHO) reference growth standards (de Onis 2007). The chapter does not cover nutrient deficiencies, such as iron and vitamin A deficiencies.
- **Inequality** – Health inequality refers to differences in health indicators in groups that raise concern about justice (equity) as they may be systematically related to social and structural conditions that are modifiable. Gender inequality in health refers to unequal distribution in health between men and women (as a social construct), whereby one group (men or women) is systematically empowered over the other by actors and institutions in society.
- **Equity and inequity** – Health equity is an ethical concept of distributive justice: implies that everyone has the same opportunities for a healthy life. Working toward health equity means minimizing inequality and that health should not be compromised because of gender, age, income, or other factors. Subgroups in populations may be treated differently to achieve equity in health.
- **Crude mortality rate and under-five mortality rate** – Mortality rates are measures of the number of deaths in a given population in a given time. Crude mortality rate (CMR) is usually defined as number of deaths per 1,000 individuals per year. The under-five mortality rate (U5MR) is usually defined as the number of deaths of children below 5 years of age per 1,000 live birth. During armed conflicts, information on live birth and mortality is often not available. The WHO Health and Mortality survey (MoH Uganda 2005) referred to in this chapter uses an alternative definition, whereby CMR and U5MR are defined as the number of death over a total person-time at risk. Being within the study population defined being at risk. Person-time was defined as the cumulative time spent in the recall period of the study (maximum 6 months) by all persons in the study sample. The CMR and U5MR were therefore expressed as the number of deaths per 10,000 persons per day. See the health and mortality report by WHO for further details (MoH Uganda 2005).
- **Syndemics** – The term syndemics refers to the existence of two of more epidemics, diseases, or other health conditions in a population that interact with each other and with social conditions which result in excess morbidity and mortality due to a synergetic effect. Hence, syndemics is a set of mutually enhanced epidemics with biologic interaction of diseases and is sustained in a population due to adverse social conditions. They involve interactions of all disease types and health conditions such as infections, noncommunicable diseases including

mental illness, malnutrition, toxic exposure, and behavioral conditions. The interaction is often caused or enhanced by health inequalities due to poverty, stigmatization, or structural violence (Lancet 2017; Singer et al. 2017).

Summary Points

- This chapter outlines the prevalence and high-risk groups for adult undernutrition and discusses the social, behavioral, and structural mechanisms that can lead to adult undernutrition in a post-conflict setting like northern Uganda.
- Undernutrition is defined as underweight, classified by body mass index (BMI [weight (kg)/height (m) ^{2}]). Adult individuals above 19 years of age are classified as underweight when their BMI is below 18.5. For young adults aged 15–19, underweight is classified by age- and sex-specific z-scores compared to the WHO reference growth standards (de Onis 2007).
- Northern Uganda experienced two decades of armed conflict (1986–2006), where 90% of the population was forced to live in internally displaced persons' (IDP) camps.
- Disruption of social structures and altered gender roles affected men more than women; trauma and loss of masculinity and identity led to humiliation and shame, which further led to high alcohol consumption, substance use, and mental illness.
- The prevalence of adult undernutrition was higher among men than women, where young men and elderly were most likely to be underweight.
- Risk factors for undernutrition among men included social and behavioral factors: being divorced, smoking, and frequent alcohol intake. Despite 17% of women were widows, social or behavioral factors were not associated with underweight among women.
- Similar factors (marital status, alcohol) have been associated with common mental illnesses in northern Uganda. However, no study has yet examined the direct link between mental health and nutrition in this setting.
- Applied gender policies in food aid distributions and development programs do not match with the high-risk groups of adult undernutrition in northern Uganda.
- Focusing on conventional target groups in health interventions and not adapting to the local context may have contributed to disruption of existing family and social structures.
- The high prevalence of undernutrition among men may be a result of a "syndemic" interaction between mental illness, HIV, substance abuse, and undernutrition itself, which further interacts with the social and structural conditions such as gender inequality, stigma, stress, feelings of disempowerment and shame, poverty, and limited access to health care.
- This emphasizes the need for better approached to consider gender norms and values within the historical and traditional aspect when implementing women empowerment strategies in post-conflict settings. Furthermore, health data should systematically be analyzed gender-disaggregated.

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Part V

Hunger and Anorexia

Initial Hunger, a Subjective, Reproducible Limit in Intake Associated with Low Blood Glucose: A Training for Malnourished Infants and Overweight Adults

29

Mario Ciampolini

Contents

Abstract

In this review, we describe the training of the passage from scheduled to demanded meals in infants and adults. Reduction in energy intake was obtained by subjectively abolishing conditioned meals and by administering food only after demand by the infant or after hunger perception by the adult (Initial Hunger Meal Pattern; IHMP). Conditioned meals were those scheduled and/or presented to the infant as well to the adult by sight, smell, mentioning, gesturing, or simply

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at a fixed mealtime. In contrast IHMP training consisted of meal suspension and of feeding after the first infant's demand or after an adult's self-noticing arousal of hunger. IHMP was checked by measuring blood glucose (MBG) and was associated with significant decreases in diary-reported energy-intake, preprandial blood glucose, glycated hemoglobin, body weight, insulin AUC in glucose tolerance tests and in days with diarrhea as compared to randomized control subjects who maintained conditioned meals. There were metabolic differences between those who received conditioned and demanded (IHMP) meals. Subjects adapted themselves to IHMP training easily. We explain characteristics like hunger equivalents, adjustments of energy intake to energy expenditure, blood glucose measurements, and characteristics that emerge in IHMP in contrast with scheduled meals. We also review previous studies in which the meals upon demand have been used. Other areas mentioned in this review include coverage on portable glucose monitoring, standard measurements of blood glucose, training protocols to remove conditioned meals and implement IHMP, the features of *Initial Hunger*, and other aspects of metabolic control that interlink hunger, meal feeding, blood glucose, and energy balance.

Keywords

Blood glucose · Diabetes · Insulin resistance · Overweight · Fattening · Energy balance · Energy intake · Limit in energy intake · Hunger · Meal onset · Energy availability · Bowel disorders · Malnutrition

List of Abbreviations

AUC	Area under curve of GTT
BG	Blood glucose, an index of energy availability in blood for the whole body
BMI	Body mass index = body weight in kg divided by squared height in meters
GTT	Oral glucose tolerance test
IH	Initial hunger
IHMP	Initial hunger meal pattern
MBG	Mean preprandial blood glucose
RMR	Resting metabolic rate
TEE	Daily total energy expenditure

Introduction

In the following text, we describe our observations and findings on undernourished infants with diarrhea, some basic facts of the intestine, the immune response, and intestinal bacteria.

The alimentary canal is exposed to a large amount of viable bacteria: about 100 trillions per gram of content. Many hundreds of species have no immune effect on the mucosa, but there are 5–15% of species that are capable of eliciting an

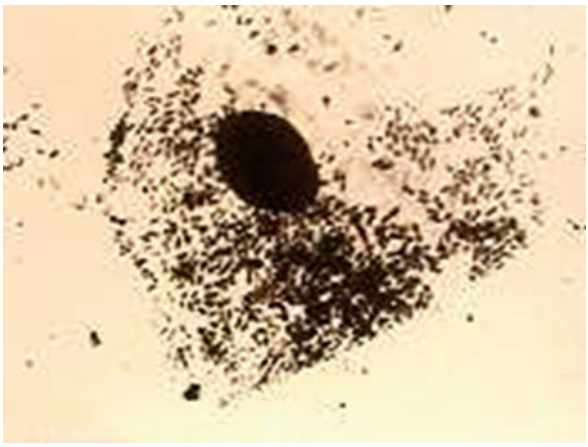

Fig. 1 Epithelial cell shed from intestinal mucosa and covered by bacteria. Magnification is 2000X. (Courtesy of Ciampolini et al. 1996, Copyright Clearance Center's Rights Link® service)

antibody response and inducing inflammation (Van der Waaij et al. 1996; Ciampolini et al. 1996, 2000). These immunogenic species alone do not provoke a general illness, but the antigen-immune elimination process damages underlying tissues (Van der Waaij et al. 1996).

The number of immune cells in the small intestinal mucosa is impressively large. Half of the immune cells of the whole body are in the small intestine (Mowat 1987; Brandtzaeg et al. 1989). The absorption of nutrients from food is thus a competition between mucosa cells and bacteria (Ciampolini et al. 1996, 2000). Figure 1 well represents the bacterial environment in intestinal lumen.

For feeding and preventing bacterial growth, in our clinic, mothers had to provide energy dense food only when necessary, i.e., food was not offered when the child was calm and happy. As soon as the baby changed their mood, the mother gave them a meal. Missing a meal reduced intestinal bacteria concentrations (Ciampolini et al. 1996). The child did not suffer hunger but only signaled it. This was an Initial Hunger Meal Pattern (IHMP). Figure 2 reports that during the associated low mean preprandial blood glucose (BG) in a week (reported as diary average glycaemia in Fig. 2), antibody response to Helicobacter pylori decreased, presumably reflecting lower H pylori growth.

In our clinic, we encountered most infants and adults with diarrhea and malabsorption who did not have Coeliac disease. We randomly assigned them to one of either two groups: (i) intervention (IHMP) or (ii) control. Controls remained on conditioned feeding. There were thus two groups in comparison for infancy and two groups for adulthood. Groups of overweight adults and malnourished infants provided further comparisons.

The intervention (Initial Hunger Meal Pattern, IHMP) consisted of suggesting subjects or mothers to suspend meals and to wait for the arousal of gastric pangs (adults), unexplainable unpleasant moods (infants), fussiness or walking in search for food, or verbal or gestural demand for food before seeing the food or laid table (Ciampolini et al. 1990).

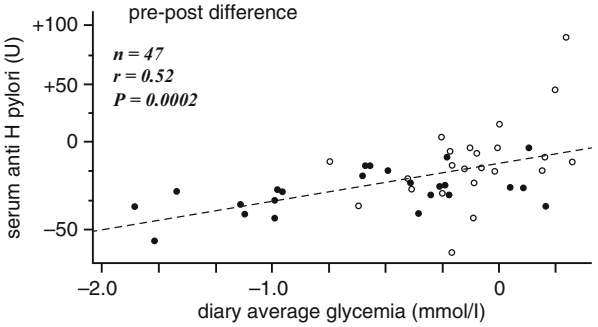

Fig. 2 Positive correlation between serum concentration of anti-*H. pylori* and diary mean preprandial Blood Glucose (MBG). Values represent the pre-/postdifferences. Black and white dots show trained and control subjects, respectively. $n = 47$, $rho = 0.52$, $p < 0.0002$. (Courtesy of Ciampolini et al. 2000; Copyright Clearance Center's Rights Link® service)

The prevalent way of eating is conditioned and sets the limit (arbitrary!) on body weight or fatness (Ciampolini et al. 2010, 2013b; Ciampolini and Bianchi 2006; Ciampolini and Sifone 2011; Fisher and Birch 2002; de Ridder et al. 2014; Herbert 2014; Melanson et al. 1999), whereas IHMP sets the limit in the arousal of subjective hunger sensations. Conditioned meals are those suggested by sight or smell of food, with company, by laid tables or by even mentioning these situations. Removing conditioned meals, i.e., implementing IHMP to achieve normative insulin sensitivity, abolished reflexes that depressed intestinal digestive functions as well as intestinal absorption (Ciampolini 1974, 1976). This was associated with a reduction of insulin resistance (Ciampolini 2013). In children, removing conditioned meals (i.e., implementing IHMP) decreased mean blood glucose (MBG) and in adults decreased the area under glucose curve (AUC) in glucose tolerance tests (GTTs) in association with lowering MBG (Ciampolini and Sifone 2011; Ciampolini 2012).

Investigating the Portable Glucose Monitor

We investigated preprandial events in blood by weekly diaries that reported blood glucose measurements with a portable device before the three main meals. Single blood glucose measurements were performed with a reliable portable potentiometer using the hexokinase method (Glucocard Memory; Menarini diagnostics; Florence, Italy). Measurements were made at 15 min before each meal. This constituted the *glucose diary*. The adults had to personally measure blood glucose in the same blood sample with the portable instrument. The reliability of the data was compared against the autoanalyzer results generated in the laboratory. **The autoanalyzer obtained a mean ± SD of 89.9 ± 11.3 mg/dL, adults measured 89.0 ± 12.5 mg/dL ($N = 85$). The mean difference (0.9 ± 7.1) was not significant. On absolute values, the mean difference was: 5.7 ± 4.3 mg/dL with no bias.** Measurements from the

blood glucose diary had a low confidence interval, 3.8 mg/dL, and after 5 months it was 6.0 mg/dL (no bias).

Measuring preprandial blood glucose is useful for the patient as they can directly notice their changes as a result of unbalanced meals (between intake and expenditure) and so may make appropriate adjustments. Moreover, he/she can learn the effects of physical exercise, changes in the immediate environment, and occasional stressful and untoward events on blood glucose (Ciampolini et al. 1990, 2010, 2013b; Ciampolini and Bianchi 2006; Ciampolini 2012). MBG characterizes the individual's meal pattern better than energy intake and much better than single fasting measurements (Ciampolini and Sifone 2011).

In the preprandial setting, BG and MBG are directly associated with insulin resistance. BG summarizes the metabolic condition that follows the previous meal and indicates energy availability (Ciampolini and Bianchi 2006; Ciampolini et al. 2010; Ciampolini and Sifone 2011). The metabolic conditions created by the previous meal ceases completely with the incoming meal. The *glucose diary* mentioned above has wide applicability and may be accurately performed by the patient under the surveillance of the family physician.

Standard Measurement of Blood Glucose

Sampling BG after an overnight fast is poorly standardized. The liver delivers glucose into blood every 12 min resulting in approximately 10% changes (Campfield and Smith 2003). Ambient temperature, physical activity, food, and stress all significantly increase blood glucose. Half of overweight subjects have high morning BG or do not consume breakfast and also those subjects who consume breakfast have unpredictable fasting BG because of variability in time, the amount of dietary energy, food composition of the previous dinner, physical activity before going to bed, and the degree to which the individual is insulated when in bed.

Sampling blood glucose within 15 min before a meal is a sufficiently reproducible (Confidence interval = 3.8 mg/dL). BG measurement at this sampling summarizes energy availability and balance for the period from last meal to the moment of sampling. This provides information about either the abundance or insufficiency (energy balance) of the last meal in comparison with the expenditure between meals. The measurements of blood glucose when receiving food energy indicate the energy available for a given situation. Being aware of this was considered the best way to adjust meal energy. As a consequence, overweight subjects lost their fear of hypoglycemic events later in the morning.

Meal onset produces a completely new metabolic condition with an increase in energy availability, a change in metabolic rate, and changes in gastric, intestinal, liver, and pancreatic functions (Ciampolini 1974, 1976; Ciampolini et al. 2013b).

The weekly MBG was calculated from 21 preprandial measurements in a week. MBG was consistent in a given subject because of the narrow confidence interval of preprandial BGs within the same person (Ciampolini and Sifone 2011). At recruitment,

the BGs of 120 investigated adults by portable device showed a mean confidence interval of ±3.84 mg/dL. By itself, this small confidence interval suggests reliability.

The normal BG range between 65 and 110 mg/dL was obtained in our studies by measuring BG in different subjects. We could stratify the 120 subjects into ten small strata. Each stratum contained subjects without differences in MBG that instead were significant from all other strata. We might say that each subject maintained a meal pattern directed by the aim of a stable level of energy availability within his/her own MBG stratum even during ad libitum meal feeding. Infants' MBGs showed stratification in less strata.

High energy availability is associated with depressed intestinal activity. To show the existence of this association, we reduced metabolic rate in experimental animals and in humans by elevating environmental temperature and tested xylose absorption in experimental animals and humans in the two conditions of energy availability: high and low (Figs. 3 and 4).

The high environmental temperature decreased the intestinal absorption rate of xylose (Ciampolini 1974, 1976). In this condition, long permanence of nutrients in the small intestine allows bacterial growth and possible diarrhea relapses. The relationship between bacterial growth and diarrhea relapses has been separately studied in children (Ciampolini et al. 1996, 2014; Ciampolini 2012).

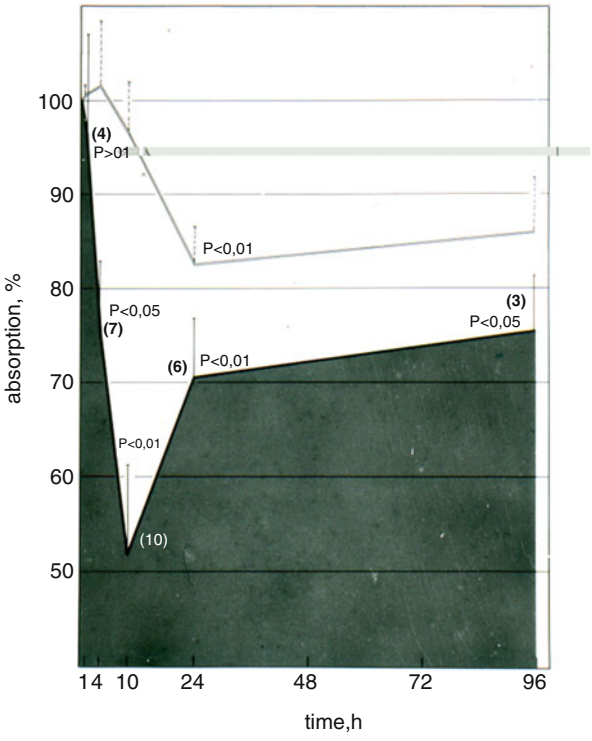

Fig. 3 Percentage of increased absorption in cold environment (6 °C) over absorption in warm environment (31 °C) of intragastric xylose in rats. (Courtesy of Ciampolini, IRSC 1974 Copyright Clearance Center's Rights Link® service)

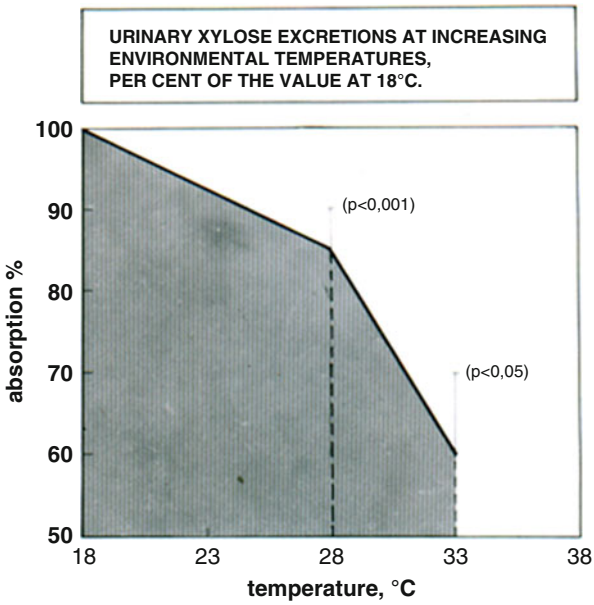

Fig. 4 Significant, negative correlation between environmental temperature and xylose absorption in man. (Courtesy of Ciampolini, Min Ped 1976 Copyright Clearance Center's Rights Link® service)

High total cholesterol, high triglycerides, low HDL cholesterol, high uric acid, high basal insulin, and high insulin responses during Glucose Tolerance Tests (GTTs) are correlated with high MBG (Ciampolini and Bianchi 2006; Reaven 2006) and contribute to the metabolic syndrome. BG assessments protect the overweight subjects against hypoglycemia and against fear of becoming hypoglycemic.

Training Protocol to Remove Conditioned Meals and Implement IHMP (Ciampolini and Bianchi 2006)

Below are the practical details of the training protocol for implementing IHMP:

1. Suspend meals for up to 48 h
2. Locate physical sensations of hunger
3. Measure blood glucose concentrations
4. Mentally associate the physical sensation with the blood glucose concentrations
5. Begin with a meal of about 300 kcal
6. Repeat 1–5 increasing the meal size in proportion to the desired inter-meal interval
7. Repeat the above procedure for 2 weeks. At each appearance of physical hunger, compare the sensations and the measured blood glucose with the previous sensations and measurement

Initial Hunger (IH)

Hunger arousal was associated with low BG, arousal of gastric activity, and a decrease in bacterial counts in the duodenum (Ciampolini et al. 1996). This suggests arousal of hunger might be the best moment for the intake of food. Initial Hunger (IH) appeared after meal withdrawal as a biophysical, subjective sensation that coincided with a recognizable (constant) physiological state of diminishing energy availability. This physiological state recurred more than once per day and suggested spontaneous energy intake in the absence of visual, olfactory, or word food cues (Ciampolini et al. 1990, 2010; Ciampolini and Bianchi 2006; Ciampolini and Sifone 2011; Ciampolini 2012; Fisher and Birch 2002). Blood glucose marked the metabolic condition at which IH could be recognized. In one study on hunger training, individualized fasting blood glucose was used to indicate when a meal could begin; hunger training was a feasible technique and was successful in over 80% of preprandial instances over 2 weeks (Jospe et al. 2015).

In home diaries, lean adults and mothers of toddlers reported IH associated with a blood glucose that was low in 90% of occasions. This percentage decreased to 60% of meals in overweight adults and to 50% in overweight children. We encountered two obese diabetic subjects who never developed hunger after meal suspension. Moreover, hunger sensations became weak and irregular in those who were years over the age of 60.

Training in Implementing IHMP

Regarding those with diarrhea (mentioned above), after the first short period of treatment, the infants and adults were trained for 7 weeks under the researchers' supervision. The first step was suspending meals until the child gave signs of hunger (Ciampolini et al. 1990, 2012). The desire for food consisted in a change of mood from tedious or restless behavior to crying. Mothers assessed the children's IH before them showing food or a laid table. On the first demand, before serving the meal, mothers were asked both to memorize the manifestations of restlessness and to measure blood glucose. On other occasions, the same child moved around in search of food or showed what he/she wanted. The first meal was postponed from 0 to 48 h, but on average 2 h after the usual schedule. At the meals that followed the mothers repeated the learned strategies. In the interval, mothers encouraged children to expend energy by physical activities rather than offering food (dietary energy). Energy-dense food with more than 60 kcal/100 g was allowed initially in a limited amount but in a growing quantity after the demand for food and after eating fruit or vegetables.

The recommended portion of fruit or vegetables was 150 g of vegetables before lunch and dinner and 200 g of fruit before breakfast. The energy-dense food was calculated in such a way as to evoke a new demand before the time fixed for the subsequent meal. Nonstarchy vegetables were recommended in large amounts (in adults, 300 g per meal or half/one kg per day). This amount prevented fainting

in overweight subjects (Ciampolini and Lovell Smith 2014). After 3–14 days training, mothers had learned how to evaluate both the energy left in blood (from blood glucose) from the preceding meal and the food energy needed to cover the gap up to the time for the following meal.

The amount of food, its content, and the timing of the meal were adjusted for each meal and situation. Mothers administered food energy which correlated with physical activity. They also found a way to make their children manifest IH (demand for food) 3–4 times a day (Ciampolini et al. 1990, 2012, 2013b). Provision of nonstarchy vegetables attenuated feeding and hunger-related behavior when food administration ceased and when energy intake was sufficient to cover the interval of time between the last and before the next planned meal.

After some days of trials and errors, and occasionally, of irregular mealtimes, mothers succeeded in obtaining the children's demand/meal 3 or 4 times per day with an average error of less than 30 min in 90% of cases. Such alimentary rhythms are called Recognizing Hunger or Initial Hunger Meal Patterns (Ciampolini et al. 1990, 2012). No food category was prohibited, except for saturated and trans fats. Nonstarchy vegetables intake was positively correlated with the intensity of hunger arousal in the same meal and intended to produce a negative hunger intensity in the subsequent meal.

The Feeding Relationship with the Mother

In the Pediatric Gastroenterology Unit, mothers of diarrheic and malnourished infants were educated to improve the health of their children and to avoid adverse health effects in their adulthood. They were also encouraged to ensure that their children avoided meals with positive energy balance (Ciampolini 2012). This requires a continuous physical monitoring of the child by the mother and for the mother to be very consciously and subconsciously mindful of the child. It requires skill and attention and the mother has to be prepared for an unremitting fight against conditioned intake of food. Mothers were the main carers in these recovering children. Some nurses were very mindful of the clinical studies and collaborated accordingly. Only one father was of help among thousands of families. Husbands however helped in the creation of a calm and serene environment.

The Timing of Blood Glucose Measurements

Subjects avoided blood glucose measurements taken less than 1 h after consuming even a few grams of food, after changes in ambient temperature, after physical activity such as walking or cycling, under behavioral stress or fever. This is because blood glucose in these circumstances is higher than 1 h after cessation of the transient metabolic condition (Ciampolini and Bianchi 2006).

The Home Diary and Blood Glucose Measurements

Seven-day home diaries were used to document and record blood glucose measurements before the three main meals, the intake of dietary energy and vegetable intake, hours in bed and hours spent during physical and outdoor activities, and the presence or absence of preprandial sensations of epigastric hunger. Infants' mothers compiled the diaries before training, after 7 weeks and at the end of investigations.

In one study, 24 similar diarrheic infant-mother pairs of the same age were trained to IHMP and the resting metabolic rate (RMR) was measured by indirect calorimetry in 14 and total energy expenditure (TEE) by doubly labeled water in 10 infants. RMR decreased by 15.4%; TEE decreased by 15.5%, from 80.1 ± 6.9 to 67.8 ± 10.0 kcal/kg/d. In the synchronous 10 day food diaries, energy intake decreased by 17.9%, from 85.7 ± 15.3 to 70.3 ± 15.8 kcal/kg/d (pre/post $P < 0.001$) after intervention. No difference was found between TEE and energy intake at recruitment, at final assessments, and in the pre-/post-decreases. The height Z-score increased significantly, while increments in body weight were normal.

We always required an objective assessment, and after the first training days, we asked adults to write the presumed blood glucose before measurement. Adults in training were often surprised in being able to predict the measured blood glucose (Fig. 5).

Fourteen out of 46 trained subjects who were not hungry had BGs below 87 mg/dL, the maximum limit of blood glucose of those who were hungry (Fig. 6). These 14 subjects showed an average estimation error of 4.5 ± 3.1% of the measured blood

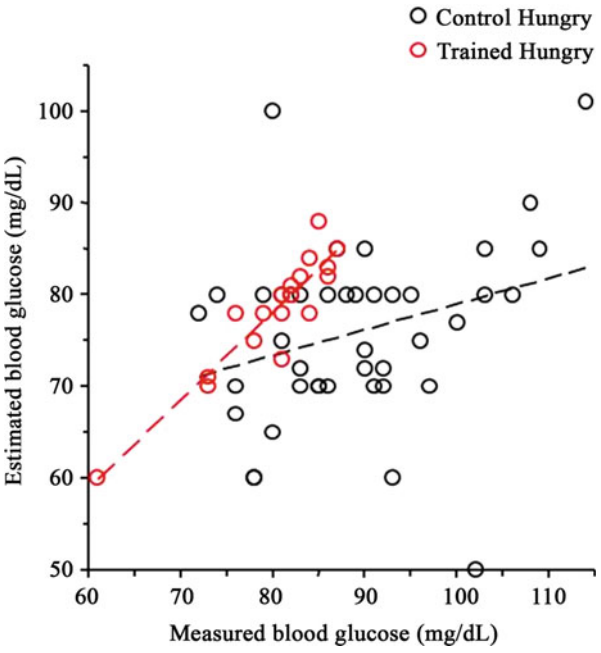

Fig. 5 Estimated versus measured blood glucose of subjects reporting to be hungry at the final laboratory investigative session. Notes: Hollow red circles, trained hungry subjects ($n = 18$); hollow black circles, control (untrained) hungry subjects ($n = 42$). Linear correlation was significant for the trained data (dashed red line; $r = 0.92$; $p = 0.0001$) but not for the control data (dashed black line; $r = 0.29$, $p = 0.06$). (Courtesy of Ciampolini and Bianchi 2006; licensee BioMed Central Ltd. under the terms of the Creative Commons Attribution License <http://creativecommons.org/licenses/by/2.0>

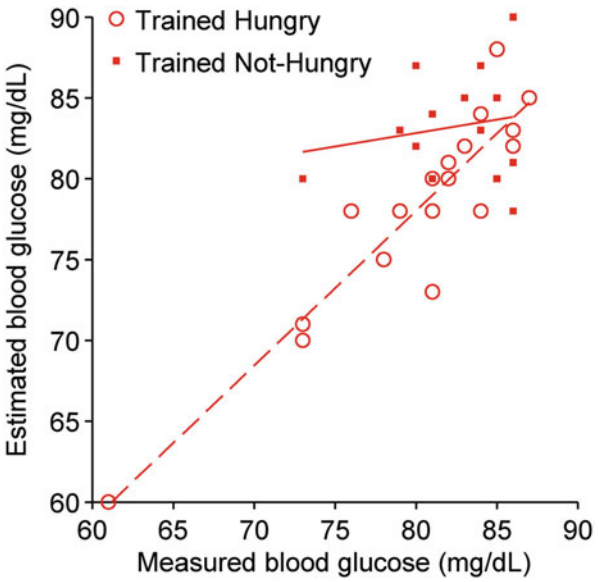

Fig. 6 Estimated versus measured blood glucose of trained subjects with levels below 87 mg/dL at the final session. Notes: The highest glycemic value measured in trained hungry subjects was 87 mg/dL. Below this value of measured blood glucose, 18 subjects reported to be hungry (hollow red circles) and 14 subjects were not hungry (full red squares). Linear regression is significant for the hungry subjects (dashed red line; $r = 0.92$; $p = 0.0001$) but not for those not hungry (solid red line; $r = 0.18$; $p = 0.54$). Courtesy of Ciampolini and Bianchi 2006; licensee Bio Med Central Ltd. under the terms of the Creative Commons Attribution License <http://creativecommons.org/licenses/by/2.0>

glucose, which did not significantly differ from the estimation error of the 18 trained subjects who were hungry (Ciampolini and Bianchi 2006).

Distinguishing Conditioned Hunger from Hunger After Meal Suspension

One question that needs to be addressed is whether adults learn to recognize children' hunger manifestations that arise after meal suspension from conditioned hunger sensations. Another question relates to the difference between the subjective blood glucose estimations and the biochemical measurements (Ciampolini and Bianchi 2006). To address this, we measured blood glucose by autoanalyzer (which has a 1% error). In our studies, adolescents have the lowest BG estimation error and people over 60 the highest. Given these observations, we later studied diarrheic infants and in adults who were less than 60 years old in the full confidence of the data derived from glucose measurements (Figs. 5 and 6).

Twenty-four diarrheic infants in their second year of life were trained to IHMP, and the resting metabolic rate (RMR) was measured by indirect calorimetry in

Table 1 Estimation of blood glucose by mothers on food demand from their infants in hospital just before blood sampling and autoanalyzer measurement: 54 infants demanded food before sampling, 16 infants did not demand food

Demanding food ($n = 54$)		With no food demand ($n = 16$)	
Estimation[a]	Measurement	Estimation	Measurement
77.4 ± 3.6[b]	74.6 ± 7.7	88.7 ± 5.9	96.3 ± 10.5[c]

[a]Mothers stated whether the infant had either demanded food or not, and estimated BG just before blood sampling
[b]mean ± standard deviation, mg/dL
[c]$p < 0.01$ versus infants demanding food. (Courtesy of Ciampolini et al. 2013a; Copyright permission granted by Dovepress). *BG* Blood glucose

14 and total energy expenditure (TEE) by doubly labeled water in 10 infants. The training consisted of blood glucose measurements at hunger arousal to validate the subjective hunger sensations and lasted 2 weeks. RMR decreased by 15.4%; TEE decreased by 15.5%, from 80.1 ± 6.9 to 67.8 ± 10.0 kcal/kg/d. In the synchronous 10 day food diaries, energy intake decreased by 17.9%, from 85.7 ± 15.3 to 70.3 ± 15.8 kcal/kg/d (pre/post $P < 0.001$) after intervention. No difference was found between TEE and energy intake at recruitment, at final assessments, and in the pre-/postdecreases.

In another study, 64 trained and 72 control subjects came to the hospital laboratory before breakfast following an overnight fast (Ciampolini and Bianchi 2006; Ciampolini et al. 2010; Ciampolini and Sifone 2011). All subjects declared current presence or absence of hunger and estimated blood glucose. A glucose autoanalyzer measured the actual blood glucose (Fig. 5 for adults, Table 1 for infants). The number of control adults (42 out of 72) stating hunger was significantly higher than the number of hungry trained subjects (18 out of 64) (Ciampolini and Bianchi 2006). All hungry adults described hunger sensations as gastric emptiness or gastric pangs. In the hungry trained group, the mean estimated blood glucose was 78.1 ± 6.7 and the mean measured value was 80.1 ± 6.3 mg/dL. This measured blood glucose was significantly lower than the measurements in hungry control subjects (89.2 ± 10.2 mg/dL) and in not-hungry subjects of both trained (90.0 ± 6.6 mg/dL) and control (90.6 ± 10.9 mg/dL) groups.

The absolute value of the difference between estimated and measured glucose (estimation error) in the hungry trained group (3.2 ± 2.4% of the measured value) was significantly lower than the one in the hungry control group (16.7 ± 11.0%). Linear regressions of the values in the hungry groups show that there was a significant correlation between estimated and measured blood glucose in the trained group ($r = 0.92$; $P = 0.0001$) but not in the control group ($r = 0.29$; $P = 0.06$). All these findings prove that control (untrained) adults do not reflect their biochemical condition when stating hunger and when estimating blood glucose.

In the hospital laboratory, control adults assessed their blood glucose and hunger state some minutes before having breakfast. Right after responding to questions about hunger, control adults focused on food and developed gastroduodenal Pavlovian reflexes. They described hunger as a tenuous, continuous state of hollow

stomach rather than intermittent sensations of wave bursts as physiological studies suggest. Their statements reflected a lack of experience in self-observations or did not have the ability to compare current sensations with past experiences. Humans tend to maintain constant blood glucose before meals (confidence interval ± 3.8 mg/dL around MBG in a week). Control adults, who asserted to be hungry, did not display blood glucose levels that were lower than their personal, habitual high BG levels (Ciampolini and Bianchi 2006; Ciampolini et al. 2010; Ciampolini and Sifone 2011). Only 8 out of 42 controls stating to be hungry had low blood glucose estimation errors (Ciampolini and Bianchi 2006, Fig. 5). Except for this minority, control adults reported conditioned hunger. All these differences were modest. The main difference consisted in the onset of hunger sensations: either before focusing on food (after meal suspension) or after focusing on food (conditioned). Low blood glucose values can confirm this distinction.

Not-Hungry Adults (Hunger Equivalents)

At the final investigative session, the trained and control adults that were not hungry significantly underestimated their glucose levels. Their estimation errors were, respectively, 4.8 ± 3.2 mg/dL and 16.1 ± 11.3 mg/dL in trained and control groups. The linear correlation between estimated and measured blood glucose was highly significant in the trained group but not significant in controls.

The difference between trained and control groups did not depend on gender, age, number of years at school, weight, or body mass index. Under 87 mg/dL, the estimation error was low in both trained and control groups apart the adult's statement on hunger (Fig. 6). In adults with values above 87 mg/dL of BG, the estimation error increased significantly. Despite them not being hungry, 12 out of 14 trained adults under 87 mg/dL and 3 out of 32 above 87 mg/dL described the slight sensations they used to estimate blood glucose concentrations. Thus, compared to controls, who did not report equivalents of hunger ($n = 30$), a significantly higher proportion out of the 46 not-hungry trained adults was able to report sensations different from gastric hunger. This was useful in estimating their blood glucoses, and this ability to report these sensations prevailed when their blood glucose was below 87 mg/dL. In their reports, 15 adults described either physical ($n = 3$) or mental weakness ($n = 10$) or abdominal changes in tension or movement ($n = 2$). Six out of 46 not-hungry trained adults, but none of the control adults, felt gastric IH before entering the hospital for the final session. However, the sensations faded while waiting for blood sampling. In the not-hungry adults' reports, the sensations of mental weakness consisted of difficulty in sustained mental concentration, impatience, irritability, drowsiness, gnawing sensation, loss of enthusiasm and effectiveness at mental work, or poor mood at their jobs.

The aforementioned mental sensations emerged alone or in addition to gastric or other sensations and ceased with the meal. Sensing impairment during physical activity was associated with heavy physical exercise outdoors and often signaled a change from a former sedentary life style. This sensation was used regularly to

indicate a meal signal with an increased requirement of high-energy-dense food for the following meal(s). The prevalence of these "hunger equivalents" ranged from an occasional occurrence to less than 15% of meals in phone reports. Two adults reported that they never sensed (gastric) hunger, but estimated blood glucose within 6% estimation error always by assessing mental or muscular weakness during training or during the final investigative session. In their reports, these adults consumed meals at blood glucose estimation between 78 and 85 mg/dL. Also Caudwell et al. (2013) found weakness and low RMR as signals for starting meals.

We recently encountered two obese adults who denied any hunger arousal after 48 h fasting. The two adults were diabetic type 2. They decreased their body weight by more than 15% and before dinner BG decreased to 75.3 mg/dL. This value is inside Low BGs in the Fig. 5. During hunger weakness, blood glucose, blood glucose estimation error, and RMR have low values (Ciampolini and Bianchi 2006; Ciampolini et al. 2010; Ciampolini and Sifone 2011; Caudwell et al. 2013). These characteristics suggest that hunger weakness is a reliable signal for intake just like hunger pangs.

One out of 89 trained adults (Ciampolini and Sifone 2011) reported not having gastric sensations at all, even long after consuming a meal. We suggested to the subject that meal should start after the estimation of blood glucose decrease to lower than 81.8 mg/dL. The adult endured physical weakness for 2 h before meal consumption, blood glucose fell from 92.5 ± 5.1 mg/dL to 80.7 ± 5.7 mg/dL, and body weight from 74.5 to 67.4 kg after 36 days. The adult coped with an open air heavy job during a cold winter. At recruitment, he was 189.5 cm tall with a BMI = 20.75. His energy intake showed a decrease from 3613 ± 615 kcal/day to 1817 ± 394 kcal/day. We suggested to this adult to use the sensation of physical weakness as IH and as an appropriate signal for meal onset. In the following 40 days, the adult increased dietary energy intake to 2380 ± 459 kcal/day, blood glucose to 85.6 ± 4.7, and weight to 70.95 kg. In the meantime, complaints reported at recruitment (abdominal pain and dermatitis) were ameliorated. This "case" suggests the need for higher blood glucose than 76.6 ± 3.7 mg/dL for subjects engaged in intense, daylong physical exercise (Ciampolini and Sifone 2011).

Overall it seems, trained subjects are able to recognize hunger by gastric sensations or by body or mental weakness. The first signal is a threshold and is quite definite. The second is gradual and unspecific.

Adapting Energy Intake to Energy Expenditure

In Table 2, we report final values in 27 adults who had a high MBG out of 89 who practiced IHMP for 5 months (Ciampolini and Sifone 2011). Six out of 27 were engaged in heavy manual work in a cold, open air climate. They asserted to comply accurately with IHMP and their high insulin sensitivity at GTT supported their assertions. Heavy physical activity in a cold climate may require high blood glucose and impose IH arousal when blood glucose is high for sedentary people. In our investigations (Figs. 5 and 6), intestinal functions and xylose absorption increased

Table 2 Heavy outdoor manual work (6 High BG) versus a sedentary lifestyle in 21 High BG of 27 (out of 89) adults who failed to reach Low BG (76.6 ± 3.7 mg/dL) at study end

	6 High BG	21 High BG ab
Final mean blood glucose (mg/dL)	86.4 ± 4.0	87.1 ± 5.3
Final insulin AUC (mU/L/3 h)	124 ± 26	207 ± 99 c
Final blood glucose AUC (mg/dL/3 h)	536 ± 56	601 ± 82 d
Insulin sensitivity index	11.4 ± 2.9	6.68 ± 4.0 e
Beta cell function index	1.29 ± 0.66	1.43 ± 1.22

[a]Six High BG subjects reported doing heavy work all day in outdoor environment during cold weather and reported accurate compliance with IHMP. No significant differences in the five parameters from recruitment. At recruitment, MBG was 86.9 ± 5.3 mg/dL in 27 High BG subjects
[b]The 21 High BG subjects included 15 that were Low BG after 7 weeks training (diary assessment) and six who had higher MBG than 100 mg/dL at recruitment
[c]$p < 0.01$
[d]$p < 0.05$
[e]$p < 0.001$. AUC: area under curve during glucose tolerance test. (Courtesy of Ciampolini and Sifone 2011; Copyright permission granted by Dovepress). *BG* Blood glucose

their rate during cold exposure in association with an increase in resting metabolic rate (Ciampolini 1974, 1976).

Adjusting energy intake according to hunger delay or anticipation is useful to maintain a steady, optimal blood glucose level. IHMP consists of a meal by meal exhaustion of nutrients in the blood with the last meal. This prevents absorption slowdowns and avoids slow intestinal absorption, bacterial growth, immune stimulation, and relapses in diarrhea.

In the first decade of our studies in a pediatric ward, we did not measure blood glucose before meals and we limited children's intake by administering food only on demand (See also: Fisher and Birch 2002). The limit in intake was not biochemical (Ciampolini et al. 1990, 2010; Ciampolini and Sifone 2011; Ciampolini et al. 2013a; Ciampolini and Bianchi 2006; Ciampolini 2012). In the occurrence of a discrepancy between hunger arousal and high blood glucose, we previously suggested mothers (and adults for themselves) to let the hunger manifestations prevail (IH arousal and IHMP). The infants did not suffer endured hunger as a consequence.

Conditioned and Demanded Meals and Validation of Diary Energy Intake

In overweight people, the only imposition they had was to approximate meal energy intake to prevent hunger arousal before the planned subsequent intake of food. We measured body weight at the beginning and the end of the 7 day diary. A decrease in body weight suggested an underreporting in overweight people who wanted to lose weight (Ciampolini et al. 2010). When subjects wanted a treatment for bowel disorders, we found no weight loss during the weekly diary, i.e., diaries of these subjects showed no underreporting (Ciampolini et al. 2010).

We also studied 24 infants with functional disorders of the bowel with regard to: (a) energy intake by a 10-day food diary; (b) RMR (indirect calorimetry) in 14 toddlers, and (c) TEE by doubly labeled water in 10 toddlers (Ciampolini et al. 2013a). Their blood parameters, anthropometry, and number of days with diarrhea were assessed before training and 50 days after training. Energy intake decreased from 85.7 ± 15.3 to 70.3 ± 15.8 kcal/kg/d (−17.9%). and TEE decreased from 80.1 ± 6.9 to 67.8 ± 10.0 kcal/kg/d (−15.5%). We found no statistical difference between intake and expenditure at recruitment, after training and in the decrease that occurred during IHMP. Dietary energy intake decreased more than expenditure (although the difference was not significant). Also fecal energy loss decreased during IHMP. This decrease in fecal energy compensated the modest difference between intake and expenditure (Ciampolini et al. 2001). RMR decreased from 58.6 ± 7.8 to 49.0 ± 9.1 kcal/kg/d (−15.4%). The height Z-score increased significantly, while weight growth was normal.

IHMP and Malnutrition

MBG is positively associated with insulin resistance, depressing intestinal functions, perturbing the microbiome, and developing an overall subclinical state of inflammation. In turn, this state produces functional diseases like asthma, arthritis, bowel disorders, and a long-term evolution to metabolic, vascular, and malignant risks. Meals with excessive energy intake are signaled by high BG before the following meal. The consequent energy availability becomes lower during IHMP than during a conditioned meal pattern. A meal pattern dictated by IH arousals lowers energy intake, BG, and insulin resistance; it maintains body weight in lean subjects and prevents risks.

A controlled study was carried out in 9 undernourished infants. Demanded meals were associated with low preprandial BG, significantly lower energy intake, prompt recovery from diarrhea, and slow recovery of lean body mass in 2 years.

Another study documented the recovery of a 12 years old boy from severe malnutrition by implementing IHMP. The MBG decreased from 98.9 ± 15.1 mg/dL to 78. 6 ± 2.9 mg/dL. This boy grew from a Gomez index of 56% (BMI 10.7) at recruitment to an index of 80% (BMI 19.6) at the age of 20. Undernourished people often have insulin resistance that impairs the passage of nutrients absorbed from blood into lean body tissues. These facts and observations suggest together that IHMP may prevent alterations of the microbiome and to avoid a deficiency in immune function from birth onwards.

Dictionary of Terms

- High Blood Glucose. This is a blood glucose of over 82 mg/dL.
- Initial Hunger: This consists of gastric pangs or physical weakness **that arise after meal suspension** and that are associated with low blood glucose.

- Initial Hunger Meal Pattern (IHMP): Energy intake is adjusted to three arousals of initial hunger per day.
- Low Blood Glucose is a blood glucose below 82 mg/dL.
- MBG = mean blood glucose: mean of BG measurements **before meals** in a week.
- Normal body weight, BMI under 25.
- Overweight = BMI > 25.
- BMI = Body weight in kg divided by the height square in meters.

Summary Points

- Initial hunger before meals protects both undernourished and overweight subjects. This may be related to the fact that more than half immune cells of the body reside in the small intestinal mucosa.
- In infancy or sedentary adulthood, humans can learn to recognize initial hunger arousal at the same blood glucose, 76.6 ± 3.7 mg/dL.
- This means that initial hunger corresponds to a reproducible metabolic condition that can be identified and recognized by humans.
- Initial hunger arises between 0 and 48 h after meals in healthy children and healthy adults but not in obese diabetics.
- An initial hunger meal pattern (IHMP) consists of initial hunger arousal three times a day. Practicing IHMP is associated with increased insulin sensitivity, lower energy intake (−18%), lower mean blood glucose (MBG) (−15 to −20%), lower resting metabolic rate (RMR)(−15%), and lower total energy expenditure (TEE)(−16%).
- The individual regression toward an even energy balance under IHMP is proportionate to the value at recruitment.
- About a third of healthy people show at recruitment (before any training) the same low MBG, low RMR, and associated insulin sensitivity that are gained by people after training IHMP.

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Restricted Temporal Access to Food and Anorexia: Modeling Systems

30

Neil E. Rowland, Dulce M. Minaya, and Kimberly L. Robertson

Contents

Abstract

The observation that the demand for a commodity decreases as its unit price increases is called demand elasticity. Applied to food, and to rodents in an operant behavior situation, we call this cost-based anorexia. We review evidence that rats and mice show cost-based anorexia when food is available in either 23-h (continuous access) protocols or as discrete food opportunities. The decrease in food intake of rats at higher unit food price is associated with sustained high operant response rates that, in the food opportunity protocol, occupy the entire time available. In contrast, mice do not show sustained high response rates and, in the food opportunity protocol, show rapid decline of food intake within a meal even when intake is very low and weight loss rapid. Differential price experiments, either for the same food at different times or for different foods, show that

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mice do not maximize their response efforts. The behavior of mice under imposed cost conditions has some similarity to the behavior of human anorexia nervosa, and potential mechanisms are discussed briefly.

Keywords
Anorexia nervosa · Activity-based anorexia · Cost-based anorexia · Elasticity of demand · Food intake · Operant behavior · Rats · Mice · Animal model · Homeostasis · Body weight · Differential food cost · Zeitgeber · Palatability

List of Abbreviations

ABA	Activity-based anorexia
AN	Anorexia nervosa
CBA	Cost-based anorexia
CC	Continuous costly
FO	Food (or feeding) opportunity
FUP	Fixed unit price
II	Intermittent inexpensive

Introduction

Famine, starvation, and/or malnutrition have been commonplace throughout evolution of most species, and it is only in the last ~10,000 years that organized agriculture has made spectacular strides in alleviating food insecurity. But these measures do not completely insulate humans from fluctuating food availability, such as those due to environmental extremes and climate change, or increased food demand by a burgeoning global population. A mismatch of supply and demand has been used historically as an agent of subjugation, and today's global market or economic forces represent centralized control of power and wealth. Less wealthy nations, or individuals of lower socioeconomic status within wealthy nations, are differentially vulnerable to economic exploitation of food resources in amount and quality (Gendron and Audet 2012). This chapter will focus on the investigation of the way in which economic or cost variables (effectively, commoditization) affect food consumption in highly controlled laboratory studies using rats and mice. Understanding these processes at a fundamental level may prove to be useful in interpreting and ameliorating food-related problems in humans, ranging from food insecurity to disorders such as anorexia nervosa (AN). Before addressing these issues, which appear to represent a breakdown of physiological regulation, it is appropriate to give a synopsis of such physiological approaches.

Homeostasis and Feeding Behavior

Homeostatic theories posit that in a system with a constantly fluctuating variable, such as energy balance (the difference between intakes and expenditures), the fluctuations will be tightly regulated in order to maintain a nearly constant

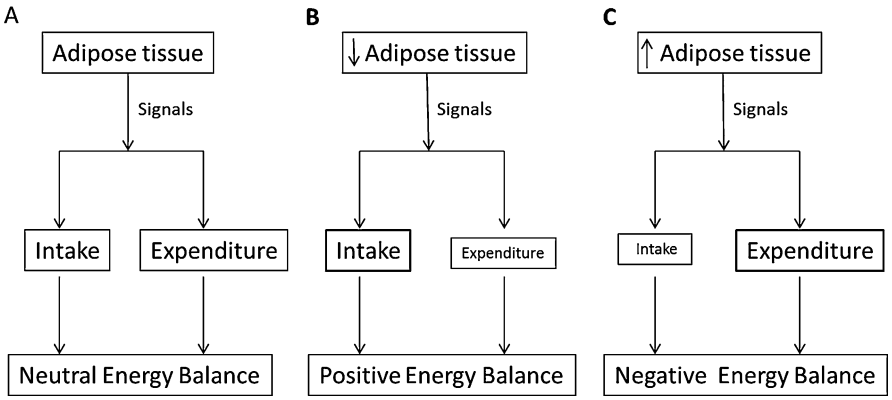

Fig. 1 Simplified diagram of homeostatic theory for energy balance of an organism. Each panel represents the sequence of events that theoretically result in (**a**) stable body weight, (**b**) body weight loss, (**c**) body weight gain

equilibrium. This model requires the existence of sensor(s) that monitor the fluctuations and activate appropriate mechanisms when suprathreshold deviations occur. Cannon's (1929) concept of homeostasis was extended to the field of feeding behavior to explain maintenance of a stable body weight. Imbalances in energy intake and expenditure result in changes in body weight. One instantiation of homeostasis uses a set point concept, a reference against which the current state of the organism is compared (Bolles 1980; Carpenter 2004). In this model, deviations from a set point (in the hypothetical case of body weight this might be mediated via a marker of body fat mass) would trigger an error which activates the system to correct the deviation (Fig. 1).

The increase in the incidence of obesity in humans over the last several decades provides evidence that any set point mechanism for body mass, or a derivative, is weak: internal signaling mechanisms are not strong enough to control feeding behavior in the face of an abundance of relatively palatable food. In addition to palatability, it is evident that environmental factors, in particular availability and cost, have to be incorporated in order to truly understand how the brain organizes food acquisition and maintenance of adequate energy balance. Consistent with this view, a multifactorial analysis of human feeding (De Castro 2010) posited both compensated and uncompensated factors, each of which could be ascribed weighting factors in terms of their actual effect on food intake. The compensated factors act in a way predicted by homeostasis, and uncompensated factors do not.

Foraging, Cost, and Convenience

In the natural environment, animals including humans forage for food. Optimal foraging theory posits that, for a given food environment, animals will maximize their gross energy gain which is the difference between the metabolic energy yield of

the food and the cost of obtaining it (Schoener 1971). Much evidence supports this general proposition, although there will be exceptions such as when a particular constituent (e.g., protein) may be more important than actual energy yield per se (DiBattista 1991). In animals, cost is measured in terms of physical exertion and time. For humans, we must add token currency (money). The primordial neurobiological algebra that maximizes gross energy gain is exploited by businesses in the form of convenience foods and large packages or portions, which are equivalent to the ecologically improbable condition of abundant food or "constant-glut" (McNamara et al. 2015).

In the animal laboratory setting, most of these variables can be controlled. Food availability and/or demand can be experimentally regulated in quality (e.g., nutritional value, energy density, or palatability), quantity (e.g., ration amount, duration of access), or both. Food may either be delivered at no cost to the animal (by far the most common procedure, although of questionable ecological significance) or the animal is required to forage in a controlled fashion. Foraging cost may be imposed by requiring repeated behavioral (operant) responses or overcoming an obstacle. Further, cost may be imposed either at the level of access in which performing the required task results in free access to a relatively large amount of food, or consumption in which each unit or morsel of food has a task requirement, or both. To simplify foraging studies in the laboratory, most have used a homogeneous nutritionally complete food (e.g., grain-based chow pellets), and this will be assumed in the following sections unless explicitly noted.

Collier and his colleagues were the first to show, in rats, that access (or procurement) cost has a markedly different effect on food demand than consummatory (or unit) cost (Collier 2005; Collier et al. 1972). In particular, they found that access cost altered the episodic organization of food intake but not the amount eaten per day. As access cost increased, rats initiated fewer sustained eating episodes or meals but with a compensatory increase in meal size. In contrast, consummatory cost altered amount eaten per day but not the pattern. As unit cost increased, rats ate less per day. This result generates an economic demand curve, in which consumption of a commodity decreases as its unit price increases. The curvature of the function is called elasticity. Essential commodities like food are expected to have relatively flat or inelastic functions compared with nonessential items (Hursh 1984; Hursh and Silberberg 2008).

Cost-Based Anorexia (CBA): Continuous Access

A result from our laboratory using young adult Sprague-Dawley rats pressing a lever for 45-mg pellets and C57BL/6 mice nose poking for 20-mg pellets in 23-h daily sessions is shown in Fig. 2 (compiled from Rowland et al. 2015). Each fixed unit price (FUP, in behavioral responses per pellet) was enforced for four consecutive days after which the price was increased. The left panel of Fig. 2 shows mean daily food intakes, expressed as % of intake at the lowest cost (actual intakes: 530 pellets or 23.8 g in rats and 200 pellets or 4.0 g in mice). The right panel of Fig. 2 shows the

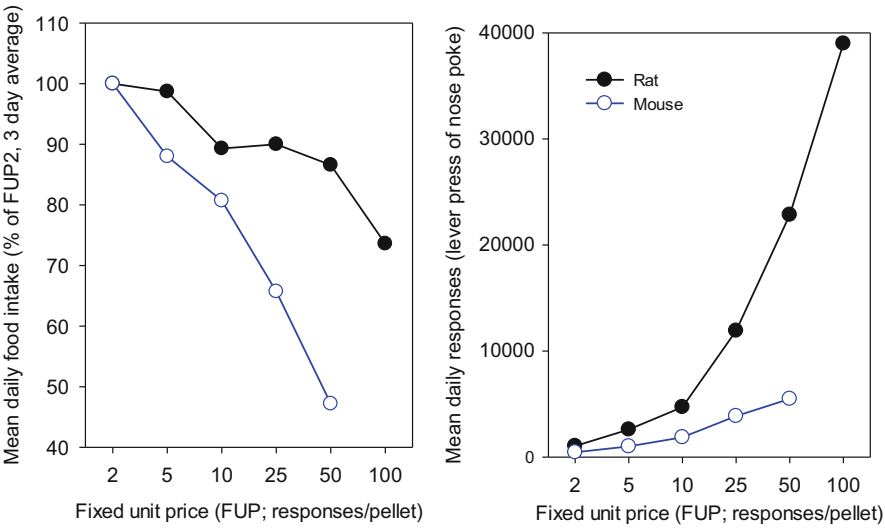

Fig. 2 Relative daily food intake or demand, and associated behavioral responses, in rats and mice as a function of the price of each pellet of food. *Left panel* shows demand curves as a function of unit price (*FUP*; categorical scale) for food pellets in groups of 6–8 male Sprague-Dawley rats (*circles*) and C57BL/6 mice (*triangles*) with 23 h per day availability to earn food. Daily intakes of 45-mg pellets (rats) or 20-mg pellets (mice) are 3-day averages shown as % of the intake at FUP2 for that group. *Right panel* shows the corresponding mean number of lever presses (rats) or nose pokes (mice) per 23-h session

corresponding mean numbers of responses emitted per day. Rats and mice generated classic demand curves, but it is evident that the elasticity in mice was higher than in rats. In fact, almost half the mice did not complete FUP 50 due to weight loss >15% (our criterion for removal from study) whereas all rats completed FUP 50 and the next highest price step (FUP 100). Under these conditions, the number of food pellets earned per day by rats is about twice the number in mice and this led us to predict that mice should show less elasticity than rats. The observed result thus was contrary to our prediction. The associated number of responses (not shown) further illustrated this unexpected species difference: the maximum daily response output by mice was only ~30% of that in rats. In another study (Atalayer and Rowland 2009), we found that lever pressing and nose poking produced identical demand curves in mice, so the difference in response type cannot explain the large species difference in Fig. 2.

Other data (Bauman 1991) show that the maximum lever press rates of rats are of the order of two per second and, at that rate, they would have taken >5 h to emit the ~40,000 daily responses at FUP 100. Given that most of the eating occurred during the artificial 12-h night, and that actual sustained rates will likely be below the maximum, rats at FUP 100 may have spent most of the night responding. To address this question, the time of food delivery was recorded throughout the 23-h sessions. At low FUP, rats acquired food in relatively discrete episodes or meals,

most of which were nocturnal, and comparable to rat meal patterns previously described without cost imposed (Le Magnen and Tallon 1966). As FUP increased, the rate of eating slowed because of the interpellet response requirement, so meals took longer and much of the night. However, meals were still evident although the number taken per day decreased as FUP increased while their average size remained constant (Rowland et al. 2015).

The corresponding calculation in mice (~6,000 responses) would predict ~2-h per day spent in responding, a relatively small fraction of the nychthemeron. Examination of response and pellet distributions throughout the 23-h session revealed a pattern completely different from rats. In particular, the first 6–8 h of the night was characterized by low rates of eating but without apparent segmentation into meals – the mice appeared to be grazing. This is consistent with findings by others (Goulding et al. 2008) that C57BL/6 mice show almost continuous locomotor activity interrupted by occasional eating during this time, a result we have subsequently corroborated in our test protocol using photobeams to measure locomotor activity. During the latter half of the night, activity was more episodic (probably with sleep episodes) and eating was correspondingly more episodic. During the daytime, both activity and amount eaten were low and episodic. There was relatively little change in this basic pattern as FUP increased. Thus, the reason that mice emit fewer responses than rats seems to be due, in part, to high levels of locomotor activity that may preclude sustained, multipellet bouts of eating. Consistent with this reasoning of a direct relationship between level of spontaneous locomotion and meal structure, Goulding et al. (2008) showed that an obese strain of mice with low spontaneous locomotor activity was observed to engage in meal-like episodes throughout the night. We (Rowland et al. 2015) have identified a somewhat more episodic pattern of feeding in an outbred strain (CD-1) of mice compared with C57BL/6, but have not compared spontaneous locomotion in these two or other strains (Kelly et al. 2011; Loos et al. 2014).

In part to amplify the time limitation on responding, as well as to emulate typical human eating patterns, we have conducted (see next section) parallel studies in which food can only be earned at discrete times or food opportunities (FOs). These are functionally equivalent to meals.

Cost-Based Anorexia (CBA): Feeding Opportunity Protocol

In our initial report of feeding opportunity (FO) protocols (Atalayer and Rowland 2012), mice were given either 4, 8, or 16 FOs per 23-h session, mostly at night, and with reciprocal duration of each FO (i.e., 40, 20, or 10 min) so that the total availability of food was always 160 min. In the 16 and 8 FO protocols, we found that mice did not eat at several FOs. All of our subsequent work in both rats and mice thus has used 4 FOs, each of 40 min duration. In both rats (unpublished data) and mice (Atalayer and Rowland 2012; Rowland et al. 2016), daily food intakes were 80–90% of those in the 23-h protocols at each corresponding FUP, so the shapes of

the daily food demand curves in 4 FO protocols are similar to those for continuous access shown in Fig. 2.

In one study, using Sprague-Dawley rats, the FOs started at 1800, 2200, 0200, and 0600 (which we will refer to ordinally as FO 1, 2, 3, and 4), with lights off 1800–0600-h. At each FUP, intake at FO 2 (midnight) was slightly greater than at FOs 1 (start night) or 3 (late night). Intake at FO 4 (dawn) was much lower even at FUP 100 when total daily intake was only ~50% of ad lib levels. Thus, at these higher FUPs, even if responses during FOs 1–3 were limited by time or fatigue (see below) the animals still had the opportunity of making up at least some of the food deficit and so obtund their rapid weight loss by eating more during FO 4, but they did not.

Assuming a ceiling response rate of ~two responses per second, then compressing the responses into 40-min episodes has the potential to limit intake at high FUP. Thus, mean numbers of pellets earned in successive 10-min segments of each FO were examined. Those for the largest meal (FO 2) are shown in Fig. 3 (left panel) for low and high FUPs (5 and 50); data for FOs 1 and 3 (not shown) were essentially identical. At low FUP, intake was high for the first ~10 min of the 40 min FO and declined monotonically thereafter, with some individuals stopping completely. At higher FUP, a high rate of responding (average 2.3 responses/sec at FUP 50) was maintained throughout the 40 min (Fig. 3). Thus, neither fatigue nor satiation from

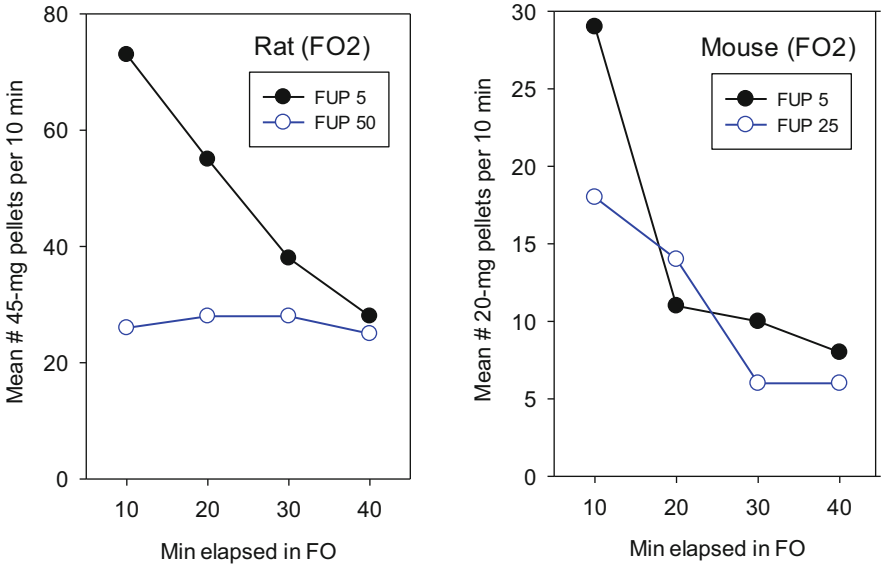

Fig. 3 Pellets earned in successive 10-min segments of a 40-min feeding opportunity (*FO*). By rats and mice. Left panel shows the mean number of 45-mg pellets earned by male rats (n=6) in successive 10-min segments of 40-min feeding opportunity #2 (FO 2) in the 4 FO protocol for the last 3 days at FUP 5 and FUP 50. Right panel shows mean number of 20-mg pellets earned by male mice (n=6) in successive 10-min segments during FO 2 of a similar protocol at FUP 5 and FUP 25

food ingested at the start of the FO were evident within the 40 min. A similarly flat function was observed in these animals at twice the FUP (100) when the ceiling response rate allowed only about half the intake in each 10-min segment. Even at these high FUPs at which intakes at FOs 1–3 were clearly limited by ceiling response rates, FO 4 (dawn) remained relatively small despite body weight loss approaching 10%.

In the 4 FO protocol, as with continuous food access, some aspects of mouse behavior differed from rats (Fig. 3, right panel). In general, as in rats, the highest intakes in mice were at FOs 1–3, and much smaller at FO 4, although modulating factors to this pattern will be discussed later. At low FUP (5), the temporal evolution of responding and intake at FO 2 (usually the largest) were similar to those in rats, with a marked reduction in pellets earned in successive 10-min segments (Fig. 3, right panel; full analysis in Rowland et al. 2016). In contrast, the maximum number of pellets obtained in the first 10 min by mice was only ~35% of the corresponding number in rats at corresponding FUPs, and the response rate was correspondingly lower. At FUP 25 (the highest ratio analyzed because, as noted before, almost half the mice did not complete higher FUPs due to excessive body weight loss) the temporal evolution of responding and intake was similar to that at lower FUP (Fig. 3, right panel). Unlike rats at higher FUP (Fig. 3, left panel), mice did not maintain their higher initial rate of responding throughout the 40-min FO, exhibiting apparent satiation even when intake early in the FO was relatively low due to the cost or response requirement. Had the mice maintained their initial rate throughout most of the 40 min then intake at FUP 25 would have been at least as high as at FUP 5 and instead showed a decline which, if the study had been continued longer, would have resulted in a self-destructive level of inanition.

Compared with rats, mice have a smaller body mass and higher surface-to-volume ratio. Thus, a given % change in food intake has a much larger effect on body weight (as % of initial) of mice than rats. By the end of the FUP 100 segment in the 4 FO protocol, rats had lost an average of 22 g body weight (~7% of initial weights) with most of that loss during FUP 50 and 100 segments. In mice, weight loss was ~8% of initial at the end of FUP 25, but 50% of animals did not complete FUP 50 due to weight loss >15% (Rowland et al. 2016). Importantly, during rapid weight loss at FUP 25 or 50, intake did not change across successive days. Individuals that ate the least day after day were the first to be removed due to weight loss. For completeness, we should note that while sex differences are sometimes evident (Minervini et al. 2015; Rowland et al. 2016), they have been omitted to simplify the foregoing section since they do not alter the main conclusions.

These data leave us with two unexpected observations in mice. First, they show satiation within a FO even when total intake is insufficient to maintain body weight. Second, within higher FUP segments, food intake does not increase while body weight decreases across days in either 23-h or 4 FO protocols. It is as if the combination of small initial amounts of food intake with the prospect of working moderately hard to obtain more food triggered satiation-like behavior even in the face of weight loss that would have proven terminal had it been allowed to go that far. We believe that this phenomenon of CBA has several of the characteristics of the

restrictive form of AN, namely, low or slow intake and premature but unforced satiation in the face of continued weight loss (Bergh et al. 2013). This is substantially different from the cause of CBA in rats which is due mainly to ceiling response rates. However, in both rats and mice, the lower intake at FO 4 cannot be so easily understood. One possibility is that of a strong circadian modulation of eating.

Effect of Zeitgeber and Differential Cost on Food Demand in Mice

In the foregoing section using the 4 FO protocol, we described how intake at FO 4, which occurred at or near the time of lights on ("dawn"), was less than half the intake at the first three (nocturnal) FOs (Atalayer and Rowland 2012; Rowland et al. 2016). It is possible that light and/or an endogenous circadian oscillator has an important inhibitory effect at FO 4.

We first tested this hypothesis by examining the effect of different timing of the FOs relative to the Zeitgeber (Minaya et al. 2016). This design used 4-h phase advanced and phase delayed groups and confirmed that light and/or an endogenous oscillator did appear to have an inhibitory effect. The most robust finding was that the characteristic variation in FO size, with intake at the smallest FO only ~30% of that at the largest FO, remained in all groups and at each of FUPs 5, 10, and 25.

It is thus possible that light and/or phase of an endogenous oscillator effectively increases the cost of food acquisition, and this is reflected in one or more small FOs; in a natural environment, such a strategy may improve inclusive fitness by foraging mainly at time(s) of low predation risk. We reasoned that by altering the price of food at different times, it may be possible to gain a better understanding of the factors causing variation in FO size and, indirectly, in satiation mechanism. We devised an economic choice protocol where animals were given access to constant costly (CC) and intermittent inexpensive (II) food to investigate whether the timing of cheap food relative to the Zeitgeber affects this decision (Minaya et al. 2016). Access to II 45-mg food pellets (FUP 5) was restricted to four 15 min opportunities that paralleled the timing of 4 FOs described above. In subsequent segments of the study using the same mice, the timing of access to II food was either advanced or delayed by 4 h such that the first FO started either 4 h prior to or 4 h after lights out. CC 45-mg food pellets (FUP 100) were available at all other times in the 23-h session, similar to the continuous access protocols discussed previously (note the larger pellet size and higher FUP).

In spite of the 20-fold difference in FUP between CC and II food, mice consumed substantial amounts from each source on a daily basis (Table 1). In the first segment, when the first 3 FOs were during the night and FO 4 was early in the day, as described before, the intake at the first 3 FOs was high and the 4th FO was very small (<20% the size). In the second segment, the first FO (in the late day) was completely omitted by all the mice, and the three nocturnal FOs were relatively large. In the third segment, the last two FOs (in the first half of the day) were very small, while the first two (at night) were large. In this latter condition, with two small II FOs, the relative amount of food taken from the CC lever was some 30% higher than in the other two

Table 1 Daily food intakes of mice from continuous costly (*CC*) and intermittent inexpensive (*II*) sources as the timing of episodes changed relative to Zeitgeber

	Total intake from CC	Total intake from II	Intake during 15 min II episodes starting at					
			D9	N1	N5	N9	D1	D5
Part 1	1.6	1.8	x	0.5	0.6	0.6	0.1	x
Part 2	1.8	1.7	0	0.4	0.6	0.6	x	x
Part 3	2.3	1.3	x	x	0.6	0.6	0.1	0

CC was fixed unit price (*FUP*) 100; II was FUP 5. Mean intakes have been rounded to the nearest 0.1 g and are for IIs starting in the ninth hour of daylight (*D9*), the first hour of the night (*N1*), the fifth hour of the night (*N5*), etc. x indicates the absence of a programmed II at that time. 0 indicates that none of the 10 mice ate in that interval (Data compiled from Table 2 and Fig. 4 of Minaya et al. (2016))

segments. Mice thus chose to expend significantly more effort to obtain expensive food and thereby maintain total intake, as more of the cheap food opportunities shifted to daytime. Further, almost all of the CC food obtained was nocturnal, and particularly during the first half of the night (Minaya et al. 2016). These data suggest that light exerts a more potent suppressive effect than price on food intake in mice. In a similar study in rats (unpublished data) we again found a difference from mice: rats ate almost all of their daily food during II opportunities.

The above study in mice did not examine the effect of changes in either the ratio of CC to II pricing (20:1) or sequential increases in both (e.g., to FUP 10 and 200). Instead, we examined the effect of diet palatability that would be expected to affect its intrinsic reward value relative to cost.

Effect of Differential Pricing on Food Demand for a Preferred Diet

This experiment in C57BL/6 mice used continuous 23-h access and a two lever arrangement. Presses on one lever delivered a highly palatable, preferred diet (either 55% dextrose or 35% fat in the form of 45-mg pellets) while presses on the other lever delivered a less preferred, grain-based diet, both delivered to a common, centrally located trough. Initially, both diets were available at the same FUP. Subsequently, FUP for the preferred diet was doubled every 4 days while the FUP of the grain-based diet was maintained constant and low.

As expected, when both foods were offered at the same low cost, intake of the more palatable diet was much greater than that of the bland grain-based food (Fig. 4). As the relative cost of the more palatable diet increased, animals took progressively less while intake of the cheaper grain-based alternative increased. The indifference point at which 50% of daily intake was from each diet was when the relative cost was, on average, five-to tenfold higher for these particular palatable diets. Both initial preference for and the indifference ratio were slightly higher for the dextrose compared with the fat diet. There was considerable individual variability in the indifference point, with some animals reaching it with only a doubling of relative price while

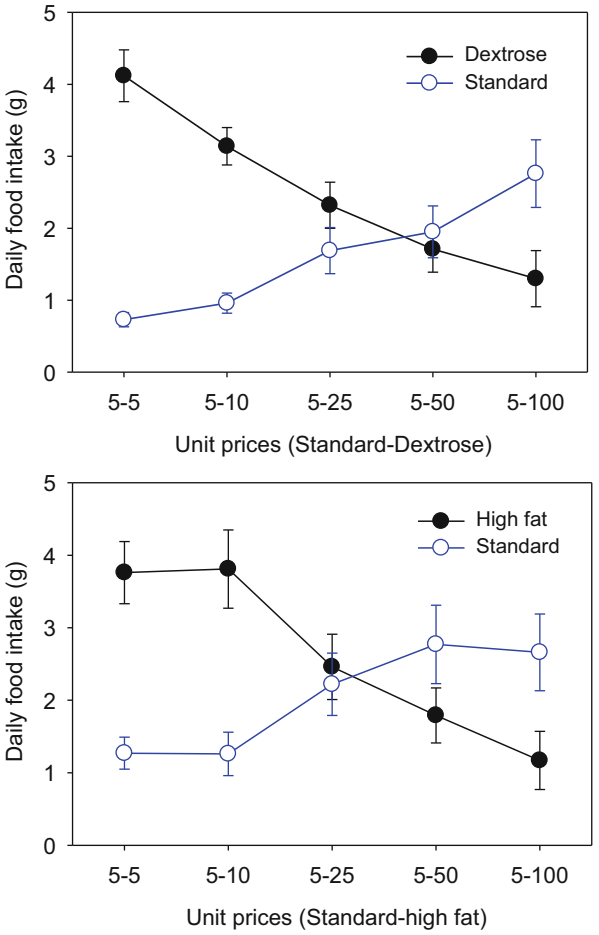

Fig. 4 Daily food intakes of mice earning a palatable diet available concurrently with standard grain-based pellets at various relative prices. Shown are M+SE for 12 C57Bl/6 mice. The *top panel* shows a choice between 55% dextrose pellets and standard diet, while the *bottom panel* shows a choice between 35% fat pellets and standard diet (Minaya and Rowland unpublished data)

others maintained preference for the palatable diet even at the highest cost. This might reflect individual differences in relative palatability, in cost sensitivity, or a combination. Although not emphasized previously, we routinely observe individual differences in demand elasticity in both 23-h and 4 FO protocols in mice using a single standard food (Rowland et al. 2015). These individual differences, as well as strain differences, remain an aspect in need of systematic investigation especially as they may relate to vulnerability of humans to AN and other eating disorders.

CBA Compared with ABA

It is relevant to compare, briefly, the current CBA protocol with the much-used model of activity-based anorexia (ABA). ABA combines severely restricted time of access to food (typically, a single 2-h access per day but without cost) with access to

running wheels which uniquely are reinforcing and increase energy expenditure (Klenotich and Dulawa 2012; Koh et al. 2000). Rats or mice without wheels eat sufficient food on this schedule to survive, albeit at reduced body weight. Restricted animals with additional wheel access do not increase their intake above this level, and are constantly in negative energy balance to the point of death, or a more humane end-point criterion of weight loss (Gutierrez 2013; Méquinion et al. 2015). The protocol has been criticized because fatal levels of anorexia and weight loss occur only in a narrow range of conditions (Boakes and Dwyer 1997; Koh et al. 2000; Wu et al. 2014), and it certainly does not emulate human AN in which food is easily available but is refused. Further, compared with our 4 FO protocol, the restriction of food to a single 2-h epoch is physiologically more challenging. If we suppose that a daily intake of 50% ad lib is a minimum compatible with survival, mice in the 4 FO protocol would need to eat ~0.5 g at each meal, which is clearly feasible (Fig 2, Table 1). In contrast, in the ABA protocol even no-wheel mice would need to eat 2 g within 2 h, which may be close to the stomach capacity (~1 cc) and/or emptying rate (half-life for a resin-based gavage = 74 min; Schwarz et al. 2002). CBA protocols thus have some degree of apparent and ultimately maladaptive choice by mice that is not obviously constrained by gastric capacity. Instead, hormonal changes that occur during the earliest stages of the FO are a candidate mechanism by which this early satiation occurs in consummatory cost protocols in mice. Identification of these or other (e.g., genetic) factors that exaggerate CBA may lead to new insights in the treatment of AN.

Policies and Protocols

Use of animals described in this Chapter was approved by the University of Florida Animal Care and Use Committee and is consistent with guidelines of the National Research Council. Animals were singly housed in standard operant behavior test chambers in daily sessions lasting 23 h at ambient temperature 23 °C and a 12:12 light cycle. They were removed to holding cages for 1 h per day while the test chambers were cleaned and serviced. The animals earned all of their food during sessions (closed economy) using the specific cost protocols mentioned in the text.

Dictionary of Terms

- **Anorexia nervosa** – A psychiatric disorder of eating in which patients excessively restrict food intake and lose excessive body weight, yet believe themselves to be heavier (distorted body image)
- **C57Bl/6** – A widely used pigmented (black coat) strain of mice; particularly susceptible to obesity when fed high fat diet and is also a background strain for most transgenic manipulations.

- **Consummatory cost** – A cost (money, time, effort) that applies to each unit of a commodity acquired; in the case of food this may refer to small units that are fractions of a meal or serving.
- **Cost-based anorexia** – Decrease in daily food intake (of animals) as consummatory cost increases, with concomitant body weight loss to dangerously low levels.
- **Demand** – In economic terms, as used herein, demand refers to the quantity of a commodity consumed in a discrete unit of time – such as number of food pellets per day.
- **Homeostasis** – For a given physiological variable, the ensemble of mechanisms within the body of a living organism which actively regulate that variable such that it remains relatively constant.
- **Nose poke response** – Rodents move their snout a small distance (<1 cm) into and out of a recess containing a photobeam or other movement sensor, so generating a (computer-acquired) record of a behavioral response.
- **Operant behavior** – Behavioral responses (either nose pokes or lever presses) on a device that cause the delivery of a reinforcer (food pellet) according to a programmed requirement.
- **Palatability** – The pleasure or hedonic reward experienced when consuming food (or fluid); unlike taste or flavor, palatability changes with the physiological state of an organism and in particular decreases during the course of a meal of that particular commodity.
- **Satiation** – The process(es) that cause interaction with a commodity to spontaneously wane or cease, for example, cessation of eating after a certain amount has been consumed.
- **Sprague-Dawley** – A widely used albino outbred strain of rat.
- **Zeitgeber** – Regular environmental cue(s), most usually the change in illumination which occurs every 24 h, that cause the internal circadian clock(s) to reset or entrain to the external world.

Summary Points

- We introduce cost-based anorexia (CBA), a new animal model of anorexia nervosa.
- Rats and mice decrease daily food intake as the cost (number of behavioral responses) per food unit increases.
- Food intake decreases despite ongoing and significant body weight loss.
- This phenomenon is exaggerated when food is available intermittently, in food opportunities (FOs) which are functionally similar to meals, compared with 23-h (continuous) availability.
- In rats, the decrease of intake occurs when responding occurs at maximum rates and occupies a substantial fraction (23-h) or almost all (FOs) of the time available.
- In mice, the decrease of food intake is not associated with sustained high rates of responding.

- In the FO protocol, mice slow eating after the first few minutes. This phenomenon appears similar to the disinterest in food shown by anorexia nervosa patients.
- Food intake across FOs show some dependence on circadian oscillators.
- In a choice situation, palatable foods are preferred by mice until their average cost is five-to tenfold higher than that of bland food, but there is substantial individual variability in this ratio.
- Future experiments might fruitfully examine genetic vulnerability to CBA and/or effects of hormones secreted during the early part of a FO as potential mechanisms by which ambient cost exerts such a profound inhibitory effect on food intake.

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Quality of Life in Adult Anorexia Nervosa

31

Enrica Marzola and Giovanni Abbate-Daga

Contents

Abstract

Anorexia nervosa (AN) is a severe mental disorder characterized by high mortality with typical onset in female adolescents. Quality of life (QoL) is strongly impaired in AN and potentially underestimated because of the ego-syntonicity of this illness which makes sufferers not fully aware of their symptoms. Taken together, eating disorders (including also bulimia nervosa and binge eating disorder) are characterized by poor QoL when compared to the general population. However, data are mixed as to whether QoL is most impaired in AN or in other eating disorders. With respect to QoL in AN subtypes, the binge-purging one resulted to have poorer QoL levels than the restricting one. Those with AN reported levels of QoL comparable to those of schizophrenic and severely

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depressed patients. It is of importance that QoL is taken into account in treatments since outpatient and inpatient interventions were shown to be able to improve it effectively.

Keywords
Anorexia nervosa · Eating disorders · Quality of life · Health-related quality of life · Hospitalizations

List of Abbreviations

AN	Anorexia nervosa
BED	Binge eating disorder
BMI	Body Mass Index
BN	Bulimia nervosa
BP-AN	Binge/purging-type AN
DSM	Diagnostic and Statistical manual of Mental disorders
EDs	Eating disorders
HRQoL	Health-related quality of life
QoL	Quality of life
R-AN	Restricting-type AN

Introduction

This chapter will describe the role of quality of life in anorexia nervosa, a serious mental illness characterized by high mortality. At the beginning of the chapter, anorexia nervosa will be in-depth described, then the methodological issues related to assessing quality of life will be pointed out and finally clinical and research implications of quality of life in anorexia nervosa will be discussed. The main findings from the field will be outlined comparing anorexia nervosa with other both psychiatric and physical disorders.

Anorexia Nervosa

According to the Diagnostic and Statistical manual of Mental disorders (DSM-5; APA 2013), Eating Disorders (EDs) are severe mental illnesses characterized by aberrant eating behaviors ranging from extreme dieting to overeating coupled with weight and body shape concerns. Additionally, harmful compensatory behaviors can occur. The official position of the Academy for Eating Disorders, a global and renowned association of researchers and clinicians with ED expertise, highlights how these disorders are as severe as other major psychiatric disorders (Klump et al. 2009). EDs include three major diagnostic categories: anorexia nervosa (AN), bulimia nervosa (BN), and binge eating disorder (BED). This chapter will focus on AN, an ED characterized by an ego-syntonic resistance to eating coupled with marked weight loss due to a relentless pursuit of thinness. Women are mostly

Table 1 Diagnostic criteria of anorexia nervosa

Diagnostic criteria of anorexia nervosa:
1. Restrictive eating and low weight (Body Mass Index <19)
2. Fear of weight gain
3. Body image distortion
Diagnostic subtypes:
• Restricting-AN: affected individuals lose weight only dieting and over-exercising
• Binge purging-AN: affected individuals periodically engage in binge eating and purging behaviors like vomiting or using diuretics and laxatives, although maintaining restrictive eating and low weight

affected and the onset of AN typically occurs in adolescence; however, age at onset has been reported to be decreasing (Favaro et al. 2009) and onsets before puberty may occur as well.

AN sufferers tend to be obsessed about food and becoming "fat" in spite of severe caloric reductions (see Table 1). Even if strongly underweight those with AN perceive themselves as overweight because of body image distortions. The body image disturbance is the key psychopathological factor and it can be present with different severity levels. In fact, some patients are aware of their emaciation, but refuse to lose weight because their self-esteem is strongly related to their body appearance while some other individuals perceive themselves as overweight or flabby to the point of resembling full-blown delusions (Konstantakopoulos et al. 2012). Two main subtypes of AN have been described: restricting-type AN (R-AN) and binge/purging-type AN (BP-AN). The first group of patients lose weight only by dieting and over-exercising. The second group periodically engage in binge eating and purging behaviors like vomiting or using diuretics and laxatives, although maintaining restrictive eating and low weight (APA 2013). Different degrees of clinical severity can be described (APA 2013) according to individuals' Body Mass Index (BMI, kg/m^2), ranging from mild (BMI > 17) to extreme (BMI < 15). Although often present, amenorrhea is no longer a criterion needed to make AN diagnosis since the lack of menstrual cycle does not correlate with psychopathological conditions (Abbate-Daga et al. 2011a).

Pathogenesis of AN is multifactorial and largely unknown, involving biological and psychosocial underpinnings. In this regard, it has been called into question the cultural pressure to be thin (Hoek et al. 2005); nevertheless, only a minority of individuals who diet end up developing AN. Therefore, other causative factors should be considered and a relevant twin study (Bulik et al. 2006) demonstrated the role of genetic vulnerability. Also psychological factors like temperament traits, perfectionism, and marked sensitivity to criticism have been identified as relevant in AN pathogenesis (Jacobi et al. 2004). Finally, as for other mental disorders, neurodevelopmental trajectories have a role in AN as well; in particular perinatal traumas (Favaro et al. 2006), neuropsychological inefficiencies (Abbate-Daga et al. 2015), and some peculiarities in brain functioning (Kaye et al. 2013). As showed by animal models, malnutrition plays a key role in maintaining and exacerbating eating symptomatology thus generating a vicious cycle of restricting and over-exercising (Gutierrez 2013).

Hence, AN is a complex illness which is difficult to fully understand; it often requires a continuum of therapeutic interventions including outpatient, day patient, and inpatient services (NICE 2004; Klump et al. 2009). Treatments imply a strong involvement of parents and families, in particular with adolescents (Murray and Le Grange 2014). Mid- and long-term sequelae are common with sufferers being impaired with respect to physical problems and social functioning. As stated at the beginning of this chapter, AN is an ego-syntonic disorder so patients' motivation is frequently poor (Abbate-Daga et al. 2013). As a consequence, lengthy and costly hospitalizations for medical care and weight restoration are often necessary to minimize the risk of complications and death (Marzola et al. 2013). Less than half of affected individuals recover and in approximately 20% of cases a chronic course takes place (Steinhausen 2002). Currently, no "gold standard" treatments exist for AN (NICE, 2004); as a result, relapse is quite common (30–50%; Guarda 2008) and mortality is high also because of heightened suicidality (Arcelus et al. 2011). In fact, the standardized mortality ratio of AN is 5.9 which is the highest mortality of any psychosomatic disorder (Erdur et al. 2012).

Why to Assess Quality of Life in Anorexia Nervosa

The World Health Organization defines quality of life (QoL) as "individuals' perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards, and concerns. It is a broad ranging concept affected in a complex way by the person's physical health, psychological state, level of independence, social relationships, personal beliefs, and their relationship to salient features of their environment" (The WHOQOL Group 1993). QoL describes a broad multidimensional construct and sometimes in medical literature it is preferred to refer to a discrete component of it, namely the health-related QoL (HRQoL). The latter is mostly subjective, multifactorial, and variable in time and represents "the functional effect of an illness and its consequent therapy upon a patient, as perceived by the patient" (Miller 2002).

QoL is key in both physical and mental health fields and it has been highlighted the need to address this issue in treatment (Jenkins et al. 2011; Sy et al. 2013). In fact, over the last two decades, the assessment of QoL has become increasingly relevant for EDs in general and AN in particular. It is noteworthy that the DSM-5 diagnostic system (APA 2013) requires individuals' functional impairment to make the diagnosis of a mental disorder. Furthermore, policy-makers became interested in this parameter when evaluating cost-effectiveness (Stuhldreher et al. 2012) and novel therapeutic interventions for those disorders that tend to have a protracted course like AN (Wonderlich et al. 2012). QoL is also an important measure of treatment effectiveness (Sy et al. 2013).

The assessment of QoL in AN is of particular interest for those patients showing malnutrition-related sequelae that affect patients not only when acutely losing weight but also during recovery; this is particularly true for those with extreme AN (i.e., BMI < 15). Consequences of malnutrition often have as a result social isolation and emotional and social avoidance (Wildes et al. 2010), thus in turn

Table 2 Usefulness of assessing quality of life in anorexia nervosa

QoL in AN is useful to evaluate:
1. Organic malnutrition-related sequelae
2. Psychological consequences of both malnutrition and illness
3. Social consequences of both malnutrition and illness
4. Clinical outcome
5. Cost-effectiveness
6. The comparison of AN with other physical and mental illnesses

impacting on QoL. Sufferers with a long duration of illness (e.g., 15–20 years) could report several sequelae like fractures, movement impairments, loss of autonomy, inability to go to work, and loneliness.

Moreover, assessing QoL allows to compare data across different diagnoses in both psychiatric and organic/medical conditions. Still, the assessment of QoL in EDs can help predict clinical outcomes in treated individuals and their risk of relapse and recurrence (Spitzer et al. 1995). Finally, from a research standpoint, QoL assessments have been increasingly considered as patient-oriented outcome measures in addition to the traditional ones (e.g., weight restoration, absence of binge-purging episodes; Bamford et al. 2015). See Table 2 for a summary of the relevance of assessing QoL in AN.

How to Assess Quality of Life in Anorexia Nervosa

The debate is currently open on how to precisely measure QoL in AN. A number of tools have been used over the past years with research studies using either generic or ED-specific instruments.

Generic instruments are represented by tools such as the EuroQoL 5D (EQ-5D VAS; EuroQoL Group 1990) or the Medical Outcome Short Form Health Survey (SF-36; Ware and Sherbourne 1992) which have been largely used in biomedical research in order to measure QoL. Such instruments have been used across different medical settings ranging from surgery (Carradice et al. 2012) to psychiatry (Maratos et al. 2012). These instruments are useful for several reasons: they are well known and broadly used, can be used by non-ED trained people, are common in literature, and most importantly make data on AN comparable with other medical and psychiatric fields. The latter aspect is of importance because it allows to compare QoL with other invalidating disorders of both psychiatric (e.g., schizophrenia and bipolar disorders) and medical pertinence (e.g., diabetes and coronary disease). However, some cons in using such assessments should be acknowledged as well. For example, anxiety and affective disorders are particularly common in AN (Swinbourne et al. 2012; Abbate-Daga et al. 2011b); since generic measures of QoL usually include questions evaluating general psychiatric symptoms, it is sometimes difficult to disentangle to what extent poor quality of life is due to the ED symptomatology or to the broader psychopathological condition.

Table 3 Main instruments used to assess quality of life in anorexia nervosa.

(a) **Generic**
• EuroQoL 5D (EQ-5D VAS; EuroQoL Group 1990)
• Medical Outcome Short Form Health Survey (SF-36; Ware and Sherbourne 1992)
(b) **Eating disorder specific**
• Eating Disorders Quality of Life instrument (Engel et al. 2006)
• Health-related Quality of Life for eating disorders questionnaire (Las Hayas et al. 2006)
• Eating Disorders Quality of Life Scale (Adair et al. 2007)
• Quality of Life for Eating Disorders measure (Abraham et al. 2006)
• Clinical Impairment Assessment questionnaire (Bohn and Fairburn 2008)

Since research interest about QoL in the ED field increased over the past years, disorder-specific measures have been developed as well. Such instruments have been pioneered in order to capture more appropriately AN-specific characteristics such as partial awareness of illness and poor motivation to change (Ackard et al. 2014; Mond et al. 2005; Muñoz et al. 2009). ED-specific instruments to assess QoL include: the Eating Disorders Quality of Life instrument (Engel et al. 2006), a 25-item scale with four subscales (psychological, physical/cognitive, work/school, and financial) and a total score, the Health-related Quality of Life for Eating Disorders questionnaire (Las Hayas et al. 2006), a 50-item instrument with 8 subscales (symptoms, restrict behaviors, body image, mental health, emotional role, physical role, personality traits, social relations), the Eating Disorders Quality of Life Scale (Adair et al. 2007), a 40-item scale with 12 subscales (cognitive, education/vocation, family and close relationships, relationships with others, future outlook, appearance, leisure, psychological, emotional, values and beliefs, physical, and eating), the Quality of Life for Eating Disorders measure (Abraham et al. 2006), a 21-item measure assessing behavior, eating disorder feelings, psychological feelings, effects on daily life, effects on acute medical status and body weight, and the Clinical Impairment Assessment questionnaire (Bohn and Fairburn 2008), a 16-item questionnaire generating a total score and three subscale scores (personal impairment, cognitive impairment, and social impairment).

Albeit precise, the disadvantage is that such instruments make it difficult to compare QoL results across physic or mental conditions (Ackard et al. 2014). The main instruments to assess quality of life in anorexia nervosa are summarized in Table 3.

Quality of Life in Anorexia Nervosa

Scientific literature has shed light on the fact that individuals affected by EDs show poorer QoL than healthy controls and other psychiatric patients (de la Rie et al. 2005; Keilen et al. 1994; Mond et al. 2005; Tirico et al. 2010) as summarized by a recent meta-analysis (Winkler et al. 2014). The impairment in QoL has been found to be comparable with that of various other serious illnesses like angina and anxiety disorders (Keilen et al. 1994; Spitzer et al. 1995). QoL has been found as even

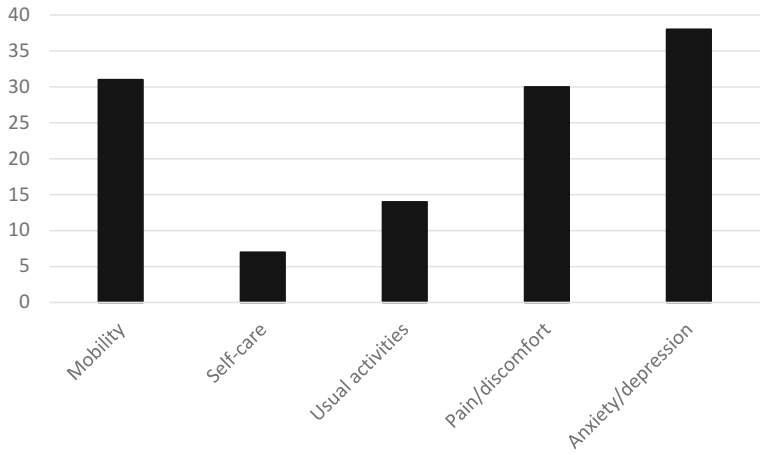

Fig. 1 Proportions of patients with anorexia nervosa reporting extreme problems in the five areas of QoL as measured by the EuroQoL 5D (Abbate-Daga et al. 2014)

more impaired in case of AN patients requiring hospitalization through the emergency department: in fact, research from our group reported scores comparable to those of patients affected by coronary disease (Goldsmith et al. 2010) but lower than that shown by psychotic and bipolar individuals (McCrone et al. 2009). In particular, patients who require an emergency hospital admission report high subjective sufferance and strong movement impairment (Abbate-Daga et al. 2014; Fig. 1). The latter finding is striking in the light of the fact that patients are usually young and starvation can be so extreme to lead to cachexia-death.

When comparing QoL across ED diagnostic subtypes, data are mixed but in spite of diagnostic differences, QoL has been consistently found to be poor mostly in case of extreme dieting (Mitchison et al. 2012) and when hospitalizations are needed (Ackard et al. 2014). Acute and chronic starvation emerges as one of the strongest factors determining poor QoL. Given such results, Hay (2003) proposed that such an impairment could be due to over−/underweight, with worse QoL for those with extremes of BMI (i.e., <20 and >30) (Hay 2003) and thus higher in those with normal weight. Several studies found no relevant differences across different ED subtypes in overall QoL (de la Rie et al. 2005; Latner et al. 2008; Winkler et al. 2014). In keeping with these findings, a recent meta-analysis confirmed the impairment of QoL across EDs, but failed to detect any differences between diagnostic groups (Winkler et al. 2014). However, other works found lower QoL in AN patients when compared to those with BN (Bamford and Sly 2010), and other reports showed higher QoL in AN (Doll et al. 2005; Mond et al. 2005; Padierna et al. 2000). Nevertheless, the latter studies used more often generic measures of QoL, thus potentially underestimating the impact of the illness on sufferers' lives (Tirico et al. 2010); additionally, increased likelihood of self-harm in this group seems to contradict such results (Doll et al. 2005) and different levels of severity of AN could have been put together in these studies further explaining such variable results. Still,

BED patients have been described as having the lowest QoL when compared to the other EDs (Baiano et al. 2014).

Physical functioning seems to be the only exception since it appeared not to be influenced by the EDs (Padierna et al. 2000; Winkler et al. 2014) although in case of emergency hospital admissions it was found to be affected as well (Abbate-Daga et al. 2014). Given the severe organic sequelae of EDs (NICE 2004) and the frequent presence of over-exercise as a compensatory behavior (Peñas-Lledó et al. 2002), this may seem a discordant result. In particular, when each component of QoL is analyzed, little impairment in physical domains is usually reported by AN patients when compared to other ED individuals (Latner et al. 2008; Tirico et al. 2010). In fact, ED patients do not report physical QoL as strongly affected (Latner et al. 2008). However, a relevant caveat should be borne in mind when analyzing research results on QoL in EDs: AN is an ego-syntonic disorder so affected individuals tend to be unaware of the burden of their symptoms and some aspects of the illness may be underestimated by sufferers. This is even more true when comparing those with AN with BN and BED sufferers since BN and BED are characterized by strongly ego-dystonic behaviors like binge eating.

Furthermore, when compared to individuals with BN, those with AN showed lower psychological and physical/cognitive QoL (Bamford and Sly 2010). In line with these findings, AN people have been also described as having poorer social skills and higher functional impairment when compared to BN individuals (Turner et al. 2010). Interestingly, recovered ED patients had a higher QoL than ill ED patients, but they still reported poorer QoL than healthy controls did (de la Rie et al. 2005).

With respect to differences in QoL between diagnostic subtypes of AN, data suggest that those with BP-AN are more impaired than R-AN (Padierna et al. 2000; De Jong et al. 2013) and this seems to correlate with clinical severity (De Jong et al. 2013). Relatedly, QoL of those with R-AN tend to improve at follow-up differently from those with BP-AN (Carter et al. 2008). However, these results may be biased by the aforementioned ego-syntonicity that characterizes mostly R-AN (Mond et al. 2005); in fact, assessing QoL in R-AN patients may pose a relevant problem because weight loss occurs without the more ego-dystonic episodes of binge eating and purging which occur in both BP-AN, BN, and BED.

Interesting data emerge also by comparing AN people with other groups of patients. For example, Carter et al. (2008) showed that AN patients scored higher on illness intrusiveness than other groups affected by either physical or psychiatric disorders. This datum highlights how AN impacts on sufferers' lives in a various and contradictory way. In fact, on one hand patients do not realize malnutrition- and illness-related consequences; on the other hand they feel overwhelmed by the disorder. Sometimes patients' thoughts about body and food are strongly time consuming and obsess affected individuals to the point of hindering food- and eating-related activities. As a result, patients maintain their suffering but at the same time would like to be free again. This ambivalence can make patients feel stuck in their illness thus heightening their demoralization (Abbate-Daga et al. 2013) and potentially induce suicidal behaviors as a way to avoid such a poor QoL. Still, ED individuals showed higher emotional reaction and social isolation than groups with physical illnesses. When AN individuals

are compared with schizophrenic and depressed patients, those with a longstanding AN scored similarly to schizophrenic patients for self-care and social contact (González et al. 2001). Also, other psychiatric patients resulted to perceive more reliably their QoL than AN people (González et al. 2001). As a result, the AN group seemed to be impaired in QoL like other severe and potentially chronic mental illnesses like schizophrenia and depressive disorder (Arkell and Robinson 2008).

Scientific literature has recently started to shed light on those factors that determine an additional impairment of QoL in AN highlighting that bingeing (Hay 2003; Latner et al. 2008; De Jong et al. 2013) and purging (Mond et al. 2005; Tirico et al. 2010; De Jong et al. 2013) behaviors influence negatively patients' QoL in both clinical and community samples. However, it remains unclear to what extent the frequency of such behaviors influences QoL (Jenkins et al. 2011). Also subjective bulimic episodes and compensatory behaviors (e.g., fasting, abuse of laxatives or diuretics, and self-induced vomiting) negatively impacted prognosis (Latner et al. 2008). Little evidence exists on the role of BMI, mostly in severely underweight samples (Jenkins et al. 2011) with data from our group showing that no correlations can be found between BMI and QoL in severe AN patients undergoing hospitalization (Abbate-Daga et al. 2014). Notwithstanding, this factor has been found to affect QoL in some reports (Bamford and Sly 2010). As aforementioned, malnutrition more than BMI impacts on QoL: in fact, patients with low QoL can have fair BMI levels and vice versa. It is well known that rapid weight loss rather than BMI per se can represent a risk factor for individuals' health and tend to impact both QoL and brain functioning (Jeejeebhoy 1998).

Also ED severity has been found to be a predictor of poor QoL (Tirico et al. 2010; Bamford and Sly 2010). Data are more mixed with regard to chronicity although reports showed its relevance in QoL (Tirico et al. 2010) with poorer QoL in individuals with illness duration greater than 5 years. It has also been found a relationship between improvement of eating symptoms and QoL, mostly with respect to family support, leisure activities, economic resources, and perceived mental health dimensions (Sy et al. 2013). Psychiatric comorbidity is endemic to AN sufferers (Swinbourne et al. 2012; Abbate-Daga et al. 2011b) and particularly major depression can influence QoL. In fact, AN people reported similar scores to those of depressed patients, with a strong correlation between depressed mood and QoL due to relational avoidance and social isolation (Arkell and Robinson 2008). After all, EDs and depressive symptomatology are strongly entrenched: recent research showed that such symptoms are correlated with persistence of body image disturbances (Junne et al. 2016). Therefore, more specific assessments of QoL should evaluate this fundamental and specific area. In this light, Stanghellini and collaborators developed an assessment (Identity and Eating disorders, IDEA; Stanghellini et al. 2012) that assesses patients' perspectives about their body and that should be compared to instruments assessing QoL.

Readiness for change is another factor which can be involved in the improvement of QoL. In fact, it has been found to be negatively correlated with the length of stay needed to achieve a positive outcome during hospitalization (McHugh 2007). Treatment motivation can be crucial concerning subjective QoL: in fact, those who are motivated to be treated hope to improve their condition and in turn have a more active and positive attitude towards their disorder. It is necessary to be strong to fight

Table 4 Main factors related to quality of life in eating disorders

Somatic area:
1. Malnutrition effects
2. Low weight
3. Somatic sequelae of the disorders
4. Limitations to movement
Clinical area:
1. Severity of eating psychopathology
2. Purgning behaviors
3. Over-exercise
4. Duration of illness
5. Body image disturbances
6. Diagnosis and diagnostic subtypes
7. Intrusiveness of symptoms
8. Depressive and anxious comorbidity
Psychosocial area:
1. Social isolation
2. Work impairment
3. Family burden
Sufferers' attitude towards their disorder:
1. Ego-syntonicity
2. Denial of illness
3. Motivation to treatments

EDs and a good therapeutic alliance is recommended (Abbate-Daga et al. 2013). Motivation is key also with long-standing patients since it can lead to a tipping point promoting patients' long journey towards recovery and QoL improvement (Dawson et al. 2014). Table 4 summarizes main factors QoL-related in EDs.

Clinical and Research Implications of Quality of Life When Treating Anorexia Nervosa

Over the past years QoL has increasingly been considered as an outcome measure, although many times as a secondary one. As a result, QoL has been studied also in a therapeutic framework with earlier data showing no differences in QoL between treated and untreated patients (Latner et al. 2008). Such findings have been partially confuted by some studies conducted with inpatients demonstrating improved QoL upon hospital discharge (Carter et al. 2008; Abbate-Daga et al. 2014). For example, data from our group (Abbate-Daga et al. 2014) showed that emergency hospitalizations in AN can be effective at improving QoL over the short run. In fact, upon discharge patients reported to be improved in mobility, pain/discomfort, and anxiety/

Table 5 Treatment for those affected by severe and enduring anorexia nervosa

• Multidisciplinary team with eating disorders-trained therapists
• Detailed anamnesis of previous treatments
• Agree upon small objectives, including medical stability
• Empathic relationship and clarity about treatment goals
• Main focus on quality of life: less attention paid to eating psychopathology and more to psychosocial functioning and to the feelings of loneliness

depression scales. Furthermore, the extent of the perceived impairment was milder after hospitalization with the latter resulting a useful intervention to improve both somatic and motor difficulties and to motivate discouraged and psychologically distressed patients. As recommended by international guidelines (NICE 2004) such QoL changes should represent only a first-step goal in treatment.

Very few data exist on the long-term effectiveness of treatments on QoL; Pohjolainen and collaborators (2016) reported that after 8 years QoL was still severely impaired, although gradually improved, in both AN and BN compared to general population.

Treating QoL can be particularly meaningful for AN patients with a severe and enduring disorder. In fact, treatments for AN focus on the resolution of medical symptoms, first of all weight gain, and this is a strong need for the vast majority of affected individuals. However, it has been proposed that those who struggled with AN for several years may need a shift in such a treatment focus (Wonderlich et al. 2012; Table 5) because the ordinary definition of recovery may be no longer adequate. A shared definition of recovery does not exist for AN, but all proposed definitions do include weight restoration. Notwithstanding, this might not be the case for those with a severe and enduring disorder, since recovery should take into account the impact of AN on QoL rather than full weight restoration. Psychotherapy was found to be effective in improving QoL of patients with a severe and long-standing disorder (Touyz et al. 2013), but research also showed that improvements in QoL can be predicted and driven by the improvement of both BMI and eating psychopathology (Bamford et al. 2015); thus, a balance between weight restoration and QoL should be achieved in treatment.

Conclusions

QoL in EDs appears overall poor because of both physical and psychosocial factors. In case of a severe ED, illness duration is usually protracted with treatment resistance and eventually generating a vicious cycle that over time impacts objective and subjective well-being with increased social isolation and poor functioning. QoL over time tends to become poorer and poorer, particularly for AN sufferers as well as that of other family members (Santonastaso et al. 1997).

Malnutrition is a core-element in deteriorating QoL for both physical sequelae and negative effects on brain functioning (Seitz et al. 2014). Furthermore, it should be also mentioned the effect of the specific psychopathology of EDs: sufferers can spend a relevant amount of time struggling with eating symptoms and eating-related thoughts and engage complex rituals checking food and body, often perceived as intrusive and oppressive. Over time, a feeling of failure can further impair QoL.

Treatments should address QoL more precisely and carefully. In this light, it is of importance to focus on patients' perspectives about better treatment and future (Malson et al. 2011) and to help sufferers understand how the improvement of eating symptoms and that of QoL can be intertwined. Finally, treating those with an enduring disorder can require a specific therapeutic effort on QoL in order to achieve the stabilization of psychiatric and eating symptoms and the improvement of social skills alike (Wonderlich et al. 2012). Although research on the effectiveness of such models is only in its infancy, consensus has been reached on the need to pay closer attention to QoL when treating EDs.

Policies and Protocols

Protocols

Protocols may include "how to" techniques such as assessing deficiency or malnutrition, measures of social factors (quality of life, food security), physical or biochemical measurements (blood, anthropometry, etc.).

In order to assess quality of life (QoL) in anorexia nervosa (AN), different approaches can be used. In fact, how to precisely measure QoL in AN is a currently debated topic. Several self-report either generic or eating disorder (ED)-specific measures have been used over the past years. Generic instruments are represented by tools such as the EuroQoL 5D (EQ-5D VAS; EuroQoL Group 1990) or the Medical Outcome Short Form Health Survey (SF-36; Ware and Sherbourne 1992) which have been largely used in biomedical research in order to measure QoL. Such instruments have been used across a variety of different medical settings and can be of help because they are well known and broadly used, can be used by non-ED trained people, and are common in literature and finally, make data on AN comparable with other medical and psychiatric fields. Nevertheless, eating disorder-specific measures have been developed as well. Such instruments have been pioneered in order to capture more appropriately AN-specific characteristics such as partial awareness of illness and poor motivation to change (Ackard et al. 2014; Mond et al. 2005; Muñoz et al. 2009). ED-specific instruments to assess QoL include: the Eating Disorders Quality of Life instrument (Engel et al. 2006), a 25-item scale with four subscales (psychological,

physical/cognitive, work/school, and financial) and a total score, the Health-related Quality of Life for Eating Disorders questionnaire (Las Hayas et al. 2006), a 50-item instrument with 8 subscales (symptoms, restrict behaviors, body image, mental health, emotional role, physical role, personality traits, social relations), the Eating Disorders Quality of Life Scale (Adair et al. 2007), a 40-item scale with 12 subscales (cognitive, education/vocation, family and close relationships, relationships with others, future outlook, appearance, leisure, psychological, emotional, values and beliefs, physical, and eating), the Quality of Life for Eating Disorders measure (Abraham et al. 2006), a 21-item measure assessing behavior, eating disorder feelings, psychological feelings, effects on daily life, effects on acute medical status and body weight, and the Clinical Impairment Assessment questionnaire (Bohn and Fairburn 2008), a 16-item questionnaire generating a total score and three subscale scores (personal impairment, cognitive impairment, and social impairment). However, these tools make it difficult to compare QoL results across physic or mental conditions (Ackard et al. 2014).

Policies

Policies can include exiting or more forward thinking guidelines and can include recommendation for workers in the field, groups, institutions, or governments. It can also include guidelines such as precautionary measures or interventions such as education.

In the ED field guidelines are lacking in several respects; for example, a formal staging of the EDs is currently not available. Nevertheless, according to the available body of evidence, it is important to provide ED patients with the assessment of quality of life (QoL). In fact, QoL is key in both physical and mental health fields and it has been highlighted the need to address this issue in treatment (Jenkins et al. 2011; Sy et al. 2013). With more detail, the DSM-5 diagnostic system (APA 2013) requires individuals' functional impairment to make the diagnosis of a mental disorder. Relatedly, policy-makers became interested in this parameter when evaluating cost-effectiveness (Stuhldreher et al. 2012) and QoL has become an important measure of treatment effectiveness (Sy et al. 2013). Assessing QoL in AN is of particular interest for those patients showing malnutrition-related sequelae, mostly in case of extreme AN (i.e., BMI < 15). Consequences of malnutrition often have as a result social isolation and emotional and social avoidance (Wildes et al. 2010), thus in turn impacting on QoL. Sufferers with a long duration of illness (e.g., 15–20 years) could report several sequelae like fractures, movement impairments, loss of autonomy, inability to go to work, and loneliness. Moreover, assessing QoL allows to compare data across different diagnoses in both psychiatric and organic/medical conditions. Still, the assessment of QoL in EDs can help predict clinical outcomes in treated individuals and their risk of relapse and recurrence (Spitzer et al. 1995). Finally, from a research standpoint, QoL assessments have been

increasingly considered as patient-oriented outcome measures in addition to the traditional ones (e.g., weight restoration, absence of binge-purging episodes; Bamford et al. 2015).

Dictionary of Terms

- **Anorexia nervosa** – Severe mental illness characterized by weight loss, obsessions about food and body shape, and body image distortions.
- **Body Mass Index** – Is a measure of body fat that applies to adult people. It is calculated as follows: kg/m^2. Commonly accepted BMI ranges are: underweight: under 18.5 kg/m2, normal weight: 18.5 to 25, overweight: 25 to 30, obese: over 30.
- **Ego-syntonicity** – It refers to behaviors and feelings considered as acceptable according to each individual's psychological needs. It is the opposite of ego-dystonicity which can be defined as the aspects of one's behavior viewed as inconsistent with one's fundamental beliefs.
- **Quality of life** – Multidimensional construct describing subjective perceptions about physical, psychological, relational, and emotional areas of individual's life.
- **Schizophrenia** – Severe mental illness that affects individuals' thoughts, feelings, and behaviors. Affected individuals tend to lose their contacts with reality. Although a rare disorders, schizophrenia is a chronic and disabling disorder.

Summary Points

- Anorexia nervosa is a severe mental disorder characterized by high mortality.
- Quality of life is strongly impaired in anorexia nervosa and potentially underestimated because of the ego-syntonicity of this illness.
- Data are mixed as to whether quality of life is most impaired in anorexia nervosa or in other eating disorders.
- Severity of malnutrition, eating psychopathology, body image distortions, psychiatric comorbidity, and social isolation seem to be mostly related to quality of life rather than Body Mass Index.
- Those with anorexia nervosa reported levels of quality of life comparable to those of schizophrenic and severely depressed patients.
- It is of importance that quality of life is taken into account in treatments.
- Preliminary data highlight that the treatment of eating disorders can improve patients' quality of life.
- The debate is open as to whether those with a chronic disorder can improve their quality of life without substantially improving their eating disorder.

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Outcomes of Severe and Enduring Anorexia Nervosa

32

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Abstract

A significant minority of individuals with eating disorders suffers with symptoms for more than 5 years (American Psychiatric Association, Diagnostic and statistical manual of mental disorders (DSM-5), American Psychiatric Pub, 2013). While most of those diagnosed with anorexia nervosa (AN) recover within 5 years of onset, others are more long-standing and have difficulty responding to established interventions (Wonderlich et al., Int J Eat Disord 45(4):467–475, 2012). Severe and enduring AN (SE-AN) is a term that refers to chronic AN, typically 7 years or longer in duration. Outcome data have shown that 7 years marks a plateau in course of illness trajectories, where recovering becomes less likely (Robinson, Severe and enduring eating disorders: a need for some new ideas, paper presented at the eating disorders international conference, London, 2010). SE-AN is by definition severe in nature, and treatment is imperative. However, research guiding the selection of targeted intervention for this population is scant. Consequently, clinicians often view individuals with SE-AN with little optimism and few positive expectations. This chapter will review the available literature to date about SE-AN. The aims of the current chapter are to: (1) empirically define SE-AN, (2) discuss the functional outcomes of those with a chronic history of AN, (3) describe various intervention strategies that show promise, and (4) summarize a randomized controlled trial (RCT) comparing two types of treatment for SE-AN (Touyz et al., Psychol Med 43 (12):2501–2511, 2013). Our review and conclusions highlight the challenges with the current literature, the importance of continuing research on this topic, and the need to set more realistic treatment goals with this population.

Keywords

Severe and enduring anorexia nervosa · Eating disorders · Anorexia nervosa · Outcomes · Quality of life · Modest weight gain · Intervention · Randomized controlled trial · Severe and enduring eating disorders · Cognitive behavioral therapy · Specialist supportive clinical management

List of Abbreviations

AN	Anorexia nervosa
ANSOCQ	Anorexia nervosa stages of change questionnaire
BDI	Beck depression inventory
CBT	Cognitive behavioral therapy
CBT-AN	Cognitive behavioral therapy for AN
CRT	Cognitive remediation therapy
DBS	Deep brain stimulation
ED	Eating disorders
EDE	Eating disorder examination
EDQOL	Eating disorder quality of life instrument
EOT	End of treatment
HRQ	Helping relationship questionnaire

QOL	Quality of life
RCT	Randomized controlled trial
SE-AN	Severe and enduring anorexia nervosa
SEED	Severe and enduring eating disorders
SF-12	Short form-12 health status questionnaire
SSCM	Specialist supportive clinical management
TMS	Transcranial magnetic stimulation
WSAS	Weissman social adjustment scale

Introduction

A significant minority of individuals with eating disorders (ED) suffers with symptoms for years. The *Diagnostic and Statistical Manual of Mental Disorders*, Fifth Edition (DSM-5), describes anorexia nervosa (AN) as a serious psychiatric and medical disorder characterized by (1) a significantly low body weight, where the individual restricts food intake to weigh less than minimally normal; (2) an intense fear of gaining weight, usually with concurrent behaviors, such as excessive exercise, that inhibit weight gain; and (3) disturbance in body experience, which often includes a preoccupation with one's weight and an inability to acknowledge or recognize one's severe thinness (American Psychological Association 2013). The criteria and specifiers are presented in Table 1. While most individuals diagnosed with AN recover within 5 years of onset, others experience a more long-standing illness and have difficulty responding to established psychological interventions (American Psychological Association 2013; Wonderlich et al. 2012).

Table 1 The DSM-5 diagnostic criteria for anorexia nervosa (American Psychological Association 2013) is shown. Additionally, the DSM-5 specifies between two subtypes, the restricting type and the binge eating/purging type, as well as by level of severity based on BMI (body mass index)

Diagnostic criteria			
A	A significantly low body weight, where the individual restricts food intake to weigh less than minimally normal		
B	An intense fear of gaining weight, usually with concurrent behaviors, such as excessive exercise, that inhibit weight gain		
C	Disturbance in body experience, which often includes a preoccupation with one's weight and an inability to acknowledge or recognize one's severe thinness		
Subtype			
Restricting type: criteria A is mainly achieved through dieting, fasting, and/or excessive exercise		**Binge eating/purging type:** criteria A is mainly achieved through self-induced vomiting or misusing laxatives, diuretics, or enemas	
Level of severity			
Mild: BMI $\geq$17 kg/m^2	**Moderate:** BMI 16–16.99 kg/m^2	**Severe:** BMI 15–15.99 kg/m^2	**Extreme:** BMI $<$15 kg/m^2

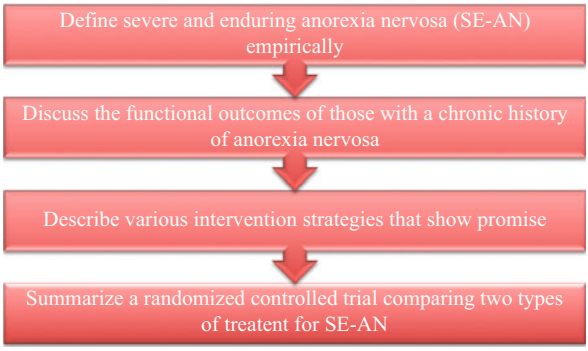

Fig. 1 Chapter goals. In discussing the SE-AN literature to date, the progression of the current chapter is shown

Severe and enduring AN (SE-AN) is a term that refers to chronic AN, typically 7 years or longer in duration. Outcome data have shown that 7 years marks a plateau in course of illness trajectories, where recovering becomes less likely (Robinson 2010). The debilitating nature of SE-AN confers an increase in disability, resulting in a higher likelihood of these patients receiving financial support and health benefits from the government and becoming a burden to their families (Touyz and Hay 2015). According to prior research analyzing chronic AN, there is a poor prognosis for those with SE-AN, as the mortality rates rise in relation to duration of illness (Steinhausen 2002).

SE-AN is by definition severe in nature, and treatment is imperative. However, research guiding the selection of targeted intervention for this population is scant. Consequently, clinicians often view individuals with SE-AN with little optimism and few positive expectations. This chapter will review the available literature on this topic. First, we will empirically define SE-AN. Second, we will describe the functional outcomes of those with a chronic history of AN. Third, we will summarize various intervention strategies that show promise. Lastly, we will describe a randomized controlled trial (RCT) that has compared two types of treatment for those with SE-AN (Touyz et al. 2013). Our review may serve as a basis for highlighting more realistic treatment goals (i.e., away from expectations of full recovery and toward improvement in quality of life (QOL) factors along with modest weight gain), which might increase patient engagement and foster treatment progression (Fig. 1).

Defining SE-AN

Prior research has indirectly studied the longitudinal outcomes of those with chronic AN. For instance, results from a 21-year follow-up study revealed that a longer duration of illness prior to first treatment, and insufficient weight gain during initial hospitalization for the illness, predicts poorer outcomes (Zipfel et al. 2000). Notably, RCTs conducted for patients with AN exhibit an extremely high attrition rate, hovering around 40%. Results suggest that only a minority of patients is willing to

stay in treatment trials; therefore, identifying those with chronic histories as early as possible is necessary for treatment engagement (Halmi 2008). The findings of prior RCTs that include chronic AN are listed in Table 2 as a reference.

More recently, as studies have evolved, the field has come to label this chronic history as SE-AN. Even though the literature has begun to focus on these more severe cases, there is considerable variability in how clinicians define chronic ED due to the lack of empirical data to support this distinction (Wonderlich et al. 2012). Researchers and clinicians often have difficulty agreeing on a common definition because chronicity and severity can relate to a variety of factors. While most use a combination of illness duration, functional impairment, and/or number of hospitalizations as indicators for differentiating those with ED from those with severe and enduring ED (SEED; Wonderlich et al. 2012), each of these individual indices also requires consensus of definition to inform the broader category.

Wildes et al. (2016) have developed an empirically derived operational definition of SE-AN. Specifically, they present a mixed categorical-dimensional model to group those with AN into two categories of severity (low versus high severity) and along three continuous dimensions: ED behaviors, life satisfaction, and indicators of chronicity (i.e., length of illness, number of hospitalizations, and severely low BMI). Results highlight that more than illness duration and number of hospitalizations should be considered when distinguishing between AN and SE-AN (Fig. 2). Specifically, understanding the patient's QOL from a holistic approach may be warranted, but further research is required to replicate and validate this model.

Functional Outcomes and QOL Factors in SE-AN

Arkell and Robinson (2008) conducted a study comparing those with SE-AN to other severe psychiatric diagnoses. The researchers collected original data for 11 participants with SE-AN to compare with preexisting data collected on 106 primary care patients with moderate-severe depression and a standardized community sample of 128 patients with schizophrenia (Parker et al. 1991; Skevington and Wright 2001). The researchers have found that those with SE-AN and severely depressed individuals are similar on measures of QOL, while those with SE-AN are similar to patients with schizophrenia on measures of life skills. These studies illustrate the severity of SE-AN in terms of QOL and functional outcomes.

In another study, Robinson et al. (2015) interviewed eight participants with an illness duration of over 20 years using thematic analysis for a qualitative description of functional impairments. They found that SE-AN appears to affect functioning across multiple domains, with particular difficulty in psychological and social realms and evidence of occupational and physical problems as well. Results suggest that these individuals often feel depressed and hopeless, as well as socially isolated and avoidant. They also tend to experience difficulties maintaining employment and have medical complications secondary to malnutrition (Robinson et al. 2015). These findings further speak to the need for a treatment model for SE-AN that focuses on

Table 2 Randomized controlled therapy trials of adults including chronic AN

Author, year	Publication title	Publication	Participants	Intervention	Findings
Dare et al. (2001)	Psychological therapies for adults with anorexia nervosa. Randomized controlled trial of outpatient treatments	Br J Psychiatry	84 (2 men) with AN (DSM-IV), mean age 26.3 years and all >18	Focal psychoanalytic psychotherapy (1 year), cognitive analytic therapy (7 months), family therapy (1 year), or routine treatment (1 year)	At 1 year, there was modest symptomatic improvement with several patients remaining significantly underweight. Focal psychoanalytic and family therapies were superior to routine treatment (control)
McIntosh et al. (2005)	Three psychotherapies for anorexia nervosa: A randomized controlled trial	Am J Psychiatry	56 females with AN (DSM-IV) or EDNOS AN (BMI 17.5–19) without consideration of amenorrhea criteria. Age range 17–40	CBT, IP, or nonspecific clinical management	Nonspecific clinical management was superior to specific therapies; 70% or participants did not complete treatment or made minimal treatment gains
Pike et al. (2003)	Cognitive behavior therapy in the post-hospitalization treatment of anorexia nervosa	Am J Psychiatry	33 females with AN (DSM-IV). Mean BMI for both groups, 16 and 15.2, age range 18–45	50 sessions of CBT or nutritional counseling over 1 year following discharge from hospitalization	CBT was found superior to nutritional counseling for preventing relapse post-hospitalization
Touyz et al. (2013)	Treating severe and enduring anorexia nervosa: A randomized controlled trial	Psychol Med	63 females with AN. Mean age 33.4 (20–62), mean BMI 16.2, mean duration of illness 16.4 years	30 sessions CBT-AN or SSCM over 8 months	85% of participants completed treatment. There were no significant differences between treatment groups; at follow-up the CBT-AN group had a better outcome

(*continued*)

Table 2 (continued)

Author, year	Publication title	Publication	Participants	Intervention	Findings
Zipfel et al. (2014)	Focal psychodynamic therapy, cognitive behaviour therapy, and optimized treatment as usual in outpatients with anorexia nervosa (ANTOP): Randomized controlled trial	Lancet	242 females with AN (DSM-IV) or AN minus one criterion, BMI 15–18.5, age ≥18	10 months of focal psychodynamic therapy, enhanced CBT, or optimized treatment as usual	At the end of treatment, all groups had increases in BMI with no significant differences between groups
Byrne et al. (2017)	A randomized controlled trial of three psychological treatments for anorexia nervosa	Psychol Med	120 (3 men) with AN. Mean age 26.2, mean BMI 16.7, median duration of illness 4 years	25–40 sessions (over a 10-month period) of SSCM, Maudsley model AN treatment for adults, or enhanced CBT	60% of participants completed treatment. All groups resulted in improvements in BMI, ED symptoms, and psychological/psychosocial well-being with no significant differences between groups at EOT and 12-month follow-up

The findings of prior randomized controlled trials that include chronic anorexia nervosa are shown. These studies illustrate the outcomes of various treatments for those with anorexia nervosa. The data exhibits extremely high attrition rates and suggests the continued limitation of losing many participants from treatment trials

patient resiliency and improvements in QOL and social functioning. That said, research on SE-AN examining the predictive association between core symptom improvement (i.e., in BMI and ED severity) and QOL shows a significant temporal relationship between the two (Bamford et al. 2015). However, even if positive changes in QOL are predicated on weight gain and behavioral symptom reduction, the imposition of full recovery as a treatment goal for those with SE-AN may compromise engagement and retention. Thus, it is important to develop interventions that directly target the psychosocial consequences of chronic AN.

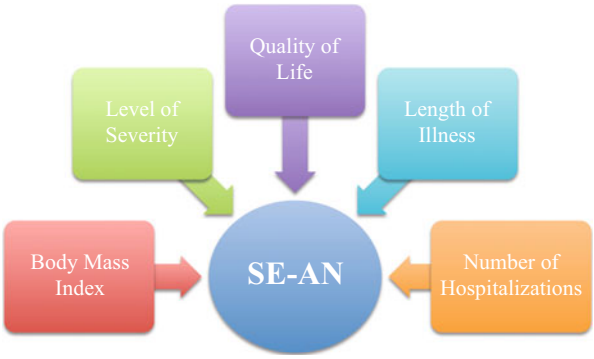

Fig. 2 Additional factors to consider for SE-AN. Wildes et al. (2016) highlight that more than illness duration and number of hospitalizations should be considered when distinguishing between AN and SE-AN, such as the factors shown here

Therapeutic Interventions for SE-AN

Psychological Interventions

To address the unique needs and differential outcome data of individuals with persistent AN (Hay et al. 2015; Zipfel et al. 2000, 2014), several in the field have proposed a clinical staging model (Touyz and Hay 2015; Treasure et al. 2015b), which acknowledges a progression of illness across the lifespan, with the necessary type and level of intervention varying as a function of degree of chronicity. Specifically, they argue that more severe neurobiological changes occur with longer illness duration, leading to an entrenched belief system that requires modification of traditional treatment goals to focus on QOL factors and habitual cognitive biases (Treasure et al. 2015a). Others suggest using supportive humanistic techniques, with a particular focus on promoting autonomy and identifying both effective and ineffective elements of past treatments (Wonderlich et al. 2012).

As described in detail below and presented in Fig. 3, in one RCT focusing on the SE-AN population, modified cognitive behavioral therapy for AN (CBT-AN) and specialist supportive clinical management (SSCM) both show promise in treating those with SE-AN. The CBT-AN manual used in the RCT, developed by Pike et al. (2003), modifies cognitive behavioral therapy (CBT) to address cognitive and behavioral distortions directly affecting AN. The authors stress a schema-focused approach to determine how the patient's internal representations of the self and others relate to their ED symptomatology. When comparing 1 year of CBT-AN to nutritional counseling for 33 weight-restored adult patients with AN, researchers found that those randomized to CBT-AN are less likely to relapse and have better outcomes as assessed by weight status, the Eating Disorder Examination (EDE; Fairburn and Cooper 1993), and the Structured Clinical Interview for DSM-IV Axis I Disorders to assess current comorbidity (Pike et al. 2003).

SSCM for AN is structured around clinical management and supportive therapy (McIntosh et al. 2010). This treatment promotes healthier eating behaviors to restore weight gain by providing clinical monitoring, nutritional information, and

CBT-AN	SSCM
Builds rapport and initiates treatment	Includes clinical monitoring, nutritional information, and psychoeducation regarding AN and healthier eating habits
Addresses cognitive and behavioral distortions directly affecting AN	Identifies and targets issues directly affecting the maintenance of the eating disorder
Utilizes a schema-focused approach to determine the patient's internal representation of self and others in relation to eating disorder symptomatology	Builds upon the patient's strengths
Plans for relapse prevention	Plans for relapse prevention
Touyz et al (2013) altered the goal for both treatments to increase quality of life factors	

Fig. 3 Comparison of CBT-AN and SSCM. A comparison of the two treatments analyzed in the Touyz et al. (2013) randomized controlled trial is shown here. In addition to the elements of the treatments described in the original protocols, Touyz et al. (2013) altered the treatment goal to focus on improving quality of life factors as opposed to full weight restoration

psychoeducation regarding AN. Simultaneously, the patient and therapist address any other relevant issues that are affecting the development and maintenance of the ED, build upon patient strengths, and ultimately plan for relapse prevention (McIntosh et al. 2010). This intervention was compared to CBT and interpersonal psychotherapy over 20 weeks of treatment for 56 women with AN and SSCM and was found to have better outcomes compared to interpersonal psychotherapy as assessed by the EDE (Fairburn and Cooper 1993) and better outcomes compared to both CBT and interpersonal psychotherapy on the Global Assessment of Functioning (McIntosh et al. 2005).

Neuropsychological Interventions

Park et al. (2014) discuss the possibility of implementing cognitive remediation therapy (CRT) for these individuals in an effort to target the severe negative habitual behavior and to improve cognitive flexibility. In a preliminary experiment with four patients with AN, implementing CRT for ten sessions improved cognitive flexibility on neuropsychological tests of set shifting and as described qualitatively by the patients after the intervention (Tchanturia et al. 2007). Abbate-Daga et al. (2012) presented a case series of 20 participants with AN that further demonstrates that CRT can increase set-shifting abilities and cognitive flexibility. Although research in this area has mostly been conducted with AN, this intervention shows promise and should be further investigated for its potential in altering severe cognitive biases that are more prominent in those with SE-AN (Treasure et al. 2015a).

Additionally, altering the attentional capacity that those with AN put on food-related stimuli has been suggested as a novel treatment and an alternative to traditional CBT (Treasure et al. 2015a). This intervention shifts attention at a nonconscious level by using a computer program (Renwick et al. 2013; Schmidt

and Campbell 2013) to redirect attention away from intrinsically rewarding illness-compatible stimuli and toward more positive health-related stimuli. In a review of the literature, Renwick et al. (2013) indicated that this process can involve simultaneously presenting neutral words with food or body-related words on a computer screen and measuring the participant's reaction time for selecting the neutral word. It is suggested that reducing attention on disorder-relevant stimuli will become more automatic as this process is repeated throughout treatment.

Conversely, in order to address the behavioral biases that maintain the rigid eating behaviors, Treasure et al. (2015a) proposed using additional implementation intentions. Specifically, an IF-THEN format might promote goal-directed behavior, such as increasing food consumption or developing healthier eating habits. Additionally, in vivo exposures might be a helpful adjunct to treatment to reduce anxiety associated with seeing and eating food (Treasure et al. 2015a).

While having intuitive appeal for their compatibility with SE-AN, most of these treatment approaches have not been specifically studied with this population, and those that have require replication (Treasure et al. 2015a; Hay et al. 2012). In an effort to establish evidence-based treatments, Hay et al. (2015) submitted a PROSPERO protocol to conduct a meta-analysis that plans to examine RCTs that include individuals with AN for at least 3 years, as well as those who are considered resistant to treatment with at least two failed attempts at treatment. They will compare outcomes across intervention groups to determine the effectiveness of various treatments for those with their determined definition of SE-AN.

Neurotechnological Interventions

Because of the severity and long-lasting changes that result from a diagnosis, new avenues for treatment are being considered (Touyz and Hay 2015). As the behaviors associated with the disorder become cognitively habituated, neuroprogressive changes occur from prolonged AN (Treasure et al. 2015a). In considering the neuroplasticity of the brain, treatments that target this mechanism include deep brain stimulation (DBS), transcranial magnetic stimulation (TMS), and pharmacological interventions (Touyz and Hay 2015; Treasure et al. 2015a); however, it is important to note that the studies analyzing these treatment interventions include small sample sizes.

Preliminary data from a small trial of six participants suggests that DBS to the subcallosal cingulate, which has been previously shown successful in treating those with resistant depression, improves weight status and QOL in half of the patients with SE-AN (Lipsman et al. 2013). Targeting the nucleus accumbens and anterior cingulate cortex with DBS should be studied more extensively as a possible intervention strategy due to the newer understanding of the role of the reward-related neurocircuitries in the etiology and maintenance of AN (Oudijn et al. 2013).

TMS, a noninvasive neuromodulatory technique that increases neural activity in a localized area of the brain through a stimulation machine held close to the head, shows potential promise in the treatment of AN (Park et al. 2014). There is some

evidence supporting that one session of this technique to the left dorsolateral prefrontal cortex reduces anxiety in those with AN due to an increase in neuroplasticity. However, the data is limited to a small pilot study, and it is still unknown whether this method leads to an increase in food consumption (Van den Eynde et al. 2013).

As for pharmacological interventions, more research with intranasal oxytocin, a hormone released naturally during social bonding, should be considered (Treasure et al. 2015a). It appears that one dose of it has been shown to reduce fixations on body image (Kim et al. 2014). While this hormone may reduce avoidance behaviors associated with AN, such as the avoidance of threatening foods and social situations, more research is necessary to determine its role in treatment of SE-AN (Treasure et al. 2015a).

Randomized Controlled Trial for SE-AN

The severity and chronicity of SE-AN make it especially difficult to treat. Few research studies have been conducted to examine targeted treatment options for those with SE-AN. Specifically, most hospital programs are ill-equipped to track patients based on severity, and, thus, individuals with SE-AN are often grouped together and treated with the same clinical goals as those with AN (Touyz et al. 2013). Only one RCT to date has examined an intervention specifically designed for patients with SE-AN. The aims of the RCT were to determine the outcomes of adapting established AN psychotherapy protocols to address the distinctive features of this subgroup (Touyz et al. 2013).

The participants of the study included females meeting criteria for AN, excluding amenorrhea, for at least 7 years. Exclusion criteria included currently experiencing a manic or psychotic episode, substance or alcohol abuse/dependence, significant medical problems unrelated to malnutrition, were in psychotherapy and unwilling to postpone treatment for the study duration, or anticipated moving too far to travel to the study site within the next 12 months. Sixty-three eligible participants (86%) were willing to participate. Participants were randomized to either 30 sessions of modified CBT-AN or modified SSCM over an 8-month period. In order to fit this population, the participants in both conditions were informed that the goal of treatment was to improve QOL across domains, instead of focusing solely on fully achieving expected body weight as described in the original manuals.

The four phases of CBT-AN included (1) building rapport and initiating treatment, (2) altering maladaptive cognitions and behaviors associated with eating, (3) analyzing overarching schemas beyond those associated with eating, and (4) promoting use of CBT-AN strategies posttreatment. For the SSCM condition, participants received more general psychoeducation and supportive psychotherapy. The sample of 63 females ranged in age from 20 to 62 ($M = 33.4$, SD = 9.6), ranged in illness duration from 7 to 49 years ($M = 16.6$, SD = 8.5), and ranged in BMI from 11.8 to 18.5 kg/m^2 ($M = 16.2$, SD = 1.3). Participants were assessed at baseline, at the end of treatment (EOT), and at 6- and 12-month follow-ups. Twenty-six of 31

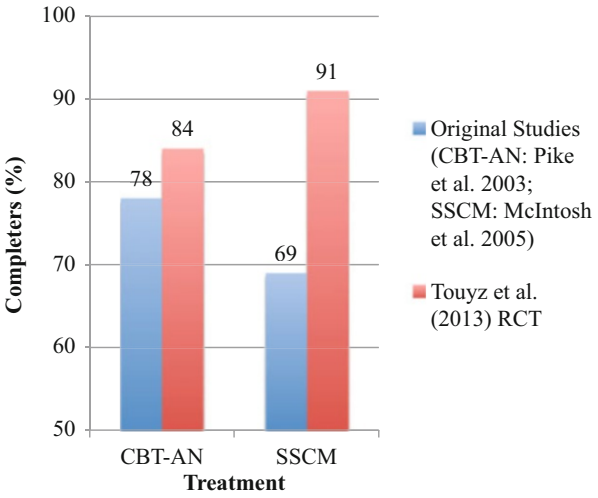

Fig. 4 Comparing percentage of completers for studies by treatment. The percentage of completers for Touyz et al. (2013) compared to Pike et al. (2003) and McIntosh et al. (2005) by treatment type is illustrated. The difference between the (Touyz et al. 2013) RCT and the original AN trials is the explicit treatment goal moving away from full weight restoration to improving quality of life factors. This appears to have a direct impact on rates of participation and completion among participants

CBT-AN (84%) and 29 of 32 SSCM (91%) participants completed treatment. Overall, 71.4% of those completed 6-month follow-up data (22 CBT-AN, 23 SSCM) and 79.4% completed 12-month follow-up data (24 CBT-AN, 26 SSCM) with no significant differences for completion rates between intervention groups.

The existing RCT and AN phenomenology literature have not specifically included, excluded, or distinguished between those with SE-AN and AN. SE-AN is considered a subset of AN samples in research to date. In comparing these modified treatments for this RCT to prior research with these protocols, more SE-AN participants completed the modified CBT-AN treatment (84%; Touyz et al. 2013) compared to all of the AN participants in the original CBT-AN trial (78%; Pike et al. 2003). Additionally, 72% of eligible participants agreed to participate in the original SSCM trial and 69% of those randomized to SSCM completed the treatment (McIntosh et al. 2005), compared to 86% that agreed to participate and 91% that completed the modified SSCM in the RCT (Touyz et al. 2013). This is shown in Fig. 4. This explicit altered treatment goal away from full weight restoration improves rates of participation and completion relative to the standard AN trials.

Participants in both treatment conditions showed significant improvements across all of the assessment time points on measures of QOL, physical and psychological well-being, BMI, ED symptomatology, and readiness to recover as assessed by the Eating Disorder Quality of Life Instrument (EDQOL; Engel et al. 2006), the Short Form-12 Health Status Questionnaire (SF-12; Ware et al. 1996), the Beck Depression Inventory (BDI; Beck et al. 1996), the Weissman Social Adjustment Scale

(WSAS; Weissman and Bothwell 1976), the EDE (Fairburn and Cooper 1993), and the Anorexia Nervosa Stages of Change Questionnaire (ANSOCQ; Rieger et al. 2002; Touyz et al. 2013). Although not statistically significant, CBT-AN produced greater social adjustment and readiness for change, as well as reduced ED symptomatology, at follow-up when compared to SSCM. Therefore, the results suggest that expanding treatment goals to focus on QOL keeps those with SE-AN involved in the treatment process and indirectly affects weight restoration.

In addition to outcome data, few RCTs for AN have examined moderators and predictors to address for whom treatment is most effective for and the mechanisms in which the treatment produces results. Consequently, researchers were interested in conducting an exploratory analysis of predictors and moderators associated with the outcomes of treatment using data collected from the RCT (Touyz et al. 2013; Le Grange et al. 2014). Of the 13 variables collected at baseline, including age, BMI, relationship status, duration of illness, subtype of illness (AN restricting type versus AN binge-purging type), employment status, education, medical concerns, psychotropic medication, EDE Global, ANSOCQ, BDI, and WSAS, four emerged as moderators at EOT. Specifically, those with higher levels of ED psychopathology, depression, older age, and the binge-purging subtype of AN were more successful in CBT-AN compared to SSCM. As for predictors, younger age, shorter duration of illness, and better interpersonal functioning predicted better outcomes at EOT for both conditions on measures of QOL, psychological well-being, and depressive symptoms, while unemployment and being on psychotropic medication predicted worse outcomes on these measures. When comparing baseline to 6- and 12-month follow-up, AN subtype also emerged as a significant predictor, such that those with the restricting subtype predicted better outcomes on measures of QOL, psychological well-being, and depressive symptoms and the use of psychotropic medication predicted worse outcomes on those measures at 12-month follow-up (Le Grange et al. 2014).

Further secondary analyses of the RCT looked at attrition and therapeutic alliance. Elbaky et al. (2014) found that dropout rates were higher among those with the binge-purging subtype and those with poorer QOL. In addition, therapeutic alliance has consistently been demonstrated to be a significant predictor of outcome for psychological interventions, including the treatment of AN (Antoniou and Cooper 2013). Stiles-Shields et al. (2013) examined therapeutic alliance in the two study treatments with the Helping Relationship Questionnaire (HRQ; Luborsky et al. 1985), finding no difference in alliance between CBT-AN and SSCM. In addition, therapeutic alliance significantly improved from treatment at week 2 compared to week 15 and EOT. While earlier development of alliance predicted lower levels on the Restraint and Shape Concern subscales of the EDE (Fairburn and Cooper 1993) at 12-month follow-up, therapeutic alliance assessed at a later time point in treatment predicted improvements on all outcome measures of ED symptomatology and depression except on the Shape Concern subscale of the EDE (Fairburn and Cooper 1993) at EOT and follow-up (Stiles-Shields et al. 2013). These results suggest that it takes time to develop the therapeutic alliance for those with SE-AN; however, the development of that relationship is important to promote and sustain positive change.

Summary and Conclusions

The goal of this chapter was to summarize the empirical evidence surrounding SE-AN. It was previously understood that treating those with chronic AN would lead to few positive outcomes. Although there is still little known about this population, the research that has been conducted shows promise with this clinical population. Specifically, setting more realistic goals away from significant weight gain to QOL factors and modest weight gain might make treatment progression and successful outcomes more feasible. A greater focus on QOL has the potential to decrease medical instabilities with the objective of spending less time in inpatient hospital care. As clinicians will continue to encounter those with chronic histories of ED, we propose that additional research with this population will contribute to clarifying the definition of SE-AN and provide more sufficient empirical evidence to support efficacious treatment intervention strategies for SE-AN.

Policies and Protocols

In this chapter we have described the available literature to date about severe and enduring anorexia nervosa. Below we describe recommendations for professionals in the field to have more success with this clinical population. We suggest the importance of:

- Early identification and intervention of eating disorders for treatment engagement and progression.
- Taking into consideration more than illness duration and number of hospitalizations when distinguishing between anorexia nervosa and severe and enduring anorexia nervosa.
- Considering the relevant treatment goals for this subgroup (i.e., away from expectations of full weight restoration and toward improvement in quality of life factors along with modest weight gain).
- Identifying strengths and weaknesses in their psychological, social, occupational, and physical functioning in order to enhance life domains.
- Remaining optimistic when working with this population as these individuals have likely experienced ineffective treatments in the past and the presented research shows promise for those with severe and enduring anorexia nervosa.
- Additional randomized controlled trials to further examine the efficacy of current treatments, including cognitive behavioral therapy for anorexia nervosa and specialist supportive clinical management, and how they can be improved for this population.
- Further investigation of the neuropsychological and neurotechnological interventions to draw empirical conclusions about their role in the treatment of severe and enduring anorexia nervosa.

Dictionary of Terms

- **Anorexia nervosa** – An eating disorder characterized by low body weight, fear of gaining weight, and body image disturbances in how the person perceives their weight.
- **Cognitive behavioral therapy for anorexia nervosa** – A therapeutic intervention designed to address cognitive distortions and behavioral disturbances associated with eating disorder symptomatology.
- **Eating disorders** – Psychological disorders characterized by disturbances in eating behaviors, such as anorexia nervosa, bulimia nervosa, binge eating disorder, and avoidant/restrictive food intake disorder.
- **Modest weight gain** – Gradual increments of weight restoration, including smaller goals of gaining 1–5 lb at a time rather than an initial goal of obtaining a weight that is considered normal for that individual.
- **Quality of life factors** – A focus on social, emotional, occupational, and physical well-being for those with severe and enduring anorexia nervosa should be considered over weight restoration.
- **Severe and enduring anorexia nervosa** – Those individuals suffering with anorexia nervosa for at least 7 years, who are unable to sustain improvement and tend to experience multiple hospitalizations due to the severity of the illness.
- **Specialist supportive clinical management** – An intervention strategy aimed at providing psychoeducation, nutritional counseling, and support to those with eating disorders as a way to gain knowledge and autonomy for addressing the illness after treatment.

Summary Points

- While most individuals with eating disorders recover within 5 years of onset, others are more long-standing and have difficulty responding to established psychosocial interventions (American Psychological Association 2013; Wonderlich et al. 2012).
- Severe and enduring anorexia nervosa (SE-AN) often refers to those who have a history of anorexia nervosa (AN) for at least 7 years because research has found that those with eating disorders reach a plateau around 7 years, where recovering becomes less likely (Robinson 2010).
- Research guiding the selection of targeted intervention for this population is scant; consequently, clinicians often view individuals with SE-AN with little optimism and few positive expectations.
- Researchers and clinicians often have difficulty agreeing on a common definition of SE-AN because chronicity and severity can relate to a variety of factors. While most use a combination of illness duration, functional impairment, and/or number of hospitalizations as indicators for differentiating those with eating disorders from those with severe and enduring eating disorders (SEED; Wonderlich et al.

2012), each of these individual indices also requires consensus of definition to inform the broader category.

- Considering that no clear cutoffs or operational definitions of SE-AN have been determined, more research that examines those with SE-AN in general, in addition to comparing this population to other eating disorders or SEED, will potentially clarify an appropriate definition.
- Robinson (2010) illustrated the severity of SEED with a study that found that those with SEED and severely depressed individuals were similar on measures of quality of life (QOL), while this group was similar to those with schizophrenia on measures of life skills.
- By interviewing eight participants with a history of AN for over 20 years, Robinson et al. (2015) found that the disorder appears to negatively impact functioning across domains, with particular difficulty in psychological and social realms.
- Although there is some inconsistency regarding the goals of treatment, the literature suggests that using humanistic techniques can be helpful for those with SE-AN, with a particular focus on promoting autonomy, as well as identifying both effective and ineffective elements of past treatments (Wonderlich et al. 2012).
- Potential psychosocial treatment avenues for those with SE-AN include cognitive behavioral therapy for anorexia nervosa (CBT-AN), specialist supportive clinical management (SSCM), cognitive remediation therapy, altering attentional capacity, and implementation interventions.
- As the behaviors associated with prolonged AN become cognitively habituated, treatments that target these neuroprogressive changes include deep brain stimulation (DBS), transcranial magnetic stimulation (TMS), and pharmacological interventions (Touyz and Hay 2015; Treasure et al. 2015a); however, it is important to note that the studies analyzing these treatment interventions include small sample sizes.
- A randomized controlled trial that analyzed treatments for those with SE-AN determined significant improvements for participants in both CBT-AN and SSCM across all of the assessment time points on measures of QOL, physical and psychological well-being, body mass index, eating disorder symptomatology, and readiness to recover. Results suggest that expanding treatment goals to focus on QOL keeps those with SE-AN involved in the treatment process and indirectly affects weight restoration (Touyz et al. 2013).
- Setting more realistic goals away from significant weight gain to QOL factors and modest weight gain might make treatment and successful outcomes more feasible.
- As clinicians will continue to encounter those with chronic histories of eating disorders, we propose that additional research with this population will contribute to clarifying the definition of SE-AN and provide more sufficient empirical evidence to support efficacious treatment intervention strategies for SE-AN.

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Endocrine Aspects of Anorexia Nervosa

33

Madhusmita Misra

Contents

Abstract

Anorexia nervosa (AN) is a common condition in adolescents and young adults associated with hypogonadotropic hypogonadism (manifesting as functional hypothalamic amenorrhea), relative hypercortisolemia, a nutritionally acquired growth hormone resistance with low insulin-like growth factor-1 levels, low levels of leptin and oxytocin, and high levels of ghrelin and peptide YY. Low body mass index, reduced lean mass, and hormonal alterations contribute to low bone mineral density in adults and reduced bone accrual in adolescents with

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anorexia nervosa leading to an increased risk of fracture. A multidisciplinary treatment team is necessary to manage anorexia nervosa, with close monitoring of eating behaviors and weight changes. Weight gain and menses recovery result in improved bone mineral density, but residual deficits may persist. Calcium and vitamin D intake should be optimized in all. Transdermal 17β-estradiol in replacement doses with cyclic progesterone may be considered in mature adolescent girls with low and decreasing bone mineral density Z-scores, or evidence of skeletal fragility. Combined oral contraceptives are not effective in increasing bone mineral density and may mask spontaneous menstrual resumption. Bisphosphonates increase spine bone mineral density in adults with anorexia nervosa, but not adolescents. Their long half-life limits their use in this age group. Teriparatide increased spine bone mineral density in one study of older women with anorexia nervosa, but these data need to be confirmed with additional studies.

Keywords

Anorexia nervosa · Growth hormone · Cortisol · Hypothalamic amenorrhea · Appetite regulating hormones · Ghrelin · Leptin · Peptide YY · Low bone density · Fracture risk

List of Abbreviations

AN	Anorexia nervosa
BMD	Bone mineral density
DSM	Diagnostic and statistical manual of mental disorders
DXA	Dual energy x-ray absorptiometry
FHA	Functional hypothalamic amenorrhea
GH	Growth hormone
GnRH	Gonadotropin releasing hormone
IGF-1	Insulin like growth factor-1
IUD	intrauterine device
LH	Luteinizing hormone
Pref-1	Preadipocyte factor-1
PYY	Peptide YY
RANK-L	Receptor activator of nuclear receptor kappa-ligand
T3	Triiodothyronine
T4	Thyroxine
TSH	Thyroid stimulating hormone
vBMD	Volumetric bone mineral density

Introduction

Anorexia nervosa (AN) is a condition of very low weight resulting from restriction of energy intake associated with an intense fear of gaining weight and altered body image (Robinson et al. 2017). DSM-5 does not define a set cut off for what

constitutes low weight and, unlike DSM-IV, does not include amenorrhea as a diagnostic criterion. However, delayed menarche in adolescents and functional hypothalamic amenorrhea are commonly associated with AN (Misra et al. 2004a; Peterson et al. 2016). Other endocrine findings in this condition of self-imposed starvation include an acquired growth hormone (GH) resistance with low insulin like growth factor-1 (IGF-1) levels, relative hypercortisolemia, altered levels of appetite regulating peptides, a state similar to the sick euthyroid syndrome, and impaired bone metabolism. Most endocrine changes are adaptive to the state of undernutrition to preserve energy for essential body functions, increase food intake, increase substrate availability for gluconeogenesis, and reduce resting energy expenditure. However, many of these changes have a deleterious effect on bone, as we will discuss.

Hypothalamic-Pituitary-Gonadal Axis

Functional hypothalamic amenorrhea (FHA) is common and in one study was reported in 38–73% of women who met AN criteria per DSM-5 (Peterson et al. 2016), which no longer requires amenorrhea as a diagnostic criterion for the disorder. The highest prevalence was noted in those with the binge-purge variant of AN and the lowest with the purge only variant. Changes in LH pulsatility (a reflection of changes in gonadotropin releasing hormone (GnRH) pulsatility) result in FHA and low estrogen levels. Women with AN also have lower levels of testosterone than controls (Miller et al. 2007). Altered GnRH secretion is a consequence of endocrine signals that reflect low energy availability. These include low levels of leptin and high levels of ghelin and cortisol (as subsequently described). Fat mass is a reflection of energy stores, and amenorrheic adolescents and women with AN have lower fat mass than those who are eumenorrheic (Misra et al. 2006b; El Ghoch et al. 2016) (Fig. 1). Further, an increase in fat mass (Misra et al. 2006b) and percent calories derived for fat (Baskaran et al. 2017a) predicts menstrual recovery. Suppression of the hypothalamic-pituitary-gonadal axis (and reproductive potential) is an appropriate adaptive response to conserve energy for vital body functions.

The hypothalamic-pituitary-gonadal axis may also be suppressed in males with AN, resulting in lower testosterone (and estradiol) levels than in controls (Misra et al. 2008a). Lower testosterone levels are associated with greater truncal adiposity (Misra et al. 2008b).

Hypogonadism and Bone

Hypogonadism is an important determinant of low bone mineral density (BMD) (Miller et al. 2006; Misra et al. 2004a, 2008a; Kandemir et al. 2017). Estrogen is primarily antiresorptive through suppression of receptor activator of nuclear receptor kappa-ligand (RANK-L) and increased production of osteoprotegerin, resulting in

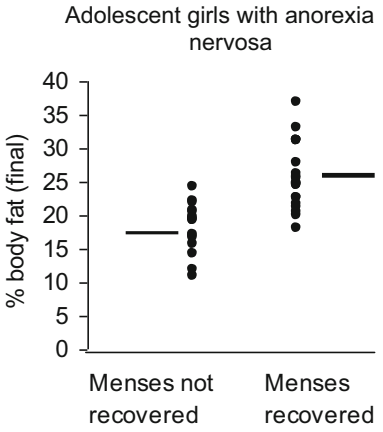

Fig. 1 Percent body fat in adolescent girls with anorexia nervosa who did or did not recover menses over 12-months follow-up. In a 12-month prospective observational study of amenorrheic adolescent girls with anorexia nervosa 12–18 years old, girls who recovered menstrual function during the follow-up period had greater percent body fat at 12-months than those who did not. (Reprinted with permission from Misra et al. (2006b). Copyright © International Pediatric Research Foundation, Inc., 2006)

inhibition of osteoclast differentiation and activation and increased apoptosis. It may also have bone anabolic effects through suppression of sclerostin, a product of osteocytes that otherwise inhibits bone formation. In women, older menarchal age and a longer duration of amenorrhea (reflecting chronicity of estrogen deficiency) are associated with lower BMD (Misra et al. 2004a). Despite low estrogen levels, osteoprotegerin levels are higher in AN than controls, indicating a compensatory mechanism to reduce bone resorption (Misra et al. 2003b). However, RANK-L is elevated, and the ratio of osteoprotegerin to RANK-L is lower than in controls, consistent with a milieu favoring resorption (Golabek et al. 2015).

Interestingly, administration of the combined estrogen-progesterone oral contraceptive pill is not effective in increasing BMD in adults or adolescents with AN (Strokosch et al. 2006; Klibanski et al. 1995), likely because oral ethinyl estradiol decreases IGF-1, an important bone trophic hormone. In contrast, a replacement dose of estrogen given as transdermal 17β-estradiol (100 mcg), a physiologic form of estrogen, with cyclic progesterone does not suppress IGF-1 in adolescents with AN and is effective in increasing bone accrual to rates observed in healthy controls (Misra et al. 2011) (Fig. 2). However, "catch-up" did not occur in this 12-month study, and girls with AN continued to lag behind controls for BMD, likely because many other endocrine changes (in addition to hypogonadism) contribute to low BMD. Transdermal 17β-estradiol did not impact sclerostin levels (Faje et al. 2012), but did decrease preadipocyte factor 1 (Pref-1) (Faje et al. 2013a). Pref-1 is an inhibitor of adipocyte and osteoblast differentiation that is increased in hypogonadal states. A reduction in Pref-1 was associated with a reduction in bone resorption markers and an increase in lumbar BMD.

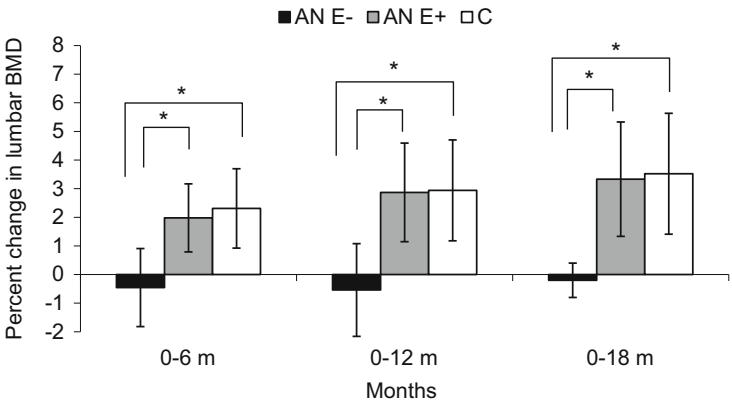

Fig. 2 Impact of physiologic estrogen replacement versus placebo on lumbar spine bone mineral density in adolescent girls with anorexia nervosa, compared to controls followed without intervention over an 18-month period. Adolescent girls with anorexia nervosa 12–18 years old randomized to physiologic estrogen administration as the 100 mcg 17β-estradiol patch and cyclic oral progesterone (AN-E+) had increases in bone density at the lumbar spine at 6, 12, and 18 months compared with those randomized to placebo patches and pills (AN-E-), to approximate bone accrual rates in healthy normal-weight controls (C) of the same age range followed without intervention. (Reprinted with permission from Misra et al. (2011). Copyright © The American Society for Bone and Mineral Research, 2011)

Testosterone is bone anabolic and antiresorptive, and in both males and females with AN, lower testosterone levels are associated with lower BMD (Misra et al. 2008a, Soyka et al. 2002). Data are lacking regarding the impact of testosterone replacement on BMD in males with AN. However, in adult women, transdermal testosterone replacement to approximate levels in the upper half of the normal range for women did not increase BMD, despite an early increase in bone formation markers (Miller et al. 2011). One group has demonstrated a maintenance of BMD Z-scores in adolescent and young adult women with AN given 50 mg of dehydroepiandrosterone daily with a combined oral contraceptive pill (Divasta et al. 2012).

Hypogonadism and Neurocognitive and Mood Outcomes

Hypogonadism is associated with impaired cognitive function, with reduced scores on verbal memory and executive function (Baskaran et al. 2017c), which improve with physiologic estrogen replacement (Baskaran et al. 2017b). Also, physiologic estrogen replacement using the transdermal 17β-estradiol patch improves trait anxiety in girls with AN and prevents the increase in state anxiety and body dissatisfaction with weight gain observed in girls randomized to placebo (Misra et al. 2013). In adult women with AN, lower testosterone levels are associated with higher anxiety and depression scores (Miller et al. 2007).

Hypothalamic-Pituitary-Adrenal Axis

The hypothalamic-pituitary-adrenal axis is in a state of overdrive in AN. Increased cortisol secretion is a consequence of increased pulse frequency and reduced clearance (Boyar et al. 1977; Misra et al. 2004b) (Fig. 3). There is also less suppression of cortisol following administration of oral glucose load in adolescents with AN (Misra et al. 2004b) and with the dexamethasone suppression test in adults with AN compared with controls (Duclos et al. 1999; Estour et al. 1990). Higher cortisol levels are associated with lower BMI and body fat, and weight gain is associated with a reduction in cortisol pulse frequency (Misra et al. 2004b). Cortisol levels are typically not elevated to any more than twice the upper limit of normal.

High cortisol levels are an appropriate adaptive response to undernutrition as cortisol is gluconeogenic, and higher cortisol levels would increase availability of gluconeogenic substrate by increasing proteolysis and help maintain euglycemia. Consistent with this, higher cortisol levels in AN are associated with lower extremity lean mass (Misra et al. 2005a).

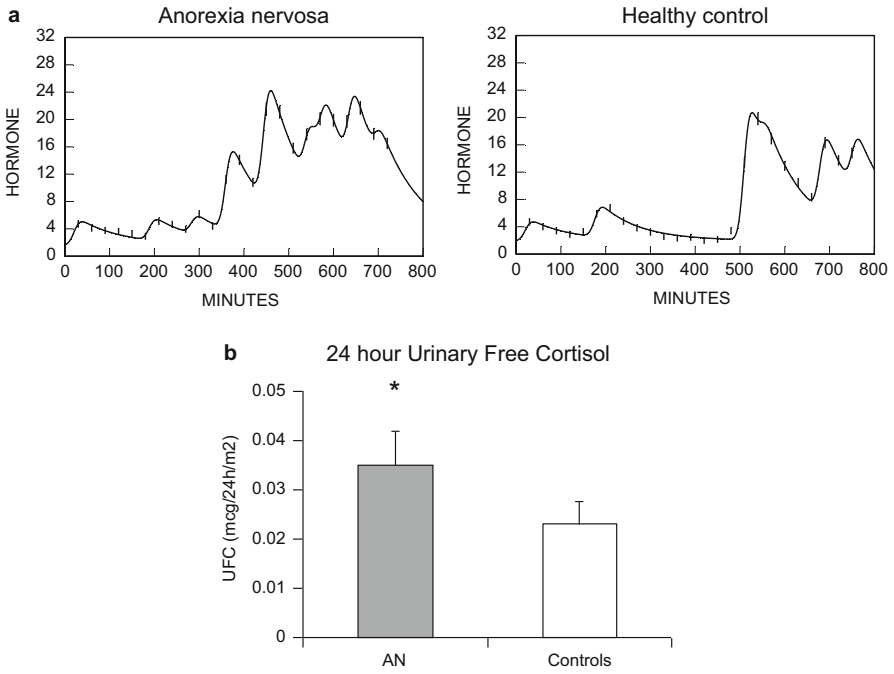

Fig. 3 Cortisol concentrations in adolescent girls with anorexia nervosa compared with healthy normal-weight controls. (**a**) Overnight serum cortisol concentrations (assessed every 30 minutes) in a teenage girl with anorexia nervosa (AN) and a healthy normal-weight control, indicating greater cortisol secretion in the girl with AN. (**b**) 24-h urinary free cortisol levels in adolescents with AN and controls 12–18 years old, showing higher mean urinary free cortisol excretion in AN. (Adapted with permission from Misra et al. (2004b). Copyright © The Endocrine Society 2004)

Hypercortisolemia is deleterious to gonadotropin releasing hormone (GnRH) pulsatility and contributes to suppression of the hypothalamic-pituitary-gonadal axis (Misra and Klibanski 2014). It also has a deleterious effect on bone by increasing bone resorption, suppressing bone formation, reducing gut calcium absorption, and impairing renal handling of calcium. In a study of adolescent girls with AN, higher cortisol levels were associated with lower levels of bone turnover markers and lower BMD (Misra et al. 2004b; Lawson et al. 2009). Normalization of cortisol following recovery should result in improved bone outcomes. In one study, cortisol levels were inversely correlated with anxiety and depression scores (Lawson et al. 2009).

Growth Hormone (GH)- Insulin-like Growth Factor-1 (IGF-1) Axis

AN is characterized by a nutritionally acquired GH resistance, associated with low IGF-1 levels despite high GH concentrations (Misra et al. 2003a, 2004c; Stoving et al. 1999) (Fig. 4). GH secretion is increased in adolescents and adult women with AN, consequent to increased GH pulse mass and frequency (Misra et al. 2003a, 2004c; Stoving et al. 1999). Lower IGF-1 (through reduced negative feedback) and glucose levels and higher ghrelin predict higher GH concentrations (Misra et al. 2003a, 2005b). An increase in GH in those with lower glucose levels is an appropriate adaptive response to increase gluconeogenesis to help maintain euglycemia. This gluconeogenic effect stems from the lipolytic effect of GH, which increases availability of gluconeogenic substrate. In girls with AN, higher GH concentrations are associated with lower trunk fat, consistent with this lipolytic effect (Misra et al. 2005a). Ghrelin is a GH secretagogue; thus, higher ghrelin levels also contribute to increased GH secretion in AN (Misra et al. 2005b). In girls with AN, there is reduced suppression of GH following an oral glucose load, compared to controls (Misra et al. 2004c).

Hepatic resistance to GH results in lower systemic IGF-1, a bone trophic hormone. This hepatic resistance may be mediated via increased fibroblast growth factor-21 (FGF-21), which is induced in the liver during fasting and inhibits IGF-1 secretion by inhibiting signal transducer and activator of transcription-5 (Fazeli et al. 2010b). GH resistance also results from downregulation of GH receptor expression in the liver, as suggested by lower levels of the GH binding protein (the cleaved extracellular component of the GH receptor).

Low IGF- 1 levels are deleterious to the hypothalamic-pituitary-gonadal axis and bone. In addition to hepatic GH resistance, AN is characterized by skeletal GH resistance. Typically, GH should increase surrogate markers of bone formation and resorption. Consistent with this, in normal-weight healthy girls, higher GH concentrations are associated with higher levels of bone turnover markers. This is not seen in girls with AN despite higher GH levels (Misra et al. 2003a). Further, administration of supraphysiologic doses of recombinant human (rh) GH to adult women with AN over a 3-month period (up to 5–6 times physiologic dosing) was not associated with a significant increase in IGF-1 or bone turnover markers, despite a reduction in

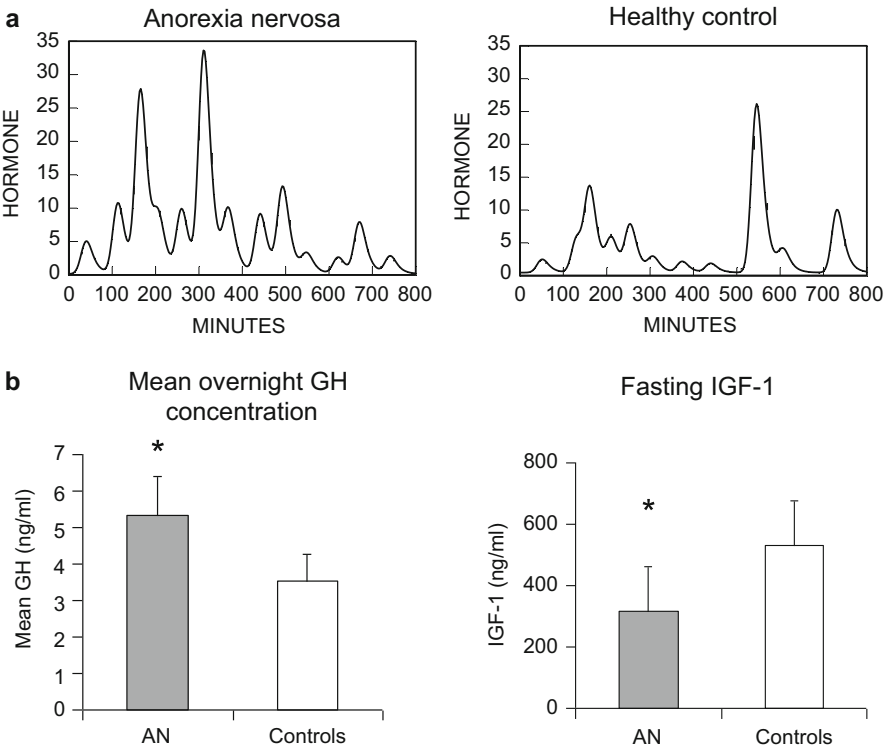

Fig. 4 Impact of anorexia nervosa on growth hormone (GH) and insulin-like growth factor-1 (IGF-1) concentrations. (**a**) Overnight growth hormone (GH) concentrations in a teenage girl with anorexia nervosa (AN) and a normal-weight healthy control indicating increased GH secretion in the girl with AN. (**b**) Mean overnight GH and fasting IGF-1 levels in adolescents with AN and controls 12–18 years old. IGF-1 levels were lower in girls with AN despite higher GH concentrations, indicative of a nutritionally acquired GH resistance. (Adapted with permission from Misra et al. (2003a). Copyright © The Endocrine Society 2003)

body fat (Fazeli et al. 2010a). In contrast, rhIGF-1 administration in replacement doses increased bone formation markers in adult and adolescent females with AN (Grinspoon et al. 1996a, 2002; Misra et al. 2009), and when administered with estrogen over a 9-month period increased BMD in adult women with AN (Grinspoon et al. 2002).

Hypothalamic-Pituitary-Thyroid Axis

Very low weight individuals with AN demonstrate a thyroid profile that resembles the sick euthyroid syndrome. Levels of total triiodothyronine (T3) and free thyroxine (T4) are low normal or low, and thyroid stimulating hormone is

typically normal or low normal (Estour et al. 2010; Misra and Klibanski 2014). Lower thyroid hormone levels should help reduce brown fat thermogenesis and resting energy expenditure, thus preserving energy for vital functions (Singhal et al. 2016). Low or low normal levels of total T3 and free T4 in AN do not require levothyroxine replacement.

Hormones Regulating Food Intake

Leptin is an anorexigenic hormone secreted peripherally by adipocytes. Levels are very low in AN and correlate with fat mass (Grinspoon et al. 1996b; Misra et al. 2005c). Leptin stimulates GnRH secretion, and low leptin levels contribute to hypogonadotropic hypogonadism in AN. In fact, administration of rh leptin (metreleptin) in replacement doses to adult women with FHA resulted in resumption of menstrual cycles in up to 70% of the women and normal ovulation in about 40% (Welt et al. 2004; Sienkiewicz et al. 2011). Leptin is also bone anabolic, and replacement doses of metreleptin increased bone formation markers (Welt et al. 2004). However, consistent with its anorexigenic effects, women receiving metreleptin had a decrease in appetite and body weight over 3 months in one study (Welt et al. 2004) and a decrease in body fat despite careful dose titration to prevent weight loss in a 9-month study (Sienkiewicz et al. 2011). This anorexigenic effect argues against its use in AN.

Ghrelin is a GH secretagogue with orexigenic effects, secreted primarily from the gastric fundus (Nakazato et al. 2001). Adolescent and adult females with AN have high levels of ghrelin compared with controls (Misra et al. 2004c, 2005b; Sedlackova et al. 2012; Stock et al. 2005), with lesser reductions following an oral glucose load (Misra et al. 2004c). Ghrelin administration inhibits gonadotropin secretion in rodents and humans (Kluge et al. 2012), and high ghrelin levels in AN likely contribute to hypogonadotropic hypogonadism. Ghrelin has stimulatory effects on osteoblasts, and in healthy controls, higher ghrelin levels are associated with higher levels of bone formation markers. This association is not evident in AN, suggesting a state of ghrelin resistance (Misra et al. 2005b).

Low levels of leptin and high levels of ghrelin are consistent with an adaptive mechanism to stimulate food intake and increase energy availability in AN. In contrast, peptide YY (PYY), an anorexigenic hormone secreted by the L-cells of the gut, fails to demonstrate this adaptive response and its levels are unexpectedly high in AN (Misra et al. 2006a). PYY is deleterious to osteoblastic activity, and consistent with this, higher PYY levels in AN are associated with lower levels of bone formation markers and lower BMD (Misra et al. 2006a; Utz et al. 2008). A PYY antagonist may thus be beneficial to bone; however, such an agent has not been studied at this time. Oxytocin is another hormone that is anorexigenic and bone anabolic. Levels are appropriately low in AN, and lower levels are associated with lower BMD (Lawson et al. 2011).

Bone Metabolism

A major consequence of the endocrine changes in AN is impaired bone metabolism. Bone metabolism is deleteriously affected because of the associated hypogonadism, hypercortisolemia, GH and ghrelin resistance, low IGF-1 and leptin, and high PYY levels (Misra and Klibanski 2014). Additional factors contributing to impaired bone metabolism are low BMI and reduced lean mass (Misra et al. 2004a). AN is also associated with reduced marrow fat at the spine and femur, which in turn is associated with lower BMD (Bredella et al. 2009). This is consistent with the bone-fat hypothesis of a common progenitor cell giving rise to both adipocytes and osteoblasts; certain conditions may induce transcription factors that stimulate one pathway at the expense of the other. Thus, an increase in marrow adipogenesis may be associated with reduced osteoblastogenesis and lower bone formation. Also, certain medications are deleterious to bone. In particular, use of selective serotonin reuptake inhibitors for more than 6 months has been associated with lower BMD in AN, even after controlling for duration of illness or amenorrhea (Misra et al. 2010).

Adults with AN have an uncoupling of bone turnover, with an increase in bone resorption and a decrease in bone formation markers (Grinspoon et al. 1996a). Conversely, adolescents with AN demonstrate a coupled decrease in bone formation and resorption markers (Misra et al. 2011, 2003b), in contrast to normal adolescence, a high bone turnover state. In adults with AN, one study reported areal BMD T-scores of <-1 at one or more sites in 92% and T-scores of <-2 in 38% of the women (Grinspoon et al. 2000). In adolescents with AN, we have reported Z-scores of <-1 at one or more sites in 52%, and Z-scores of <-2 in about 11% (Misra et al. 2004a). The lumbar spine appears to most severely affected in females with AN, though total hip, femoral neck, and whole body BMD Z-scores are also low in these women. One study (Schorr et al. 2017) examined BMD outcomes in women with AN and atypical AN using both DSM-IV and DSM-5 criteria. BMD Z-scores were <-1 in 78% of AN diagnosed by DSM-IV, 82% of those diagnosed by DSM-5, and 69% of atypical AN.

Recent studies have examined bone geometry and microarchitecture in AN using high resolution peripheral quantitative computed tomography (Faje et al. 2013b; Lawson et al. 2010; Milos et al. 2005; Singhal et al. 2018). At the nonweight bearing radius, compared with controls, adolescents with AN have lower total and trabecular volumetric BMD (vBMD), cortical thickness, trabecular number and thickness, and higher cortical porosity associated with reduced estimated strength (Faje et al. 2013b). At the weight-bearing distal tibia, they have greater cortical porosity, lower total and cortical vBMD, cortical area and thickness, trabecular number, and strength estimates than controls (Singhal et al. 2018). In another study of adolescents with AN, trabecular bone score (measure of bone texture) showed evidence of degraded microarchitecture in over 40% of study participants and correlated with BMD and strength estimates (Donaldson et al. 2015). In adult women with AN, at the distal radius, total, cortical and trabecular vBMD is lower than in controls. They also have lower cortical thickness, trabecular number and thickness, and increased trabecular separation, associated with lower estimated strength (Milos et al. 2005).

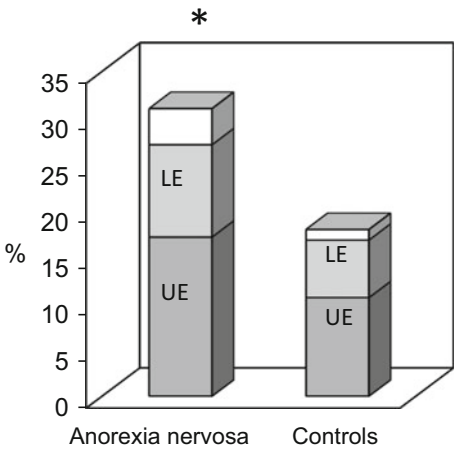

Fig. 5 Prevalence of fractures in adolescent and young adult women with anorexia nervosa (AN) 12–22 years old compared with healthy normal-weight controls. Adolescent and young adult women with anorexia nervosa (AN) 12–22 years old had a higher prevalence of fractures (31%) than normal-weight controls of comparable age (18%); however, the proportion of upper extremity (UE), lower extremity (LE), and nonextremity fractures did not differ between groups. (Adapted with permission from Faje et al. (2014). Copyright © 2014 Wiley Periodicals, Inc.)

Further, vertebral integral vBMD and estimated vertebral strength are lower in AN than in controls, associated with lower BMI and longer duration of amenorrhea (Bachmann et al. 2017).

Lower BMD and impaired bone geometry and structure translate to increased fracture risk in both adolescents and adults with AN (Faje et al. 2014; Vestergaard et al. 2002). One study reported a 31% prevalence of fracture in adolescent girls with AN compared to 19% in controls (60% increase) (Fig. 5), with fracture rates peaking after diagnosis. In adults, the incidence rate ratio of fracture was 1.98 and also increased after diagnosis. This increased risk persisted for 10 years postdiagnosis (Vestergaard et al. 2002). The distribution of fractures is similar to controls, with extremity fractures being the most common (Faje et al. 2014).

Management of Low Bone Mineral Density

Individuals with AN should be assessed for areal BMD using dual energy x-ray absorptiometry (DXA) (Gordon et al. 2017). Because adolescence is a critical window in time to optimize bone accrual towards attainment of peak bone mass, and because deficits incurred during this time may well be permanent, it is important to monitor BMD at least annually in adolescents with AN. However, DXA measurements may also be repeated every 6 months, if clinically necessary (Gordon et al. 2017).

Calcium and vitamin D intake should be optimized in all patients. Vitamin D intake should be at least 600 IU daily and adjusted to maintain 25(OH) vitamin D

levels in the 32–50 ng/ml (80-125 nmol/L) range. Calcium intake should be at least 1300 mg daily. Although studies have shown that in general adolescents and adults with AN do better for calcium and vitamin D intake than controls (Hadigan et al. 2000; Misra et al. 2006c), serum 25(OH) vitamin D levels of <50 nmol/L are associated with lower BMD Z-scores (Gatti et al. 2015).

A critical aspect of management of low BMD in AN is weight gain and menstrual recovery. All individuals with AN should have a treatment team in place that includes an adolescent medicine physician or internist with expertise in managing eating disorders, a dietician, and a psychologist or psychiatrist. An increase in caloric intake is critical to recovery, along with curtailing of exercise activity (Gordon et al. 2017). The goal is to get to a weight that corresponds to >90% of median BMI for age. Even after the patient gets to a "healthy" weight, it may require 6–12 months of weight maintenance or continued weight gain before menses resume. At the time of menstrual recovery, girls are about 2 kg heavier than when they lose their periods (Golden et al. 1997). It is thus important to set realistic expectations of when to expect menstrual recovery.

Weight gain and menstrual recovery are associated with improved bone accrual in adolescents with AN, although residual deficits may persist (Misra et al. 2008c). One study suggests that the effect of weight gain on BMD may be attenuated when 25 (OH) vitamin D levels are < 30 ng/ml (75 nmol/L) (Giollo et al. 2017). In adults, weight gain alone leads to improved hip BMD, whereas menstrual recovery alone results in improved spine BMD. Women gaining weight and resuming menses demonstrate improved BMD at all sites (Miller et al. 2006).

In girls whose BMD Z-scores are <−2 and decreasing over time, and in those with evidence of skeletal fragility, one may consider short-term physiologic estrogen replacement to improve bone accrual (Misra et al. 2011), after a reasonable trial (6–12 months) of nutritional, psychological, and modified exercise intervention (Gordon et al. 2017). When the bone age is at least 14 years, we use the 100-mcg transdermal 17β-estradiol patch applied continuously (changed weekly or biweekly depending on the preparation) with 12 days of cyclic progesterone every month. For the progesterone component of therapy, we prefer 200 mg of micronized progesterone given daily for 12 days of each month; however, a progestogen such as medroxyprogesterone acetate or norethindrone could also be used. Before starting estrogen replacement therapy, contraindications to estrogen administration should be ruled out, and patients counseled about the risks of estrogen and progesterone/progestogens.

Combined estrogen-progesterone containing oral contraceptive pills should not be given for the purpose of achieving regular menses or improving bone outcomes. Regular menses induced by such medications do not help recovery and may mask return of spontaneous menses. Thus, bone loss may persist, particularly if energy deficit is not addressed (Gordon et al. 2017). Further, randomized controlled studies have clearly shown that combined oral contraceptive pills do not increase BMD in AN (Klibanski et al. 1995; Strokosch et al. 2006), although those with very low BMD have gain some benefit. However, adolescents and young women desiring oral contraceptives for birth control should not be denied this option. At the same time, consideration should be given to other forms of contraception, such as an intrauterine

device (IUD). Progesterone-releasing IUDs have gained popularity in recent times; however, they can lead to irregular menses or amenorrhea, masking the timing of menstrual resumption.

When adult women with AN have BMD Z-scores of <-2 or evidence of skeletal fragility, a question is whether they should be treated with pharmacologic options such as bisphosphonates, teriparatide, or denosumab. One study in adults reported a beneficial effect of risedronate (35 mg weekly), with 3–4% an increase in spine BMD and 2% increase in hip BMD over a year compared to placebo (Miller et al. 2011). In contrast, a 12-month study in adolescents reported no increase in lumbar spine BMD and only a small increase in femoral neck BMD with weekly alendronate therapy compared to placebo (Golden et al. 2005). Concerns persist regarding the use of bisphosphonates in adolescents and adult women of childbearing age given their very long half-life, although available data thus far are reassuring.

In a 6-month randomized controlled trial of teriparatide versus placebo in older adult women with AN, 20 mg teriparatide (PTH_{1-34}) SC daily was successful in increasing BMD by 6–10% at the AP and lateral lumbar spine, but not at the hip (Fazeli et al. 2014). The increase in BMD was associated with an increase in bone formation markers. More and longer-term studies are necessary to confirm these findings. In adolescents with AN, teriparatide should not be used, because of the black box warning it carries with respect to risk for osteosarcoma (based on rodent studies). The Endocrine Society guidelines for hypothalamic amenorrhea suggest that short-term use of teriparatide is an option in the setting of delayed fracture healing and very low BMD (Gordon et al. 2017). Studies of denosumab are currently lacking in AN.

Conclusion

AN is associated with alternations in multiple endocrine axes, an adaptive mechanism in this state of severe undernutrition. However, these endocrine changes (together with low BMI and lean mass) contribute to impaired bone metabolism and increased fracture risk. Neuroendocrine changes may also impact neurocognitive and mood outcomes. A multidisciplinary treatment team is necessary for management of AN and to optimize nutritional repletion. Management should include optimizing vitamin D levels. Options for treatment of low BMD in AN are few and include physiologic estradiol replacement with cyclic progesterone (in adolescents with AN), and rarely bisphosphonates or teriparatide (in adults with AN).

Policies and Protocols

In this chapter, we have described the endocrine and bone complications that are associated with anorexia nervosa, and their management. Here we highlight three key aspects of management of low bone density in anorexia nervosa:

1. A multidisciplinary team is necessary to manage the psychological, nutritional, hemodynamic and endocrine consequences of anorexia nervosa. This team includes a psychologist+/- psychiatrist, dietician, eating disorder specialist (adolescent medicine physician/pediatrician/internist), and an endocrinologist. Regular and frequent follow-up appointments are necessary to ensure that weight gain goals are being met. Weight gain and menstrual resumption are a critical first step to optimizing bone accrual and bone density in anorexia nervosa. It may take 6-12 months of being at or above target weight (as determined by the eating disorder specialist) before menses resume.
2. It is important to optimize calcium and vitamin D intake as low vitamin D levels may impair bone accrual during weight regain. Calcium intake should be in the range of 1200-1500 mg/day (dietary and supplements). Vitamin D intake should be adjusted to maintain 25(OH) vitamin D levels between 32-50 ng/ml (80-125 nmol/L).
3. Transdermal 17-β estradiol (100 mcg administered continuously) with cyclic progesterone/progestogen (for 12 days of every month) may be necessary to improve bone accrual rates in adolescent girls whose bone mineral density Z-scores are low and decreasing over time, or if there is evidence of skeletal fragility. Combined estrogen-progestogen containing oral contraceptive pills are not effective in increasing bone density in adolescent or adult women with anorexia nervosa, and should not be administered to 'regulate' menstrual periods. They mask return of spontaneous menses without any beneficial bone effects. Oral contraceptive pills should not be denied to women requesting these for contraception, if other strategies for contraception are contraindicated or not desired. In these situations, women should be informed that oral contraceptive pills may mask the return of spontaneous menses without improving bone health.

Dictionary of Terms

- **Functional hypothalamic amenorrhea** – This is a form of hypogondatropic hypogonadism caused by conditions such as undernutrition, excessive exercise, and emotional stress, where there is no organic pathology. It is a diagnosis of exclusion.
- **Physiologic estrogen replacement** – This refers to replacement of estrogen in doses that mimic physiological levels, with estrogen administered as 17β-estradiol, the natural form of estrogen
- **Combined oral contraceptives** – This refers to oral contraceptive pills that contain ethinyl estradiol (10–35 mcg), and a progestogen (such as norethindrone, levonorgestrel, desogestrel, norgestimate, and ethynodiol diacetate (among others)). Neither the estrogen nor the progestogen is a physiologic form of the hormone.
- **Bone anabolic hormone** – One that increases bone formation
- **Antiresorptive effect** – Effect resulting in decreased bone resorption
- **Anorexigenic** – Appetite suppressing
- **Orexigenic** – Appetite stimulating

Summary Points

- Anorexia nervosa is associated with alterations in several neuroendocrine axes (adaptation to the state of undernutrition)
- Endocrine changes include functional hypothalamic amenorrhea (hypogonadotropic hypogonadism), relative hypercortisolemia, a nutritionally acquired growth hormone resistance with low insulin like growth factor-1 levels, low levels of leptin and oxytocin, and high levels of ghrelin and peptide YY.
- Low body mass index, reduced lean mass, and hormonal alterations contribute to low bone mineral density in adults and reduced bone accrual in adolescents with anorexia nervosa leading to suboptimal peak bone mass acquisition and increased fracture risk.
- A multidisciplinary treatment team is necessary to manage patients with anorexia nervosa.
- Weight gain and menstrual recovery improve bone mineral density in adults and bone mineral accrual in adolescents with anorexia nervosa at multiple sites (though residual deficits persist).
- Calcium and vitamin D intake should be optimized.
- Transdermal 17β-estradiol in replacement doses (100 mcg) with cyclic progesterone or a progestogen may be considered in adolescents with a bone age of at least 14 years, whose bone mineral density Z-scores are low and decreasing over time, or if there is evidence of skeletal fragility.
- Combined oral contraceptives are not effective in increasing bone mineral density and mask spontaneous resumption of menses. They should be avoided except for reasons of contraception.
- Bisphosphonates increase spine and hip bone mineral density in adults with anorexia nervosa, but benefits in adolescents are limited only to the hip; if considered for treatment, they should be administered with great caution given their long half-life.
- Teriparatide increased bone mineral density at the spine in older women with anorexia nervosa in one small study – these data need to be confirmed with additional studies.

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Male Anorexia as an Eating Disorder: Similarities and Differences with Anorexia Nervosa in Women

34

Karin Sernec and Špela Brecelj

Contents

Abstract

Anorexia nervosa is a chronic mental disorder manifested as persistent restriction of energy intake leading to significantly low body weight, intense fear of gaining weight or becoming fat, and disturbance in body schema. The title of this chapter, which so explicitly relates to men, aims at overcoming the myth that eating disorders affect only female patients; at the same time, it also suggests that eating disorders, including anorexia nervosa, are nevertheless gender-related. According to some recent studies, men represent as much as one fourth of all anorexia patients. Male anorexia nervosa is similar to female anorexia nervosa in many

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respects, but there are also gender- and sex-specific traits related to the history of body weight fluctuations, the type of compensatory behavior used to achieve weight loss, the patient's motivation for weight loss, the frequency of recorded sexual abuse, sexual identity, and sexual orientation, any recognized comorbidity, and the body schema and body image, in conjunction with the increasing frequency of objectification and sexualization of the male body in the media. Since amenorrhea is no longer an essential diagnostic criterion for anorexia nervosa, the diagnosis of this disorder in male patients has become easier. This may be one of the reasons for the increasing incidence of this disorder among men in recent years. It makes sense for this trend to be accompanied by increased attention from researchers and clinicians if they are to meet this population's specific therapeutic needs.

Keywords

Males · Anorexia nervosa · Eating disorders · Energy restriction · Diagnosis · Psychotherapy · Comorbidity · Gender identity · Compulsive exercise · Body schema · Body image

List of Abbreviations

AN	Anorexia nervosa
ASD	Autistic spectrum disorder
BMI	Body mass index
DSM-V	Diagnostic and statistical manual of mental disorders, 5th edition
ED	Eating disorder(s)
EDE	Eating disorders examination
EDE-Q	Eating disorder examination questionnaire
EDI-3	Eating disorder inventory-3
EKG	Electrocardiogram
ICD-10	International statistical classification of diseases and related health problems, 10th revision
IGT	Iowa gambling task
MPMW	Matched population mean weight

Introduction

Anorexia nervosa (AN) is a chronic mental disorder manifested as a dysfunctional attitude toward food. It represents an external expression of a profound emotional distress and a lack of self-acceptance. Through food restriction and/or purgative behavior, patients maintain an excessively low body weight (below 85% of their ideal weight). This is accompanied by a strong fear of weight gain, along with the signs of an endocrine dysfunction. Clinical experience shows that various eating

disorders (ED) are parts of the same spectrum, they can morph into each other in the same patient and ultimately they all lead to undernourishment. This chapter focuses on AN, in which undernourishment results primarily from restricted intake of micro- and macronutrients. It should be noted that the name anorexia nervosa (lat. from gr. *anorexis* = loss of appetite) is a misnomer, because patients do experience hunger and have appetite. The causes of undernourishment are much more complex and should be sought in a specific interweave of biologic, psychodynamic, and socio-cultural factors.

The title of this chapter, which so explicitly relates to males, aims at overcoming the myth that ED affect only female patients. At the same time, it also suggests that ED, including AN, are nevertheless gender-related. Epidemiological data has shown a significant percentage of men among patients with AN; according to some studies, it is as high as one fourth of all patients (Hudson et al. 2007). The finding that ED are not related exclusively to the female gender goes hand in hand with the finding that ED in males differ from those in females in many respects, and that gender plays a significant role in the development of such disorders (Stanford and Lemberg 2012).

With the present discrepancy between the quantity and quality of literature on AN in male and female patients, it is interesting to note that the first medical description of AN was gender-balanced: In 1689 Richard Morton described two cases of a "Nervous Consumption as a result of Sadness and Anxious Cares" (Silverman 1990, p. 3), one in Mr. Duke's daughter and one in Minister Steele's 16-year-old son (Silverman 1990). The description of a melancholic boy who fasts and starves himself to the extreme limits of his body can also be found in the records of eighteenth century physicians Robert Whytt and Robert Willan (Silverman 1990). The asceticism – which in terms of eating is very close to AN because of its restrictive nature, the fear of gaining weight, and the related disgust – can be recognized in both men and women through a significant part of history (Banks 1996). Siddhartha Gautama, Zarathustra, and Christian hermit saints come to mind. Furthermore, AN can also be recognized in the diary entries of Lord Byron (Morgan 2008) and Franz Kafka (Morgan 2010). In the nineteenth century, AN was not named "anorexia hysterica" exactly because this disorder was also recognized in men, who traditionally could not be characterized as hysteric (Crisp and Burns 1983).

Modern epidemiological studies state a 0.2–0.3% lifetime prevalence of AN among males, whereby the ratio of the genders ranges between 1:3 and 1:12 (Raevuori et al. 2014). It is probable that the differences in data and differences in prevalence rates between the genders at least partially reflect insufficient recognition of AN among male patients, as well as the stigma and shame which prevent many men from seeking medical help for this disorder which is still considered "female" among both lay persons and medical professionals (Wooldridge and Lytle 2012; Raevuori et al. 2014). The difficulties experienced by young men in recognizing their own ED because of inadequate, female-oriented information have been reported (Raisanen and Hunt 2014).

The Problem of Diagnosis

The diagnostic questionnaire EDE-Q proved to have a lower reliability when applied to men (Reas et al. 2012). Similar findings were also noted in the case of EDI-3 (Stanford and Lemberg 2012) and EDE (Darcy et al. 2012). Women received a significantly higher number of points for items related to the desire for being thin and for dissatisfaction with their bodies (Stanford and Lemberg 2012). The majority of diagnostic tools for ED is adjusted to the female population and the specifically female set of symptoms; they are also validated only in women (Boerner et al. 2004). This is because, until recently, it was believed that men represent less than 10% of all persons suffering from ED (Crisp and Burns 1983).

Studies addressing the validity of diagnostic questionnaires for the male population have revealed a need for designing questionnaires that would contain specifically male topics: instead of focusing on the desire to lose weight, they should concentrate more on the patient's desire to increase muscle mass; instead of questions about satisfaction with the appearance of their hips, buttocks, and legs, questions on the patient's satisfaction with his stomach, pectorals, and arms are more informative. Regarding the symptoms of an ED, questions that are more relevant to men are related to compulsive overexercising (Fig. 1), fear of gaining weight on account of fatty tissue (Figs. 2 and 3), and preoccupation with the lean, muscular body ideal (Fig. 4). As proposed by the authors, if such questions were included, diagnostic questionnaires would become more sensitive to men (Darcy et al. 2012; Stanford and Lemberg 2012; Bjork et al. 2012).

The insistence on keeping amenorrhea among the essential diagnostic criteria for AN may be one of the main reasons for the blind spot which developed in the field of male AN in the middle of the previous century (Crisp and Burns 1983).

Fig. 1 Compulsive exercise. Patients feel compelled to overexercise and struggle with guilt and anxiety if they are not able to do so (Illustration by Alba Korošec 2017, published with permission)

Fig. 2 Food restriction without loss of appetite. Due to distorted body schema and morbid fear of gaining weight patients refrain from eating most foods while fantasizing about food most of the time (Illustration by Alba Korošec 2017, published with permission)

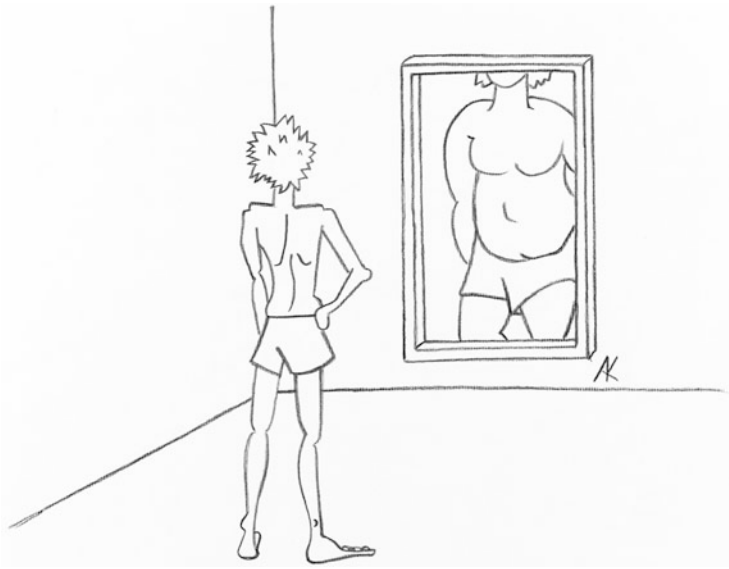

Fig. 3 Distortion of body schema. Patients erroneously perceive themselves as well fed or obese while often being dangerously undernourished (Illustration by Alba Korošec 2017, published with permission)

Fig. 4 Preoccupation with a muscular, lean body ideal. Exposure to the thin ideal and sexual objectification of males in mass media may lead to body dissatisfaction, internalization of the thin ideal, and disordered eating among young men (Illustration by Alba Korošec 2017, published with permission)

Table 1 Comparison of anorexia nervosa in male and female patients: History and presentation

	Male	Female
Pre-morbid history of body mass index	Commonly overweight or obese	Normal range body mass index
Compensatory behaviors	Overexercising	Self-induced vomiting, restriction
Motivation for dieting	Avoidance of obesity-related disease and lean, muscular body ideal	Thinness
Clinical signs of endocrine dysfunction	Loss of sexual interest and potency	Amenorrhoea
Comorbidity	Substance abuse	Depression
	Autistic spectrum disorder	Anxiety
	Underdiagnosed depression	Sexual abuse
	Unrecognized sexual abuse	Underdiagnosed substance abuse

The revised section on Feeding and Eating Disorders, DSM-V (American Psychiatric Association 2013) describes AN as a disorder which develops primarily in young girls, but the criterion of amenorrhea is no longer essential. While stating the frequency of AN in young women, it is added in the latest version of the ICD-10 (World Health Organisation 2016) that this disorder can also be found in adolescents and young men. In addition to amenorrhea, the male correlates of an endocrine disorder, namely loss of sexual interest and potency, have been added to the diagnostic criteria. In prepubescent boys, an endocrine disorder within the scope of AN is indicated by underdeveloped, juvenile genitalia (Table 1).

Clinical Presentation

In the majority of boys with AN, this disorder develops in early puberty, when major shifts in weight are common. Gaining more fatty tissue than muscles appears to be a risk factor for AN in boys and girls alike (Scott 1986). Not all men with AN are obese before the onset of this disorder; however, there are many who are indeed overweight (Fernandez-Aranda et al. 2004; Wooldridge and Lytle 2012). Unlike men, women with AN seldom have a history of obesity, they only feel overweight (Anderson 1990).

It is reported that men hospitalized for AN are on average older than women with the same disorder, they have a higher BMI upon admission, and also higher minimum and maximum BMIs, along with a higher ideal BMI (Raevuori et al. 2014). In men with AN, the duration of hospitalization is usually shorter than in women. Fewer suicide attempts are also reported, along with less somatization, and less frequent obsessive-compulsive symptoms or social anxiety (Raevuori et al. 2014). The same authors also found no differences between the genders in terms of mortality, binging and purging symptoms, and the abuse of laxatives, weight loss products, and psychoactive substances. For both sexes, the possibility of developing AN is higher in twins, triplets, and children with low gestational age at birth. Unlike women with AN, no correlation with the educational degree of the parents or the mother's higher body weight before pregnancy was found in men with this disorder (Raevuori et al. 2014).

Family Environment, Personality Traits, and Psychiatric Comorbidity

One of earlier descriptions of male AN states surprisingly varied family circumstances among adolescent men with AN (Beumont et al. 1972). Even decades ago, studies of family background in young men with AN showed a uniform distribution among all social classes, while women with AN were still perceived to originate from higher social classes (Scott 1986). More recent studies report certain similarities between the families of adolescent men with AN: they often come from single-parent families, and their fathers often live elsewhere or are deceased, while the mothers tend to be excessively controlling and protective, preventing their sons from developing full independence and autonomy, and having high expectations of their sons (Wooldridge and Lytle 2012).

Gila et al. (2005) found that there were no differences between young men with AN and healthy controls in terms of perfectionism and fear of adulthood (both characteristic of women with AN). Yet another group of researchers found a statistically significant association between perfectionism and starving in men (Forbush et al. 2007). Based on the differences in personality traits and comorbidity among women with the restrictive and purgative form of AN (Lavender et al. 2013), one might expect similar differences to be exhibited in men, but such specific studies are yet to be conducted. There are reports of less frequent abuse of laxatives in men, who

rather resort to compulsive physical activity, while the data on the frequency of other purgative behavior in men is not uniform (Murray et al. 2010; Nunez-Navarro et al. 2012, Gueguen et al. 2012).

Based on the mirror-image distribution by gender between autistic spectrum disorders (ASD) and AN, and a similar pattern of social and cognitive functioning, Zucker et al. (2007) posed a hypothesis that autistic spectrum traits are manifested as AN in female patients and ASD in males. Koch et al. (2015) found a higher cooccurrence of AN and ASD in male than in female patients. The cognitive profile of men with AN was also studied by Tchanturia et al. (2012), and they found a higher level of impulsivity in males than in female patients with AN, while compared to healthy controls both genders achieved much lower IGT scores, indicating poor decision-making.

In both genders, AN commonly presents along with depression, anxiety disorders, obsessive-compulsive disorders, personality disorders, and substance abuse. Researchers have found more similarities than differences between the genders in terms of psychopathology and comorbidity (Fernandez-Aranda et al. 2004; Ulfvebrand et al. 2015), but this finding is not universally accepted among researchers. A study on American veterans with ED included 25 men with AN, of whom 92% had at least one other psychiatric diagnosis. The frequencies of mood disorders was comparable to the values for female patients, but there was more abuse of alcohol and other substances, as well as more concomitant schizophrenia or psychotic disorders; this is consistent with earlier reports (Striegel-Moore et al. 1999). In men with ED, Bean et al. (2005) found less marked symptoms of psychopathological comorbidity, but in female patients more successful remission from these symptoms was noted during hospitalization. Study results are therefore not uniform, certainly also because of the relatively low prevalence of AN among men. Strother et al. (2012) consider the related stigma – which compels men to transform their depression and shame into auto- and heteroaggressive behavior and to abuse psychoactive substances – as the possible reason for meager data on the frequency of depression among males with ED. Data on different suicidality rates between the genders is contradictory; Bramon-Bosch et al. (2000) recorded a higher suicidality rate on a sample of 10 men with AN, while others have noted fewer suicidal attempts among males with AN (Gueguen et al. 2012).

Gender Identity, Sexual Orientation, and History of Sexual Abuse

Male AN is probably much more frequent than indicated by research data. One of the reasons why men find it more difficult to seek professional help might be the fear of being incorrectly characterized as homosexual (Birmingham et al. 2004). However, among men suffering from AN there are more heterosexual than homosexual men, and naturally, among homosexual men there are more of those who do not have AN than those who have it. ED thus do not only affect women and men who have sexual relations with men. But the percentage of homosexual, bisexual, and transsexual men among males with AN is indeed higher than in the general population, which

indicates a special vulnerability of these groups to develop ED (Ewan et al. 2014; Wooldridge and Lytle 2012). Wooldridge and Lytle (2012) and Dakanalis et al. (2012) have summarized the possible reasons for such vulnerability: like women, homosexual men are often exposed to greater pressures to maintain an attractive, lean, and muscular body. This population more frequently uses dieting for weight loss and exhibits more depression and dissatisfaction with one's own body. These individuals are also more prone to perceive themselves as sexual objects, and the history of childhood sexual abuse is more common among them. As summarized by Strother et al. (2012), in boys, the history of childhood sexual abuse is frequently associated with issues in forming their gender identity and sexual orientation, because the perpetrators are mostly male. Due to the stigma and shame which are disproportionally greater in male than in female victims of sexual abuse, the frequency of such trauma in males with AN is underestimated. Among patients with ED, approximately 30% have experienced sexual abuse, and a disproportionally greater number of abuse cases is recorded in females than in males (Strother et al. 2012).

Male AN is characterized by low serum testosterone with the resulting loss of sexual desire and impaired sexual function. This state of asexuality can serve to avoid taking on male sexual and social roles (Romeo 1994). The loss of interest in sex may in some cases persist in spite of appropriate body weight during remissions of AN (Beumont et al. 1972). In their study of sexual identity disorder in conjunction with AN in male monozygotic twins, Hepp et al. (2004) posed a hypothesis that starving and maintaining a very low body weight may be how these individuals inhibit their libido, limit the expression of secondary sex signs of their biological sex, and try to come closer to the female ideal of attractiveness. Similar findings have been reported by Ewan et al. (2014) regarding transsexual men in whom AN serves the function of preventing male-pattern weight gain. For this reason, authors recommend that sex change procedures, when appropriate, might be the only way towards remission of AN (Ewan et al. 2014).

Body Schema, Body Image, and Ideals

The problematization of to the objectification and sexualization of the female body in the media did not yield the desired results. The notorious "male gaze," which has transformed women into third-party observers of their own image, is now also directed against men. The number and explicit nature of objectification of men as sexual objects is increasing, and because of this the male body ideal moves even farther away from any realistic standards. Large shifts in the male body ideal are illustrated by action figure design that in the last 30 years developed to produce figures far more muscular than any human bodybuilder at their peak (Pope et al. 1999). Such sociocultural changes are not without consequences; a meta-analysis of 15 studies has shown a small, but statistically significant relationship between exposure to images of ideal male bodies among young men and their body dissatisfaction (Blond 2008).

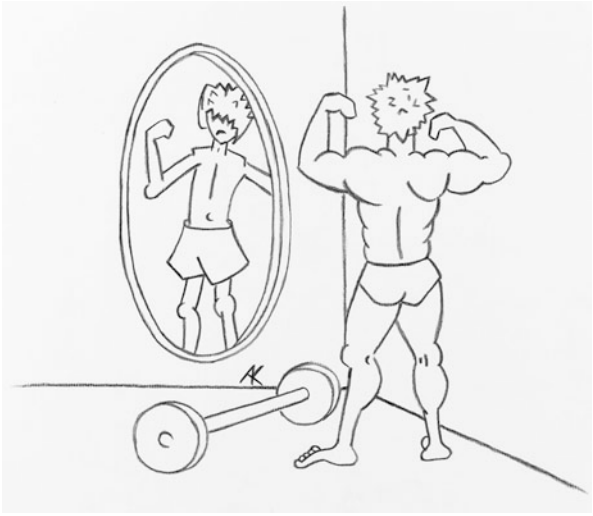

Fig. 5 Reverse anorexia as a body dysmorphic disorder. Unlike anorexia nervosa, bigorexia is characterized by a drive to be muscular, "big," while perceiving oneself as skinny, scrawny. This often leads young men to abuse anabolic steroids (Illustration by Alba Korošec 2017, published with permission)

The level of dissatisfaction with their own bodies among men has been on an increase in the past decades and has reached the level of dissatisfaction observed in the female population (Murray et al. 2010). Unlike women, who wish to be thinner, men more often desire larger and highly muscular bodies. This is often complicated by a disordered body schema. A study that included 17 men with AN, showed that these men do not differ significantly from healthy controls in terms of their ideal body type, but men with AN perceived themselves to be almost twice as fat as they objectively were (Mangweth et al. 2004). A study which compared the symptoms of bigorexia (muscle dysmorphia) and AN found that the only significant difference between these two groups was the marked motivation to gain muscle mass in the case of bigorexia, in which the abuse of laxatives and anabolic steroids is more frequent, while both groups experienced the same level of dissatisfaction with their body image (Fig. 5; Murray et al. 2010). In a study which compared male adolescents with AN to a healthy control group, Gila et al. (2005) found that both groups of young men overestimated the dimensions of their bodies, but those with AN exaggerated much more when estimating the circumference of their shoulders, hips, and thighs. The authors interpreted this difference as an indicator of a special, socioculturally determined meaning of these body parts for men with AN, in particular because these body parts are the same that worry women with AN (Gila et al. 2005).

Peer bullying and criticism regarding body weight is another common risk factor for developing ED. Young men with AN may be especially sensitive to teenage bullying, which targets their once excessive body weight (Sterling and Segal 1985).

Unlike female patients who are driven by a desire for thinness, according to research results summarized by Strother et al. (2012), the main motivation of male

patients to lose weight is to avoid diseases associated with obesity and to achieve better sports results. "Antigravitational" sports pose a high risk for the development of AN, so-called anorexia athletica (Sudi et al. 2004; Weltzin et al. 2012). In addition to sports in which a low body weight and a low body fat constitute a competitive advantage (ski jumps, running, horse riding, wrestling, diving, etc.), those sports in which the athlete's aesthetic appearance is very important (e.g., gymnastics, figure skating, dancing etc.) also represent a risk factor (Sudi et al. 2004; Baum 2006).

Biological Characteristics

Undernourishment during childhood and puberty has many consequences, the most readily visible being growth retardation. In AN, there are many hormonal changes which accompany starving and contribute to growth retardation: low levels of thyroxine (T_4) and triiodothyronine (T_3), increased cortisol levels, and low levels of sex hormones (Modan-Moses et al. 2003). In distinction from undernourishment during childhood, in which restoring of adequate nourishment is followed by accelerated bone growth, through which the patient can achieve the anticipated height (catch-up growth), undernourishment during puberty will lead to permanently reduced height. Since growing boys have greater caloric needs and a longer bone growth period than girls, undernourishment as part of AN may limit growth more in male than in female patients, although study results regarding this difference are not uniform (Modan-Moses et al. 2003).

By measuring bone mineral density in men and women with AN, Mehler et al. (2008) found that osteoporosis is disproportionally more common and more pronounced in men than in women, while the risk for it was proportionate to the patient's age, duration of the disease, and lower body weight. Like the reduced level of serum albumin, pancytopenia, electrolyte disturbances in the purgative type of AN, sinus bradycardia, other ECG abnormalities, and the starving-induced autophagia, which leads to increased serum transaminase levels, osteoporosis is also a common finding in both male and female patients with AN (Table 2; Sabel et al. 2012; Foppiani et al. 2014).

AN is characterized by hypogonadism, and the level of serum testosterone in males may not return to normal even after weight restoration, suggesting a need for testosterone replacement in such patients (Wooldridge and Lytle 2012). In males with AN, markedly reduced testosterone levels can be proved in urine; clinically, this is usually manifested as cessation of spontaneous morning erections, strongly reduced libido, and cessation of masturbation (Crisp and Burns 1983). The results of a longitudinal study monitoring serum leptin levels during weight gain and body fat gain within the scope of AN treatment of three young men (Wabitsch et al. 2001) support the hypothesis that leptin as the regulator of the hypothalamic-pituitary-gonadal function is responsible for normalizing gonadotropin and testosterone levels in men.

Table 2 Some of the relevant biological abnormalities found at admission in addition to low body weight with depleted body fat mass and muscle mass in males with anorexia nervosa

Abnormality at admission	% of patients	Total No. of male anorexia nervosa patients included in the study	Study
Reduced testosterone levels	100	14	Sabel et al. (2012), mean age 24 years, BMI range 11.5–15.9 kg/m^2
	66	3	Wabitsch et al. (2001), mean age 15,3 years, BMI range 12.5–17.3 kg/m^2
Growth retardation	92	12	Modan-Moses et al. (2003), mean age 14.3 years, BMI range 13.5–18 kg/m^2
Leukopenia	79	14	Sabel et al. (2012)
Cortical atrophy	70	10	Siegel et al. (1995), mean age 16 years, BMI range 10.9–16.9 kg/m^2
Anemia	70	10	Siegel et al. (1995)
	57	14	Sabel et al. (2012)
Increased transaminase levels	66	14	Sabel et al. (2012)
Reduced leptin level	66	3	Wabitsch et al. (2001)
Vitamin D deficiency	65	14	Sabel et al. (2012)
Mild-to-moderate hypophosphatemia	50	14	Sabel et al. (2012)
Thrombocytopenia	7	14	Sabel et al. (2012)

Therapy and Prognosis

The course of recovery from ED is slow and repeated admissions to treatment programs are common. The basic therapeutic approach in all AN patients involves the following main objectives: stabilization of the patient's somatic status with weight normalization and alleviation of permanent consequences of undernourishment; changing dysfunctional beliefs and behavioral patterns through the use of cognitive-behavioral therapeutic interventions; and overcoming obstacles to achieve remission of the ED by providing relief from affective disorders, anxiety, and other psychiatric comorbidities using pharmacotherapy in addition to psychotherapeutic management of past trauma, victimization, and any relationships which maintain the disorder (Weltzin et al. 2012). Through the prism of dynamic-analytical psychotherapy, the symptoms of AN are viewed as expressions of the patient's needs and issues, concerning control over one's life, power in interpersonal relationships, self-punishment,

and avoidance of the social role of a grown-up of one's own gender. Because the symptoms of AN are initially usually quite effective in fulfilling these needs, the disorder is egosyntonic, i.e., in harmony with one's own goals and consistent with one's own self-image. It is only after the symptoms become too destructive that the patient begins to be motivated for therapy (Sernec 2012). The management of AN can be done on an outpatient basis, in a hospital or a day hospital, within a live-in facility, or in group or individual psychotherapeutic setting.

Weltzin et al. (2012) suggest male-only therapeutic groups and emphasize that a separate male treatment program helps men overcome the biases of EDs being "female" disorders, and expressing of emotions being a sign of weakness.

In the available literature, less information can be found regarding motivation for treatment in males with AN than one would expect for such an important topic determining treatment outcome (Wade et al. 2009). In addition to insufficient motivation for treatment, resulting in high rate of drop-out (Sly et al. 2013), a possible source of complications in treating severe and enduring AN is also the potentially fatal refeeding syndrome, for which no differences in frequency have been found between the sexes (Sabel et al. 2012).

The full remission of AN, marked by the return of spontaneous menstrual activity in women, is less well defined in men. The increase in body weight to the range within 85% of the expected value may be too mild for the male population. This is because in the male body, fatty tissue accounts for a smaller percentage of total body weight and recovery would therefore be indicated by a weight which is much closer to the ideal body weight (Burns and Crisp 1990). In their study of treatment outcomes in 28 males with AN, Burns and Crisp (1990) posed the criterion of potency, which is indicated by sexual activity (intercourse, masturbation), as the equivalent of the menstrual activity criterion. According to their criteria, good treatment outcomes were achieved in those patients who were regularly sexually active and who restored their body weight to at least 85% of the MPMW (Table 3). Achieving only one of the two remission criteria signified intermediate outcome and failing to achieve either of the two criteria meant poor outcome (Burns and Crisp 1990).

Among 24 male patients included in their retrospective cohort study, Gueguen et al. (2012) reported three deaths, all of which were due to complications of the restrictive subtype of AN, and all of the three patients also discontinued their treatment early. In this study, long-term survival in the severe form of AN did not show any differences between the genders, while higher age, lower BMI upon

Table 3 Comparison of anorexia nervosa in male and female patients: Treatment outcome and recovery

	Male	Female
Indicators of good outcome	Normal weight Regular sexual activity and spontaneous morning erections	Normal weight Regular menstrual activity
Patient's experience of recovery	Exercise without compulsion Less worried about relapsing	More worried about relapsing

hospital admission, and the restrictive subtype of AN were shown to predict poor treatment outcomes (Gueguen et al. 2012).

Bjork et al. (2012) detected a difference between the sexes in how they experienced recovery from ED. Both shared a common understanding of recovery in the sense of acceptance of one's own body, improved confidence, better handling of emotions, better problems solving skills, more relaxed attitude toward food, and improved social life. However, men more often reported being able to exercise without compulsions, and they expressed less concern about relapsing than women (Bjork et al. 2012).

Conclusion

The growing rate of AN among young men is likely to continue in the future. In many respects, male AN is similar to that in women, but it also shows gender- and sex-specific issues that should be addressed in order to provide a comprehensive treatment for this population. The increasing scope of research in this complex field provides ever more pieces of this puzzle, although the pieces are often contradictory and rather than contributing to its understanding, they tend to exacerbate the enigmatic nature of this disorder. The initiatives for more intense research of ED in men, which would shed more light on this particular topic and enable timely, appropriate treatment for men with AN, reflect the current situation in the field of medical research in general, wherein up until recently researchers acted as if sex or gender did not matter and viewed a large majority of disease entities and treatment modalities as directly transferrable from men to women. The pluralism of gender identities, which is manifested as a real factor in the development of ED including AN, may show that the understanding of this phenomenon cannot be adequately approached by merely comparing the two traditional biological sexes and social genders and that it might be prudent to look beyond the dichotomy.

Policies and Protocols

A distinction should be made between life-saving interventions, in which motivation is not crucial, and comprehensive psychosocial management of anorexia nervosa, for which motivation is vitally important.

In men with anorexia nervosa, shame is one of the prevailing emotions, and it partially originates from the general belief that eating disorders are "female disorders." This is why staff working with these patients should be experienced in working with males and should show particular empathy and understanding of their specific issues.

It is important to pay special attention to any masked comorbidities, such as depression and anxiety. The possibility of concurrent abuse of both legal and illegal substances should be considered. The patients should be evaluated for concurrent overexercising and gently asked about their gender identity. In the case of transgender issues, management should include a broad team of specialists needed to lead the patient through the sex change process if appropriate.

Sexual abuse in males is often overlooked and therapists should always ask about it.

The level of sexual activity is an indicator of the seriousness of this disorder and of treatment outcome. Regular sexual activity which includes desire, fantasizing, masturbation, and intercourse reflects the level of testosterone and predicts a good outcome.

Family environment plays a significant role in the development of anorexia nervosa. Family involvement in the treatment of this disorder has beneficial effects on patient motivation and reflects the level of support available, so family members should be invited to participate whenever possible.

Dictionary of Terms

- **Body image** – A person's perception of the attractiveness of one's own body.
- **Body schema** – An internal awareness of the body and the relative shape and position of body parts.
- **Cognitive behavioural therapy** – A therapeutic approach that aims to modify distorted beliefs and cognitive processes that maintain disordered behavior.
- **Compulsive exercise** – Exercise that is continued despite overuse symptoms, injury, or social isolation.
- **Eating disorder** – A chronic mental disorder defined by abnormal, harmful eating habits that impact on daily functioning, usually accompanied by a high degree of anxiety around food and eating, body image distortions, and a preoccupation with food.
- **Impulsive behaviours** – Behaviors that are characterized by little or no forethought, i.e., explosive anger, binging and purging, unprotected casual sex, self-harming behaviors, substance abuse, excessive money spending, etc.
- **Manorexia** – A reference to anorexia nervosa in males.
- **Muscle dysmorphia** – Also known as reverse anorexia or bigorexia, a form of body dysmorphic disorder, but also classified as an eating disorder characterized by a pathological preoccupation with muscularity.
- **Perfectionism** – A multidimensional personality trait characterized by a strong drive for flawlessness, setting excessively high goals and performance standards, being overly self-critical, and concerned about others' evaluation.
- **Psychodynamic psychotherapy** – This therapeutic approach builds on the belief that all behaviors are derivatives of internal conflicts and unconscious motives, while symptoms are assumed to disappear with the completion of working through these issues.
- **Purgation** – Compensatory behavior undertaken in order to "un-do" eating or to compensate for the calories consumed, i.e., self-induced vomiting, misuse of laxatives or diuretics, enemas, and overexercising.
- **Restriction** – The reduction or elimination of certain foods or food groups from one's diet, usually the ones perceived as fattening or unhealthy.

Summary Points

- Anorexia nervosa is a mental disorder which develops mainly in women, although more recent studies have shown that up to 25% of such patients are male.
- Since anorexia nervosa is still considered a "female" disorder by lay persons and frequently also by medical professionals, this bias prevents men and their doctors/therapists from properly recognizing this disorder and providing timely and appropriate treatment.
- In both genders, its clinical presentation is manifested as undernourishment and gonadal dysfunction. However, there are also gender-specific traits that have to be considered in order to offer comprehensive therapeutic management.
- Males with anorexia nervosa commonly struggle with gender identity and sexual orientation issues, but this is not a rule and should not be made into a new stereotype.
- Males with anorexia nervosa more commonly than women have a history of being overweight and were exposed to peer bullying on account of obesity.
- The most common type of compensatory behavior in male anorexia nervosa is compulsive physical activity.
- In addition to an eating disorder, depression and sexual abuse are often overlooked in men.
- More often than in women with anorexia nervosa, male patients also suffer from the abuse of alcohol and other substances, as well as autistic spectrum disorders, schizophrenia, or psychotic disorders.
- In the media, increasing objectification and sexualization of the male body is observed. As the level of dissatisfaction with one's own body increases among young men, it approaches the level observed in women.
- The modern ideal male body involves an extremely lean and muscular physique. Male patients with anorexia nervosa share this ideal, but due to a disturbed body schema, they perceive themselves as much more overweight and as having more fat than is objectively true.
- Appropriate body weight, positive body image, and regular sexual activity indicate successful recovery from male anorexia nervosa.

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Part VI

Screening Tools, Classifications, and Applications

Subjective Global Assessment (SGA) of Malnutrition

35

Narayan Prasad and Archana Sinha

Contents

Abstract

Malnutrition is a highly prevalent and well-recognized problem in hospitalized as well as in general population but often goes unrecognized. Nutrition assessment is a comprehensive approach to define nutritional status using various methods.

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Subjective Global Assessment (SGA) is one of the most commonly used methods to assess nutritional status. SGA is a semiquantitative tool to assess nutritional status based on the history and physical examination. Data are scored subjectively and determine nutritional status in three major SGA categories as well nourished (A), mild to moderately malnourished (B), or severely malnourished (C). It is simple, noninvasive, inexpensive, and quickly executable and can be performed bedside by any skilled and trained health-care professional after brief training. It also agreeably goes with the objective methods of evaluating nutritional status. Various modified versions of SGA have been proposed since its conception to improve its precision. It helps in identifying malnutrition, predicting outcomes, and making appropriate recommendations in hospitalized patients as well as in general population.

Keywords

Malnutrition · Subjective Global Assessment · Nutritional status · Hospitalization · Mortality · Morbidity · Malnutrition and infection · Malnutrition and inflammation · Malnutrition in dialysis

List of Abbreviations

ASPEN	American Society for Parenteral and Enteral Nutrition
BIA	Bioimpedance analysis
BMI	Body mass index
CANUSA	Canada-USA
CKD	Chronic kidney disease
DEXA	Dual energy X-ray absorptiometry
DMS	Dialysis malnutrition score
ESPEN	European Society for Clinical Nutrition and Metabolism
ESRD	End-stage renal disease
KDOQI	Kidney Disease Outcomes Quality Initiative
MIS	Malnutrition-Inflammation Score
NKF	National Kidney Foundation
NRI	Hemodialysis
NRI	Nutritional Risk Index
PD	Peritoneal dialysis
PG-SGA	Patient-Generated-Subjective Global Assessment
SGA	Subjective Global Assessment
TIBC	Total iron-binding capacity

Introduction

Malnutrition is caused by an imbalance between nutrient intake and demand. “The deficient nutrient intake or uptake results into altered body composition in form of decreased fat-free mass and body cell mass leading to reduced physical and mental function and impaired clinical outcome from disease” (Sobotka 2012). Jensen et al.

(2009) defined malnutrition as "decline in lean body mass with the potential for functional impairment." Malnutrition in hospitalized patients may be different from general population. Butterworth defined malnutrition as the "skeleton in the hospital closet" (Butterworth 1974). It is an unrecognized or poorly identified ailment in hospitals (Waitzberg et al. 2001).

A little improvement has been observed in prevalence of malnutrition reported in 1970–1980s (Bistrian et al. 1974; Weinsier et al. 1979) as compared to few recent reports (Waitzberg et al. 2001; Kyle et al. 2003; Prasad et al. 2008). We have also observed that majority of ESRD patients (74.9%) had some degree of malnutrition at the initiation of dialysis in 1 of our study of 283 patients (Prasad et al. 2008). Exact estimation of the prevalence of malnutrition is challenging. Prevalence of in-hospital malnutrition has been estimated between 15% and 60% at admission (Amaral et al. 2010), and the prevalence goes up to the tune of 75% in patients with prolonged length of stay in hospital (Wysynski et al. 2003). However, this scenario may be different in developed and developing countries. In estimations from developed countries, it has been observed that malnutrition impacts at least one in three patients upon hospital admission (Tappenden et al. 2013; Lim et al. 2012). Two of three experiences further decline if left untreated during the hospital stay. At least, one in three *may become* malnourished who were not malnourished at admission during their hospital stay (Tappenden et al. 2013; Braunschweig et al. 2000). Overall varying from 20% to 50% of hospitalized adult patients may be malnourished (Kirkland et al. 2013).

Assessment of Malnutrition and SGA

Despite high prevalence and a well-recognized problem in hospitalized patients as well as in general population, there is lack of uniform definition and universal standard tool for estimating malnutrition. It poses challenges before clinicians and clinical nurses in quantifying malnutrition, and the assessment of the nutritional status of patients is not routinely carried out (Mowe et al. 2006; Elia et al. 2005). Nutrition assessment is a comprehensive approach to define nutritional status using medical, nutrition, and medication histories, physical examination, anthropometric measurements, and laboratory data (ADA 1994). ASPEN and the ESPEN have made recommendations for good clinical practices in screening of malnutrition (Ukleja et al. 2010; Kondrup et al. 2003).

The exact assessment of nutritional status is cumbersome in day-to-day practice. Severe degree of malnutrition may be clinically obvious, while mild degree of malnutrition is difficult to assess without objective parameters. Diverse nutritional assessment tools varying from anthropometry and biochemical parameters like serum albumin to sophisticated techniques DEXA and BIA to assess body composition are available. Although newer methods like BIA and DEXA give more comprehensive and objectivize values in assessment of nutrition status, however, these methods are technology dependent, time-consuming, and cost-effective in day-to-day practice and as a bedside tool is questionable. The anthropometric tool

(Ulijaszeki and Kerr 1999) and traditional biochemical indices (e.g., albumin, transferrin, retinol-binding protein) become less reliable and often invalid due to the influence of hydration status and coexisting inflammation, particularly in case of critical illnesses. Many non-nutritional factors affect the conventional nutritional tools resulting in their poor sensitivity and specificity (Gibson 1990; Jeejeebhoy 2000). The assessment of nutritional status should be performed with an amalgamation of valid, complementary measures rather than any single measure as no single parameter is sufficiently sensitive or specific to identify malnutrition. The assessment tools become more effective when used in combination (Gibson 1990).

This assessment can be a brief screening, when the patient is comparatively well nourished, or more comprehensive when the patient appears to be malnourished or at risk for malnutrition. It is recommended that nutritional assessment should be performed with easy, simple, rapid, inexpensive, and convenient methods especially when a large number of patients need to be evaluated within a short time. SGA is one of the most commonly used bedside methods to assess nutritional status.

Conventional Subjective Global Assessment

Conventional SGA is semiquantitative tool to assess nutritional status based on the history and physical examination. To surmount the shortcomings associated with traditional nutritional tools like only anthropometry, Baker et al. (1982a) developed this simple tool to assess nutritional status that used a questionnaire encompassing clinical history and physical examinations and classified nutritional status in three different categories well nourished, moderately malnourished, or severely malnourished (Baker et al. 1982a, b). Detsky et al. (1987) further standardized this tool naming it as SGA which encompasses five components of a medical history (weight change in previous 6 months, dietary intake, gastrointestinal symptoms continued for more than 2 weeks, functional capacity, comorbid illness and its relation to nutritional requirements) and three components of a brief physical examination (signs of fat, muscle wasting, and nutrition-associated alternations in fluid balance (presence of pedal/sacral edema/ascites) to assess nutritional status (Fig. 1a). Data are scored subjectively, and patients are classified in terms of the three major SGA categories determining nutritional status as well nourished (A), mild to moderately malnourished (B), or severely malnourished (C) just by subjective consideration of the data collected in the eight areas, without adhering to a rigid scoring system (Detsky et al. 1987). It provides a comprehensive appraisal of nutrition status just based on the features of a medical history and physical examination.

Benefits of SGA

SGA is simple, noninvasive, inexpensive, and quickly executable and can be performed bedside by any skilled and trained health-care professional after brief training. It is capable of identifying malnutrition (K/DOQI-NKF 2000) and gives a

global score of malnutrition. It correlates well with the subjective and objective aspects of medical history and physical examination (McCann 1996). It also agreeably goes with the objective methods of evaluating nutritional status (Enia et al. 1993). SGA can be easily used in routine clinical practice to assess the nutritional status of the patients. Simultaneously, it can also be used in making appropriate recommendations and strategies to prevent malnutrition.

Disadvantages/Limitations of SGA

Subjective nature of SGA limits its precision depending on the experience and skill of the observer in assessing malnutrition (Barbosa-Silva and Barros 2006). The visceral protein levels are not incorporated in this assessment. In spite of extensive use of SGA for initial nutrition assessment, very few studies have reported its use to assess changes in nutrition status over time (Baldwin and Weekes 2012).

a Features of subjective global assessment (SGA) adopted from Detsky et al , 1987

(Select appropriate category with a checkmark, or enter numerical value where indicated by "#.")

A. History

1. Weight change

Overall loss in past 6 months: amount = # _____ kg; % loss = # _____

Change in past 2 weeks: _____ increase,

_____ no change,

_____ decrease.

2. Dietary intake change (relative to normal)

_____No change,

_____Change _____duration = # _____ weeks

_____type: _____ suboptimal liquid diet, _____ full liquid diet

_____ hypocaloric liquids, _____ starvation.

3. Gastrointestinal symptoms (that persisted for >2 weeks)

_____none, _____nausea, _____vomiting, _____diarrhea, _____anorexia.

4. Functional capacity

_____ No dysfunction (e.g., full capacity),

_____ Dysfunction _____ duration = # _____ weeks.

_____ type: _____working suboptimally,

_____ambulatory,

_____bedridden.

5. Disease and its relation to nutritional requirements

Primary diagnosis (specify) _____

Metabolic demand (stress) : _____ no stress, _____low stress,

_____moderate stress, _____high stress.

B. Physical (for each trait specify: 0 = normal, 1+ = mild, 2+ = moderate, 3+ = severe).

_____loss of subcutaneous fat (triceps, chest)

_____muscle wasting (quadriceps, deltoids)

_____ankle edema

_____sacral edema

_____ascites

C. SGA rating (select one)

_____A = Well nourished

_____B = Moderately (or suspected of being) malnourished

_____C = Severely malnourished

Fig. 1 (continued)

b The seven point SGA with more objectivise global assessment (adopted from Lim et al, 2016)).

	RATINGS (circle one rating for each category)		
Ratings / Weight Loss 7 / 0% 6 / <3% 5 / 3<5% 4 / 5<7% 3 / 7<10% 2 / 10<15% 1 / ≥15% If weight trend, add 1 point, if weight trend within 1 month, minus point	**7 6**	**5 4 3**	**2 1**
If weight trend, add 1 point, if weight trend within 1 month, minus point **Dietary Intake (past 2 weeks)** 7) Good (full share of usual meal) 6) Good (>$^3/_4$ -<1 share of usual meal) 5) Borderline ($^1/_2$ - $^3/_4$ share usual meal), but increasing 4) Borderline ($^1/_2$ - $^3/_4$ share usual meal), no increasing or decreasing 3) Poor (<$^1/_2$ share of usual meal), but increasing 2) Poor (<$^1/_2$ share of usual meal), no increasing or decreasing 1) Starvation (<$^1/_4$ of usual meal)	**7 6**	**5 4 3**	**2 1**
Gastrointestinal symptoms (that persisted for > 2 weeks) Nausea:_____ vomiting: _____Diarrhea:_____ 7) NO symptom 6) Very few intermittent symptoms (1x per day) 5) Some symptoms (2-3x per day)—improving 4) Some symptoms (2-3x per day)—no change 3) Some symptoms (2-3x per day)—getting worse 1-2) Some or all symptoms (>3x per day)	**7 6**	**5 4 3**	**2 1**
Functional Status (nutrition related) 6-7) Full functional capacity 3-5) Mild or moderate loss of stamina 1-2) Severe loss of functional ability (bedridden)	**7 6**	**5 4 3**	**2 1**
Disease state affecting nutritional requirements 6-7) No increase in metabolic demand (no or low stress) 3-5) Mild to moderate increase in metabolic demand (moderate stress) 1-2) Drastic increase in metabolic demand (high stress)	**7 6**	**5 4 3**	**2 1**
Muscle wastage: 6-7)No deletion in all areas 3-5) Mild to moderate depletion 1-2) Severe depletion	**7 6**	**5 4 3**	**2 1**
Fat stores 6-7) No depletion in all areas 3-5) Mild to moderate depletion 1-2) Severe depletion	**7 6**	**5 4 3**	**2 1**
Edema: 6-7) No edema (Nutrition related) 3-5) Mild to moderate edema 1-2) Severe edema	**7 6**	**5 4 3**	**2 1**
Nutritional status: Well-nourished / Mildly to Moderately Malnourished / Severely Malnourished **Overall SGA Rating:** 7 6 5 4 3 2 1 (Circle one)			

Fig. 1 (**a**) Features of subjective global assessment. (SGA) (Adopted from Detsky et al. 1987). (**b**) The seven point SGA with more objectivize global assessment. (Adopted from Lim et al. 2016)

Modifications of SGA

Various modified versions of SGA have been proposed since its conception (Detsky et al. 1987). Several attempts were made to convert conventional SGA into a semiquantitative or quantitative tool in order to improve its ability to assess and recognize petite yet significant changes in varying grades of malnutrition in differing clinical scenario.

7-Point SGA

The shortcoming of conventional SGA is that it cannot detect small yet significant changes in nutrition status during follow-up (Visser et al. 1999; Kalantar-Zadeh et al. 1999). To circumvent this drawback of traditional SGA, Churchill et al. (1996) expanded it into 7-point scale to assess the changes in nutrition status of 680 ESRD patients commencing peritoneal dialysis (PD). The 7-point SGA is a modification of the original 3-point ordinal scale SGA (A, B, and C) initially illustrated by Detsky et al. (1987) and Baker et al. (1982a) to 7 points (rating 1 severely malnourished to 7 well nourished (Churchill et al. 1996; McCusker et al. 1996). In this system, four items are scored on a 7-point Likert-type scale, with lower values signifying inferior nutritional status. Parameters were weight loss during the past 6 months, anorexia, loss of subcutaneous fat, and muscle mass. These four items are given subjective weights to produce a global assessment. Scores of 1–2 indicates severe malnutrition, 3–5 mild to moderate malnutrition, and 6–7 normal nutrition. Nutrition assessment performed using the 7-point scale can be aligned with the conventional SGA (i.e., well nourished, moderately malnourished, or severely malnourished).

The 7-point SGA has more potential for classifying patients than does the conventional SGA (de Mutsert et al. 2009). The weight loss pattern has been given more significance in SGA scoring (Detsky et al. 1987). A small improvement or worsening within each category may be identified earlier which can be easily assessed using the 7-point SGA. The 7-point SGA may be much more time sensitive than the conventional SGA in identifying small changes in nutrition status (Campbell 2007a; Lim et al. 2016). Lim et al. (2016) (shown in Fig. 1b) used the 7-point SGA as well as the conventional SGA for nutritional assessment of 67 patients at baseline and reassessed it at 1, 3, and 5 months. It took significantly shorter time to see a 1-point change using 7-point SGA (median value 1 month) as compared to conventional SGA (median, 3 months). The likelihood of at least a 1-point change is 6.74 times greater in 7-point SGA compared to conventional SGA after controlling for age, gender, and medical specialties. Fifty-six percent of patients who had no change in conventional SGA score had changes detected using 7-point SGA. The level of agreement was 100% between 7-point SGA and 3-point SGA and 83% between two-blinded assessors for 7-point SGA.

The overall SGA scoring is not merely a score; it also includes an evaluator's clinical judgment. If the patient deteriorates or improves, the clinician may apply different score to each section to reflect these changes.

Patient-Generated-Subjective Global Assessment

PG-SGA was adapted from the conventional SGA and had been particularly designed for cancer patients in 1994 by Ottery and colleagues. In PG-SGA system, the components of the medical history are completed by the patient himself, and physical examination is performed by health professional either nurses/technician or healthcare professionals. The scored PG-SGA is a further advancement of PG-SGA which provides numerical scoring as well as a global rating in form of well nourished, moderately malnourished, or severely malnourished. For each component of the scored PG-SGA, points (0–4) are awarded depending on the impact of the symptom on nutritional status. A sum of total score provides a guideline to the level of nutritional intervention. The scoring system also allows triaging of the patients. It has been accepted by the Oncology Nutrition Dietetic Practice Group of the American Dietetic Association as the benchmark for malnutrition assessment in oncology patients. A score $\geq$9 indicates the requirement for nutritional intervention (Ottery 1994). The PG-SGA score has also been regarded as one of the most suitable method for identifying nutritional derangements in gynecological cancer patients (Laky et al. 2008). Bauer et al. (2002) reported a sensitivity and specificity of 98% and 82%, respectively, of PG-SGA score for predicting SGA in their study on 71 cancer patients.

Dialysis Malnutrition Score

ESRD patients on dialysis may be different clinically than patients with other medical and surgical illnesses. Kalantar-Zadeh et al. (1999) proposed a modified version of SGA for patients on dialysis which was initially named as modified quantitative SGA and later on as DMS (Kalantar-Zadeh et al. 2001a, 2004b).

All seven features of SGA are evaluated: (1) weight change, (2) dietary intake, (3) gastrointestinal symptoms, (4) functional capacity, (5) comorbidity, (6) loss of subcutaneous fat (triceps, biceps, chest, and below the eyes), and (7) signs of muscle wasting (temples, clavicle, scapula, quadriceps, knee, and interosseous). A score on scale of 1 (normal) to 5 (very severe) is awarded to each of the seven features of SGA. Thus, a total score ranging from 7 (normal) to 35 (severely malnourished) are achieved in each case. Subsequently patients are categorized into (1) normal nutritional status (score 7), (2) mild to moderate malnutrition (score 8–14), and (3) severe malnutrition (score 15–35). The ascites and edema have been deleted from conventional SGA, and dialysis vintage has been added into the comorbidity component.

Malnutrition-Inflammation Score

In order to make DMS scoring system more robust, comprehensive, and quantitative, the evaluation criteria for the seven DMS components were revised in MIS. Three new items BMI, serum albumin level, and TIBC were added. It differs from DMS scoring system in the sense that the number of severity levels of each component was reduced from five to four levels. Thus, the MIS has three

components, each with four levels of severity, from 0 (normal) to 3 (severely abnormal). The sum of all ten MIS components ranges from 0 (normal) to 30 (severely malnourished). A higher score reflects a more severe degree of malnutrition and inflammation. Few studies have reported that MIS score is better correlated with actual nutritional status of patients as compared to the conventional SGA (Kalantar-Zadeh et al. 2001a, 2004a). The MIS is more comprehensive and quantitative and found to be significantly correlating with hospitalization, mortality, and indices of nutrition, inflammation, and anemia as well (Kalantar-Zadeh et al. 2001a; Afsar et al. 2006).

Validity and Reliability of SGA

SGA in either conventional or modified form has been validated as reliable tool for assessing malnutrition in various clinical conditions, cancer patients, postsurgical patients, hospitalized patients, and ESRD patients on dialysis (K/DOQI- NKF 2000; Visser et al. 1999; Ottery 1994; Hirsch et al. 1991; Sacks et al. 2000; Thoresen et al. 2002). SGA gives reproducible results with more than 80% agreement when two-blinded observers have assessed the same patient (Detsky et al. 1987; Mc Cann 1996). The Veterans Affairs perioperative total parenteral nutrition (1991) randomized trial also reported the predictive validity of SGA in malnourished patients of different degrees. Lim et al. (2016) in their study on 67 patients have observed that the 7-point SGA was positively correlated with body mass index and mid-arm circumference with a level of agreement of 100% between the 7-point SGA and 3-point SGA. The inter-rater agreement between two assessors for the 7-point SGA was good, at a rate of 83%.

Hirsch et al. (1991) have validated SGA in a study on 175 patients admitted in medical-surgical gastroenterology and reported a 79% inter-rater reliability between assessments done by residents and nutrition specialists and also confirmed the expediency of SGA even when used by inexperienced professionals. Sacks et al. (2000) have reported a significant correlation between SGA scores and nutritional complications in their study from long-term care facilities. Visser et al. (1999) have found that the 7-point SGA scale has sufficient interobserver and good intra-observer reliability in dialysis patients. Moreover, it also correlates with objective nutritional factors like anthropometric measures and biochemical parameters including prealbumin. The sufficient intra-observer and interobserver reliabilities of the SGA have been documented in various studies regardless of its subjective nature (Visser et al. 1999; Steiber et al. 2007). However, it has been recommended that one observer or a select group of observers should execute SGA to maximize the reliability of SGA (Visser et al. 1999).

Clinical Uses of SGA

SGA can be quickly used in a wide variety of health-care settings such as geriatric patients (Christensson et al. 2003; Stephenson et al. 2001), cancer patients (Bauer

et al. 2002), patients on radiotherapy (Isenring et al. 2003), patients who have liver transplants and chronic liver disease, patients who experience stroke (Davis et al. 2004), pregnant patients, and adult patients undergoing maintenance dialysis (Enia et al. 1993; Visser et al. 1999; Churchill et al. 1996; Cooper et al. 2002). Studies have documented the association of one time SGA on various clinical outcomes including morbidity, hospitalization, and mortality (Steiber et al. 2007; Pifer et al. 2002; Kalantar-Zadeh et al. 2001b). Shirodkar and Mohandas (2005) in their study on 266 cancer patients observed that SGA scores were significantly associated with adverse outcomes like mortality and hospital stay following cancer surgery. In a study on advance colorectal cancer patients, Gupta et al. (2005) reported SGA as an important predictors of survival and also showed that the median survival of patients with SGA-A, SGA-B, and SGA-C was 12.8 months, 8.8 months, and 6 months, respectively. SGA was recommended as the method of choice as compared to Nutritional Risk Index (NRI), anthropometry, and laboratory data in a study on 100 patients undergoing major abdominal surgery (Sungurtekin et al. 2004). In a study (Detsky et al. 1984) in which SGA was initially meant for use in surgical inpatients for assessing malnutrition and predicting postoperative infection, SGA was found to be a better predictor of these complications compared to serum albumin level, delayed skin hypersensitivity, mid-upper arm circumference, creatinine height index, and other prognostic nutritional index. It has also been observed that SGA had the best sensitivity and specificity for predicting infection in post-surgery period (Detsky et al. 1984, 1987). SGA as an independent predictor of mortality in dialysis patients have been reported in several studies (Kalantar-Zadeh et al. 2001a; de Mutsert et al. 2009). It has been also successful in predicting complications and death in ESRD patients on dialysis including HD (Kalantar-Zadeh et al. 1998) and PD (Churchill et al. 1996).

We have observed that peritonitis rate can be predicted as per nutritional status based on SGA. Mean peritonitis rate per patient-year was significantly higher in patients with malnutrition (mean 0.99 episodes per patient-year) compared to patients with normal nutritional status (mean 0.18 episodes per patient-year). Peritonitis-free survival in patients with normal nutrition was significantly higher compared to patients with malnutrition (42 months vs. 21 months) based on SGA (Prasad et al. 2007).

The famous multicenter Canada-USA (CANUSA) study showed that 1-unit decrease in SGA is associated with a 25% increase in mortality risk of PD patients (Churchill et al. 1996). SGA predicts the outcome and the 2-year patients survival rate which was 80.5%, 77.3%, and 47.2% ($P = 0.01$), respectively, in patients with normal nutrition, mild to moderate malnutrition, and severe malnutrition categories assessed by SGA (McCusker et al. 1996). Several other studies also documented that SGA is an independent predictor of patient's survival (Ottery 1994; Kalantar-Zadeh 2001a; Pifer et al. 2002; Chung et al. 2000).

In a different study of 342 patients on PD, authors have observed that the relative risk of death in patient with malnutrition was 3.2 times higher as compared to the patients with normal nutritional status. The 5-year survival of patients with

malnutrition was significantly poor (33%) as compared to well-nourished patients (58%) (Prasad et al. 2010).

SGA has been recommended by K/DOQI- NKF (2000) as one of the panel of nutrition indicators for longitudinal monitoring of CKD patients in view of its strong association with malnutrition (Cooper et al. 2002) and clinical outcomes (Yang et al. 2007). Since initial validation of SGA in HD patients (Enia et al. 1993), SGA has been used as benchmark tool for nutritional assessment in pre-dialysis (K/DOQI-NKF 2000; Cupisti et al. 2004), patients initiating dialysis (Prasad et al. 2007, 2008; Lim et al. 2016; Asgarani et al. 2004; Heimburger et al. 2000) and maintenance dialysis patients as well (Churchill et al. 1996; Pifer et al. 2002; Stenvinkel et al. 1999; Raslan et al. 2010). Malnutrition predicted on SGA has been associated with increased risk of prolonged hospital stay and hospital readmission in a prospective cohort study. Sacks et al. (2000) in their study on 53 facility residents from long-term care facilities have reported a significant association between SGA score and other nutrition-associated complications including death, with severely undernourished residents having the highest mortality rate. The clinical utility of SGA in assessing clinical outcomes on long-term follow-up of dialysis patients is also emerging in a multicenter study (de Mutsert et al. 2009) of incident dialysis patients in which nutritional status was assessed on 7-point SGA at 3 and 6 months after commencing dialysis and every 6 months afterward during 7 years of follow-up. Baseline 7-point SGA was found to be associated with a twofold increase in mortality risk. Time-dependently, the mortality rate in 7 years of follow-up was 11/100 person-years in patients with a normal nutritional, 30/100 person-years with moderate, and 69/100 person-years with severe malnutrition. The stronger time-dependent association indicates that malnutrition was associated with high short-term mortality. SGA appears to be a semiquantitative tool to assess the effect of nutritional status on short-term as well as on long-term outcomes of patients.

Policies and Protocols

Assessment of Components of SGA

All following items of SGA are allocated with a subjective weight: A or 6–7, or normal (well nourished); B or 3–5, or mild-to-moderate malnutrition; and C or 1–2, or severely malnourished.

Weight loss: A score is assigned on a scale from 1 to 7 based on the amount of weight lost or gained compared to previous measurements. A weight loss of >10% over the last 6 months is considered as severe, 5–10% mild to moderate, and <5% normal. Rate of weight loss and its pattern should also be taken into consideration. A recent stabilization or increase in weight after suffering a net weight loss is regarded as well nourished.

Food intake: Food intake of patient is assessed based on the dietary intake compared to patients' usual dietary pattern and intake. Lower scores indicate decreased intake. History of adequate dietary intake meeting nutritional needs with no change or slight change for a short duration is categorized as normal (6 or 7), borderline or decreasing intake as mild to moderately malnourished (3, 4 or 5), and condition of inability to eat and starvation as severely malnourished (1 or 2). The patient is evaluated in detail to quantify and qualify the change in the oral intake.

Gastrointestinal symptoms: Gastrointestinal symptoms (diarrhea, anorexia, nausea, and vomiting) are appraised based on the frequency, duration, and severity. Severe symptoms indicate lower rating. Symptoms persisting on daily basis for >2 weeks is considered to be severe (1 or 2); no/minor/occasional symptoms are considered normal nutrition (6 or 7); degree and symptoms between the two categories is considered as mild-moderate malnutrition (3, 4, 5). Intermittent changes are not regarded as significant.

Physical examination includes loss of subcutaneous adipose tissue and muscle. The tips on the physical examinations are shown in Table 1. Figure 2a–f demonstrates loss of subcutaneous fat, A–C showing changes in subcutaneous fat below the eye and D–F at level of biceps and triceps. Figure 3 shows muscle wasting at level of temple in A, B, and C; at clavicle D, E, and F; at shoulder G, H, and I; at scapula J, K, and L; and at level of interosseous muscle in M, N, and O. Figure 4 shows muscle wasting around knee A, B, and C; quadriceps D, E, and F; and calf G, H, and I.

The different modifications of SGA had been widely used in CKD and dialysis patients. The summary of comparison of description, advantage, and disadvantages of different modifications of SGA has been shown in Table 2.

Dictionary of Terms

- **Nutrition** – It is the science dealing with the role of various foodstuff and various types of nutrients in order to safeguard and maintain human health.
- **Nutrients** – Are the fundamental elements in food (e.g., protein, fat, carbohydrate, vitamins, and minerals) that must be supplied in sufficient quantity to the body to maintain health
- **Malnutrition** – It is the state of imbalance between the nutrient intake and demand. It can be a result of a relative or complete insufficiency or excess of one or more essential nutrients.
- **Nutritional status** – The condition of health of an individual that indicates the balance among the various nutrient intake and their utilization in various health, growth, development, and diseased conditions.
- **Nutritional assessment** – It is a comprehensive and in depth evaluation to determine an individual person's health and health-related issues using different parameters like anthropometry, medical, dietary, clinical history, physical examination, laboratory data, etc.

Table 1 Physical examination consists of subcutaneous fat and muscle wasting. (Adopted from Asgarani et al. 2004)

Physical examination consists of subcutaneous fat and muscle wasting				
Areas of exam	Tips	Severe malnutrition	Mild-moderate malnutrition	Well nourished
Subcutaneous fat				
Below the eye	Look at patient straight on	Hollow look, depressions, dark circles, loose skin (Fig. 2a)	Slightly dark circles, somewhat hollow look (Fig. 2b)	Slightly bulged fat pads. Fluid may mask loss (Fig. 2c)
Triceps/biceps	Arm bent, do not include muscle in pinch, roll skin between fingers	Very little space between folds, fingers touch (Fig. 2d)	Fingers almost touch, some depth to pinch (Fig. 2e)	Ample fat tissue obvious between folder of skin (Fig. 2f)
Muscle wasting				
Temple	Observe straight on, have patient turn head side to side	Hollowing, scooping, depression (Fig. 3a)	Slight depression (Fig. 3b)	Can see/feel well-defined muscle (Fig. 3c)
Clavicle	Look for prominent bone	Protruding, prominent bone (Fig. 3d)	Some protrusion (Fig. 3e)	Not visible in male, visible but not prominent in female (Fig. 3f)
Shoulder	Arms at side, look at shape	Shoulder to arm joint looks square (Fig. 3g)	Acromion process may protrude slightly (Fig. 3h)	Rounded, curves at junctions arm/shoulder/neck (Fig. 3i)
Scapula	Have patient push hands against solid object	Prominent, visible bones, depressions below ribs/scapula or shoulder/spine (Fig. 3j)	Mild depression or bone may show slightly (Fig. 3k)	Lines of bones not prominent, no significant depressions (Fig. 3l)
Interosseous muscle	Pads of thumb/forefinger touching	Depressed area between thumb forefinger (Fig. 3m)	Slightly depressed or flat (Fig. 3n)	Muscle bulges, could be flat in well nourished (Fig. 3o)
Knee, note: lower body is less sensitive to change	Have patient sit with leg propped up, bent a knee	Bones prominent, little sign of musculature around knee cap (Fig. 4a)	Knee cap less prominent more rounded (Fig. 4b)	Muscle protrudes, bones not prominent (Fig. 4c)

(*continued*)

Table 1 (continued)

Physical examination consists of subcutaneous fat and muscle wasting				
Areas of exam	Tips	Severe malnutrition	Mild-moderate malnutrition	Well nourished
Subcutaneous fat				
Quadriceps	Not as sensitive as upper body	Depression on inner thigh, obviously thin (Fig. 4d)	Mild depression on inner thigh (Fig. 4e)	Well rounded developed (Fig. 4f)
Calf	Observe side and front view	Thin, minimal or no muscle definition (Fig. 4g)	Not well developed (Fig. 4h)	Well-developed bulb of muscle (Fig. 4i)
Edema, ascites (HD only) R/O other causes of edema, patient at dry weight	View sacrum in activity restricted patient, ankle in mobile patient	Significant swelling	Mild to moderate swelling	No sign of fluid accumulation

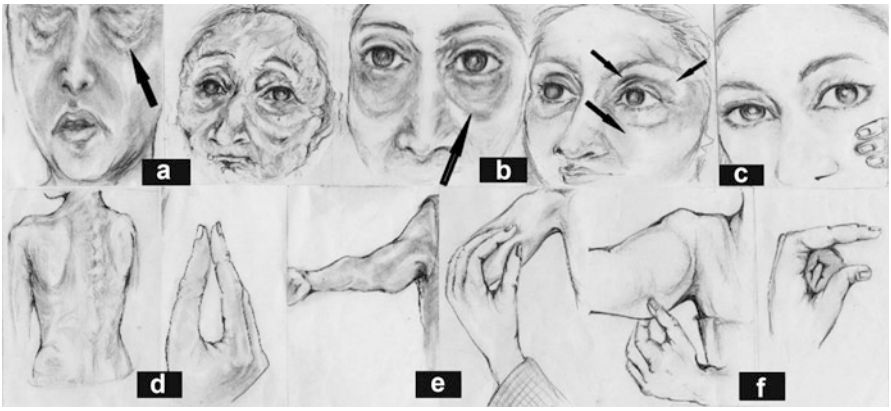

Fig. 2 (**a**–**f**) Figure shows the assessment of the loss of subcutaneous fat. Depression under the eye, darkened area "hollow eyes" (**a**) severe malnutrition; moderate fat loss and slightly dark circle and hollow look (**b**) Mild-Moderate malnutrition; appears slightly bulge (**c**) well nourished. Assessment of the fat stores at triceps/biceps: Small amount of space between fingers. Severe loss of subcutaneous fat tissue, fingers touch (**d**) severe malnutrition; moderate loss of subcutaneous fat tissue, fingers almost touch (**e**) mild-moderate malnutrition, fingers do not touch, ample of subcutaneous fat tissue (**f**) well nourished

- **Glomerular filtration rate (GFR)** – It is estimating the amount of blood that passes through the glomeruli each minute. It is defined as the flow rate of filtered fluid through the kidney and is the indicator of kidney function.

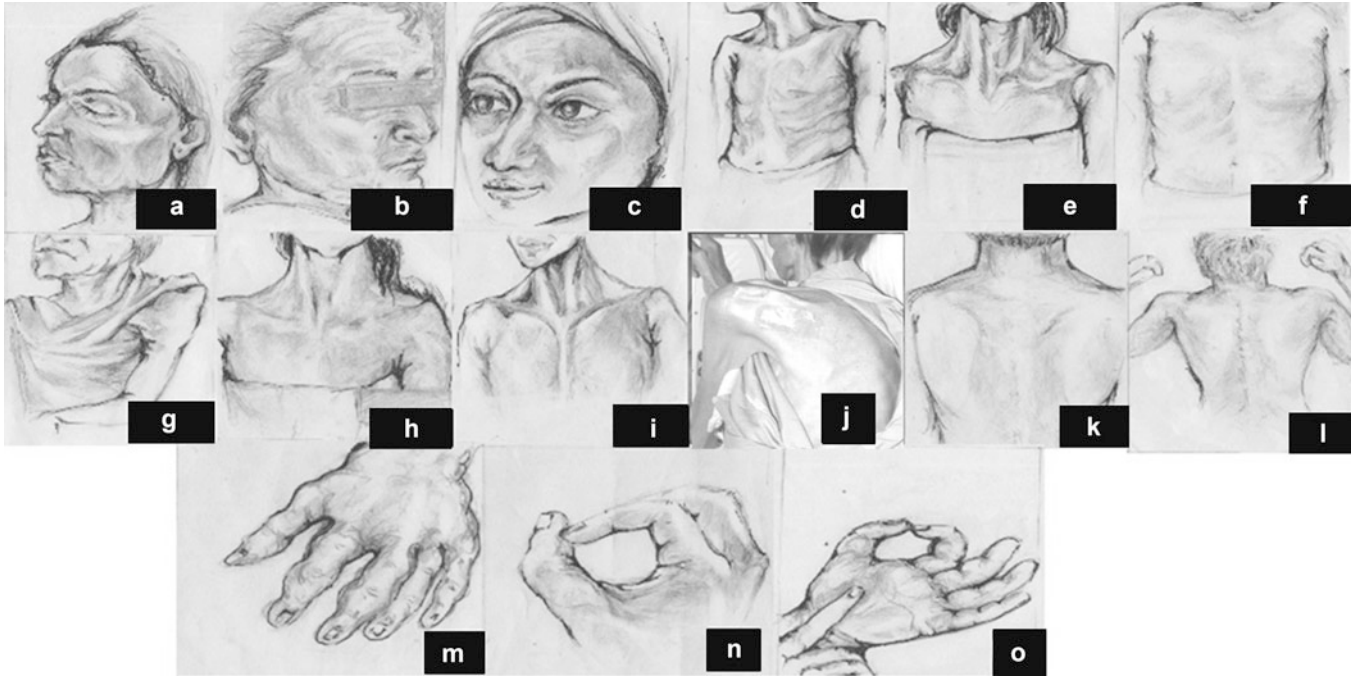

Fig. 3 Muscle wasting at temple shows hollowing and depression (**a**) severe malnutrition; slight depression (**b**) mild-moderate malnutrition; well-defined muscle/flat (**c**) well nourished. At level of clavicle showing protruding, prominent clavicle bone (**d**) severe malnutrition; partially protruded (**e**) mild-moderate malnutrition; barely visible (**f**) well nourished. At level of shoulders showing square shoulders prominent acromion protrusion (**g**) severe malnutrition; acromion protrusion can be evident (**h**) mild-moderate malnutrition; rounded, curves at junctions between the neck and the shoulder joint (**i**) well nourished. At level of scapula showing visible scapula prominent ribs with depressions between ribs/scapula (**j**) severe malnutrition; moderate depression around the bone (**k**) mild-moderate malnutrition; scapula is not prominent (**l**) well nourished. Muscle stores revealing interosseous muscles between thumb and forefinger showing significant wasting and scooping (**m**) severe malnutrition; slightly depressed/scooping (**n**) mild-moderate malnutrition; flat or raised (**o**) well nourished

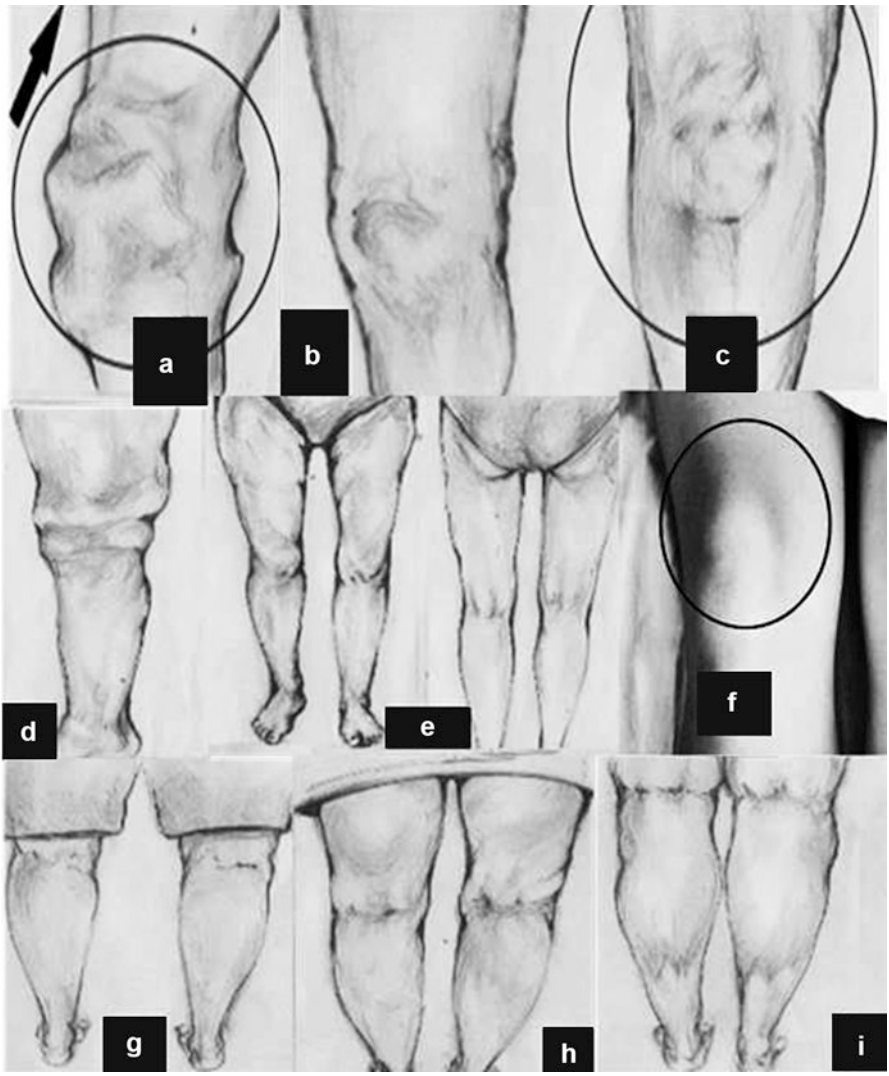

Fig. 4 Muscle stores at knee showing prominent bones and severe muscle wasting (**a**) severe malnutrition; knee cap less prominent – mild-moderate malnutrition; bones not prominent, muscle protruded (**c**) well nourished. At level of quadriceps showing depression, thin (**d**) severe malnutrition; mild depression' (**e**) mild-moderate malnutrition; well rounded, no depressions (**f**) well nourished. At level of calf showing protruded knee bone, and thin calf (**g**) severe malnutrition; protruded knee bone and calf not well developed (**h**) mild-moderate malnutrition; full and solid, calf well developed (**i**) well nourished

- **Chronic kidney disease (CKD)** – It is the condition when kidney is not able to function normally and the GFR value falls either below 60 ml/min/1.73^2 for three or more than 3 months or GFR value is above 60 ml/min/1.73^2 with kidney damage as indicted by presence of albumin in the urine

Table 2 Description, advantages, and disadvantages of SGA-based nutrition assessment tools utilized in CKD. (Adopted from Campbell et al. 2007b)

Tool	Method	Modification from SGA	Advantages	Disadvantages
Retrospective mSGA	Rating A, B, C	Retrospective "self-rating" on A, B, C scale	Conducted as a survey (self-report)	Relies on self-report and carer's physical assessment
7- point SGA	Rating 7–1	Expands the 3 categories of the original SGA to 7 on a Likert-type scale	May delineate levels of nutrition status	May increase interobserver variation
Dialysis Malnutrition Score (DMS)	Scored 7–35	Scores 7 components of the SGA as 1 (normal) to 5 (very severe)	Scored so less subjectivity	Allocation of scores not based on evidence
Malnutrition-Inflammation Score (MIS)	Scored 0–30	10 components, the DMS with BMI, serum albumin, and total iron-binding capacity, scored according to severity 0 (normal) to 3 (very severe)	Includes objectives categories – less reliance on subjectivity	Requires biochemistry (albumin and iron studies), and weight/height measures for BMI
Patient-Generated-Subjective Global Assessment (PG-SGA)	Scored 0–35 and A, B, C	Provides a numerical score, dependant on the impact of each SGA component on nutrition status	Patient completes medical history, scored so less subjective	May require more patient input

- **End-stage renal disease** – It is a stage of gradual and progressive loss of renal function (GFR <15 ml/min/1.73^2) when kidney is unable to function and sustain life, and there is need for long-term dialysis or transplantation.
- **Dialysis** – The procedure done via a machine for removing the excess waste products (toxins) and excess fluid from the body. It is used as a modality to treat renal failure in case when the kidneys are not able to function adequately.
- **Body mass index** – It is the measure of body fatness based on height and weight that applies to adult men and women. It is calculated by dividing the weight in kilograms by the square of height in meters.

Summary Points

- Malnutrition is highly prevalent and a well-recognized problems in hospitalized patients as well as in general population.
- This chapter illustrates the SGA method for assessing malnutrition. SGA in either conventional or its modified form has been validated as reliable semiquantitative tool for assessing malnutrition in various clinical conditions.
- It is designed for the overall evaluation of nutritional status on the basis of history and physical examination on a scale ranging from well nourished to severely malnourished.

- Various modified versions of SGA have been proposed since its conception. The 7-point SGA is a modification of the original SGA in which four items are scored on a 7-point Likert-type scale, with lower values signifying inferior nutritional status (rating 1 severely malnourished to 7 well nourished). The 7-point SGA may be much more time sensitive than the conventional SGA in identifying small changes in nutrition status.
- It helps in identifying malnutrition, indirectly differentiating malnutrition from disease states, predicting outcomes and requirement of nutritional intervention to improve the outcomes of patients. Its applicability has been widely studied in surgical and cancer patients, patients in intensive care unit, patients with CKD, and patients on dialysis.
- It can also be used as tool in making strategies for prevention of malnutrition in general population as well.
- SGA is simple, noninvasive, and inexpensive tool which can be quickly executed in routine clinical practice to assess the nutritional status of the patients.

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Nutritional Screening Tools for Malnutrition in Pediatrics

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Abstract

Malnutrition in pediatrics remains a cause for concern due to its considerably high prevalence and deleterious effects on growth, development, and overall health. Early identification of malnutrition risk may prevent nutritional deterioration during hospitalization. There are currently a number of suggested screening tools for use in pediatrics; however, there is no consensus on a single tool that is favorable over others. Thus selection of a screening tool for implementation is perplexing. Presented here is an overview of the screening tools available for use in pediatrics and further actions needed in order to implement the use of screening tools in different settings in pediatrics. Seven screening tools that are intended for use upon admission to the hospital were identified. Two screening tools were designed for specific medical conditions. One screening tool was designed for sole use in ambulatory settings. Of the seven tools identified for use upon hospital admission, some tools were also validated for use in specific medical conditions and one tool was also validated for use in ambulatory settings. Comparison between screening tools failed to offer one tool favorable to others. A model for implementation of nutritional screening in pediatrics in terms of policy change is suggested. In conclusion, there is currently no single nutritional screening tool that is superior to others. When selecting a screening tool, one should consider the setting in which screening will take place, in terms of purposes and applications. Governments and healthcare providers should promote implementation of nutritional screening in pediatrics in all healthcare facilities.

Keywords

Malnutrition · Malnutrition risk · Nutritional status · Nutritional assessment · Nutritional screening · Screening tools · Pediatrics · Children · Hospitalization · Undernutrition

List of Abbreviations

ASPEN	American Society of Parenteral and Enteral Nutrition
BIA PhA	Bioelectrical Impedance Phase Angel
BMI	Body Mass Index
CDC	Center for Disease Control
ESPEN	European Society of Enteral Nutrition
ESPGHAN	European Society of Pediatric Gastroenterology Hepatology and Nutrition
GI	Gastro Intestinal
ICD-10	International Classification of Diseases
LOS	Length of (hospital) Stay
MUAMC	Mid Upper Arm Muscle Circumference
NRI	Nutritional Risk Index
NRS	Nutrition Risk Score
nutriSTEP	Nutritional Screening Tool for Every Preschooler
PeDiSMART	The Pediatric Digital Scaled Malnutrition Risk Screening Tool

PICU	Pediatric Intensive Care Unit
PNRS	Pediatric Nutrition Risk Score
PYMS	Paediatric Yorkhill Malnutrition Score
SCAN	Screening tool for childhood CANcer
SGNA	Subjective Global Nutritional Assessment
STAMP	Screening Tool for the Assessment of Malnutrition in Pediatrics
STRONGkids	Screening Tool for Risk on Nutritional status and Growth
UK90	United Kingdom (growth charts)
WHO	World Health Organization

Introduction

Studies published over the past decade show that malnutrition prevalence upon admission to pediatric hospitals remains considerably high, ranging from 6% to 14% in developed countries (Joosten and Hulst 2011) with higher prevalence rates in infants and toddlers (Hecht et al. 2015). In specific medical conditions the described prevalence is much higher (Joosten and Hulst 2008). Hospital stay has deleterious impact on the nutritional status leading to weight loss during hospitalization even in children affected merely by mild clinical conditions (Campanozzi et al. 2009; Pacheco-Acosta et al. 2014). Early detection of children at malnutrition risk has been discussed by international organizations such as ASPEN (Corkins et al. 2013), ESPGHAN (Agostoni et al. 2005), and ESPEN (Kondrup et al. 2003).

Nutritional screening is a process aimed to identify an individual who is malnourished or who is at risk for malnutrition, to determine if a detailed nutritional assessment is indicated (Teitelbaum et al. 2005). An ideal screening tool should demonstrate good ranking at concurrent validity (the extent to which screening tools agree with each other), predicative validity (the extent to which screening tools predict certain outcomes), reproducibility (agreement between users of a given tool), and applicability in terms of ease and speed of administration (Elia and Stratton 2011).

However widely used in adults, nutritional screening is not routinely conducted in children because of the lack of a simple, properly validated screening tool (Hartman et al. 2012). Also, since there is no one universally accepted definition of malnutrition, there is also lack of consensus on a single definition for nutritional risk (Huysentruyt et al. 2015). As a result, different screening tools were developed for different purposes using different methodological methods (Elia and Stratton 2011), making it nearly impossible to compare between tools in order to favor one tool over the others. Thus, adding to the difficulties to choose an appropriate screening tool there is lack of agreement between tools (Chourdakis et al. 2016) and evidence for an impact of the screening on long-term outcome is lacking. In this chapter we will review the literature on pediatric screening tools and discuss the complexity of choosing a single tool. We will also discuss the policy needed to ensure implementation of nutritional screening in pediatrics.

Nutritional Screening Tools Currently Available for Use in Pediatrics upon Hospital Admission

The main components of the following screening tools are described in Table 1.

1. Nutrition Risk Score (NRS) – Developed by Reilly et al. (1995), the NRS is a screening tool validated for use by the nursing staff to assess the risk of nutritional depletion in hospitalized patients, both adults and pediatrics. The NRS collects data on weight loss (amount and duration), BMI for adults or percentile charts for children, food intake (appetite and the ability to eat and retain food), and stress factors (effect of medical condition on nutritional requirements). Each parameter is given a score affected by the severity of the condition described. The scores are summed and patients are allocated into nutritional risk groups. A course of action is then advised accordingly. The tool was validated on a sample of 20 patients ranging from 6 weeks of age to 79 years. The NRS scores were assessed in comparison to Nutritional Risk Index (NRI) ($r = 0.68$, $p < 0.001$) and a dietitian's clinical impression of the patient ($r = 0.83$, $p < 0.001$). Inter-rater reliability was also assessed by comparing NRS scores conducted by two dietitians ($r = 0.91$, $p < 0.001$). It should be noted that this tool was validated by comparison to the NRI (Wolinsky 1990) which was originally designed for use in geriatrics. Moreover, this tool is not specifically intended for use in pediatrics and the study's sample size was exceptionally small with a large age range. No data on sample size calculations was mentioned in the text.
2. Pediatric Nutritional Risk Score (PNRS) – Originally developed by Sermet-Gaudelus et al. (2000), this tool is designed to identify children at risk of losing 2% or more of their admission weight, during hospitalization. Nutritional risk was

Table 1 Main components of screening tools. Different screening tools take into consideration different nutritional related data when assessing nutritional risk. The table describes the different screening tools with regards to their components

Tools	Effect of disease	Current nutritional intake	Anthropometry measurements	Weight loss	Other components
NRS	٧	٧	٧	٧	GI symptoms, ability to eat
PNRS	٧	٧			Pain
STRONGkids	٧	٧		٧	
PYMS	٧	٧	٧	٧	
STAMP	٧	٧	٧		
PNST		٧		٧	
PeDiSMART	٧	٧	٧		GI symptoms

Key: *NRS* nutrition risk score, *PNRS* pediatric nutrition risk score, *STRONGkids* screening tool for risk on nutritional status and growth, *PYMS* paediatric yorkhill malnutrition score, *STAMP* screening tool for the assessment of malnutrition in pediatrics, *SGNA* subjective global nutritional assessment, *PeDiSMART* the pediatric digital scaled malnutrition risk screening tool, *GI* gastro intestinal

assessed prospectively in 296 children within the first 48 h of admission. Various nutritional-related factors were assessed regarding their ability to predict weight loss during hospitalization. Multivariate analysis indicated that food intake below 50% of dietary allowance, pain, and the severity of the disease were found to be the most significant predictors of weight loss greater than 2% during hospital stay ($p = 0.0001$ for each). It should be noted that although authors described this tool as simple and easy to use, it has some downfalls. First, the tool is not applicable upon admission since it requires monitoring nutritional intake over the first 48 h of hospitalization. Thus, by using this tool, children who could benefit from early intervention may be overlooked. Second, authors does not specify the resources needed to implement the screening procedure; however, it appears trained personnel is required to implement the tool, if not only to assess dietary intake compared with recommended allowance (Hartman et al. 2012). Third, there is no data on reproducibility, inter-rater reliability, and the sensitivity of the tool (Hartman et al. 2012; Joosten and Hulst 2014).

3. Screening Tool for Risk on Nutritional status and Growth (STRONGkids) – Developed and tested by Hulst et al. (2010) in a prospective observational multicenter study including 424 children admitted to 7 academic and 37 general hospitals across the Netherlands. The STRONGkids consists of four items each given a numeric score: subjective clinical assessment, high-risk disease, nutritional intake, and weight loss. Weight and height were also measured and z-scores were calculated. Risk scores were then compared with z-scores. Hulst et al. showed that z-scores decreased with the rise in risk score ($rs = -0.25$, $p < 0.001$), suggesting the higher the score according to STRONGkids, the greater the risk according to anthropometric measurements. In addition, length of hospital stay (LOS) was significantly shorter in children in the low-risk score group compared to children with a moderate or high risk score ($p < 0.001$). This was also demonstrated by Cao et al. (2014) who used STRONGkids to analyze nutritional risk in hospitalized children and its relationship with clinical outcome. Cao et al. showed that children in the high-risk group demonstrated higher complication rate, longer hospital stays, greater weight loss, and greater hospital expanses, compared with children with moderate or low risk ($p < 0.001$).
   Hulst et al. (2010) described this tool as practical and easy to use; however, it should be noted that both subjective clinical assessment and determining the severity of the disease rely on expert knowledge and experience and thus might not be suitable for use by all healthcare workers in different settings.
4. Paediatric Yorkhill Malnutrition Score (PYMS) – Developed by Germasimidis et al. (Gerasimidis et al. 2010), the PYMS consists of four parameters: BMI, weight loss history, changes in nutritional intake, and the severity of the underlying disease and its impact on nutritional status. The tool was validated by comparing its allocation to nutritional risk groups, with that determined by full nutritional assessment. Inter-rater reliability was determined by comparing the PYMSs' scores as rated by the nursing staff with those rated by dietitians. PYMSs' allocation into nutritional risk groups was also compared with other commonly used tools (STAMP and SGNA). The study included 247 children, of whom

nurse-rated PYMS identified 59% of those rated at high risk by full nutritional assessment. Of those rated at high risk by nurse-rated PYMS, 47% were confirmed at high risk by the full nutritional assessment. These results could be interpreted that approximately 40% of children considered at high risk were not identified by nurse-rated PYMS. Moreover, 53% of children were falsely identified at high risk by nurse-rated PYMS and inadequately referred to dietitians (Hartman et al. 2012). PYMS demonstrated a moderate agreement with full dietitians' assessment ($k = 0.46$) and inter-rater reliability ($k = 0.53$) when nurse-rated PYMS was compared with dietitian-rated PYMS.

5. Screening Tool for the Assessment of Malnutrition in Pediatrics (STAMP) – Developed by McCarthy et al. (2012), STAMP consists of three steps and gathers information aimed to detect low percentile weight for age, reported weight loss, discrepancy between weight and height percentile, recently changed appetite, and the expected nutrition risk of clinical diagnoses. All were identified as predictors of nutrition risk, in the development phase of the tool. Nutritional risk is translated into the need for a referral to a full nutritional assessment. No outcomes were evaluated in the validation study (Hartman et al. 2012). The tool was validated in a cohort of 238 children upon admission to hospitalization. STAMPs' allocation to nutritional risk groups was compared with a classification determined by a registered dietitian. The tool demonstrated fair to moderate reliability ($k = 0.54$), and sensitivity and specificity were estimated at 70% and 91%, respectively. It should be noted that this tool assesses growth parameters using specific charts that were developed for this purpose (based on either UK90 or CDC charts). Therefore one might claim implementation of such tool requires resources, such as time and money, in order to train healthcare staff accordingly. However, McCarthy et al. (2012) claim STAMP utilizes information that should be routinely collected by nursing staff upon admission, thus introduction of the tool required minimal training. Moreover, in the validation study, STAMP was completed by different members of the nursing staff, including student nurses and nursing supporting staff, suggesting implementation of the tool requires no specific training.
6. Pediatric Nutrition Screening Tool (PNST) – Developed by White et al. (2016), PNST consists of four dichotomous (yes/no) nutritional screening questions and requires no data collection on medical condition or anthropometric measurements. The PNST was validated on 295 hospitalized children from birth to 16 years in Australia. The pediatric SGNA and anthropometry were chosen as the gold standards in defining nutritional risk. The sensitivity and specificity for the PNST compared with the pediatric SGNA were moderate and high, scoring 77.8% and 82.1%, respectively. However, it should be noted that screening procedure was performed by the same investigator using both PNST and SGNA. No clinical outcome was investigated.
7. The Pediatric Digital Scaled Malnutrition Risk Screening Tool (PeDiSMART) - Developed and validated by Karagiozoglou-Lampoudi et al. (2015), the PeDiSMART is a software that consists of four elements: weight for age z-scores, nutrition level intake, overall disease impact, and symptoms affecting intake.

Other than anthropometric data, all other parameters are described as categorical variables rated 0–4. In order to validate the tool, PeDiSMART was compared to bioelectrical impedance phase angle (BIA PhA) on 161 hospitalized children aged 1 month to 17 years in Greece. Data showed inverse correlation between the tool and PhA values ($R = -0.582$, $p < 0.001$). PeDiSMART was then compared to STAMP, PYMS, and STRONGkids screening tools in 500 hospitalized children. Patient allocation to nutritional risk groups on admission was associated with clinical outcomes such as weight loss or nutritional support and LOS. ROC curves showed sensitivity of 87% and specificity of 75%, ranking better than STRONGkids and STAMP. In regards to outcome measurements, PeDiSMART accuracy in predicting weight loss/nutritional intervention was higher than PYMS and comparable to STAMP and STRONGkids. PeDiSMART accuracy in predicting LOS > 7 days was higher than STAMP and STRONGkids and comparable to PYMS. Inter-rater reliability was evaluated in 57 patients by two dietitians and showed moderate agreement of $k = 0.474$. Authors claim PeDiSMART is rapid, easy to use, and suitable for use by all clinical staff members. Since the software incorporates information documented on electronic medical files automatically, the authors suggest the use of this technology may facilitate and possibly improve the prediction of nutritional risk.

It should be noted that although some may refer to the Subjective Global Nutritional Assessment (SGNA) tool published by Secker and Jeejeebhoy (2007) as a nutritional screening tool, we chose not to describe this tool as it resembles a nutritional assessment tool rather than a nutritional screening tool. In fact, it was sometimes used as a gold standard for validation of a new tool, as was performed in the validation of the PNST (White et al. 2016).

While a number of screening tools had emerged in the field of nutritional screening upon hospitalization in the last decade, very few focused on nutritional screening in community settings. Randall Simpson et al. (2008) developed the nutriSTEP, a parent-administered questionnaire intended for nutritional risk screening in preschoolers. However, we choose not to focus on this tool since it is intended to be solely used in community settings and was validated for that purpose only. Despite Rub et al. (2016) validated STAMP for ambulatory use in pediatrics, further research in the field is necessary before a definitive recommendation can be made.

Comparison Between Different Screening Tools

Since nutritional screening is widely recommended by numerous organizations such as ASPEN (Corkins et al. 2013), ESPGHAN (Agostoni et al. 2005), and ESPEN (Kondrup et al. 2003) a number of papers were recently published comparing different screening tools in the hopes of identifying one tool that can be considered superior to others. Examining the volume of these papers fails to yield consistent results. The comparison between the tools brings forward the complexity of prioritizing one tool over the other due to the different design and evaluation methods

Table 2 Aims of the nutritional screening tools. Different screening tools were developed for different aims, thus providing different uses. Table 3 describes the different screening tools with regards to their aims

Tools	Determine nutritional status	Identify need for nutritional intervention	Predict clinical outcome without nutritional intervention
NRS		٧	
PNRS		٧	٧
STRONGkids		٧	٧
PYMS	٧	٧	٧
STAMP	٧	٧	
PNST	٧	٧	
PeDiSMART	٧	٧	

Key: *NRS* nutrition risk score, *PNRS* pediatric nutrition risk score, *STRONGkids* screening tool for risk on nutritional status and growth, *PYMS* paediatric yorkhill malnutrition score, *STAMP* screening tool for the assessment of malnutrition in pediatrics, *SGNA* subjective global nutritional assessment, *PeDiSMART* the pediatric digital scaled malnutrition risk screening tool

used. As was previously proposed by (Elia and Stratton 2011), different screening tools were designed for diverse purposes, for use by people with different backgrounds and for application by one or more settings, one or more age groups, and one or more disease groups.

With regards to the aims of the tools, while NRS, STAMP, PYMS, and STRONGkids are designed to be completed upon admission, PNRS requires the assessment of nutritional intake over the first 48 h of hospitalization. Ergo, using the PNRS requires time and resources spent on nutritional intake documentation and analysis, suggesting this tool has greater resemblance to a nutritional assessment tool rather than a nutritional screening tool. The aims of the aforementioned screening tools are displayed in Table 2.

Within the aforementioned tools STAMP, SGNA, and PYMS include anthropometric measurements thus identifying nutritional status upon admission, while the other tools merely provide the perceived risk of deterioration. PNRS, PYMS, and STRONGkids were also designed to prognostically predict clinical outcomes (without nutritional intervention) such as weight loss of >2% or LOS. However, it should be noted that LOS may not function as a direct assessment of nutritional risk because it may be influenced by many other factors and a causative relation has not been shown (Huysentruyt et al. 2015).

Another complexity arising from comparison is the validation methods used for each tool. There is currently no agreed upon "gold standard" for the assessment of malnutrition and malnutrition risk (since there is no universally accepted definition of malnutrition). In the absence of a nutrition screening tool that can act as a "gold" standard, information on the agreement between tools (concurrent validity) is used, especially when the comparison involves tools developed for the same purpose and when no judgment is made about the superiority of one tool over another (Elia and Stratton 2012). For example, the NRS was compared to NRI for validation, the

Table 3 Studies comparing different screening tools with regards to clinical outcomes. The table presents studies recently published comparing different screening tools in regards to their ability to predict clinical outcome, in the hopes of identifying one nutritional screening tool superior to other. Different studies compared different tools with regards to different outcomes

	NRS	PNRS	STRONGkids	PYMS	STAMP	PNST	PeDiSMART	Clinical outcome
Chourdakis et al. 2016			٧	٧	٧			Anthropometry, LOS, infection rates
Huysentruyt et al. 2015		٧	٧	٧	٧			>2% weight loss, referral to dietitian, dietitians' assessment, nutritional intervention
Karagiozoglou-Lampoudi et al. 2015			٧	٧	٧		٧	Weight loss, nutritional support, LOS
Galera-Martinez et al. 2017			٧		٧			Anthropometry, LOS
Thomas et al. 2016				٧	٧			Anthropometry
Ling et al. 2011			٧		٧			Anthropometry, nutritional intervention

Key: *NRS* nutrition risk score, *PNRS* pediatric nutrition risk score, *STRONGkids* screening tool for risk on nutritional status and growth, *PYMS* paediatric yorkhill malnutrition score, *LOS* length of (hospital) stay, *STAMP* screening tool for the assessment of malnutrition in pediatrics, *SGNA* subjective global nutritional assessment, *PeDiSMART* the pediatric digital scaled malnutrition risk screening tool

STRONGkids was compared to WHO cutoff reference for malnutrition, STAMP was compared with a dietitian's nutritional assessment, and PYMS was compared to a dietitians' assessment as well as to both STAMP and SGNA.

In the absence of a universally agreed upon reference, attempts have been made to rank nutritional screening tools by their ability to predict outcome. Summary of the studies comparing the different tools with regards to clinical outcome measured is displayed in Table 3. For instance, the PNRS was designed to predict weight loss of more than 2% during hospitalization. Also, as part of its validation study, STRONGkids' allocation into nutritional risk groups was compared to LOS showing higher risk group had longer hospitalizations. Chourdakis et al. (2016) recently evaluated PYMS, STAMP, and STRONGkids compared with and were related to anthropometric measurements and clinical variables such as LOS and infection rates. Children categorized in medium and high-risk groups according to all tools were found to have significantly longer LOS compared with children in low-risk group. However, authors stated that a considerable portion of children with subnormal anthropometric measures were not identified by neither tool. Thus the use of these tools is not recommended by the authors. Using LOS as a means to rank nutritional screening tools raises some reservations. First, LOS as well as other clinical outcomes is subjected to many confounders and can be influenced by the medical staffs' policy or even by work load and availability of medical staff to discharge patients. This was also supported by Huysentruyt et al. (2015) who chose not to consider LOS as a direct assessment of nutritional risk, in their systematic review comparing PNRS, STAMP, PYMS, and STRONGkids. Moreover, when LOS as an outcome measure was controlled for confounders in the PeDiSMART validation study (Karagiozoglou-Lampoudi et al. 2015), it was found not to be significantly associated with nutritional risk. Another reservation is that a tool that is good at predicting outcomes in the absence of nutritional interventions is not necessarily good at predicting outcomes induced by nutritional interventions (Elia and Stratton 2012). Karagiozoglou-Lampoudi et al. (2015) used weight loss or nutrition support during hospitalization as an outcome measure and showed it was independently associated with the malnutrition risk groups' allocation on admission, regardless of the tool used for allocation.

In terms of practicality, a screening tool should be fast and easy to use and should be suitable for use by untrained personnel. In terms of time needed for administration, original validation studies did not report speed of administration. Ling et al. (2011) reported time of administration by two trained investigators, and found that while STAMP took 10–15 min due to anthropometric measurements, STRONGkids took merely 5 min. These findings were supported by Huysentruyt et al. (2013) that reported median time of 3 min for administration of STRONGkids in a validation study in Belgium. In terms of personnel needed to perform nutritional screening, while STAMP and PYMS were developed for use by nurses, the PNRS requires qualified personnel to assess nutritional intake, and STRONGkids was originally developed to be completed by junior physicians or pediatricians. Nevertheless, in a different validation study (Huysentruyt et al. 2013) STRONGkids was administered by nurses and was found to be easy to

use with substantial intra- and inter-rater reliability rates. Furthermore, in another very recently published study by Galera-Martinez et al. (2017), STAMP and STRONGkids were assessed for reproducibility and inter-rater reliability between expert staff specialized in pediatric nutrition (physicians and dietitians) and clinical staff nonexpert in nutrition. Agreement between expert and nonexpert staff was good: 94.78% for STRONGkids ($k = 0.72$ [$p < 0.001$]) and 92.55% for STAMP ($k = 0.74$ [$p < 0.001$]). These findings suggest whether STRONGkids was originally developed for administration by qualified personnel, it can be used for practice by clinical staff, as it is already widely used in current clinical practice by nurses (Joosten and Hulst 2014). The PeDiSMART was claimed to be appropriate for use with all clinical staff members. Nonetheless, it was validated for use by certified dietitians (Karagiozoglou-Lampoudi et al. 2015). Further research is needed to assess PeDiSMARTs' inter-rater reliability between a dietitian's assessment and other members of the clinical staff.

Not all nutritional screening tools describe the course of action that is advised according to screening results, and the ones who do, describe different follow-up care plans. The care plans advised by the different nutritional screening tools are displayed in Table 4.

Nutritional Screening Tools for Specific Medical Conditions

Different screening tools are designed for different purposes, to be used on one or more underlying disease (Elia and Stratton 2011). Some tools were originally designed to be used upon specific conditions such as SCAN, nutritional screening tool for childhood cancer (Murphy et al. 2016), or the nutrition risk screening tool in cystic fibrosis (McDonald 2008), while other tools were later on validated for specific purposes. For example, PeDiSMART was validated in 30 children with chronic kidney disease (Apostolou et al. 2014). Moderate inverse correlation was found between PeDiSMART score and PhA ($p = 0.001$), MUAMC ($p = 0.008$) as well as protein intake ($p = 0.016$). STAMP was also validated in 51 pediatric spinal cord injury (SCI) patients admitted to a tertiary SCI center (Wong et al. 2013). STAMP had moderate agreement with dietitians' assessment ($k = 0.507$). STAMP, PYMS, and PMST (modified STAMP) were tested in acute pediatric setting in 300 children (Thomas et al. 2016).

The tools were compared to WHO growth reference cutoffs for malnutrition. Those who scored medium or high risk by the tools were compared with those who could be considered malnourished or at risk of malnutrition using the WHO's definitions. The results showed poor sensitivity and specificity rates; however, it should be noted that WHOs' definitions for malnutrition assess current state rather than the risk for malnutrition. Thus it may not be the most suitable reference to test validity. Moreover, the majority of children at PICU are at nutritional risk of some degree, thus it may be more effective to directly perform nutritional assessment in the form of growth and intake assessment rather than nutritional screening.

PNRS, PYMS, STAMP, and STRONGkids were also tested in children with IBD (Wiskin et al. 2012). The tools were tested on 46 children and risk score was

Table 4 The care plans advised by the different nutritional screening tools according to screening result. Each screening tool offers a specific care plan according to the allocated risk group. The table presents the course of action advised by each tool according to the different risk groups allocated

Tools	Low risk	Moderate risk	High risk
NRS	N/A	N/A	N/A
PNRS	None	Weight surveillance, report intake, consider dietetic consult	Nutritional assessment, monitor intake, consider nutritional intervention
STRONGkids	Repeat screening weekly	Check weight twice a week, consider referring the child to a dietitian	Refer the child to a dietitian
PYMS	Repeat screening weekly	Repeat screening after 3 days	Refer the child to a dietitian
STAMP	Repeat screening weekly	Monitor the child's nutritional intake for 3 days, repeat screening after 3 days	Refer the child to a dietitian/ nutritional support team/ consultant
PNST	None	Not relevant (moderate risk category does not appear in the PNST)	Refer the child for further nutritional assessment, check if child was previously cared for by a dietitian, measure weight and height, commence food and fluid intake record
PeDiSMART	Check weight status weekly	Check weight status twice a week, repeat the screening after a week	Refer to a dietitian

Key: *N/A* no care plan available, *NRS* nutrition risk score, *PNRS* pediatric nutrition risk score, *STRONGkids* screening tool for risk on nutritional status and growth, *PYMS* paediatric yorkhill malnutrition score, *LOS* length of (hospital) stay, *STAMP* screening tool for the assessment of malnutrition in pediatrics, *SGNA* subjective global nutritional assessment, *PeDiSMART* the pediatric digital scaled malnutrition risk screening tool

compared to the degree of malnutrition according to WHO's definition (as expressed in ICD-10). The tools showed good agreement with one another ($k = 0.6$); however, no agreement was found between each tool and anthropometric measures ($k < 0.1$). Nevertheless, it should be noted that authors compared nutritional risk as assessed by the aforementioned tools, with criteria of nutritional assessment such as set by the WHO's definition for malnutrition.

Policies and Protocals

Implementation of Nutritional Screening in Pediatrics

In this chapter we have described recently published research in the field of nutritional screening in pediatrics, and the complexity of comparing the different tools in the hopes of identifying one screening tool that can be considered superior to others.

Below we describe the detailed policies that should be adopted in order to make implementation of nutritional screening in pediatrics feasible in various settings. Policy should acknowledge a number of main areas:

- The government's role in prioritizing nutritional screening and allocating resources for that matter
- The standardization of nutritional screening across different healthcare facilities
- The role of healthcare facilities in implementation of nutritional screening
- The need to establish follow-up protocol when advised and the means to do so
- The assessment of long-term nutritional outcomes

As nutritional screening in pediatrics is recommended by international organizations (Corkins et al. 2013; Agostoni et al. 2005; Kondrup et al. 2003), it should be made into a governmental policy in order to reduce disease complications (Sermet-Gaudelus et al. 2000; Cao et al. 2014), LOS (Hulst et al. 2010; Cao et al. 2014; Karagiozoglou-Lampoudi et al. 2015; Chourdakis et al. 2016), and overall economic burden on the healthcare system (Ahmed et al. 2012). To this date the majority of nutritional screening tools in pediatrics are suitable for use in specific healthcare facilities. Ergo, some tools are suitable for use in primary healthcare centers while other tools are suitable for ambulatory setting. Government should allocate resources to validate one nutritional screening tool for pediatrics that can be feasible for use in different healthcare settings. The use of a single screening tool in different healthcare settings will allow follow up and monitoring of nutritional status when child is being transferred from one healthcare facility to another. Government should also instruct that nutritional screening become mandatory upon arrival or admission to healthcare facilities, and should enforce implementation upon periodic inspection. Nutritional screening should be named as a quality measure at the national state, to ensure quality of care.

Different healthcare facilities should incorporate nutritional screening as part of their routine patient care. In order to do so, healthcare facilities should introduce nutritional screening tools and initiate training sessions on performance of such tools to all healthcare staff. All healthcare staff should be educated on the importance of nutritional screening in order to promote adherence. Healthcare facilities should develop a follow-up protocol which will define tendency required and the actions taken to ensure follow-up takes place. It is advised that each child will be assigned a case manager (such as the primary physician), who will supervise the process. Healthcare facilities should create a system that will allow physician minimal time consuming, easy and effective means to supervise nutritional status of children under their care, such as a computerized alert system for instance.

Only after nutritional screening is widely implemented, research can focus on long-term effects and assess whether early recognition of malnutrition risk produced appropriate nutritional intervention. Resources should be allocated, in the national and institutional level, to the study of nutritional risk screening effect on clinical outcomes such as rate of childhood infections, readmissions to hospitals, LOS as well as long-term outcomes such as long-term health parameters, social and academic achievements, and overall economic burden.

Dictionary of Terms

- **Malnutrition** – A state of over- or undernutrition that is accompanied by micro- and or macronutrient deficiencies, and causes malfunction at the level of the cell and/or the organ and/or the body.
- **Malnutrition risk** – The risk of deterioration into a state of over- or undernutrition.
- **Nutritional assessment** – The assessment of current nutritional status of a subject. Full nutritional assessment is usually a subjective process performed by a clinical dietitian and includes nutritional intake, review of nutrition-related blood tests, and anthropometric measurements.
- **Screening tool** – A questionnaire, form, or method that enables early identification of subject with specific conditions within a large group.
- **Length of hospital stay (LOS)** – Duration of hospitalization to a healthcare institute. This term is commonly used to represent clinical outcome of assigned intervention.
- **Anthropometry** – A term that describes measurements of the human body such as weight, height, mid-upper arm, or waist circumferences. Anthropometric indices are usually used for the purpose of nutritional assessment. In children anthropometric measurements are vital to assess growth and development.
- **Inter-rater reliability** – The degree of agreement between different raters when performing the same procedure.
- **Intra-rater reliability** – The degree of agreement between repeated tests performed by the same rater.
- **Concurrent validity** – The degree of agreement between a method and previously described methods aimed at testing the same thing.
- **Predictive validity** – The degree in which a certain test corresponds with measurable clinical outcome.
- **Reproducibility** – The degree in which a certain test can be reproduced to yield the same results either by the same rater or by different raters.
- **Sensitivity** – The proportion of positive observations recognized by the screening process among all the true positives in the population. For instance, the proportion of children with high risk for malnutrition as recognized by a certain screening tool applied, among all the children with high risk for malnutrition (combination of the children who were recognized by screening and those who were not).
- **Specificity** – The proportion of negative observations recognized by the screening process among all the true negatives in the population. For instance, the proportion of children with low risk for malnutrition as recognized by a certain screening tool applied, among all the children with low risk for malnutrition (combination of the children who were recognized by screening and those who were not).
- **Z-scores** – A term that describes distance from the mean as expressed by standard deviations.

- **Clinical outcome** – The effect of a certain intervention on the clinical state of a patient, as expressed by infection rate, comorbidity, duration of hospital stay, and so forth. The effect can be either short or long term.
- **Subjective clinical assessment** – An assessment of the clinical state of a subject which is determined by subjective impression of physical examination. In nutritional assessment this can include signs of physical wasting such as muscle or fat atrophy.
- **Gold standard** – The best available test/method/practice in a specific field.
- **Bioelectrical impedance phase angle (BIA PhA)** – A measurement used to assess muscle mass as part of the nutritional assessment procedure. The device applies a low-frequency current to the body and assesses the proportion of reactance and resistance. The result reflects the ratio of the body's cell mass to fat-free mass.

Summary Points

- Nutritional screening is recommended by multiple international organizations.
- While widely used in adults, nutritional screening is not routinely practiced due to the lack of a universally accepted, validated, easy-to-use screening tool.
- Over the past two decades many different screening tools emerged. Each tool designed for different purposes, to be used by different members of the clinical staff, on different healthcare settings.
- Comparison between the tools is complex due to the different methodology methods used, thus recent research was unable to properly compare between tools or demonstrate the superiority of one tool over the other. In choosing a screening tool one should take into account predictive and concurrent validity, reproducibility, and applicability in terms of ease and speed of application. Tools should also specify advised course of action.
- Further research should validate the use of a single screening tool in different healthcare facilities in order to facilitate continuous patient care between different settings.
- Nutritional risk screening should be administered on admission to healthcare facilities.
- Outcome measures should integrate the screening process and the nutritional interventions that follow the screening process.
- Follow-up of nutritional risk screening should be performed at the frequency suitable for the healthcare facility (frequently performed during hospitalization and less frequent on community settings).
- The implementation of nutritional risk screening in pediatrics should be made into governmental policy as a primary prevention strategy.
- Following implementation, research should focus on nutrition-related outcomes to investigate cost-effectiveness.

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Prealbumin and Retinol Binding Protein as Screening Tools for Malnutrition

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Contents

Abstract

Prealbumin (transthyretin) and retinol-binding protein are mainly secreted from the liver for what they are also called as hepatic proteins. Different hepatic or visceral proteins are increasingly used for the screening of malnutrition, according to many national and international guidelines from 1995 up to now. On the other hand, the screening is a clinical process and cannot be based only on

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a single marker. The clinical and economic impact of the application of different biochemical markers for malnutrition assessment in a variety of clinical settings has been extensively studied. Several previously published nutrition screening tools have been validated in a variety of clinical conditions and patient populations. The current approach to malnutrition screening encompasses a combination of screening tools with laboratory investigations.

Keywords

Prealbumin · Transthyretin · TTR · Retinol-binding protein · RBP · Malnutrition · Hepatic proteins · Biochemical screening

List of Abbreviations

AGP	α1-acid glycoprotein
AMA	Automated malnutrition assessment
APACHE II	Acute Physiology and Chronic Health Evaluation II (scoring system)
ASPEN	American Society for Parenteral and Enteral Nutrition
BMI	Body mass index
CBG	Corticosteroid-binding globulin
CPK model	Corey-Pauling-Koltum model
CRABP	Cellular retinoic acid-binding protein
CRBP	Cellular retinol-binding protein
CRP	C-reactive protein
ELISA	Enzyme-linked immunosorbent assay
HR	Hazard ratio
IL	Interleukin
IGF-I-BP	Insulin-like growth factor I-binding protein
LBM	Lean body mass
mRNA	Messenger ribonucleic acid
PA	Prealbumin
PEM	Protein energy malnutrition
PINI	PROGNOSTIC Inflammatory and Nutrition Index
RARs	Retinoic acid receptors
RBP	Retinol-binding protein
RXRs	Retinoic X receptors
SGA	Subjective Global Assessment
SIRS	Systemic inflammatory response syndrome
SOFA	Sequential Organ Failure Assessment (scoring system)
STRA 6 receptor	Stimulated by retinoic acid 6 receptor
TBG	T4-binding globulin
T3	Triiodothyronine
T4	Thyroxine
TNF α	Tumor necrosis factor alpha

TTR	Transthyretin
VA	Vitamin A
γ-interferon	Gamma interferon
TPBA	Thyroxine-binding prealbumin

Introduction

Prealbumin is also called transthyretin (TTR). It was first recommended as a malnutrition marker in 1972 by Ingenbleek et al. in a clinical observation of 40 children with pre-kwashiorkor. Several years later, the same author published the results of an observation which studied the use of retinol binding protein as a malnutrition marker. The authors investigated and compared the use of albumin, prealbumin, and transferrin and retinol-binding protein (RBP) for the biochemical screening of malnutrition (Ingenbleek et al. 1975). In 1995, the first set of recommendations regarding the application of TTR were published in the consensus document, "Measurement of visceral protein status in assessing protein and energy malnutrition: Standard of care: Prealbumin in nutritional care consensus group" (Bernstein et al. 1995). This document defined the necessity of applying some laboratory indices in the malnutrition screening and monitoring. The First International Transthyretin Congress dedicated to the clinical use of transthyretin took place as recently as in 2001 in Strasbourg, France.

According the WHO definition, malnutrition is a highly prevalent condition in developing countries (Blössner and de Onis 2005). In western countries, malnutrition is also found frequently, especially in some vulnerable populations – elderly people, patients admitted to hospital, patients with chronic diseases, etc. This strongly underlines the need for separate diagnostic approaches and screening tools for the different patient populations.

There is a huge amount of research aimed at describing the development of malnutrition in different diseases and its long- and short-term effects on potential complications and clinical outcomes (Jensen 2006). ASPEN provided an etiologically based definition of malnutrition (Jensen et al. 2010). As the screening per se is a process for the assessment groups of individuals to identify a disease, the clinicians should use a number of tools, methods, and markers for the clinical assessment of malnutrition, especially in risk groups. Guidelines recommend the use of different screening tools, such as Malnutrition Universal Screening Tool, Nutritional Risk Screening 2002, Mini Nutritional Assessment®, Short Nutritional Assessment Questionnaire©, Malnutrition Screening Tool, and the Subjective Global Assessment. Biochemical screening performed through the analysis of TTR, independently or in combination with RBP, albumin, and transferrin, is recommended as an additional test (Jensen et al. 2010). Contrary to the screening applied to big cohorts, there is a need for an individual approach to evaluate the specificities in each patient. From this point of view, we could say that there is no universal marker serving any case. In

order to explain properly the biochemical screening markers, it might be necessary to look back into the works of the pioneers in experimental medicine. What, and who discovered the structure and properties of our proteins of interest, and when?

TTR and Retinol-Binding Protein Structure and Functions

TTR was discovered in human cerebrospinal fluid in 1942. The name prealbumin originated from its place just before albumin on electrophoresis (Kabat et al. 1942). Promptly afterwards, it was also identified in serum samples. German authors described the main physicochemical characteristics of TTR. They also found a high proportion of tryptophan in its structure (Schönenberger et al. 1956). In the 1950s and 1960s, it was found that prealbumin, like albumin, participates in thyroxine transport at a considerably lower degree than the thyroxine-binding globulin. This discovery led to modification in the name of prealbumin to thyroxine-binding prealbumin TPBA (Ingbar 1958). By the end of this period, it was found that prealbumin has another transporting function (Kanai et al. 1968). Prealbumin forms a complex with a smaller molecule carrier of vitamin A and for this reason received the name retinol-binding protein (Fig. 1). The discovery of the TTR physiological role of thyroxine- and retinol-transporting protein led to the acceptance of the modified name trans-thy-retin in 1981 (Robbins 2002). Prealbumin and transthyretin are used as synonyms in literature. In 1974, Kanda et al. described the amino acid sequence of transthyretin. The gene related to TTR synthesis was identified on the long arm of the chromosome 18q23 (Wallace et al. 1985). The mature TTR molecule comprises four identical subunits (Fig. 2), each of them of 127 amino acids, which are noncovalently linked (Sasaki et al. 1985). The molecular mass of prealbumin is 55 kDa. In physiological conditions, the central part of transthyretin molecule has two identical thyroxine linking sites (Robbins 2002). The RBP-retinol complex binding parts are situated in the lateral parts of the

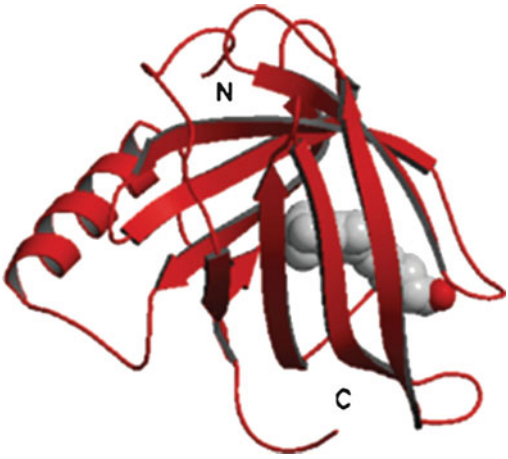

Fig. 1 Ribbon diagram of the retinol-binding protein molecule. The retinol molecule is represented as a CPK model (Corey-Pauling-Koltum model) (Monaco 2002 with permission)

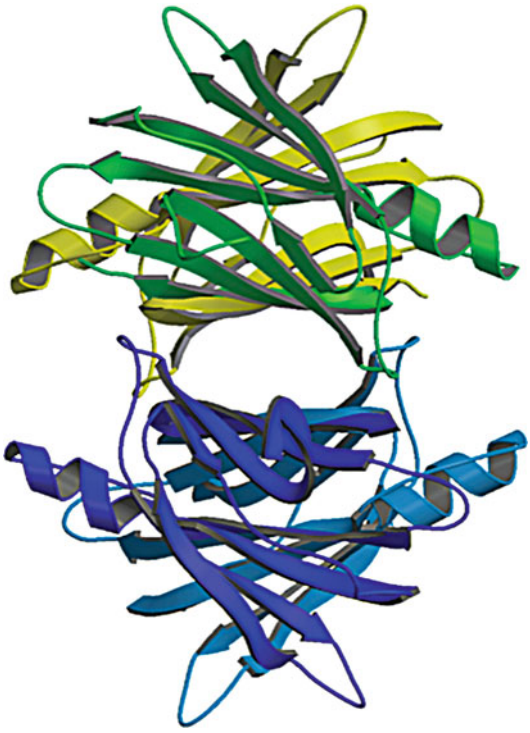

Fig. 2 Ribbon representation of the transthyretin (TTR) tetramer. This is the view down the z axis of the TTR tetramer (Monaco 2002 with permission)

transthyretin molecule. The RBP-retinol complex interacts with only one of the sites at any moment. The three components of the retinol circulating complex have a total molecule mass of 76 kDa and they circulate bound in the proportion of 1:1:1. Crystallographic studies showed that the in vitro binding capacity of one molecule TTR is with maximum two RBP molecules (Monaco 2002), Fig. 3. The molecule mass of the retinol binding protein (RBP) is approximately 21 kDa. RBP belongs to the lipocalin protein family, known for its capacity to bind hydrophobic ligands (Newcomer and Ong 2000). RBP is produced mainly in the liver, where it is released linked with retinol. In normal conditions, RBP is completely saturated with retinol. The expression of RBP in the liver is dependent on vitamin A and the nutritional status (Ronne et al. 1983; Noy and Xu 1990). Retinol is bound to RBP in the Golgi apparatus of the liver cell, from where it is secreted, bound to a second protein TTR (Noy 2000). In the case of vitamin A deficiency, RBP is accumulated in the endoplasmic reticulum of the liver cell. The presence or absence of retinol determines only the secretion, but not the synthesis of RBP (Ronne et al. 1983). More recently other sites of RBP synthesis were identified. These are the kidneys, peritubular and Sertoli cells in the testicles, the retina epithelium, the choroid plexus of the brain (Newcomer and Ong 2000), and adipose tissue (Burtis et al. 2012, p. 526) among others. RBP in blood circulation (named also RBP4) ensures the provision of tissues with retinol. In target tissues, RBP binds to a membrane receptor identified as STRA 6 (Fig. 4),

Fig. 3 Quaternary structure of the (RBP)2-TTR hexamer present in the crystals of the chimeric complex chicken RBP human TTR. The TTR molecule is oriented as in Fig. 2 (Monaco 2002 with permission)

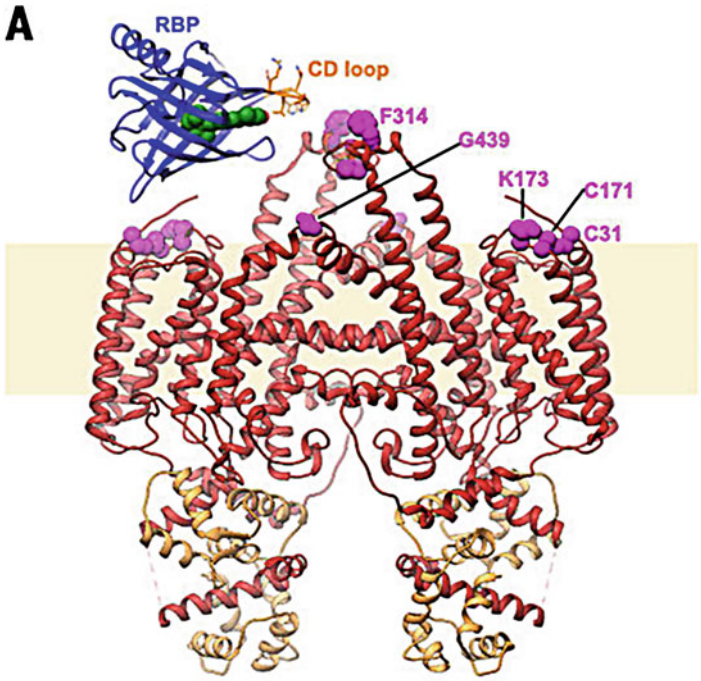

Fig. 4 Possible mechanism for STRA 6-mediated retinol uptake. Ribbon representation of the STRA6-CaM complex, viewed from the plane of the membrane, with STRA6 colored in *red* and CaM in *gold*. Residues previously shown to be important for RBP binding are shown as *magenta spheres*. *Green spheres*, retinol. The abbreviations used are the following: *STRA 6* stimulated by retinoic acid 6, *CaM* calmodulin, *RBP* retinol-binding protein (Chen et al. 2016 with permission)

which promotes the cellular adhesion of retinol (Sundaram et al.1998; Kawaguchi et al. 2015; Chen et al. 2016). Cellular retinol-binding proteins (type I, II, III, and IV) facilitate the intracellular transport of retinol (Noy 2000). Figure 5 represents a scheme of vitamin A absorption, digestion, and transport to the liver and delivery to target tissues together with main sites of cellular RBPs. After the retinol is released from the protein complex, it is quickly dissociated and the residual RBP rapidly catabolized in the kidneys. Depending on whether it is bound to retinol or not, in the circulation both forms, holo-RBP (bound to retinol) and apo-RBP (not bound to retinol), can be observed (Zanotti and Berni 2004).

The biological half-life of TTR is 2 days; in contrast to it, the half-life of RBP is 12 h. The half-life of apo-RBP, released from the retinol ligand, is only 3.5 h (Burtis et al. 2012, p. 522).

Reference Ranges and Cutoff Points

Ritchie et al. (1999) found that TTR levels in adults are different according to gender and age. Currently proposed reference ranges for adults for TTR are 0.2–0.4 g/L (Burtis et al. 2012, p. 527; Ledue and Collins 2011) and 0.03–0.06 g/L for RBP respectively. Children's reference ranges for TTR are presented in Table 1. RBP levels at birth are 0.011–0.034 g/L (1.1–3.4 mg/dL), and at 6 months they increase to 0.018–0.05 g/L (1.8–5.0 mg/dL) (Burtis et al. 2012, p. 527).

Bernstein and Ingenbleek (2002) suggested the criteria (cutoff points) for TTR interpretation to determine the malnutrition risk in the initial assessment. They are presented in Table 2.

Analytical Methods

TTR can be measured by nephelometric or by turbidimetric assays. The same methods are applicable to modern automated biochemical analyzers routinely used in practice.

RBP can be also measured by nephelometry, or by ELISA. The radial immunodiffusion methods are not currently preferable in routine practice (Burtis et al. 2012, p. 526).

Factors Influencing the Transthyretin and Retinol-Binding Protein Levels

Hepatic Function

TTR is produced mainly in the liver and the choroid plexus. Small amounts of TTR are synthesized in the beta-cellular structures in the pancreas, and retina. It is known that the control of the liver and the choroid plexus TTR production is independent (Buxbaum and Reixach 2009).

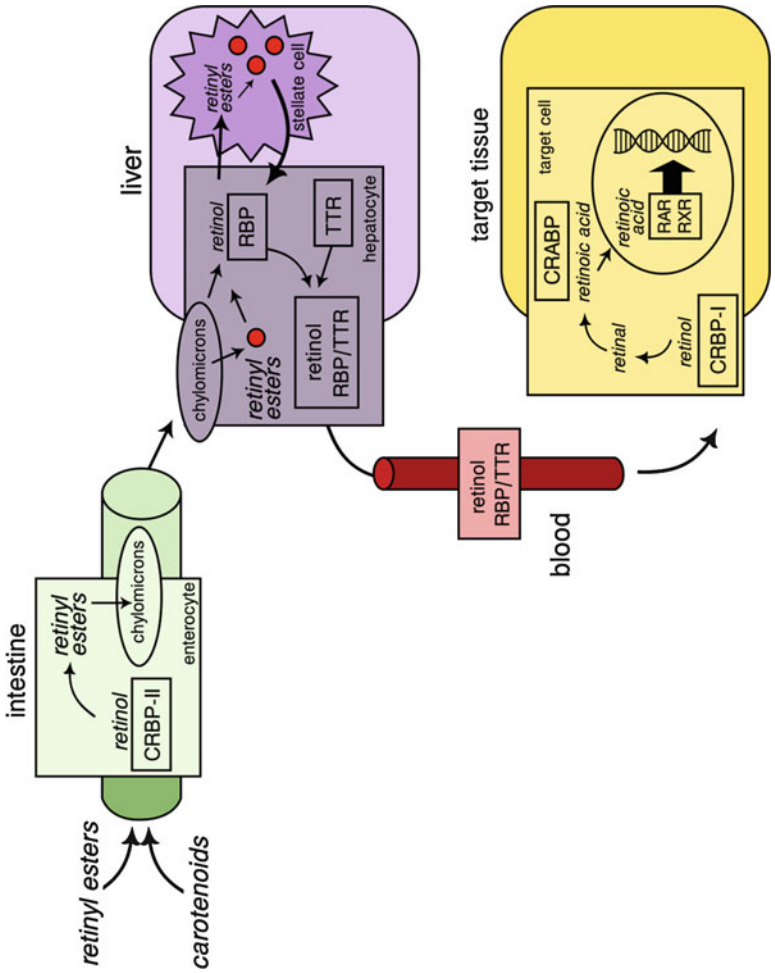

Fig. 5 Schematic drawing representing vitamin A absorption, digestion, transport to the liver and delivery to target tissues. Vitamin A metabolism can be classified into three major processes – intestinal uptake, hepatic storage, and tissue-specific metabolism. Cellular retinol-binding proteins (CRBPs) are important for the vitamin A metabolism. CRBPs have a similar structure and size ~14.6 kDa. Each of them contains a single binding site for one molecule retinoid (retinol,

Table 1 Reference ranges for transthyretin

	Reference ranges transthyretin	
Age	mg/dL	g/L
<1 month	7–39	0.07–0.39
1–6 months	8–34	0.08–0.34
7 months–6 years	12–36	0.12–0.36
Adults	20–40	0.2–0.4

Adapted from Davis et al. (1996) and Burtis et al. (2012, p. 527)

Table 2 Transthyretin levels for nutritional assessment

Transthyretin		Interpretation
mg/dL	g/L	
<5	<0.05	Critical
5–10	0.05–0.1	High risk
11–19	0.11–0.19	Mild
20–40	0.2–0.4	Normal

Bernstein and Ingenbleek (2002) with permission

Low serum levels of transthyretin and RBP are accepted as signs of liver injury, and a primary liver disease should be ruled out in case of low levels. Albumin is the most frequently used protein to assess the hepatic function and has a survival prediction role (Castera 2011; Dooley et al. 2011, p. 110). Albumin is part of the Child-Pugh scoring system for severity of liver disease. The progression of liver disease (i.e., decompensated cirrhosis, acute alcoholic or toxic hepatitis) is accompanied with reduced hepatic synthetic function. The consequence is the reduction of the values of all visceral proteins. The nature of the changes observed in the protein levels (albumin, TTR, and others) during the progression of a hepatic disease reflects mainly its severity (Yovita et al. 2004; Dooley et al. 2011, p. 21). On the other hand, hepatic insufficiency in liver cirrhosis is associated with prevailing catabolic processes, persistence of subacute or chronic inflammations, and clinically overt malnutrition in different grades (Toshikuni et al. 2014).

Fig. 5 (continued) retinal, or retinoic acid). CRBP-I is expressed in the adult in multiple tissues: liver, kidney, lung, reproductive organs, choroid plexus in the brain, and in pigment epithelium cells in the eye. In contrast, in the adult, CRBP-II is restricted to the small intestine, where it is located in mature enterocytes on the villi of the mucosal epithelium (Noy 2000). CRBP-III and CRBP-I have overlapping expression in heart, muscle, adipose, and mammary tissue. Cellular retinol-binding proteins (type I, II, III, and IV) facilitate the intracellular transport of retinoid inside the cell. Retinol is transformed in the target cell through a series of specific reactions to the retinoic acid, which interacts in the nucleus via specific transcription factors, called retinoic acid receptors (RARs) or retinoic X receptors (RXRs). The abbreviations used are the following: *CRBP-I and -II* cellular retinol-binding protein I and II, *RBP* retinol-binding protein, *TTR* transthyretin, *CRABP* cellular retinoic acid-binding proteins, *RAR* retinoic acid receptors, and *RXR* retinoic X receptors (Bellovino et al. 2003 with permission)

TTR and RBP Against the Background of Protein Insufficiency

Many experimental studies in the 1980s and 1990s support the idea that the limitation of the protein import to the body leads to reduction of the protein synthesis (Smith and Lunn 1984). The limited intake of amino acids in the context of protein-energy malnutrition without any juxtaposed inflammation leads to reduction of the nitrogen pool. It is also accompanied by suppressed hepatic production of transthyretin mRNA (De Jong and Schreiber 1987) and corresponding reduction of the export of TTR molecules to the circulation (Straus et al. 1994). On the other hand, the total nitrogen pool in the human body correlates closely to the lean body mass (LBM) or fat free mass. Ingenbleek and Young (2002) concluded that transthyretin could serve as a marker for the LBM which is linked with the free nitrogen in average relationship equal to 6.25. This data undoubtedly support the hypothesis that the circulating proteins produced by the liver cells reflect the dynamic changes in energetic and protein metabolism of the body.

Reduced Values Due to Inflammation

Several immunological and biochemical phenomena play a key role with respect to the TTR and RBP levels in acute or chronic inflammation, regardless of whether overt of clinically subtle.

Role of Cytokines

The inflammation, irrespective of the underlying causes, usually begins with leukocytes activation and cytokine release (Bienvenu et al. 2000). The influence of cytokines on protein metabolism correlates with the durability and severity of the main process. A variety of systemic diseases leads to enhanced secretion and/or release of interleukin-1, tumor necrosis factor alpha, interleukin-6, and gamma interferon. Cytokines are known to rearrange the protein metabolism (Gabay and Kushner 1999). It was previously reported that cytokines could control and stimulate the production of acute-phase proteins (CRP, α1-acid glycoprotein (AGP), fibrinogen, haptoglobin, α1-antitrypsin, antichymotrypsin, and others (Bienvenu et al. 2000) frequently called positive acute-phase proteins. Simultaneously with the enhanced synthesis of positive acute-phase proteins, cytokines could also cause a decrease in albumin, transferrin, prealbumin, and retinol-binding protein synthesis. The latter proteins are called negative acute-phase proteins. Interleukin-6 (IL-6) is accepted as a key mediator in acute and chronic inflammation. Its serum level is accepted as a reliable marker in determining the inflammatory state (Ohzato et al. 1992; Clarke et al. 2011). It was shown in animal models that the stress-induced increase of IL-6 led to rise of acute-phase reactants levels with the reciprocal suppression of TTR synthesis (Murakami et al. 1988). Further clinical experiments confirmed these observations (Banks et al. 1995).

Hormonal Alterations

Proinflammatory cytokines stimulate the supersecretion of regulatory hormones, such as glucocorticoids, cathecolamines, glucagon, and growth-hormone. This could happen in response to the hypoglycemic and anabolic effect of the secreted insulin and therefore contribute to insulin resistance in healthy tissues (Gelfand et al. 1984). Independently from the moderate hyperglycemia, glucose and amino acids are redistributed to the damaged tissues having protective and restorative functions. TTR, RBP, T4-binding globulin (TBG), and CBG have an effect on the overall early endocrine reaction during the inflammation. Within the first 3–4 days of sepsis or severe injury, plasma concentrations of TTR, RBP, CBG, and TBG decrease by half. These low plasma values are also associated with decreased binding capacity for FT4, retinol, and cortisol. On the other hand, in the extracellular fluids elevated concentrations of free cortisol, FT4, and free retinol occur, together with the increased intracellular levels of the free mediators. All T4-, retinol-, and cortisol-dependent processes are magnified during the inflammation, which enhance the immune response in the areas with inflammation and regulate energy expenditure and anabolic processes in healthy tissues (Ingenbleek and Bernstein 1999; Fig. 6 and Table 3).

Prevailing Catabolic Processes and Development of Negative Nitrogen Balance

Acute infections like SIRS, for example, lead to predominantly protein catabolism, which affects the muscular tissue and results in amino acids release (Arnold et al. 1993) The peak of increased urine excretion of nitrogen-containing substances (urea, ammonia, and creatinine, together with trimethylhistidine, hydroxyproline, and free amino acids) is usually detected between the 3rd and 5th (8th) day. These changes correspond to the low transthyretin levels. Urine nitrogen secretion and transthyretin levels return to their normal levels when the inflammatory process declines. In the case of persisting inflammation, sepsis, or metabolic complications, the catabolic processes substantially prevail and transthyretin levels do not return to normal ranges (Ingenbleek and Bernstein 2015).

ASPEN's recommendation does not actively support the application of transthyretin for nutritional status assessment in intensive care units. Nevertheless, some clinical observations have proven that transthyretin negatively correlated with the length of stay, APACHEII score, SOFA score, and mortality (Devakonda et al. 2008).

In many cases of clinical practice, the influence of nutritional inflammatory factors on the hepatic synthesis of TTR and RBP is simultaneous. Therefore, serial measurements were recommended, rather than single determination of biochemical malnutrition markers. Routine daily dynamic monitoring by serial measurements is preferable in severely ill patients. Twice weekly biochemical monitoring would be wise for those with moderate inflammation. Adequate therapeutic and nutritional intervention corresponded with the gradual trend of transthyretin increase, compared to the lowest measured value. On the other hand, the maintenance of low transthyretin concentrations over days and weeks indicates ongoing catabolic processes or the presence of underlying comorbidity (Ingenbleek and Bernstein 2015).

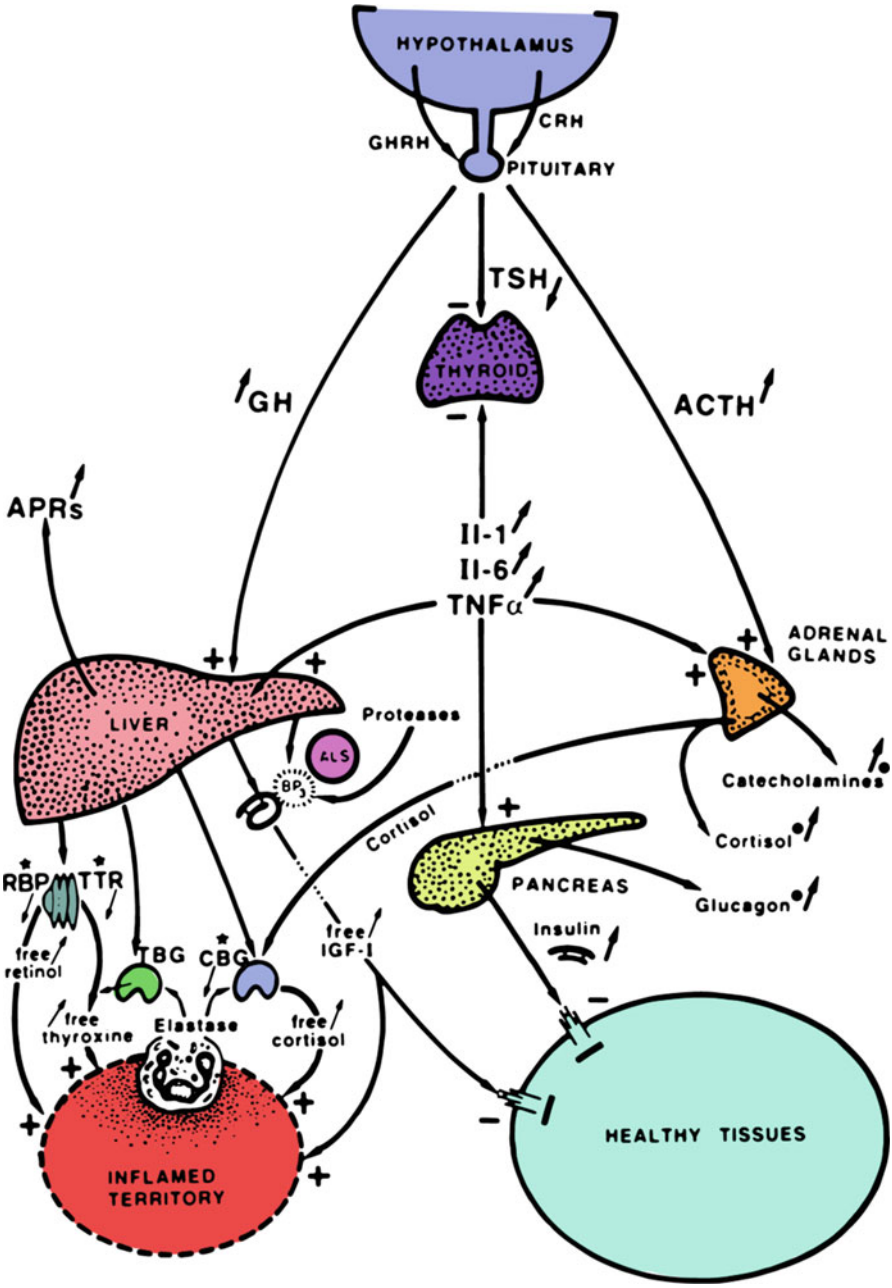

Fig. 6 Schematic diagram of the metabolic dichotomy found during stress. The three main cytokines (interleukin-1 [IL-1], IL-6, tumor necrosis factor-α [TNF-α]) stimulate the hypersecretion of insulin and glucagon by the pancreas and that of cortisol and catecholamines by the adrenal glands. In healthy tissues, stress-induced insulin postreceptor defect (—) creates a stage of insulin

Table 3 Main physicochemical and metabolic characteristics (healthy adult man) of three carrier proteins involved in the stress response

	CBG	TTR	RBP
Molecular mass (Da)	42,650	54,980	21,200
Conformation	Monomeric	Tetrameric	Monomeric
Amino acid sequence	383	4 × 127	182
Carbohydrate load	23% glycosylated	Unglycosylated	Unglycosylated
Hormonal binding sites	One for cortisol	Two for TH	One for retinol
Association constant ($M^{-1)}$	3×10^7	7×10^7 (T4)	1.9×10^7
Normal plasma concentration	30 mg/L	300 mg/L	50 mg/L
Biological half-life	5 days	2 days	14 h
Bound ligand concentration	120 μg/L	80 μg TT4/L	500 μg/L
Free ligand concentration	5 μg/L	20 ng FT4/L	1 μg/L
Ratio free:bound ligands	4%	0.034%	0.14%
Distribution volume of free moieties	18 L	12 L	18 L

Ingenbleek and Bernstein (1999) with permission
CBG corticosteroid-binding globulin, *RBP* retinol-binding protein, *TTR* transthyretin

Influence of Renal Function on TTR and RBP Levels

It is known that the chronic kidney disease (CKD) affects TTR and RBP levels, due to their delayed elimination (Cano 2002). Therefore, the assessment of the renal function is the third important factor to be taken into account in order to interpret the results correctly.

Objective evaluation, concordant with clinical observations in predialysis and dialysis patients, illustrates how the adoption of strict criteria compliant with a specific biochemical profile of a disease contributes to the correct interpretation and clinical

Fig. 6 (continued) resistance and hyperglycemia strengthened by counterregulatory hormones (•). Inhibition of thyroid activities, working in concert with insulin resistance and rising growth hormone (GH) levels, downregulates most anabolic pathways, allowing them to reduce protein breakdown and to drive the whole body economy upon lipolytic dependency (RQ ~ 0.7). Inflamed territory is characterized by upregulation of anabolic processes aimed at promoting defense mechanisms and tissue repair, grounded on increased requirements of energy from glycolytic sources (RQ ~ 1). Cytokines rearrange the liver synthetic priorities, favoring overproduction of acute-phase reactants (*APRs*) at the expense of acute-booster reactants (*ABRs*) (★). Following the free hormonal theory, depressed secretion of transthyretin (*TTR*), retinol-binding protein (*RBP*), and corticosteroid-binding globulin (*CBG*) releases substantial amounts of ligands readily available to cells, enhancing thyroid-, retinoid-, and steroid-dependent pathways at the site of inflammation. The local endocrine tone is still reinforced by the elastase-induced digestion of specific carrier proteins (*CBG*, thyroxine-binding globulin [*TBG*]) belonging to the serpin superfamily. Enzymic cleavage of insulin-like growth factor-binding protein-3 (*IGFBP-3*) in the bloodstream increases the supply of free insulin-like growth factor 1 (*IGF-1*) and potentiates mitogenic processes and N accretion. These last effects fail to develop in healthy tissues due to cytokine-induced IGF-1 postreceptor defect (—). Symbols: Increased (↑) or decreased (↓) concentrations. Stimulation (+) or inhibition (−) of effector tissues (Ingenbleek and Bernstein 1999 with permission)

use of the transthyretin values. Current studies (Rambod et al. 2008; Lee et al. 2016) mainly support earlier recommendations for transthyretin use as a malnutrition and prognostic biomarker for CKD patients (Sreedhara et al. 1996; Mittman et al. 2001).

Other Factors

While discussing the main factors which influence TTR and RBP serum levels (gender, age, limited protein administration, hepatic synthetic function, inflammation, zinc deficiency, and renal function) we have to mention additional factors. Nonsteroid anti-inflammatory medicines, cortisol, and anabolic steroids, among others lead to an increase in TTR and RBP levels (Burtis et al. 2012, p. 526).

Interestingly, obesity also influences RBP levels. RBP is secreted not only by the liver but also by the adipose tissue. In cases of obesity, impaired glucose tolerance, and diabetes mellitus type 2, the RBP levels measured in serum correlate with the grade of insulin resistance (Graham et al. 2006). In obese or diabetic patients, RBP levels could be increased. These observations might be of importance in the obesity epidemic in recent years.

TTR and RBP as a Part of an Integrated Approach to Diseases

The application of TTR and RBP screening alone or in combination with various inflammatory markers such as CRP, IL-6, procalcitonin, and anthropometric data with malnutrition screening tools could add a lot, depending on specific clinical context. Practically, it is impossible to make a correct interpretation of TTR and RBP values out of the specific clinical context of the disease. In many cases, malnutrition and inflammation are mutually potentiating factors, exerting a synergic influence on the outcome of the disease (Jensen 2006).

An example of a complex approach to the disease is the application of TTR as a screening and prognostic marker in cancer patients. A number of studies support earlier data that TTR levels between 180 and 200 are critical. Lower levels imply a higher risk of fatal outcome. Levels below 110–100 reveal depletion of LBM stores (Ho et al. 2003). A study in renal cancer patients reveals that low basic levels of prealbumin dependently predict the overall survival (HR 1.963; 95% CI, 1.140–3.381; $P = 0.015$) and the progression free survival (HR 2.021; 95% CI, 1.227–3.329; $P = 0.006$, Cai et al. 2017). Similar results were also shown for patients with gastric (Esfahani et al. 2016; Zhou et al. 2017), colorectal (Shimura et al. 2016), and other types of cancers.

Elderly people are also a target group which may benefit from the use of transthyretin as a screening tool for malnutrition assessment. The prevalence of malnutrition in this group is between 29% (Guigoz et al. 1994) and 50% (Visvanathan et al. 2004). In elderly population, the latent malnutrition associated with low or reduced transthyretin levels is related and potentiates the increase of homocysteine. In the last decade, extensive research has analyzed the correlation between transthyretin and homocysteine, as well as between lean body mass and

homocysteine (Ingenbleek et al. 2002). As the link between homocysteine and vascular disease is known, the above data features a new discussion on the relationship between malnutrition and the increased thrombovascular events, i.e., cardiovascular risk in the elderly.

A classic example for the use of transthyretin and retinol-binding protein as malnutrition markers is the studies of vitamin A levels and its deficiency. Retinol-binding protein has been recommended for individual application as a marker that reflects the vitamin A status (Almekinder et al. 2000). Serum RBP levels strongly correlate with serum retinol ($r = 0.88$) in healthy individuals. This correlation remains significant in different conditions like HIV-1 infection, protein-energy malnutrition (PEM), and acute inflammatory response (Baeten et al. 2004). The clinical scientists involved in vitamin A deficiency research in developing countries in the 1990s underlined that PEM always accompanied the deficit of micronutrients to different extents. That leads to the conclusion that the control of both should be simultaneous (McLaren and Kraemer 2012, p. 148). In this context, the concomitant analysis of RBP and TTR for the diagnosis of the vitamin A deficiency plays a key role (Ingenbleek and Young 1994).

TTR and RBP in Indices and Score Systems

Calculating CRP and TRP ratio is among the easiest and most accessible methods of assessing malnutrition and inflammation effects. Férard et al. suggested in 2002 a cutoff CRP/TTR value = 1 in acute inflammatory conditions. Earlier an AGP and TTR ratio was proposed for assessment of the chronic inflammatory diseases (Charet et al. 1996). These indices could give an approximate estimate of the impact of inflammation on the changes in the biochemical markers. Although not widely accepted, they are quite convenient for routine clinical practice.

It was previously discussed that in cases of vitamin A deficiency the molar ratio RBP:TTR contributes to the evaluation of the inflammatory effect on serum vitamin A levels (Rosales and Ross 1998). The suggested cutoff level is 0.36 or less for the RBP/TTR ratio. Accordingly, the value could be used to monitor the efficacy of vitamin A supplementation and also to select persons with critical vitamin A deficiency. Later study validated a cutoff value of 0.37 for the RBP/TTR ratio in adults (Zago et al. 2002).

Prealbumin is a part of several score systems for the assessment of malnutrition:

- Maastricht Index (21 − 0.24 × albumin − 19 × transthyretin (prealbumin) − 1.9 × lymphocytes − 0.04 × percentage of ideal weight) proposed initially by De Jong et al. in 1985.
- Prognostic Inflammatory and Nutrition Index (PINI) (Ingenbleek and Carpentier 1985)

$$\mathrm{PINI} = \frac{\mathrm{CRP(mg/L)} \times \mathrm{AGP(mg/L)}}{\mathrm{SA\ (g/L)} \times \mathrm{TTR(mg/L)}}$$

PINI is a coefficient, integrating two of the most frequently used markers for the assessment of inflammation grade CRP (mg/L) and AGP (mg/L). AGP is an acute-phase protein, whose production depends mainly on the concentration of interleukin-1. AGP's half-life is between 5 and 7 days. CRP is a nonglycosylated protein, whose production depends mainly on the presence of interleukin-6. The half-life of CRP is much shorter: 4–6 h. Normally, the values of PINI are below 1. Values exceeding 30 indicate serious and life-threatening conditions (Ingenbleek and Young 1994 with permission).

David et al. (2013) suggested the use of a combination of laboratory and anthropometric data in AMA. In the initially published model, the main visceral protein was albumin, but the authors also reported results with prealbumin.

Transthyretin is often included in panels of biomarkers, developed by proteomic analysis (Bakry et al. 2011). A new generation surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) mass spectrometry (MS) was used to discover the specific and unique combination of serum biomarkers characteristic for different diseases. In the next step, the markers were identified by matrix-assisted laser desorption/ionization (MALDI-) coupled with time-of-flight mass spectrometry (TOF-MS) and finally immunologically verified. The aim was to develop panels of new prognostic biomarkers able to improve the diagnosis and to predict the likelihood of different clinical outcomes. This approach has been already applied in ovarian cancer (Zhang et al. 2004), esophageal cancer (Kelly et al. 2012), and breast cancer (Chung et al. 2014) patients, as well as in other nonmalignant diseases like chronic pancreatitis (Hartmann et al. 2007), etc.

Conclusion

Malnutrition, in particular the reduction of LBM, can be a consequence of insufficient protein and calorie intake, inevitably leading to reduced protein synthesis. It could be also cytokine-induced, a result of a tissue-associated proteolysis, negative nitrogen balance, and prevalence of catabolic processes. Malnutrition might be a combination of these two factors acting to different extents. TTR level depends on its hepatic production which, in return, is influenced by protein intake and by inflammation. Since the initial experimental and clinical observations of transthyretin functions in the 1990s, many nutritionists and physicians successfully apply this marker in routine practice as a screening and predictive tool. With this review, which does not claim to be fully detailed, we have tried to demonstrate the application of transthyretin in the daily clinical practice of different medical specialities. Any screening tool, including one used in malnutrition, has its advantages and limitations. In this context, we cannot recommend a universal approach. Due to its unique biochemical nature, transthyretin is a marker that reflects protein losses in a variety of diseases. It is worth using it for establishing of predictive disease models based on the protein analysis, but also in software-based models and formulas, such as the AMA case.

Polices and Protocols

In this chapter, we have described the significance of biochemical screening via assessment of transthyretin and retinol-binding protein and the main factors influencing their levels. Application depends on national policies and local guidelines. It is important to know that measurement of transthyretin and retinol-binding protein is useful for malnutrition screening, prognosis, and monitoring of therapeutic interventions in combination with other data. The additional data set depends on the screened population, cohorts, and disease-specific characteristics.

Dictionary of Terms

- **Biochemical screening for malnutrition** – The serum concentrations of several proteins that are synthesized in the liver are used as indicators of protein-calorie status – albumin, prealbumin (transthyretin), transferrin, and retinol-binding protein.
- **Hepatic synthetic function** – The ability of the liver to produce proteins including coagulation factors, lipids, bile acids, and other molecules. The most common used hepatic synthetic function tests are albumin and prothrombin.
- **Negative acute phase response proteins** – A group of proteins whose levels decrease during inflammation. This includes albumin, prealbumin, retinol-binding protein, transferrin, and apo-AI lipoprotein.
- **Positive acute phase response proteins** – A group of proteins whose levels increase during inflammation. This includes C-reactive protein, α1-acid glycoprotein, interleukin-1, interleukin-6, tumor necrosis factor-α, γ-interferon, and others.
- **Visceral proteins** – These are also named as hepatic proteins (which are produced in the liver) and include albumin, prealbumin, retinol-binding protein, and transferrin.

Summary Points

- Prealbumin, also called transthyretin, is a protein with a molecular weight of 55 kD. It transports around 10% of thyroid hormones and more than 90% of retinol.
- The three components of the retinol circulating complex which include transthyretin, retinol-binding protein, and retinol have a total molecular mass of 76 kDa. They also circulate in blood, bound in a proportion of 1:1:1.
- Both transthyretin and retinol-binding protein are produced mainly by the liver cells. Together with albumin and transferrin, transthyretin and retinol-binding protein are used for biochemical screening for malnutrition. Current guidelines suggest application of biochemical laboratory data, together with other screening tools for malnutrition.

- Many factors influence levels of transthyretin and retinol-binding protein: gender and age, liver function, starvation and nutrient deprivation, acute or chronic inflammation, renal function, drugs (such as nonsteroid anti-inflammatory drugs), cortisol, and anabolic steroids. All these influencing factors should be taken into account when interpreting the clinical values of TTR and RBP levels. Because of their short half-lives, transthyretin and retinol-binding protein are accepted as rapid responders to therapeutic interventions.
- Serial measurements reflect more correctly the balance between catabolic and anabolic processes and lean body mass fluctuations.
- Incorporation of transthyretin or retinol-binding protein into panels of biomarkers, developed by proteomic analysis or software based models, will direct future research in this area.

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Using Mid-Upper Arm Circumference to Detect High-Risk Malnourished Patients in Need of Treatment

38

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Abstract

Measurement of mid-upper arm circumference (MUAC) was originally proposed in the 1960s to assess the nutritional status of children between the age of 1–5 years. It was based on the assumption that it was closely related to muscle mass and also that it varied little with age, making rapid nutritional assessment possible in populations where age is not known with precision. Development of MUAC growth reference curves later showed that the assumption of age independence was not correct and MUAC was progressively abandoned. Weight-for-height z-score (WHZ), which is determined independently of age, became the gold standard for the assessment of acute malnutrition. However, more recently, results of several community studies comparing anthropometric indices to identify children with highest risk of dying have renewed interest in MUAC. Comparison of the receiver operator characteristic (ROC) curves of different indices showed that MUAC was consistently superior to WHZ in identifying children with a high risk of dying. These studies also showed that correction of MUAC for age or for height did not improve its prognostic value. The superior performance of MUAC to identify high-risk children could be due to its preferential selection of younger and/or more stunted children, or to the close relationship of MUAC with muscle and fat mass. These two explanations are not mutually exclusive as young and stunted children tend to have a low muscle mass in relation to body weight which makes them more vulnerable to malnutrition. The performance of WHZ can also be affected by differences in body shape not linked to wasting, by hydration status or by measurement errors. Following these mortality studies, a paradigm shift has taken place in populations with high malnutrition-related mortality, such as in famine situations. The priority then is to identify high-risk children in need of urgent treatment to prevent short-term death. In this context, identification by WHZ, measuring a statistical deviation from a standard, has less practical relevance. Available evidence also suggests that children with a low MUAC can be rapidly identified by community- or facility-based health workers, or by mothers and that children identified by MUAC put on weight rapidly when treated. They should be the priority target for nutritional programs.

Keywords

Mid-upper arm circumference · MUAC · Nutritional status · Risk · Mortality · Children

List of Abbreviations

AUC	Area Under the Curve
BMI	Body mass index
CMAM	Community-based management of acute malnutrition

DEXA	Dual-energy X-ray absorptiometry
MUAC	Mid-upper arm circumference
ROC	Receiver operating characteristics
RUTF	Ready-to-use Therapeutic Foods
SAM	Severe acute malnutrition
WHO	World Health Organization
WHZ	Weight-for-height z-score

Introduction: Assessing Nutritional Status by Measuring Arm Circumference

The idea of using mid-upper arm circumference (MUAC) for nutritional status assessment was first put forward in a World Health Organization (WHO) monograph published in 1966 (Jelliffe 1966). The rationale was that MUAC was highly correlated with muscle mass, which was then regarded as the body component most relevant to assess nutritional status. Measures of creatinine excretion and limb circumference, which are related to muscle mass, suggested that muscle mass increased proportionately more than body weight during malnutrition treatment (Standard et al. 1959). This suggested that measure of limb circumference was a more reliable measure of nutritional status than body weight. The 1966 WHO Monograph suggested a simple equation to measure muscle arm circumference as a proxy of muscle mass and the idea was later developed to estimate muscle arm area and validated by dual-energy X-ray absorptiometry (DEXA) (Rolland-Cachera et al. 1997) (Fig. 1). The 1966 WHO monograph presented in its annex the expected MUAC in children under 5 years old in relation to age based on a sample of Polish children.

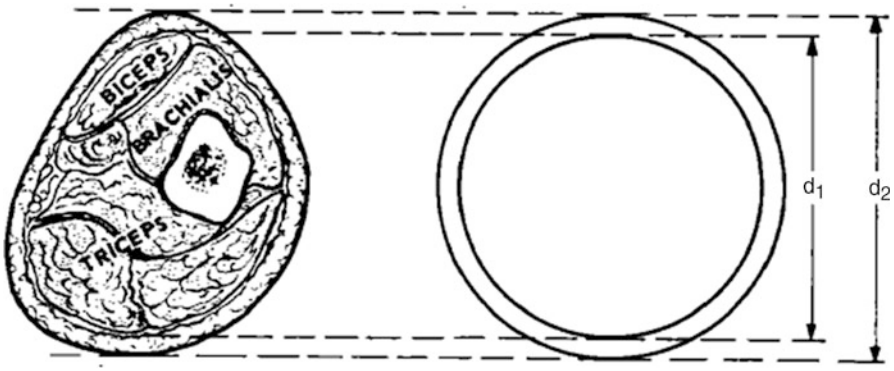

Fig. 1 Schematic cross section of the upper arm. Muscle circumference (MC) can be estimated from MUAC and skinfold thickness (SF) by the formula: MC = MUAC – π SF (Jelliffe 1966). The muscle arm area (MA) can be calculated with the formula (Rolland-Cachera et al. 1997). $MA = (MUAC)^2/4\pi - MUAC \times SF/2$

Using MUAC to assess nutritional status looked simple and quickly attracted attention. In 1969, in a special section of the December issue of the Journal of Tropical Pediatrics, surveys which assessed nutritional status with MUAC were reported from Ethiopia, Uganda, Tanzania, Zambia, Caribbean, Malaysia, Tunisia, Nigeria, Lebanon, Congo, and Haiti. In the same issue, MUAC growth data from Polish children were presented with a smoothed curve which suggested that between the ages of 1–5 years, MUAC grows only by 1 cm (Burgess and Burgess 1969). This led to the suggestion to use MUAC with a single threshold in this age range to detect malnutrition in situations where age is not known with precision (Jelliffe and Jelliffe 1969). In the same issue of the Journal of Tropical Pediatrics, it was proposed to adjust MUAC for height using a specially designed measuring height stick called the "QUAC stick" (Rainer 1969). This method of nutritional assessment was used extensively during the Nigeria civil war of 1969 and led to a rapid mapping of zones most affected by famine (Davis 1971). Such a mapping would have been logistically impossible using weight-for-height z-score (WHZ) and has never been repeated to such an extent in more recent crises.

To further simplify nutritional assessment, Shakir and Morley later suggested using the same MUAC cut-offs to define three degrees of malnutrition in children aged 12–60 months (Shakir and Morley 1974). The proposed cut-offs, 125 and 135 mm, were chosen to be consistent with the then existing classification of malnutrition based on weight-for-age (Shakir 1975). Shakir and Morley also suggested to use a tricolored cord with a green, yellow, or green zone, and Zerfas proposed to use an "insertion tape" (Zerfas 1975). This led to the development of currently used tapes.

The enthusiasm for using MUAC as a simple measuring tool declined when new MUAC growth curves were developed showing that MUAC increases with age more than initially estimated. The MUAC reference curves of the National Center for Health Statistics and also data from Malawi showed that in the age range 1–5 years, the total increase is about 2 cm compared to 1 cm in the Polish sample (World Health Organization 1995). Also, several studies had reported that MUAC gave discordant results compared with weight-for-height, which at that time was the reference criterion to diagnose acute malnutrition in hospital settings (Lindtjørn 1985;Carter 1987; Bern and Nathanail 1995). The 1995 WHO Technical Report on nutritional assessment noted that MUAC selects more children as malnourished compared to WHZ in younger age groups and recommended MUAC-for-age for nutritional assessment (World Health Organization 1995). Progressively, MUAC was abandoned in favor of WHZ, which was perceived to be a more rigorous measure of nutritional status. In its 2000 report on Management of Nutrition in Major Emergencies, WHO recommended to measure of MUAC adjusted for age to assess nutritional status only in situation where weight and height cannot be measured and advised not to use MUAC uncorrected for age (World Health Organization 2000). The rationale for rejecting MUAC was that it did not identify the same children diagnosed as malnourished when using WHZ, although previous reports had shown that WHZ itself did not reflect the clinical judgement of nutritional status any better than MUAC (McDowell and King 1982; Van Loon et al. 1987).

Identifying High-Risk Children with Anthropometric Indices

In the 1980s and 1990s, several studies aimed to determine which nutritional index would best predict children with a high risk of death. The objective was to select a small group of children who would benefit most from a nutritional intervention. All these studies had the same design. After enumerating all children in a community usually within the age range of 6–60 months by an exhaustive census, a full set of anthropometric measures was taken and after a follow-up of several months, survival was assessed. In most cases, this follow-up after nutritional assessment was repeated several times, including in each round all children who reached 6 months and excluding those above 60 months. All these studies took place before the development of community-based management of acute malnutrition (CMAM) and reflect outcome in absence of treatment. At the end of the follow-up, receiver operatic curves (ROC) plotting sensitivity against 1-specificity of each nutritional index to detect children who died were used to compare the value of each nutritional index to identify children with a high risk of death (McNeil et al. 1975). In practical terms, 1-specificity is the proportion of children below a given cut-off who will survive, and sensitivity is the proportion of these children who will die absence of treatment. The most useful anthropometric index will classify below any cut-off the largest number of children who are at risk of dying and the smallest number of children who will survive. In other words, the most useful anthropometric index will have the highest ROC curve.

Several community studies examining the relationship between anthropometry and mortality in untreated children (Table 1). They took place in Bangladesh (2 studies) (Bairagi 1981; Alam et al. 1989), Uganda (Vella et al. 1994), and Senegal (Briend et al. 1989). A review of these studies published in 1994 concluded that "in comparing across studies and across the various comparative criteria, the most consistent observation is that WHZ is the least effective predictor of mortality. For the criterion that is common to all studies (ROC curves), it appears that at high specificities simple MUAC is superior to height-for-age and weight-for-age" (Pelletier 1994, 2073–2074). Another study from Malawi did not include MUAC, but MUAC for age and also found it superior to all other indices (Pelletier 1994; Pelletier et al. 1994). These findings were reconfirmed in two later studies, one in Democratic Republic of Congo (Van den Broeck et al. 1996) and another one in the Gambia in children aged less than 6 months (Mwangome et al. 2012) (see section below).

These studies also showed that correcting MUAC for age did not improve identification of high-risk children. This was shown for the first time by Bairagi in Bangladesh who showed that ROC curves for MUAC and MUAC-for-age were virtually identical (Bairagi 1981). Later on, with a different data set, also from Bangladesh, a logistic regression model showed that correcting MUAC for age or height for did not improve the assessment of the risk of dying (Briend and Zimicki 1986). This suggested that using the QUAC stick was no more effective than using simple MUAC to identify high-risk children.

In 2006, WHO released new growth standards, with potential changes in the relationship between different anthropometric indices and the risk of dying. A reanalysis of the original data of the studies from Senegal and from Congo confirmed the

Table 1 Community studies examining the relevance of different anthropometric indices to identify children with a high risk of death

Study	Country	Age range (months)	Follow-up period (months)	Ranking of ROC curves at high specificity levels
Bairagi 1981	Bangladesh	12–23	24	MUAC > WA > HA > WFH
Alam et al. 1989	Bangladesh	12–59	6	MUAC > HA > WA > WHZ
Briend et al. 1989	Senegal	6–59	6	MUAC > WA > HA > WHZ
Vella et al. 1994	Uganda	6–59	12	MUAC > WA > HA > WHZ
Pelletiet al. 1994	Malawi	0–60	6	MUACA > WA = WHZ > HA
Van den Broeck et al. 1996	Democratic Republic of Congo	12–30	6	MUAC > WA > WHZ
Mwangome et al. 2012	Gambia	1.5–3	Up to the age of 12 months	MUAC > WHZ

Adapted and updated from Pelletier 1994. WA: weight-for-age; HA: height-for-age; MUACA: MUAC-for-age. All these studies used NCHS references to calculate anthropometric indices

superiority of MUAC over other indices to assess the risk of dying (Briend et al. 2012) (Schwinger et al. 2016). The lack of effect of age on risk assessment was also re-examined by a study from Guinea Bissau using MUAC-for-age calculated with the 2006 WHO growth standards. This study confirmed that using MUAC-for-age did not improve the detection high-risk children compared to MUAC (Rasmussen et al. 2012).

Community studies to determine the relationship between different anthropometric indices and the risk of death among untreated children can no longer be ethically repeated now that CMAM is available. Importantly, studies among children who have been selected for treatment by one anthropometric criterion cannot give information on the mortality relationships of other indices.

Hospital based studies, with a recruitment independent of initial WHZ or MUAC, have also shown that MUAC is superior to other nutritional indices in predicting inpatient death (Briend et al. 1986) (Berkley et al. 2005) (Sachdeva et al. 2016). However, hospital-based studies, while potentially informative for that setting, are to be interpreted with caution with respect to understanding the general relationships between anthropometry and mortality, as they are receiving treatment based on the anthropometry and are a sample of children unrepresentative of the general population.

Balancing Benefits and Risk of Treatment

Ideally, screening of children should aim at identifying among high-risk children those with the highest chance of benefiting from an intervention. This information is now difficult to obtain, as it would require comparing groups of treated and untreated

children, and in practice, the risk-based approach is used to identify individuals who may be eligible for interventions.

Selected children should also not be exposed to undesirable secondary effects of treatment. In this respect, fear of treating low-MUAC children with a WHZ above the usual definition of malnutrition with high-fat Ready-to-Use Therapeutic Foods (RUTF) has led some experts to advise not to treat young or stunted children (Myatt et al. 2006). The concern was that these children were not acutely malnourished, that RUTF will fail to generate rapid weight gain and may lead to excessive fat deposition. These concerns, however, were not based on evidence. In programs where short children received RUTF on the basis of their low MUAC independently of their height, weight gain was independent of height (Dale et al. 2013) (Binns et al. 2016) (Fabiansen et al. 2016). Fat deposition is highly energy demanding, and the high weight gain seen in short children suggests it represents only a small portion of weight gain during treatment. In the long term, children treated for SAM often have metabolic abnormalities associated with a reduced proportion of lean mass which may predispose them to chronic diseases (Lelijveld et al. 2016), but there is no evidence this is a special concern for children selected by MUAC.

Towards a Paradigm Shift: From Detecting Malnourished Children to Those with a High Risk of Death

The finding that MUAC was better than other nutritional indices to detect high-risk children has the potential to facilitate the roll out of CMAM programs aiming at reducing the malnutrition related mortality. Children with a low MUAC are easy to identify, have a high risk of death, and respond to treatment, and they should be treated independently of their WHZ when the objective is to reduce malnutrition-related mortality. Such a MUAC-based approach, however, raised concern as children with a low MUAC are not the same as those with a low WHZ. Among children who have either a WHZ less than −3 or a MUAC less than 115 mm, which is the current WHO definition of non-edematous severe acute malnutrition (SAM) (World Health Organization 2009) about 45% only have a WHZ less than −3 and will not be identified by a screening based only on MUAC less than 115 mm. Also, this degree of overlap varies between populations (Grellety and Golden 2016). That these two indices do not identify the same children, however, also explains why MUAC is better that WHZ at identifying high-risk children. If the selected children were the same, the risk would be the same. The move to MUAC requires a paradigm shift, i.e., one has to abandon the objective of detecting the most "malnourished" children, as defined by statistical deviation from a standard, in favor detecting those with the highest risk of death.

The lack of agreement between MUAC and WHZ led most national programs and some nongovernmental organizations to strictly follow the WHO definition of SAM (MUAC <115 mm or WHZ < −3 or edema) and use these two criteria to select high-risk children. Using MUAC alone, however, seems more effective than using a combination of MUAC and WHZ for detecting high-risk children. This is suggested by a reanalysis of the Senegal data which showed that a screening scheme using

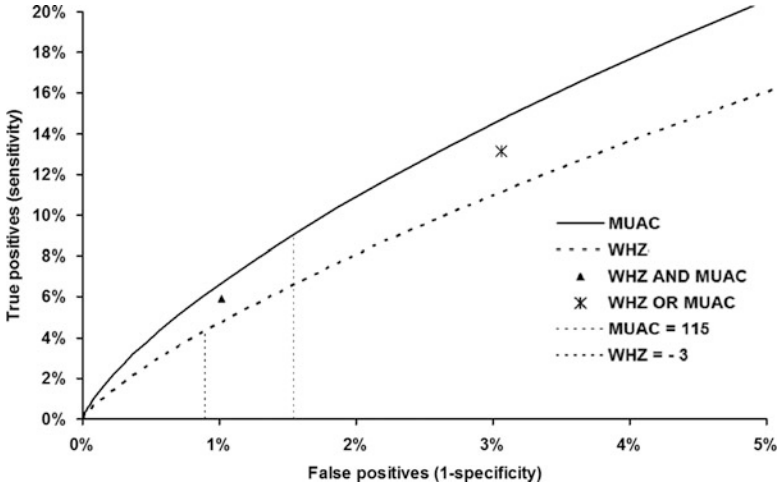

Fig. 2 ROC curves of MUAC, WHZ, and their combinations for assessing the risk of death. ROC curves show sensitivity (% of children below cut-off among those who died) plotted against false positives (1-specificity = % of children below cut-off among those who survived). Data from the Senegal study (Briend et al. 2012). The combination WHZ < −3 OR MUAC <115 mm (*) is more sensitive (i.e., detected more children who died) than using MUAC <115 mm alone. However, this combination is located below the MUAC curve, which means that using MUAC only with a slightly higher cut off (119 mm) selected more children who died while selecting the same number of children who survived. MUAC only is better at detecting high-risk children than combining MUAC and WHZ. WHZ calculated with WHO growth standards

MUAC <115 mm OR WHZ < −3 had a greater sensitivity for death than using MUAC only, i.e., identified more children who eventually died, but this was at the expense of specificity, i.e., the number of selected children greatly increased. This combination of these two diagnostic criteria has a specificity and a sensitivity located under the ROC curve of on MUAC only. This suggests that instead of adding children with a WHZ < −3 to those with a MUAC <115 mm, it is better to include all children with a MUAC <119 mm, irrespective of their WHZ. This will lead to the same number of children to be referred to treatment but among them more would die in absence of treatment (Briend et al. 2012) (Fig. 2).

Protocol: Measuring Arm Circumference

When the measure of MUAC was first proposed, it was advised to do the measurement on the left arm and that the mid-peer arm should be precisely located at mid-distance between the tip of the acromion process and the olecranon process of the ulna (Jelliffe 1966). The rationale for this recommendation was that subcutaneous fat around the arm is not uniformly distributed, and also, it was assumed that the left arm is less influenced by physical activity and would better reflect nutritional status. Although it is not clear that these considerations are relevant in children, these

recommendations have been regularly reproduced in most training documents for health workers. This practice has been challenged recently by a study showing that choosing the left arm and measuring exactly the mid-point did not change significantly the measure in children (Blackwell et al. 2015). Also, in the study showing the closest relationship between MUAC and the risk of death, MUAC was not measured systematically on the left arm and the mid-upper arm not determined precisely as recommended in the initial guideline (Briend et al. 1987). The choice of the tape to measure MUAC is important. It should be done in thin flexible and nonstretchable material. Preference is given to colored insertion tapes, with a window where the precise circumference can be read easily (Zerfas 1975). These tapes usually have three colors corresponding to the locally used cut-off to define severe and moderate malnutrition.

Why is MUAC Better than WHZ to Identify High-Risk Children?

The constant finding that MUAC is better than WHZ at identifying high-risk children can be explained in different ways. Possible explanations are not mutually exclusive and may all be related to the closer link between MUAC, muscle mass, and survival (Briend et al. 2015).

Relationship Between MUAC, Muscle and Fat Mass, and Survival

MUAC is related to arm muscle circumference and subcutaneous fat, and to a lesser extent to bone circumference (Fig. 1) (Jelliffe 1966). This close relationship between MUAC and muscle and fat was one of the main reasons for introducing MUAC as an indicator of nutritional status (Jelliffe and Jelliffe 1969). This relationship has been confirmed by DEXA among healthy well-nourished children (Rolland-Cachera et al. 1997; Jensen et al. 2015) and seems closer for MUAC than for WHZ. Muscle in children represents only 23% of body weight (FAO-WHO-UNU Expert Consultation 1985) and variations in muscle mass will have only a small effect on WHZ compared to its effect on MUAC. Also, weight-based indices may not reflect well muscle wasting in malnourished children who often have an excess of body water (Waterlow 1992).

In absence of infection, fat stores seem to be the main determinant of survival. Carbohydrates disappear after a few hours of fasting, and proteins provides only about 4% of energy in fasting malnourished children. Fatty acids and ketone bodies produced by the metabolism of triglycerides stored in body fat become the main fuel for most organs (Cahill 2006) (Kerr et al. 1978). When malnutrition is associated with infection, or any other aggression (injury, trauma), muscle proteins are mobilized to make free amino-acids available for acute phase proteins synthesis (Reeds et al. 1994). In these clinical situations, muscle mass seems to be the main factor limiting survival (Heymsfield et al. 1982; Briend et al. 2015).

Only two epidemiological studies attempted to assess the respective role of muscle and fat mass as determinant of survival. In the first study in Senegal, triceps skinfold thickness was associated with survival in univariate analysis but this association disappeared in multivariate analysis when arm circumference was introduced in the model. The authors concluded that survival is mainly determined by muscle mass (Briend et al., 1989). A second study examined this association in different times of the year using indices of "fatness" and "muscularity" derived from MUAC and skinfold thickness and found that fat was the main determinant of survival in the dry hungry season whereas muscle was more important in other seasons with a higher prevalence of infections (Van den Broeck et al. 1998). This observation fits well with the hypothesis that whereas fat is the main factor limiting survival in starvation, muscle may be more important for survival when malnutrition is associated with infection.

The Age Effect

MUAC increases continuously with age and selection of children based on a fixed cut-off will always select preferentially younger children. This bias in favor of young children is well described in the 1995 WHO report on the use and interpretation of anthropometry (World Health Organization 1995). In all populations, mortality declines with age in children and this selection bias in favor of young children tends to select a group with a higher mortality when exposed to malnutrition. This effect, however, is likely to be minimal. First, the decline in mortality with age is slow after the first 6 months, and is unlikely to explain the high gradient of mortality associated with low MUAC. Also, the correction of MUAC for age does not affect the value of MUAC to identify high-risk children, whereas it should remove it if the main effect of MUAC was only to select younger children. Importantly, the good prognostic value of MUAC is also observed in adults, when it cannot be due to an age effect. Age may have an effect of muscle mass. Infants and young children, however, also have a low muscle mass compared to adults: about 23% vs 43% (FAO-WHO-UNU Expert Consultation 1985) and this different body composition is likely to make them more likely to die from the association of malnutrition and infection. This suggests that for deciding about an intensive nutritional support, this selection bias in favor of young children with a low muscle mass is advantageous.

The Effect of Body Shape

Differences in body shape can also explain why some children can have a low WHZ while having a MUAC above the malnutrition threshold. This has been well described in the case of leg length: children with long legs have a lower WHZ on average, which may be common in some parts of Africa (Myatt et al., 2009). This can lead to classifying wrongly as malnourished children who have leg length above average, as children with long legs are usually in better health and nutritional status

than average (Bogin and Varela-Silva 2010). On the opposite, stunted children who usually have shorter legs can have an artificially high WHZ although they are likely to have a low muscle mass. Children treated for SAM and who remain stunted have a smaller arm and calf circumferences compared to sibling controls, suggesting a lower muscle mass (Lelijveld et al. 2016).

The effect of leg length on WHZ was highlighted in the context of diagnosing malnutrition in some parts of Africa but is presumably not the only factor related to body shape affecting the relationship of WHZ with nutritional status. Chest and abdomen circumference are also known to influence WHZ and could explain the low prevalence of low WHZ observed in Brazil (Post and Victora 2001). This effect will influence WHZ independently of muscle mass as there are only few muscles in chest and abdomen. Other subtle differences in body shape, not well described by simple length or circumference measures, may also influence WHZ independently of nutritional status and this would warrant further investigation using modern 3D modelling of body volumes.

MUAC in Other Age Groups

Infants Less than 6 Months

The risk of mortality associated with MUAC in early infancy is less well understood. In the half a century (1966–2016) of studying MUAC, most studies and surveys have systematically excluded infants under 6 months and as a result little data is available to describe MUAC's and other anthropometric indices performance in this age group. The main reason for this exclusion has been the perception that malnutrition is rare within this age group because optimal breastfeeding is practiced universally. With the introduction of the WHO growth standards in 2006, data was reanalyzed and this perception was challenged: studies observed a much higher proportion of undernutrition in the under 6 months than previously reported. Using the WHO standards increased the prevalence of severe wasting in the under 6 months by a factor of 3.5 (de Onis et al. 2006; Kerac et al. 2011).

Although limited, there is strong evidence for introducing the use of MUAC in the under 6 months as well. Recent studies of MUAC in the under 6 months have attempted to replicating experimental designs reproduced observations made with previously used in studying MUAC in older children (6–59 months). They have compared MUAC to WHZ performance to assess the risk of death and used similar analysis methods used in the historical studies. Using a ROC curve analysis method, in a cohort of community Gambian infants, MUAC taken at the point of vaccination between 6 and 14 weeks better predicts mortality occurring before 12 months of age than WHZ (Mwangome et al. 2012). The reported Area Under the Curve (AUC) of the ROC curves for predicting mortality for MUAC was 0.64 (95% CI 0.55 to 0.73) compared to 0.55 (95% CI 0.46 to 0.65) for WHZ. In this study a MUAC cut-off of $<$110 mm was proposed for diagnosis of SAM in infants under 6 months. The poor outcome associated with a MUAC less than 110 mm in children less than 6 months

has been confirmed in a clinical trial in Kenya which examined the effect of cotrimoxazole prophylaxis on posthospital mortality in children with SAM. In this sample, 24.5% of children aged 2–5 months with a MUAC less than 110 mm died during the 6 month follow-up, with no beneficial effect of cotrimoxazole prophylaxis (Berkley et al. 2016).

Adults

There are no community studies which examined the respective value of different anthropometric indices to identify malnourished patients with a high risk of death in absence of nutritional support. Several studies, however, suggest as well that MUAC is a good prognostic indicator, and better than body mass index (BMI). A study done among patients admitted in emergency to a London hospital found that MUAC was a significant predictor of mortality either alone ($P = 0.002$) or after adjustment for BMI ($P = 0.007$), but that BMI was not (Powell-Tuck and Hennesy 2003). Another study done in a therapeutic feeding center in South Sudan found that the AUC of ROC curves for MUAC to predict death was 0.71 for MUAC compared with 0.57 for BMI and 0.51 for weight (Irena et al. 2013). As patients in this study were selected with MUAC, extrapolation of this result to the community should be cautious.

A third study also examined MUAC and WFH in adult patients with tuberculosis in Guinea Bissau and found MUAC to be a good predictor of death. Although this study did not formally compare the ROC curves of these indices to identify high-risk patients, they advised the use of MUAC as it was not possible to measure height in some patients who were unable to stand up (Gustafson et al. 2007). Outside the context of malnutrition, muscle arm area was found to better identify elderly patients at a high risk of death in Australia (Miller et al. 2002). Eventually, in patients with Chronic Obstructive Pulmonary Disease, arm muscle area was also found to be a better predictor of the risk of death than BMI (Soler-Cataluña et al. 2005).

Conclusion: Towards a Frequent Decentralized Nutritional Status Assessment with MUAC to Identify Children in Need of Treatment

MUAC is comparatively easy to measure and requires only a simple tape. This contrasts with WHZ which requires the presence of two trained people for measuring height. Also, MUAC does not require calculations, which beyond simplicity has the advantage of not adding errors on different measures. This is a problem especially in children aged less than 2 years, for WHZ is strongly affected by measurement errors on height which is difficult to measure (Mwangome and Berkley 2014). Illiterate mothers given colored tapes can correctly classify children in different MUAC categories (Blackwell et al. 2015) and using mothers to measure MUAC has the huge advantage of making possible frequent screening, which is more difficult with community health workers, and impossible outside research context with WHZ. The association between anthropometry and the risk of death is closer for shorter duration

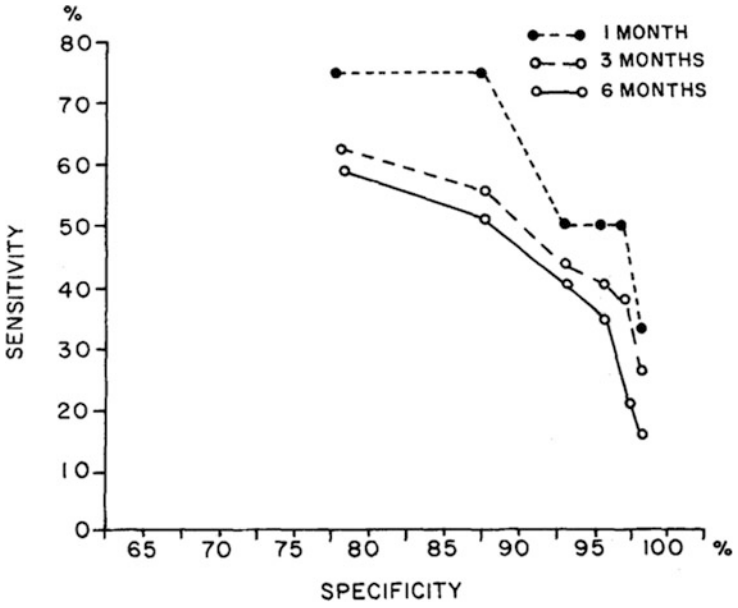

Fig. 3 Effect of durations of follow-up from 1 to 6 months on the position of the ROC curve of MUAC to identify children with a high risk of death. The higher position of the ROC curve for shorter follow-up duration indicates a better identification of high-risk children. This suggests that the best identification will be obtained by frequent measures. The same phenomenon is likely to occur with other nutritional indices, in particular WHZ, but their frequent measure in the community is not possible in practice (Data from Bangladesh (Briend and Zimicki 1986))

of follow-up for all nutritional indices (Pelletier et al. 1994). This has also been shown for MUAC with an improvement of the ROC curves when duration of follow-up is shortened to 1 month (Briend and Zimicki 1986) (Fig. 3). Pilot programs with detection of SAM carried out by mothers with MUAC have shown that this approach resulted in a decreased hospital admission (Alé et al. 2016), suggesting that early detection of high-risk children allowed an early initiation of treatment before the occurrence of complications. This decentralized detection approach seems the way forward for early detection and treatment of these high-risk children.

Summary Points

- MUAC was initially proposed to assess nutritional status as it was believed to be closely related to muscle mass
- It was also initially assumed that MUAC varied little between 12 and 60 months and that it could be used without correction for age in nutritional assessment in populations where age is not known with precision but this assumption is not correct.

- MUAC increases with age and does not identify as malnourished the same children as WHZ which is often considered as a "gold standard" for nutritional assessment
- All community studies examining the relationship between anthropometry and mortality in absence of treatment showed MUAC is better than WFH to identify children in need of treatment to prevent death.
- The superiority of MUAC to identify children at risk of inpatient mortality was confirmed in hospital-based studies using a sample of children selected independently of anthropometry
- Early work suggests that MUAC identifies high risk infants less than 6 months
- The relationship between MUAC and risk of death may be due to its association with body composition or to a preferential selection of younger and more stunted children with a higher risk of death; these two explanations are not mutually exclusive
- Identification of high-risk children seems more effective for short follow-up periods, suggesting that frequent screening would reduce risk.
- MUAC can be measured frequently by minimally trained health workers or by mothers, which allows the rapid evaluation of high-risk children.

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Nutrition Screening and Assessment in Hip Fracture

39

Jack Bell

Contents

Abstract

Protein-energy malnutrition is prevalent in one in two patients admitted with a hip fracture; commonly observed inadequate postoperative protein/energy intake leads to further incident inpatient malnutrition. This condition is reported as the most costly comorbidity in hip fracture. The complex, multimorbid population limits the usefulness of routinely applied malnutrition screening tools. Given the

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high cost, prevalence, and incidence of malnutrition, and poor sensitivity of most screening tools, routine comprehensive nutrition assessment of all acute hip fracture inpatients is recommended. There is no gold standard for malnutrition diagnosis in the clinical setting in hip fracture. Malnutrition diagnosis is complex in this patient population, and often requires triangulation of multiple nutrition assessment measures from a variety of sources over multiple time periods. Single variable measures should not be relied upon to diagnose malnutrition, and commonly applied index measures may still require a subjective clinical judgment to inform a final diagnosis.

Keywords
Malnutrition · Hip fracture · Screening · Assessment · Nutrition · Orthopedic · Neck of femur · Protein · Energy

Malnutrition in Hip Fracture

In the UK alone, hip fractures annually cost £1.1 billion in the acute care setting; estimates suggest a doubling or tripling of this figure after accounting from broader health and social care costs (Hernlund et al. 2013; Leal et al. 2016). Forward estimates suggest an increase in annual UK hip fracture rates from 79, 000 to 104, 000 within the next decade (Hernlund et al. 2013).

Protein/energy malnutrition or undernutrition (malnutrition) is the most costly comorbidity in hip fracture inpatients, the comorbidity most likely to prolong hospital stay, and is an independent predictor of increased morbidity and 12-month mortality (Table 1).

One-third to half of hip fracture inpatients admitted to hospital are malnourished (Avenell et al. 2016; Bell et al. 2014a, 2016). Inadequate inpatient oral intake is

Table 1 Reported impact of malnutrition on patient and healthcare outcomes in hip fracture (A sample of references reporting the impact of malnutrition on patient and healthcare outcomes in hip fracture is provided)

Outcomes measure	Example
Delirium	Juliebo et al. 2009
Pressure injuries	Lindholm et al. 2008
Wound healing	Guo et al. 2010
Functional status	Goisser et al. 2015; Inoue et al. 2016; Koren-Hakim et al. 2012
Mobility	Gumieiro et al. 2013; Helminen et al. 2017; Nuotio et al. 2016
Length of stay	Correia and Waitzberg 2003; Nikkel et al. 2012
Discharge destination	Bell et al. 2014b; Nuotio et al. 2016
Treatment costs	Correia and Waitzberg 2003; Nikkel et al. 2012
Unplanned readmissions	Koren-Hakim et al. 2012
Quality of life	Hoekstra et al. 2011
Mortality	Bell et al. 2016; Correia and Waitzberg 2003; Nuotio et al. 2016

common and resistant to intervention; this has been observed to lead to further nutrition decline in the inpatient setting (Bell et al. 2013b).

Despite demonstrated impact on patient and healthcare outcomes, malnutrition has not been prioritized in international registry data or guidelines until recently (Australian and New Zealand Hip Fracture Registry (ANZHFR) Steering Group 2014; Johansen et al. 2017.; National Institute for Health and Clinical Excellence 2011; Scottish Intercollegiate Guidelines Network 2009). Limitations in malnutrition screening and assessment processes are considered to have contributed to this limited attention toward clinical nutrition care (Avenell et al. 2016; Bell 2014).

Nutrition Screening in Acute Hip Fracture Inpatients

Nutrition screening should be a quick and easy process able to be applied to large numbers of people to identify those at significant risk of malnutrition (Elia and Stratton 2011; Mueller et al. 2011). In the hospital setting, a positive nutrition screen should routinely lead to further detailed nutrition assessment, diagnosis, and initiation of medical nutrition therapy where significant patient and cost benefits are expected (Jensen et al. 2013; Mueller et al. 2011). There is neither a gold standard nor a single purpose for inpatient nutrition screening; the usefulness of each tool is dependent on the population, nutrition issue(s), context, and treatment aims and outcomes (Elia and Stratton 2011; Rasmussen et al. 2010). A broad number of validated screening tools have been applied across hip fracture settings; these report substantially different proportions of "at-risk" patients (Table 2).

The Malnutrition Screening Tool (MST) (Ferguson et al. 1999), Nutrition Risk Screen (NRS) 2002 (Kondrup et al. 2003), and Malnutrition Universal Screening Tool (MUST) (Elia 2003) are applied in a number of studies, although have recently been reported as inadequately sensitive to identify malnutrition in a representative cohort of acute hip fracture inpatients (Bell et al. 2014c). The comprehensive Mini Nutritional Assessment requires considerable time to complete and may be less suitable for application in routine clinical nutrition care than the short-form version (Kaiser et al. 2009). The Mini Nutritional Assessment Short-Form (MNA-SF), although reported as poorly specific in hip fracture, is adequately sensitive, and consequently may be best placed to apply if screening is to be relied on rather than proceeding directly to nutrition assessment (Bell et al. 2014c; Koren-Hakim et al. 2016).

Factors adversely affecting nutrition screening tool acceptability, sensitivity, specificity, reliability, and completion rates, when applied in hip fracture inpatients in routine clinical practice, are highlighted in Fig. 1 (Bell 2014).

Nutrition Assessment in Hip Fracture

A comprehensive nutrition assessment for the purpose of diagnosing malnutrition should consider a systematic approach to collecting and recording the data obtained from patients, clients, family members, caregivers, and/or other individuals and

Table 2 Nutrition screening tools applied in acute hip fracture populations and proportion of "at-risk" patients (The substantial variance in reported malnutrition risk both within and across commonly reported malnutrition risk measures is highlighted (Adapted from Bell 2014; used with permission))

Tool	% At risk[a]	Examples
Mini Nutritional Assessment[b]	29–89	Guo et al. 2010; Hoekstra et al. 2011; Goisser et al. 2015; Helminen et al. 2017; Gumieiro et al. 2013
Mini Nutritional Assessment-Short Form[c]	39–73	Bell et al. 2014a; Formiga et al. 2005; Helminen et al. 2017; Inoue et al. 2016; Koren-Hakim et al. 2016; Nuotio et al. 2016
Nutrition Risk Screening 2002[d]	23–37	Bell et al. 2014a; Gumieiro et al. 2013; Koren-Hakim et al. 2016
Malnutrition Universal Screening Tool[e]	18–20	Bell et al. 2014a; Koren-Hakim et al. 2016; Myint et al. 2013
Malnutrition Screening Tool[f]	49–56	Bell et al. 2013a; Bell et al. 2014a
Simplified Nutrition Appetite Questionnaire[g]	70	Villani et al. 2013
Rainey-MacDonald Nutrition Index[h]	–	Guo et al. 2010
Prognostic Nutrition index[i]	52	Foster et al. 1990
Hammersmith Hospital[j]	56	Nematy et al. 2006

[a]Combined data for at risk/high risk/malnourished
[b]Guigoz et al. (1994)
[c]Kaiser et al. (2009)
[d]Kondrup et al. (2003)
[e]Elia (2003)
[f]Ferguson et al. (1999)
[g]Wilson et al. (2005)
[h]Rainey-Macdonald et al. (1983)
[i]Buzby et al. (1980)
[j]Peak (2000)

groups (Cederholm et al. 2017; White et al. 2012). Commonly applied assessment measures and markers are listed in Table 3.

Clear nutrition diagnoses are considered helpful to guide nutrition treatment goals, interventions, and monitoring and evaluation strategies (Mueller et al. 2011). A detailed diagnosis of malnutrition should include the problem (protein/energy malnutrition) and ideally should also be accompanied by one or more etiologies (root causes) along with documentation of supporting signs and/or symptoms (White et al. 2012).

The overarching etiology of malnutrition in acute hip fracture patients can be attributable to wasting, cachexia, or a combination of the two (Bell 2014; Cederholm et al. 2017; Jensen et al. 2013). Diagnostic nomenclature may consider acute and chronic disease-related malnutrition (with or without inflammation), and/or malnutrition without underlying disease (Cederholm et al. 2017; White et al. 2012). The hip fracture population demography can make the challenge of differentiating

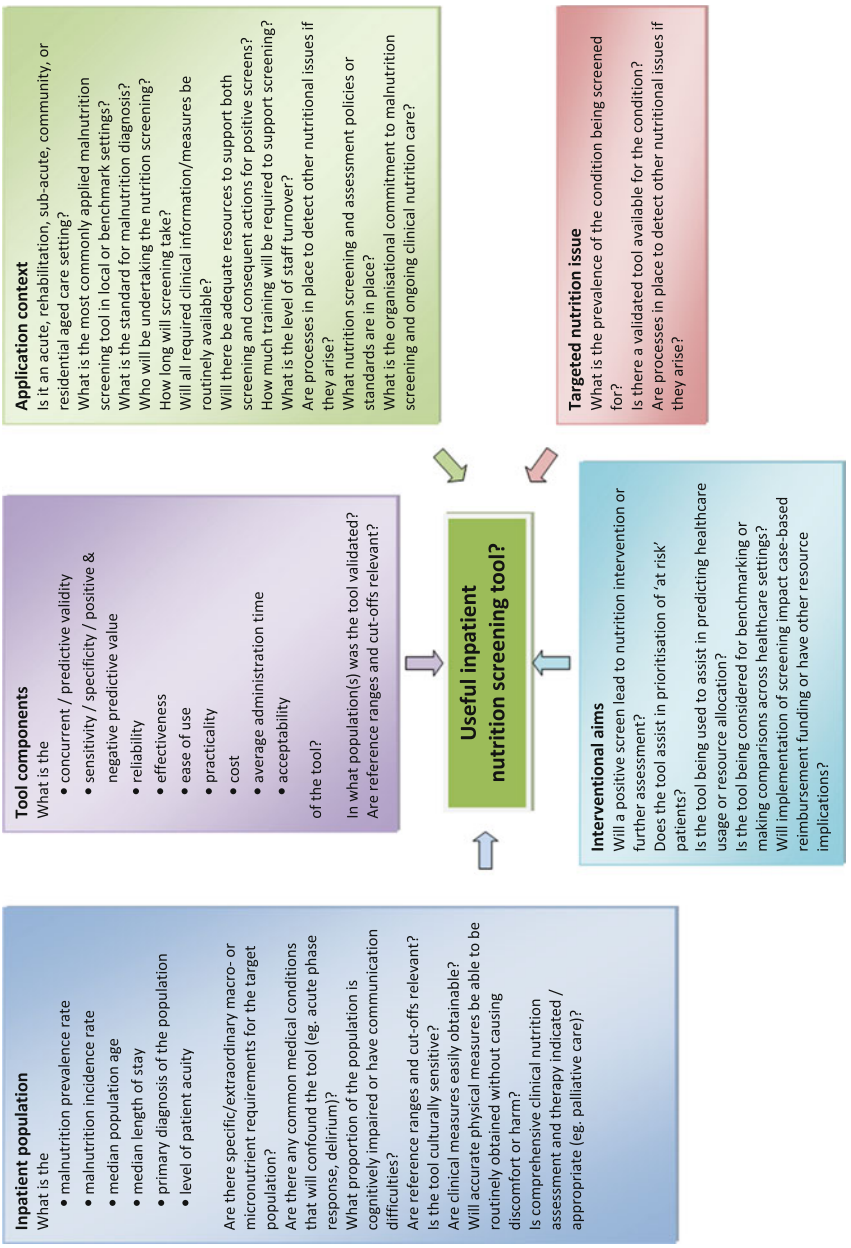

Fig. 1 Factors affecting the usefulness of nutrition screening tools in hip fracture (Source: Adapted from Bell 2014; used with permission)

Table 3 Malnutrition diagnostic measures and markers commonly reported in hip fracture (Malnutrition diagnostic measures and markers commonly reported in hip fracture within an ABCDE clinical assessment format are summarized (Source: Adapted from Bell 2014; used with permission))

Malnutrition diagnostic measure or marker
A – Anthropometric, physical and functional measures
Weight/weight changes/percent weight loss Height/calculated height BMI/Demiquet/Mindex Circumference measures
Skinfold/combined skinfold/other caliper measures
Bioelectrical impedance analysis
Dual-energy X-ray absorptiometry
B – Biochemical markers
Albumin Prealbumin, total protein, transferrin, and retinol-binding protein
Insulin-like growth factor-1
Creatinine height index/nitrogen balance Vitamin and mineral assays
Clinical assessments and measures
Clinical background Estimates of energy expenditure Clinical/bedside assessment Clinical history
Physical and functional measures
D – Dietary history
Recall methods
Food records
E – Environmental factors **Index Scores/global assessment measures**
Mini Nutritional Assessment[a]
Mini Nutritional Assessment-Short Form[b] Subjective Global Assessment[c]
ICD Criteria[d] ASPEN/AND Consensus Statement for the Identification and Documentation of Adult Malnutrition[e]

[a]Guigoz et al. (1994)
[b]Kaiser et al. (2009)
[c]Detsky et al. (1987)
[d]National Centre for Classification in Health (2006)
[e]White et al. (2012)

between malnutrition, frailty, and ageing and disease processes difficult (Cederholm et al. 2017). A diagnosis of protein/energy malnutrition should only be made in cases where there is either insufficient intake or uptake of protein/energy (wasting), increased metabolic requirements for protein/energy, which are not being met (cachexia), or both (Cederholm et al. 2017; White et al. 2012).

The many underlying potential contributors to malnutrition observed in hip fracture are diverse, as highlighted in Table 4 (Bell et al. 2013a).

Table 4 Factors contributing to wasting and cachexia in hip fracture (Commonly observed factors that contribute to wasting and cachexia in hip fracture are detailed (Source: Adapted from Bell 2014; used with permission))

Physiological barriers	Psychosocial	Workplace cultural	Environmental barriers	Perceptions, biases, and misconceptions
Acutely unwell Anorexia of ageing Aspiration bedbound Cachexia and increased losses Communication impairment Delirium, dementia, and cognitive impairment Dysphagia Edentulism Fine and gross motor control Frailty Gastrointestinal issues Impaired ADLs Malnutrition Medical comorbidities and complications Medications Memory impairment Micronutrient deficiencies Pain/discomfort Reduced level of consciousness/ drowsiness Unclear medical trajectory/ fluctuating medical condition Urinary frequency/need for toileting	Affect optimization and differentiation *Alea iacta est* – the die has been cast Burden of care Depression Disordered eating patterns Fear Food habits Gastronomy and pleasure Generational characteristics Loss of control Loss of dignity Poor motivation Poor or fair emotional well being Quality of life Reduced hardiness Resistance to change Social isolation Stoicism/ independence Unaccepted societal norms	Deferral of accountability Encouragement, assistance, and time to eat Meal and snack-time interruptions Role delineation, role redefinition, and role accountabilities Staff rights Status quo Task minimization Therapeutic negativity Transition of knowledge to practice Work prioritization	Clinical environment Clinician skills and capacity Diagnostics and therapeutics Diagnostic inaccuracies Hospital foodservice policy and systems Increased risk of harm/ exposure Information overload or overwhelming Knowledge deficits Lack of clear process Misreporting Medico-legal issues Short length of stay Tethering devices Time and resources Tube intolerance/ dislodgement Unclear purpose/benefit	Clinician resistance to tube feeding Community perceptions Ethical perceptions Iatrogenic diets Informed consent Normalization or lack of insight Patient perceptions Staff perceptions Tube feeding is the last resort Tube feeding is not kind Value of nutrition

Nutrition Assessment Parameters Applied in Hip Fracture: An ABCDE Approach

A – Anthropometric: Physical and Functional Measures

A variety of anthropometric measures are applied for the purpose of nutrition assessment in hip fracture. A recent review detailed clinical considerations regarding application and interpretation of these measures when applied in hip fracture patients (Bell 2014). These are summarized in Table 5.

The association between weight loss and poor patient and healthcare outcomes are broadly reported following acute hip fracture (Nikkel et al. 2012). However, factors detailed in Table 5 require attention to avoid misclassification of nutrition status based on weight or weight changes.

Stadiometer height should not be considered the gold standard in hip fracture; the significant underestimate of height related to kyphosis is considered to increase the likelihood of BMI misclassifications and underdiagnosis of malnutrition (Bell 2014). Recently documented or self-reported height may be appropriate where considered realistic; however, the accuracy of self-reported estimates in hip fracture inpatients needs to be carefully considered (Bell et al. 2013a). Consideration should also be given to the overreporting of height in older patients attributed to age-related height reduction. Calculated heights or surrogate measures of height including knee height, demi-span, half-arm span, arm-span, ulna, humeral, tibia, and fibula lengths, and combinations of these, are also reported across hip fracture studies (Bell 2014). Ulna measurements, demi-span, and half-arm span are most commonly recommended and reported. In a patient population prone toward osteoarticular disease, kyphosis, and cognitive impairment, calculated heights from ulna measurements may provide the most practical alternative to apply in clinical practice (Auyeung et al. 2009).

BMI (body mass index) is the second most commonly applied nutrition assessment measure applied for the purpose of describing malnutrition in hip fracture populations after albumin (Bell 2014; Rasmussen et al. 2010). Many prognostic studies identify a positive association between weight-for-height measures and poor patient and healthcare outcomes following hip fracture (Bell 2014). BMI is reliant on accurate height and weight data; underreporting of height may underestimate true BMI in hip fracture inpatients (Bell 2014). This measure fails to differentiate between adiposity and lean body mass, or fluid shifts. Identifying the appropriate cutoff points for individuals and population groupings using weight-for-height measures remains a challenge in response to ethnic variances, the obesity paradox, and age- and disease-related recommendations. Most importantly, reliance on BMI will fail to identify healthy weight or overweight malnutrition (Bell et al. 2014a). The lower than median reported prevalence for BMI-reported malnutrition versus other measures may suggest that healthy weight or overweight malnutrition is masking the true prevalence of malnutrition in hip fracture inpatients (Bell 2014; Bell et al. 2014a).

Calf circumference or mid-upper arm circumference measures may provide useful alternatives to BMI in hip fracture (Kaiser et al. 2009; Rasmussen et al.

Table 5 Clinical considerations or concerns when applying weight, BMI, and other anthropometric measure applied in routine clinical evaluation of acute hip fracture patients (A number of clinical factors that should be considered when collecting or interpreting weight, BMI, and other anthropometric measure applied in routine clinical evaluation of acute hip fracture patients are highlighted (Source: Adapted from Bell 2014; used with permission))

Consideration/concern	Potential cause
No measures easily identified	Devolution of accountability High measurement cost High patient acuity Inadequate access to equipment or trained operators Lack of accountability Lack of clear process Limited patient mobility Pain Poor documentation processes Poor attention to regular measurements by health professionals or facilities
Incorrect or unreliable measures	High interobserver variability Inadequate training/skills/practice Inappropriate equipment Incorrect documentation Incorrect, inaccurate, or misleading patient reported data Kyphosis Limited attention to calibration of equipment Physiological/physical limitation Poor technique
Fluid shifts	Postoperative overhydration/fluid overload Dehydration Multimorbid population (e.g., Chronic kidney disease, Heart failure, liver disease) Medications, e.g., diuretics
Fat versus fat free mass redistribution	Ageing Disease processes Physical (in)activity Hormonal/metabolic changes Medications Rapid weight gain or loss Insufficient protein versus total energy intake
Reference values	Lack of suitable age, gender, ethnicity, or disease-specific reference ranges
Healthy/overweight/obese malnutrition	Wasting/cachexia observed in patients with higher BMIs

2010). However, circumference measures also appear to report lower malnutrition prevalence data when compared with other measures, and appear less likely to prognosticate negative nutrition related outcomes post hip fracture (Bell 2014; Bell et al. 2014a).

Triceps skinfolds measures are often combined with the mid-upper arm circumference to calculate the mid-arm muscle circumference to estimate total body protein stores (Bishop et al. 1981; Blackburn et al. 1977). These measures need to be

cautiously applied in routine clinical practice due to interobserver variation, limited sensitivity in articulating acute nutrition status changes, the need to account for edema, and limited availability of population-specific reference ranges (Bell 2014). Studies in hip fracture tend to associate nonsignification over positive associations between low skinfolds/arm circumference values and poor outcomes (Bell 2014).

Bioelectrical impedance analysis studies in hip fracture report high exclusion criteria resulting in a research population not considered representative of those found in routine clinical practice. This may result from difficulty in applying, for example, to hip fracture patients with agitated delirium (Bell 2014). This method may provide an underestimate of malnutrition in hip fracture patients prone to overhydration. Differences in individual body morphology and fat distribution can also impact accuracy (Hoekstra et al. 2011). Finally, the requirement for specialized equipment and trained operators may limit applicability in some clinical settings.

Dual-energy X-ray absorptiometry measures total body protein, total body fat, electrolyte levels, and water content. Although both considered gold standards for research purposes, these methods are not routinely applied in clinical practice assessment in hip fracture.

Even basic anthropometric data can be difficult to obtain and interpret in the multimorbid hip fracture inpatient. Basic pragmatic measures should consider measured weight and weight changes, ulna length or patient/first degree relative reported heights, and BMI or calf circumference measures. However, insightful clinicians will avoid routine reliance on single anthropometric measures as stand-alone markers of malnutrition.

B – Biochemical Markers

Albumin synthesis is compromised in nutrition depletion, and is the most highly reported nutrition marker in hip fracture studies (Weimann et al. 2017; Yuwen et al. 2017). The need to consider the limitations of this measure is highly disregarded across many studies (Table 6) (Bell 2014).

Although "quick and easy" to access and report, there is a growing call for clinicians and researchers to discontinue relying on albumin as a stand-alone marker of nutrition status in hip fracture, particularly in older patients with acute infection or inflammation (Bouillanne et al. 2011; Marshall 2008).

Prealbumin (transthyretin), total protein, transferrin, and retinol-binding protein are also reported as markers of nutrition status across hip fracture studies. Low levels of these markers are generally nonsignificantly or negatively associated with poor outcomes (Bell 2014). However, sensitivity to the acute phase response, cost of testing, and limited availability of these measures should be considered as limitations to reliance on these proteins as single-point malnutrition diagnostic measures in clinical practice (Marshall 2008).

A decline in insulin-like growth factor (IGF-1) is inconsistently associated with reduced nutrition status, sarcopenia, postoperative outcomes, and increased risk of hip fracture, and may be positively influenced by nutrition intake (Chevalley

Table 6 Advantages and disadvantages of albumin as a nutrition marker in hip fracture (Commonly reported advantages and limitations of albumin as a marker of malnutrition in patients following an acute hip fracture are highlighted (Source: Adapted from Bell 2014; used with permission))

Perceived/reported advantages	Limitations
Associated with patient, clinical, and healthcare outcomes Easy to access retrospectively Inexpensive to test Routinely available Simple quantitative measure	Does not routinely deplete in long-term malnutrition Impacted by synthetic function Impacted by fluid shifts/blood product support Poorly sensitive to short-term nutrition changes Reduced levels in acute phase/inflammation/infection Reduced levels with renal losses Sensitive to fluid shifts and redistribution between intracellular/extracellular compartments

et al. 2010; Myint et al. 2013). However, cost and availability of testing combined with suppression in the acute inflammatory state may impact the utility of this measure as an independent measure of malnutrition in routine clinical practice.

Creatinine height index and nitrogen balance studies appear limited to highly controlled, efficacy-focused hip fracture studies (Bell 2014).

The multimorbid nature of hip fracture patients necessitates regular consideration of a broad variety of additional laboratory measures to assess vitamin and mineral deficiency or redistribution states, and to support supplementary nutrition diagnoses in addition to that of protein/energy malnutrition.

Many laboratory measures provide useful support in facilitating comprehensive nutrition assessment, monitoring, and evaluation. However, the proclivity of clinicians and researchers toward application of laboratory measures as independent malnutrition diagnostic measures, with lack of attention to confounders, requires attention.

C – Clinical History and Physical/Functional Assessment

Current and previous medical and surgical conditions, medications, and identification of barriers and facilitators to nutrition care should be considered (Bell 2014). Patient perceptions of nutrition and their opinions regarding nutrition support options should also be sought (Bell et al. 2013a). A multidisciplinary orthogeriatric service has been considered best placed to source this data, although further work is required to establish the usefulness of interdisciplinary or transdisciplinary models of care (Bell et al. 2014b; Hoekstra et al. 2011; Choi and Pak 2007).

Indirect calorimetry is increasingly applied across research and clinical settings, although remains outside the scope of most clinicians and their patients in acute hip fracture settings (Bell 2014). Estimates of energy expenditure suggest an increased metabolic requirement in hip fracture patients; however, reported estimates do not routinely account for additional thermic effects of food, physical activity, or multimorbid states (Bell 2014). Study populations also appear highly selected and may not be reflective of those encountered in current routine clinical practice (Bell 2014).

Complex equations to estimate energy do not always relate to measured requirements or the actual requirements of individuals observed through careful monitoring and evaluation processes. For this reason, it may be more judicious to choose a simple estimation method, such as 25–35 cal/kg or 1.2–1.5 g/kg protein, and focus resources toward monitoring and evaluation strategies instead (Dietitian/Nutritionists from the Nutrition Education Materials Online team 2014).

Direct observation of muscle wasting and fat stores, skin integrity, wound healing, hair and nail deformities, and other specific bedside observations will facilitate identification of macro- and micronutrient deficiencies.

Physical and functional measures applied to facilitate nutrition assessment in hip fracture include muscle strength, exercise tolerance, and immune response. Handgrip strength or dynamometry may be useful in some hip fracture settings (Duncan et al. 2006; Gumieiro et al. 2012). However, the heterogeneity of findings combined with a population demographic prone to delirium, dementia, cognitive impairment, and osteoarticular disease may limit the routine application of this technique in clinical practice (Bell 2014).

Numerous hip fracture studies report associations between lymphocyte count and/or delayed cutaneous hypersensitivity as markers of nutrition status (Bell 2014; Foster et al. 1990). Although associated with nutrition depletion and morbidity and mortality, the use of lymphocyte count and delayed hypersensitivity as independent measures of nutrition status in routine clinical practice is not recommended (Marshall 2008).

D – Dietary Information

Dietary intake assessment of hip fracture inpatients will need to consider preadmission intake for the purpose of nutrition risk screening and diagnosing malnutrition prevalence, and inpatient intake for the purpose of ongoing nutrition risk screening, nutrition support, and diagnosis of malnutrition incidence.

Adapted visual estimate methods recording quartile or proportional intake of served meals are the most commonly reported nutrient intake estimation method applied in hip fracture settings (Duncan et al. 2006; Eneroth et al. 2005). Highlighted limitations have included difficulties capturing intake between main meals, limited training of data collectors, and inconsistent approaches to describing attribution of macronutrient values (Bell 2014).

Chronological 24-h recalls are also commonly reported, in addition to multiple pass 24-h recalls, recorded or photographed intake data, food frequency questionnaires, and diet history interviews (Chevalley et al. 2010; Hoekstra et al. 2011; Rutishauser 2005). However, the accuracy and reliability of dietary recall data obtained from elderly inpatients prone to delirium, dementia, cognitive impairment, and affect optimization should not be assumed (Bell et al. 2013a; Sivakumar et al. 2013).

Although weighed food records are considered as gold standard intake measures, these are considered both time and resource intensive (Williamson et al. 2003). Menu records, estimated records (or unweighed food records), and adapted weighed food records may be more useful in the clinical and pragmatic research environments (Rutishauser 2005).

E – Environmental

A robust nutrition assessment will consider social, psychological, and environmental information; however, in a patient population where the majority may have delirium, dementia, or cognitive impairment, accurate and adequate collection of historical data may be difficult to achieve (Hoekstra et al. 2011; Sivakumar et al. 2013). Triangulation of data from a variety of sources should be routinely undertaken where required. This will help to guide a final clinical judgment diagnosis inclusive of the problem, etiology, and signs and symptoms (White et al. 2012).

Index Scores or Global Assessment Measures

In the absence of a single measure "silver bullet" diagnostic measure, index scores or global assessment methods may be considered more appropriate for the purposes of diagnosing malnutrition in hip fracture. These are described in Table 7.

Nutrition Screening and Assessment: Summary

Barriers to appropriate nutrition screening and assessment measures in hip fracture inpatients are numerous (Fig. 2). These have contributed to the highly divergent reported prevalence of malnutrition risk or malnutrition of 0–99% across studies and measures (Bell 2014). This justifies the need for a more considered approach toward nutrition screening and diagnostic tool selection and application in hip fracture. Conflicting and lacking data will challenge nutrition assessment processes in an acutely unwell, multimorbid population prone to delirium, dementia, cognitive impairment, affect optimization, and frailty. There is no malnutrition screening or diagnostic gold standard. However, the lack of a gold standard should not be considered an opportunity to seize single "quick and easy" techniques that do not adequately assess individuals for malnutrition or consider well-established confounders. Hip fracture patients are multimorbid and require multimodal, multidisciplinary care. Routine nutrition assessment will require triangulation of multiple nutrition assessment measures from a variety of sources over multiple time periods. Many cases will still, however, necessitate a clinical judgment diagnosis of malnutrition which may require some divergence from standardized criteria

Table 7 Key advantages and limitations of index scores reported across hip fracture studies (Reported advantages and limitations of index scores when applied in hip fracture patients are outlined)

Index score	Description	Observed/ reported advantages	Potential or perceived limitations	Examples
Mini Nutritional Assessment[a]	Numerical scoring system for both nutrition screening and malnutrition diagnosis No nutrition risk Nutrition risk Malnutrition	Demonstrated predictive and concurrent validity Broadly recommended, implemented, and reviewed across settings Highly sensitive screening component Allows for international benchmarking Objective numerical measure	Some parameters difficult to obtain/ confirm in hip fracture inpatients Length of time to administer Low specificity reported across a number of studies Low completion rates reported in a number of studies	Helminen et al. 2017; Gumieiro et al. 2013; Guo et al. 2010; Hoekstra et al. 2011; Duncan et al. 2006
Mini Nutritional Assessment-Short Form[b]	Numerical scoring system for both nutrition screening and malnutrition diagnosis (short-form version) No nutrition risk Nutrition risk Malnutrition	Less than 5 min to administer Limited training required Ease of use by a variety of health professionals Objective numerical measure	Some parameters difficult to obtain/ confirm in hip fracture inpatients Limited application in hip fracture with mixed predictive/ concurrent validity reported May underidentify malnutrition in hip fracture	Nuotio et al. 2016; Formiga et al. 2005; Koren-Hakim et al. 2016; Bell et al. 2014a
Subjective Global Assessment[c]	Subjective assessment completed by trained assessor. Includes weight, intake, GI symptoms, functional capacity, and physical evaluation components. Categorizes patients as:	Demonstrated predictive and concurrent validity Broadly recommended, implemented, and reviewed across settings Allows for international benchmarking Reported good intra- and	Some parameters difficult to obtain/ confirm in hip fracture inpatients Requires training to administer Subjective interpretation necessitates clinical judgment capacity and evaluation of reliability for study purposes	Eneroth et al. 2006; Bell et al. 2014a; Bell et al. 2016

(*continued*)

Table 7 (continued)

Index score	Description	Observed/ reported advantages	Potential or perceived limitations	Examples
	SGA A (well nourished) SGA B (suspected or mild-moderate malnutrition) or SGA C (severe malnutrition)	interrater reliability Relatively easy to administer by trained clinicians Subjective nature allows clinical judgment diagnosis Takes 5 - 10 minutes to complete	Categories may require adaptation to meet diagnostic criteria for case-based reimbursement funding	
International Statistical Classification of Diseases and Related Health Problems Criteria[d]	Subjective assessment completed by trained assessor. Includes BMI or weight, intake, and physical evaluation components.	Allows clinical judgment diagnosis Demonstrated predictive and concurrent validity Linked to case-based reimbursement funding	Less commonly reported in hip fracture Some parameters difficult to obtain/ confirm in hip fracture inpatients	Bell et al. 2014a; Nikkel et al. 2012
ASPEN/AND Consensus Statement[e]	Subjective assessment completed by trained assessor. Includes assessment across six domains with two or more domains to be met for diagnostic purposes	Allows clinical judgment diagnosis Includes consideration of etiology of malnutrition within diagnostic component	Limited application or evaluation in hip fracture settings	Bell et al. 2014a

[a]Guigoz et al. (1994)
[b]Kaiser et al. (2009)
[c]Detsky et al. (1987)
[d]National Centre for Classification in Health (2006)
[e]White et al. (2012)

or index measures. Consultation and confirmation with other members of the multidisciplinary team prior to documentation may be helpful in such cases. Malnutrition is the most costly comorbidity in hip fracture; the additional effort required to secure a correct diagnosis should be considered time well spent.

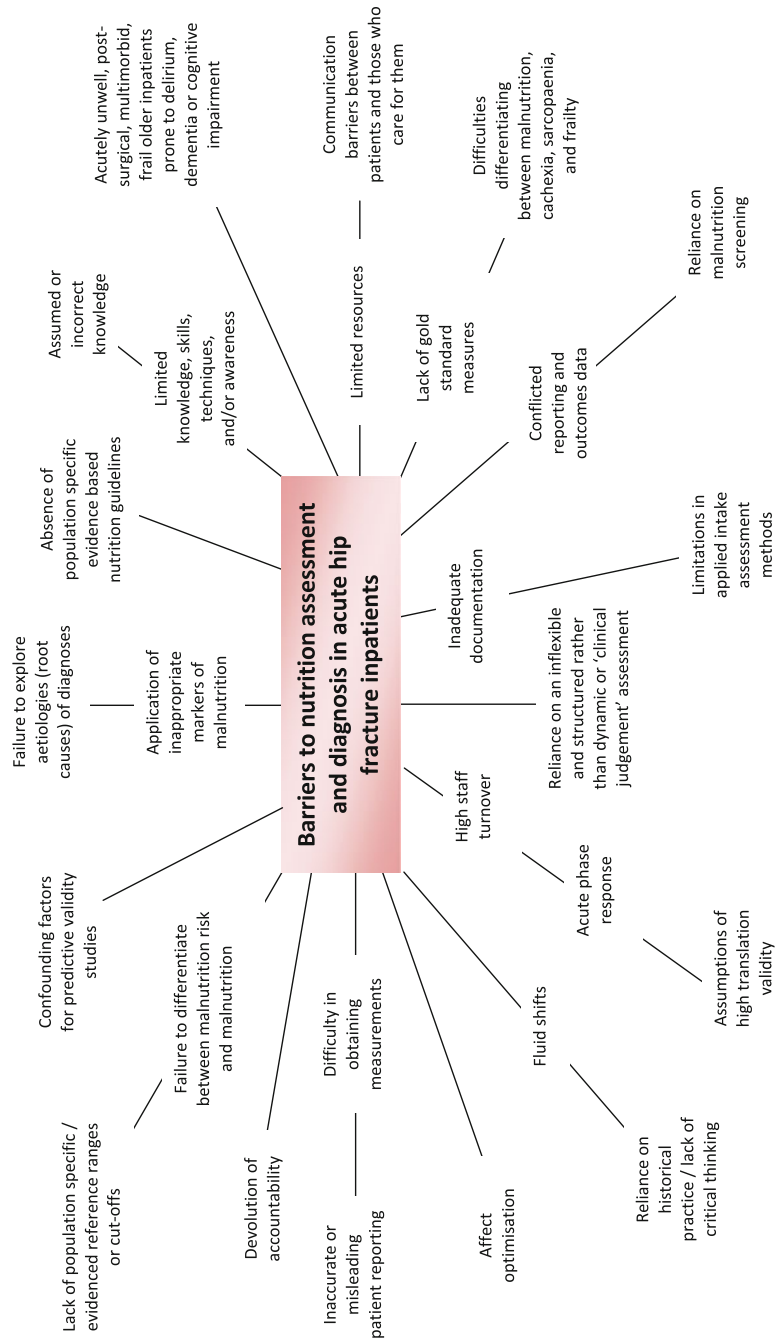

Fig. 2 Potential barriers to nutrition assessment and diagnosis in hip fracture inpatients (Source: Adapted from Bell 2014; used with permission)

Protocol: Nutrition Screening and Assessment in Hip Fracture

Purpose

Identify hip fracture inpatients at risk of malnutrition or with a malnutrition diagnosis to facilitate timely delivery of clinical nutrition care

Procedure

- Systematic, multidisciplinary nutrition assessment and intervention should be undertaken on admission for all hip fracture inpatients wherever resources allow
- Malnutrition diagnosis criteria should be applied by a trained medical officer, dietitian, or delegate, using a tool that:
- has demonstrated adequate predictive and concurrent validity in hip fracture
- is not limited to a single measure or marker
- allows for clinical judgment
- If screening is to be relied upon, a screening tool should demonstrate adequate sensitivity in the population in which it will be applied to minimize false negative, missed diagnoses. This should be conducted by a trained member of the multidisciplinary team
- A thorough nutrition assessment should apply the ABCDE format prior to informing a nutrition diagnosis
- Physiological barriers, psychosocial and workplace cultural factors, environmental considerations, and patient/healthcare provider perceptions, biases, and misconceptions should be considered within scope of nutrition assessment
- Nutrition assessment should include consideration of the patient's treatment goals and intentions
- All malnutrition diagnosis are to be clearly documented, including the problem, etiology, and justifying signs/symptoms
- Patients identified as at risk or malnourished should routinely receive clinical nutrition support that combines systematized, multidisciplinary processes with individualized nutrition care where appropriate
- Processes should be in place for weekly nutrition rescreening, reassessment, and monitoring and/or evaluation

Dictionary of Terms

- **Cachexia** – Sarcopenia resulting from an unmet increased metabolic demand associated with acute or chronic clinical condition(s)
- **Malnutrition** – Malnutrition broadly envelopes undernutrition, overnutrition, or nutrient imbalance states, across macro- and micronutrients. The definition in this chapter is limited to protein/energy malnutrition or undernutrition as defined by a tool broadly recognized and validated for that purpose in the population being applied

- **Multidisciplinary** – For the purpose of this review "multidisciplinary" is inclusive of engagement of team members from more than one professional discipline, either within a multidisciplinary, interdisciplinary, or transdisciplinary context
- **Multimorbid** – Having multiple medical conditions or disease states which are considered to have substantial impact on patient and/or healthcare outcomes
- **Nutrition assessment** – A systematic approach to collecting and recording the data obtained from patients, clients, family members, caregivers, and/or other individuals and groups across a number of domains for the purpose of informing a nutrition diagnosis, clinical nutrition interventions, and/or monitoring and evaluation purposes
- **Nutrition screening** – A quick and easy process able to be applied to large numbers of people, with limited training, to identify those at significant risk of malnutrition
- **Sarcopenia** – Loss of skeletal muscle mass, strength, and/or function that can be attributable to either nutritional or nonnutritional factors
- **Wasting** – Sarcopenia resulting from insufficient protein and/or macronutrient intake or uptake, or increased losses

Summary Points

- Malnutrition diagnosis in hip fracture is a key predictor of poor patient and healthcare outcomes
- The majority of hip fracture patients are at high risk of malnutrition and inadequate oral intake
- Where resources allow, nutrition screening should be replaced with routine assessment
- There is no gold standard for nutrition screening or diagnosis of malnutrition
- There are multiple barriers and confounders to commonly applied nutrition screening and nutrition assessment measures and markers
- Multimorbid hip fracture patients require a comprehensive nutrition assessment triangulating data from multiple sources across multiple time points
- A clinical judgment diagnosis of malnutrition may require some divergence from standardized criteria

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Growth of Skinfold Thickness in the Undernourished Santal Children: A Focus on the Purulia District of India

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Abstract

The skinfold thickness in different locations like tricep, bicep, subscapular, suprailiac, and calf generally measures the subcutaneous fat deposition in the body. These skinfold parameters may be used to assess nutritional status in children as the subcutaneous fat deposition varies with the changes in nutritional status of children. Santals are backward tribal community of India, and in the present review the growth of the skinfold thickness of Santal children has been considered to evaluate the status of skinfold in undernutrition. Undernutrition does not affect the skinfold thickness in different locations uniformly, and the sensitivities of skinfold thickness of some locations are good markers (triceps skinfold, TRSF, and subscapular skinfold, SBSF), while others may not be changed with undernutrition (suprailiac skinfold, SPSF; biceps skinfold, BCSF; and calf skinfold, CSF). SBSF and TRSF were strongly associated with the nutritional status (HAZ) in Santal boys, whereas in Santal girls, TRSF appeared to be strong predictor to assess nutritional status. TRSF is a sensitive indicator of undernutrition in both the boys and girls, and SBSF is less sensitive to identify undernutrition in girls but not in boys. The triceps-for-age z-score (TAZ) appears to be better indicator of undernutrition than that of height-for-age z-score (HAZ) and weight-for-age z-score (WAZ) in Santal children. The growth and velocity of growth of different skinfold thickness in well-nourished Santal children was found to be changed in undernourished Santal boys and girls.

Keywords

Santal · Undernutrition · Nutritional status · Skinfold thickness · Nutritional anthropometry · Body fat · Growth curve · Body mass index · Upper arm fat area

List of Abbreviations

BCSF	Biceps skinfold
BMI	Body mass index
CSF	Calf skinfold
FFMI	Fat-free mass index
HAZ	Height-for-age z-score
MUAC	Mid-upper arm circumference
NCHS	National Center for Health Statistics
NHANES	National health and nutrition examination survey
PTG	Primitive Tribal Groups'
SAZ	SBSF-for-age z-score
SBSF	Subscapular skinfold
SPSF	Suprailiac skinfold
STSSF	Sum of triceps and subscapular skinfold
STSSFAZ	Sum of triceps and subscapular skinfold thickness-for-age z-score
TAZ	TRSF-for-age z-score
TRSF	Triceps skinfold
UAFA	Upper arm fat area

UAMA	Upper arm muscle area
UAMAH	Upper arm muscle area by height
UAMC	Upper arm muscle circumference
WHO	World Health Organization
WHZ	Weight-for-height z-score

Introduction

The nutritional status of children can be assessed by different methods such as nutritional anthropometry (measuring anthropometric parameters), biochemical estimation, clinical diagnosis, and dietary measurements (Lee and Nieman 2007). Nutritional anthropometry is one of the simple methods for measuring the nutritional status. It comprises of the measurements of physical dimensions and the gross composition of the body (Gibson 1990). As the growth is a major characteristic of childhood and it is dependent on adequate supply of nutrients, the measurement of growth by anthropometric measurements usually represent an idea about the nutritional status of a child (Jelliffe 1996).

The growth and development of children depend both on exogenous (nutrition, socioeconomic factors, geographical environment, etc.) and on endogenous (genetic, hormonal, etc.) factors. The favorable environmental conditions help the child to grow fullest, whereas unfavorable conditions try to depress it. Height is a measure of linear growth of the body and the degree of the skeletal development, and weight is a measure of total body mass including sum of protein, fat, water, and bone mineral mass. Hence, height and weight are sensitive to changes in muscle mass, fat, body fluid, and the skeleton of the body (Shils and Shike 2006). Weight reduction can easily occur as the result of impairment of metabolism, decrease in nutrient intake, or increase requirement of the same. Immediate decrease in weight reflects current nutritional status (Gibson 1990). Another anthropometric parameter, mid-upper arm circumference (MUAC), has been used for nutritional anthropometry as it assesses the degree of muscle and fat around the bone in the mid-upper arm region. A decrease in MUAC may reflect a reduction in muscle mass, a reduction in subcutaneous tissue, or both. In developing countries, where the amount of subcutaneous fat is frequently small, the changes in MUAC reflect the parallel alteration in muscle mass, and hence MUAC is particularly useful in the assessment of nutritional status (Lohman et al. 1988). The measurement of head circumference is important for nutritional assessment because it is closely related to brain size and can be used as an index of chronic undernutrition. Chronic undernutrition during the first few months of life, or intrauterine growth retardation, may decrease the number of brain cells and may result in an abnormal low head circumference. If undernutrition continues, it affects the total head circumference in later ages (Gibson 1990). The body mass index (BMI) proved to be useful in ascertaining the change in nutritional status over a period of time, particularly when dietary indicators could not be relied on to reflect the change(s) because of their large variations. BMI can be a reasonably good substitute not only for assessing energy status of the subject but also for evaluating

the nutritional status of the child. Moreover, BMI has been used to diagnose undernutrition, controlling for age and gender (Cederholm et al. 2015; Bailey and Ferro-Luzzi 1995). It has been opined that BMI is closely associated with the weight rather than the height of the individual because weight is the responsive variable to energy balance (NIN 1990). The measurement of chest circumference of young children is useful parameter for assessing the nutritional status (Jelliffe 1996). The chest, thigh, and mid-calf circumferences are most useful measurements for grading of body fat. In girls, thigh circumference is highly correlated with fat mass and body fat percentage (Konstantynowicz et al. 2011).

Skinfold thickness measurements are said to provide an estimate of the size of the subcutaneous fat deposition, which in turn provides an estimate of the total body fat. The variations in distribution of subcutaneous fat in different locations like triceps, biceps, subscapular, suprailiac, and calf occur with changes in nutritional status (Lee and Nieman 2007). The skinfold thickness is affected within a short duration of inadequate nutrient intake and is responsive to acute nutritional deficiency or acute undernutrition. Hence, these parameters are sensitive makers and good indicators to assess nutritional status in children. Several indices of body fat/fat-free mass like upper arm muscle circumference (UAMC), upper arm muscle area (UAMA), fat-free mass index (FFMI), and upper arm fat area (UAFA) are calculated from MUAC and skinfold measurement, and these are useful parameters for nutritional measurement in children (Chowdhury and Ghosh 2009; Chowdhury et al. 2006; Frisancho 1990). Nutritional anthropometry has been applied widely to assess the magnitude of undernutrition in children as the effect of undernutrition on different anthropometric parameters is well documented and has been classified in standard grades. But the influence of undernutrition on the growth of skinfold thickness in children has not been studied adequately. Santals are backward tribal community of India, and in the present review, the growth of the skinfold thickness of Santal children has been considered to evaluate the status of skinfold in undernutrition.

The Santals

There are several socially backward communities in India such as scheduled castes and scheduled tribes. The term “scheduled tribe” refers to specific indigenous people whose status is acknowledged to some formal degree by national legislation. A tribe viewed historically or developmentally consists of a social group existing before the development of states (Bagchi 1981). There are 645 types of scheduled tribes or tribal populations in India, and few groups were identified as more backward communities among the tribal population. These groups have been categorized as “primitive tribal groups” (PTGs) by the Government in the Census of 1975. So far, 75 tribal communities have been identified as “primitive tribal groups” in different states of India (Sanyal 2006). Santals have been recognized as one of the primitive tribes of India. Santals, the third largest tribe of India, live in remote places of different states. In West Bengal, they are spread over in vast areas of Purba and

Paschim Medinipur, Bankura, and Purulia. This tribe is characterized by poverty, illiteracy, and nutritional problems.

Nutritional Status of Santal Children

The nutritional and health status of Santals were measured long time ago in Midnapore of West Bengal (Bagchi 1981) by using anthropometric parameters like height and weight. It was reported that more than 60% of Santal children suffered from undernutrition in this study (Bagchi 1981). Another study conducted in Purnia district of Bihar reported that 6% of Santal children were found in severe grade of acute undernutrition (Rao and Vijay 2006). The nutritional status of Santal children in some areas of Purulia district of West Bengal has been measured on the basis of their height and weight, and a high percentage of undernutrition (42.27% stunting, 66.81% underweight, and 61.95% wasted) has been reported in this population (Chowdhury et al. 2008). In further study, about 17.13% Santal boys and 20.63% Santal girls were identified as truly undernourished based on height-for-age, weight-for-height, and upper arm muscle area by height (UAMAH) (Chowdhury and Ghosh 2009). In addition, the growth pattern of Santal children as assessed from height-for-age and weight-for-age growth curves showed that undernutrition prevailed in these children compared to international standard (NCHS). The poor growth pattern of Santal children on the basis of NCHS data of height and weight was further supported from other anthropometric parameters like MUAC in these subjects (Chowdhury and Ghosh 2009).

Nutritional Status of Other Tribal Communities

The undernutrition in children of different countries has been assessed in terms of stunting (from height-for-age) and underweight (from weight-for-age). The prevalence of stunting and underweight showed wide variation, in the children of different countries like Mexico (Tejas et al. 2001), Chile (Ivanovic et al. 1995), Malaysia (Marjan et al. 1998), and Nigeria (Ukoli et al. 1993) and Nepal (Ghosh et al. 2009). Even reports from different regions of India indicate the variability in stunting and underweight among children (Joseph et al. 2002; Laxmaiah et al. 2002; Abel and Sampathkumar 1998) and thus indicate regional difference in the nutritional status of the children. The prevalence of undernutrition among the tribal children in India has not been investigated sufficiently except in few studies (Table 1). Rao et al. (2005) reported a widespread undernutrition (51.6% stunting and 61.6% underweight) among the preschool children of Gond tribe of Madhya Pradesh. Mitra et al. (2007) showed that Kamar children (4–12 years) of Chhattisgarh suffered from extensive undernutrition (63.5% stunting, 57.8% underweight, and 57% wasting). A large percent of undernutrition (54.9% stunting and 62.8% underweight) had been reported in Saharia preschool children of Rajasthan (Rao et al. 2006). Similarly, children of Sugali tribe of Andhra Pradesh are also found to be suffering from

Table 1 Stunting, underweight, and wasting in different tribal children of India. Severe stunting/underweight/wasting < -3 z-score, moderate stunting underweight/wasting < -2 z-score

Authors	Year	Tribe	State	Age Gr.	Height-for-age	Weight-for-age	Weight-for-height
Chowdhury and Ghosh	2008	Santal	West Bengal	5–12	$< -3 = 13.82\%$	$< -3 = 20.4\%$	$< -3 = 15.01$
					$< -2 = 31.73\%$	$< -2 = 47.69\%$	$< -2 = 33.39$
Mitra et al.	2007a	Kamar	Chhattisgarh	4–12	$< -2 = 63.5\%$	$< -2 = 57.8\%$	$< -2 = 57\%$
Rao et al.	2005	Gond	Madhya Pradesh	1–5	$< -3 = 30.1\%$	$< -3 = 27.7\%$	$< -3 = 6.5\%$
					$< -2 = 51.6\%$	$< -2 = 61.6\%$	$< -2 = 32.9\%$
Rao et al.	2006	Saharia	Rajasthan	0–5	$< -3 = 29.3\%$	$< -3 = 19.9\%$	$< -3 = 2.3\%$
					$< -2 = 54.9\%$	$< -2 = 62.8\%$	$< -2 = 12.9\%$
Mitra et al.	2007b	Gond	Chhattisgarh	1–5	$< -3 = 13.3\%$	$< -3 = 15.5\%$	$< -3 = 15.5\%$
					$< -2 = 29.4\%$	$< -2 = 33.3\%$	$< -2 = 29.4\%$
Mitra et al.	2007b	Kawar	Chhattisgarh	1–5	$< -3 = 11.6\%$	$< -3 = 7.1\%$	$< -3 = 6.5\%$
					$< -2 = 28.2\%$	$< -2 = 25.2\%$	$< -2 = 25.1\%$
Laxmaiah et al.	2007	Mandal	Andhra Pradesh	1–5	$< -3 = 20\%$	$< -3 = 17.7\%$	$< -3 = 3.6\%$
					$< -2 = 46.4\%$	$< -2 = 65.4\%$	$< -2 = 21.3\%$
Rao and Rao	2006	Santal	Bihar	6–9	–	Severe = 6%	–

undernutrition (46.4% stunting and 65.4% underweight) (Reddy and Rao 2000). In West Bengal, the children (6–12 years of age) of Oraon tribe were getting less energy from their food even after their enrolment in the midday meal program at the local primary school (Mittal and Srivasatava 2006). As a result, 54% of the children were suffering from severe malnutrition as measured by BMI. The variability of undernutrition in different regions may be due to socioeconomic conditions, environmental factors, and ethnic differences (Frongillo et al. 1997). The number of stunted and underweight (moderate and severe grade) Santal children of Purulia district is less than that of many tribes (e.g., Gond (Madhya Pradesh), Saharia, and Oraon tribes) (Chowdhury et al. 2008). It seems that the Santals children of Purulia district are not affected maximally in their nutritional status among the different tribes of India. The socioeconomic status of Santals was found to be in lower and upper-lower category (Chowdhury et al. 2008), and they send their children to local primary schools where midday meal program is in operation. The different anthropometric parameters (e.g., height, weight, BMI MUAC) of Santal children have been studied to evaluate the grade of undernutrition in this population. Hence these Santal children are suitable subjects to evaluate the influence of undernutrition on the skinfold thickness.

Growth Curve of Skinfold Thickness in Santal Children

The assessment of nutritional status of Santal children from skinfold thickness was based on the changes of subcutaneous fat in different locations. The calorie reserve of the body is reflected on the subcutaneous fat. The growth curves of skinfold-for-age from different locations were employed to assess the nutritional status of Santal children (Chowdhury and Ghosh 2013).

TRSF-for-Age and SBSF-for-Age Growth Curve

The nutritional status of Santal children was assessed from the location of the TRSF-for-age and SBSF-for-age growth curve in the respective skinfold-for-age reference growth curve of NHANES (Lee and Nieman 2007). A poor nutritional status of Santal children was evident from the location of growth curves of TRSF-for-age and SBSF-for-age in the reference values of NHANES, and the effect of undernutrition on these two skinfolds was almost similar. It was observed that SBSF values of Santal boys remained around 5th–10th percentile and TRSF values remained between 5th and 10th percentile of the reference. The poor growth pattern of skinfold thickness was also observed in girls, but unlike boys the TRSF was affected more (below 5th percentile) than that of SBSF, i.e., around 15th percentile (Chowdhury and Ghosh 2013). Previously, it was reported that the height-for-age and weight-for-age growth curves of Santal children remained at 5th–10th percentile of the WHO reference (Chowdhury et al. 2008). Therefore, the age-related growth curves of TRSF and SBSF were affected in undernourished children in a similar way to that of height-for-age and weight-for-age growth curves.

Growth of SPSF, BCSF, and CSF

As the reference values of SPSF, BCSF, and CSF for NHANES are not available, the growth pattern of these skinfold parameters cannot be assessed like that of TRSF and SBSF in Santal children. The effect of undernutrition on these skinfolds was measured by the difference of the mean values between well-nourished and undernourished children (which were identified on the basis of height-for-age z-score) in different ages.

Skinfold Thickness Between Well-Nourished and Undernourished Santal Children

It has been widely accepted that height-for-age z-score (HAZ) is one of the sensitive parameters for measuring chronic undernutrition (WHO 1986). According to criteria of WHO, children with HAZ below 1 were considered as undernourished (WHO 1986). This criterion has been used in skinfold, and the age-related mean values of skinfold parameters were compared between well-nourished ($HAZ > -1$) and undernourished ($HAZ < -1$) Santal boys and girls to find out the effect of undernutrition on the growth of different skinfold thickness (Chowdhury and Ghosh 2013). It was found that the mean values of BCSF (except 6 and 12 years) and CSF (except 10–12 years) in the well-nourished and undernourished groups of boys and girls were similar, and there was no significant change between these two groups of children having different nutritional status. These results probably indicate that these skinfolds are less sensitive in undernutrition. However, the skinfold thickness in SPSF showed significant difference between well-nourished and undernourished groups of boys and girls. Thus, the undernourished children ($HAZ < -1$) showed the lower thickness of TRSF, SBSF, and SPSF compared to that of well-nourished children. Similar difference in the thickness of TRSF and SBSF between well-nourished and undernourished groups of boys and girls was also reported in American black children (Curran and Barness 2000) and in Turkish children (Yuca et al. 2011). It appears that these skinfolds are sensitive parameters for assessing undernutrition. Therefore, undernutrition does not affect the skinfold thickness in different locations uniformly. The sensitivity of skinfold thickness of some locations are identified as good markers (TRSF, SBSF, and SPSF), while others may not be changed with undernutrition (BCSF and CSF).

Growth Velocity of Different Skinfold Thickness in Santal Children

Measurement of growth velocity of skinfold parameters is important to evaluate the growth of skinfold thickness of a subject. Growth velocity curves for skinfold thicknesses represent the increase of these parameters during a fixed period of time. Skinfold thickness velocity curves depict the age-dependent changes in velocity that characterize the postnatal growth of these children. Pubertal growth spurt was also observed in the velocity growth curves of different skinfold thickness.

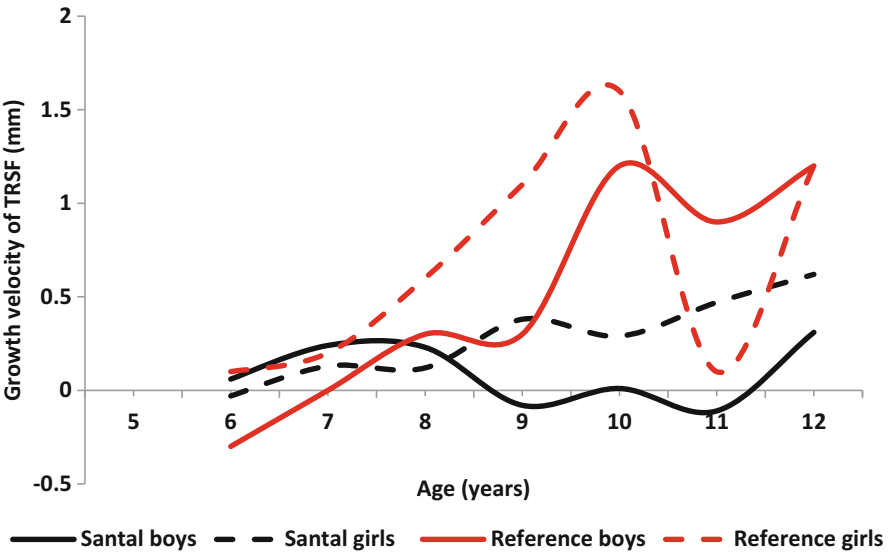

Fig. 1 Velocity of growth curve in triceps skinfold (TRSF) thickness for Santal children and reference children (Frisancho 1990)

Velocity of Growth in TRSF Thickness

The velocity of growth in TRSF thickness for Santal boys (9–12 years of age) was found to be lower than that of reference curve (Frisancho 1990). In Santal girls, velocity of growth in TRSF thickness was lower than that of reference values, except in 11 and 12 years of age. The velocity curve of TRSF thickness for Santal boys and girls showed a progressive growth from 6 years of age, followed by decrease in boys at 8 and 11 years of age, and in girls the velocity was lower at 8–10 years of age than that of the reference curve (Fig. 1). The total gain of growth (from 6 to 12 years) in TRSF of Santal boys and girls was about 0.25 mm and 0.58 mm, respectively. The growth spurt in TRSF was started at 11 years in boys and at 10 years of age in girls.

Velocity of Growth in SBSF Thickness

The velocity of growth in SBSF was lower at 6–7 years of age in boys compared to reference curve, and then the velocity was decreased at 10–11 years of age. In girls, the velocity was lower at 8–10 years of age compared to that of reference curve (Fig. 2). The velocity of growth in SBSF thickness of Santal boys and girls was comparable in 6–10 years of age, but thereafter the velocity was steeply increased in girls at 10 years of age and in boys at 11 years of age, which might be the indicator of growth spurt (Fig. 2). The total gain of growth in SBSF of Santal boys and girls (from 6 to 12 years) was about 0.63 mm and 0.32 mm, respectively.

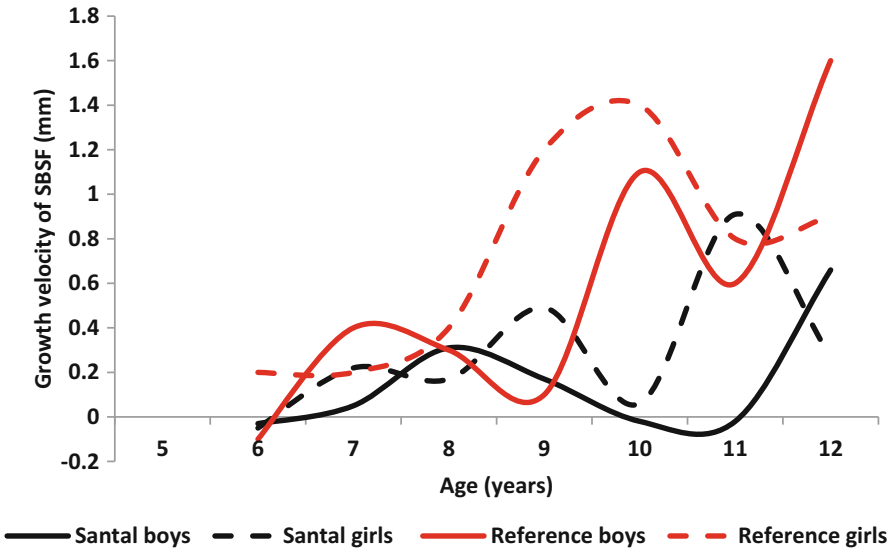

Fig. 2 Velocity of growth curve in subscapular skinfold (SBSF) thickness for Santal children and reference children (Frisancho 1990)

Velocity of Growth in SPSF Thickness

The velocity of growth in SPSF thickness in Santal boys and girls is shown in Fig. 3. As the reference values of SPSF thickness are not available, the velocity of growth in SPSF thickness is shown in well nourished and undernourished in Santal boys and girls. The nutritional status was identified from height-for-age z-score of reference (WHO 2007). The velocity of growth in SPSF of well-nourished and undernourished girls was not markedly different from each other at 6–9 years of age, and thereafter the velocity in undernourished girls was decreased; however at 11 years of age an increase of growth velocity was noted which might be indicator of growth spurt. In Santal boys, the velocity of growth in SPSF was totally different at 6–12 years of age in well- and undernourished boys; probably the growth spurt in SPSF is absent in undernourished boys. Santal girls gained higher SPSF (0.37 mm) than that of boys (0.18 mm) between 6 and 12 years of age groups.

Velocity of Growth in BCSF Thickness

The velocity of growth in BCSF thickness is shown in well-nourished and undernourished Santal boys and girls in Fig. 4. The pattern of velocity of growth in BCSF during 6–12 years of age is similar in well- and undernourished Santal girls, but the velocity values were lower at 8–9 and 11 years of age (Fig. 4). Though the velocity

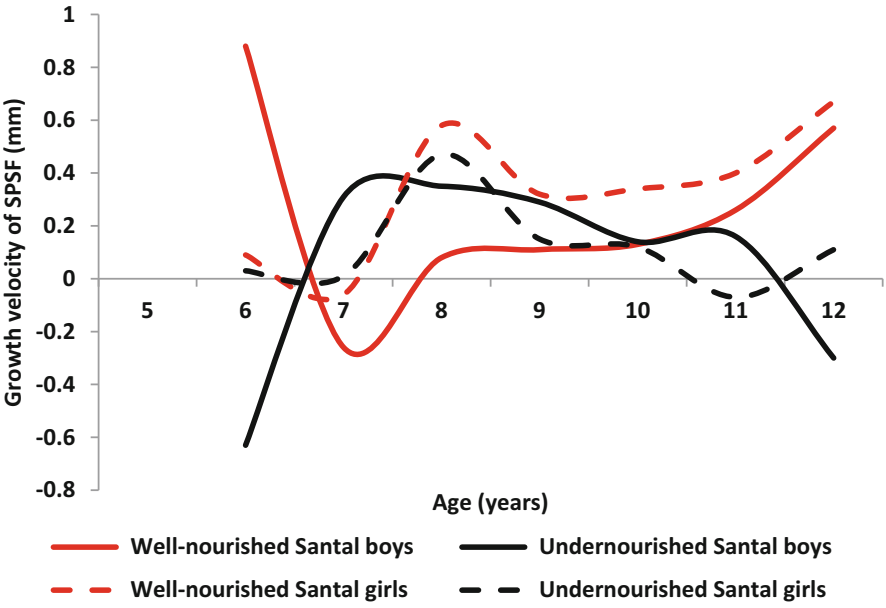

Fig. 3 Velocity curve for growth of suprailiac skinfold (SPSF) for well-nourished and undernourished Santal children

of growth in BCSF thickness showed undulation during 6–10 years of age in undernourished Santal boys, it took higher velocity at 11 years of age indicating the growth spurt. The velocity of growth in BCSF thickness in well-nourished Santal boys was steady during 6–10 years of age, and velocity was increased at 11 years of age. The total gain of growth in BCSF thickness in Santal girls was about 0.25 mm, but in boys it is negligible.

Velocity of Growth in CSF Thickness

The velocity of growth in CSF thickness in well-nourished boys showed steady velocity during 6–10 years of age after which the velocity was increased but again attained steady level at 12 years of age (Fig. 5). In undernourished boys, the velocity was always lower than that of well-nourished boys though the trend was similar. The velocity of growth in CSF thickness in well- nourished Santal girls showed undulation during 6–10 years of age, and then a steep increase in velocity was noted at 11–12 years of age. In undernourished girls, the velocity showed variation during the observed period (6–12 years of age), and the trend was different from that of well-nourished girls. The total gain of growth in CSF in Santal boys and girls was about 0.28 mm and 0.72 mm, respectively, during the observed period (6–12 years of age).

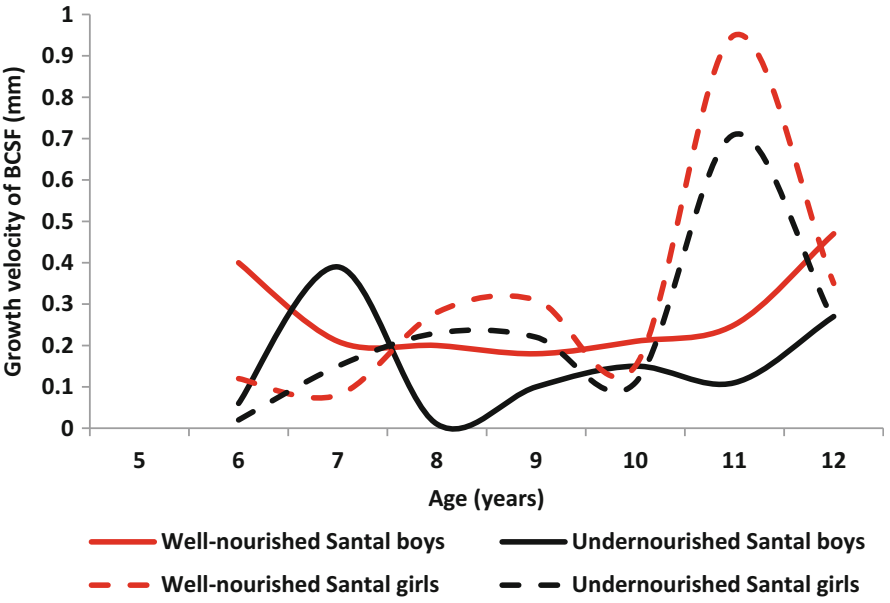

Fig. 4 Velocity curve for growth of biceps skinfold (BCSF) for well-nourished and undernourished Santal children

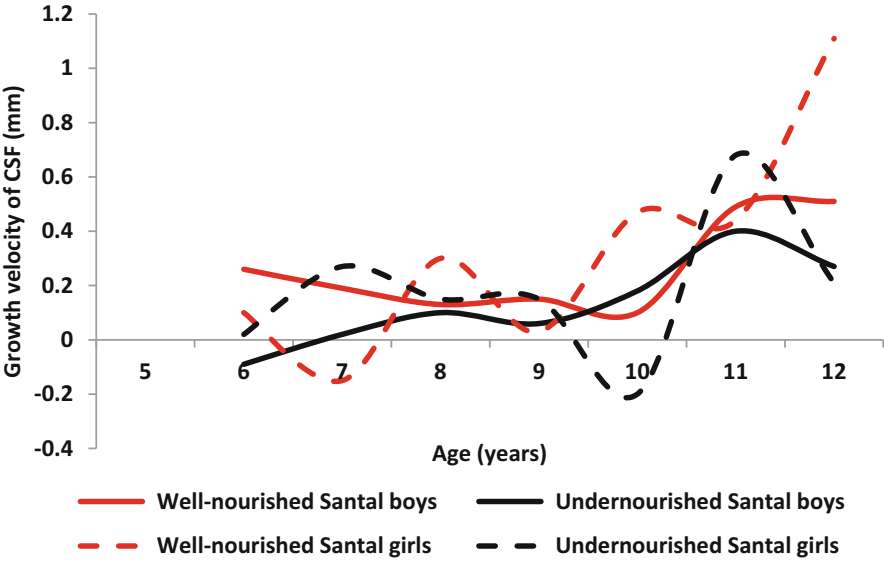

Fig. 5 Velocity curve for growth of calf skinfold (CSF) for well-nourished and undernourished Santal children

Correlation Study Among Skinfold Thickness

Pearson's correlation analyses were used to evaluate the relationship among skinfold parameters in well-nourished and undernourished children separately (Chowdhury and Ghosh 2013). In well-nourished (HAZ > -1) boys, four skinfold thicknesses (TRSF, BCSF, SBSF, and SPSF) showed significant correlation with each other but not with CSF. Such correlation was not observed in the undernourished (HAZ < -1) boys. BCSF showed significant correlation with SPSF and SBSF, and SBSF showed significant correlation only with CSF in undernourished boys. In well-nourished (HAZ > -1) girls, all the five skinfold parameters (TRSF, BCSF, SBSF, SPSF, and CSF) showed significant correlation with each other. The undernourished girls on the other hand showed different types of correlations than that of undernourished boys. In undernourished (HAZ < -1) girls, the four skinfold thickness (TRSF, BCSF, SBSF, and SPSF) showed significant correlation with each other, but CSF was not significantly correlated with SBSF and SPSF. Chowdhury and Ghosh (2013) concluded from these results that the growth of different skinfold thickness in well-nourished children was found to be changed in undernourished boys and girls. Additionally, it was observed that the pattern of growth in skinfold thickness of two locations may not be similar in well-nourished or undernourished boys and girls, and therefore significant correlation in some skinfold parameters between children of two nutritional statuses was not observed.

Association Between Nutritional Status and Skinfold Thickness

The association between nutritional status (well-nourished and undernourished according to the height-for-age z-score) and five different skinfold parameters (TRSF, SBSF, SPSF, BCSF, and CSF) was analyzed by multiple regression using four incremental models where the outcome variable was nutritional status (Chowdhury and Ghosh 2013). The results of regression analyses suggested that SBSF and TRSF were strongly associated with the nutritional status (HAZ) in boys, whereas TRSF and SPSF in girls appeared to be strong predictor to assess nutritional status. In the Santal girls, thickness of SBSF was not associated with their nutritional status suggesting, a poor indicator of undernutrition. It may be noted that the SBSF-for-age growth curve was also less affected than that of TRSF-for-age in girls. The association of BCSF and CSF with nutritional status (HAZ) seems to be much weaker as its association with nutritional status failed to show significant involvement after their inclusion in the models of regression.

Body mass index (BMI) has been used to measure the nutritional status in children of different countries (Nething et al. 2007; Wolney and Carlos 2006). BMI proved to be useful in ascertaining the change in nutritional status over a period of time, when dietary indicators could not be relied on to reflect the change(s) because of their large variations. BMI can be a reasonably good substitute not only for assessing energy status but also for evaluating the nutritional status of the child. BMI is closely associated with the weight rather than the height of the

Table 2 Regression analyses between different skinfold parameters and BMI-for-age z-score (dependent variable) in boys

	Beta (95% CI)	p-value	R^2	Change in R^2 from previous model
Model 1			0.426	0.426
SBSF	0.477	0.001		
Model 2			0.445	0.019
SBSF	0.538	0.001		
TRSF	0.342	0.012		
Model 3			0.458	0.013
SBSF	0.524	0.001		
TRSF	0.318	0.042		
SPSF	0.058	0.547		
Model 4			0.463	0.005
SBSF	0.503	0.027		
TRSF	0.317	0.305		
SPSF	0.042	0.243		
BCSF	0.027	0.574		
CSF	0.024	0.819		

TRSF triceps skinfold, *BCSF* biceps skinfold, *SBSF* subscapular skinfold, *SPSF* suprailiac skinfold, *CSF* calf skinfold

individual because weight is the responsive variable to energy balance (NIN 1990). In the present study, regression analyses were done to find out the association between skinfold thickness and nutritional status in terms of BMI-for-age z-score (BMIAZ). Results showed a similar outcome in case of association between BMIAZ and skinfold thickness as it was found in the association between HAZ and skinfold thickness. It has been observed that SBSF for boys and TRSF and SPSF for girls were significantly associated with the BMI-for-age z-score (Tables 2 and 3). Researchers have already established that skinfold thickness is also a good predictor for assessing nutritional status in terms of assessment of energy balance like BMI (Chowdhury et al. 2013; Booyens et al. 1997) and both of these parameters are strongly associated with body fatness (Freedman et al. 2007; Freedman and Sherry 2009).

Z-Score Analyses of Skinfold Thickness for Assessing Nutritional Status

The nutritional status of Santal children was assessed by the nutritional categories based on z-scores of skinfold thickness of TRSF and SBSF (Frisancho 1990). The subjects having the thickness of the skinfolds above +1 z-score and below −1 z-scores were identified as well-nourished and undernourished, respectively. A high prevalence of undernutrition was found in Santal children (Fig. 6). Percentage of undernourished boys and girls (<−1 z-score) was found to be higher in SBSF (75.1%) and TRSF (92.3%), respectively. Similarly, percentage of severe

Table 3 Regression analyses between different skinfold parameters and BMI-for-age z-score (dependent variable) in girls

	Beta (95% CI)	p-value	R^2	Change in R^2 from previous model
Model 1			0.547	0.547
TRSF	0.514	0.001		
Model 2			0.578	0.031
TRSF	0.531	0.001		
SPSF	0.221	0.023		
Model 3			0.561	0.017
TRSF	0.543	0.001		
SPSF	0.183	0.041		
SBSF	0.094	0.241		
Model 4			0.552	0.009
TRSF	0.538	0.001		
SPSF	0.164	0.037		
SBSF	0.081	0.177		
BCSF	0.037	0.351		
CSF	0.026	0.542		

TRSF triceps skinfold, *BCSF* biceps skinfold, *SBSF* subscapular skinfold, *SPSF* suprailiac skinfold, *CSF* calf skinfold

undernutrition (<−1.6 z-score) for boys and girls was also found to be higher in SBSF (35.9%) and TRSF (70.1%), respectively. Results of Figs. 1 and 2 pointed out that TRSF for girls and SBSF for boys seemed to be sensitive marker for assessing nutritional status in Santal children. This conclusion is also supported from the observation made by Chowdhury and Ghosh (2013) as well as from the observation of regression analysis for the association between BMIAZ and skinfold thickness (Tables 2 and 3).

The nutritional categories of Santal boys and girls were further analyzed on the basis of z-score for TRSF-for-age (TAZ), SBSF-for-age (SAZ), and sum of triceps and subscapular skinfold thickness-for-age (STSAZ) (Chowdhury and Ghosh 2013) as per Frisancho (1990). The results indicated that the percent of undernourished boys according to TAZ (61.5%) and SAZ (61.3%) were similar, but in the girls TAZ (90.8%) was higher than that of SAZ (44%). Therefore, this analysis (TAZ and SAZ) further supports the previous conclusion that SBSF is less sensitive to identify undernutrition in girls but not in boys, and TRSF is a sensitive indicator of undernutrition in both the boys and girls. The number of undernourished children according to STSAZ (66.3% boys and 66.5% girls) was similar in boys and girls, and hence this parameter may be good to assess undernutrition in both boys and girls. The number of undernourished children according to STSF-for-age z-score (STSAZ, 66.3% boys and 66.5% girls) were again little bit higher than that of height-for-age (HAZ < -1, 52.9% boys and 62.6% girls) and weight-for-height (WHZ < -1, 57.5% boys and 63.6% girls). Therefore, STSAZ appears to be good indicator for assessing undernutrition in children and may be equally potent in this regard to HAZ and WAZ. It may be

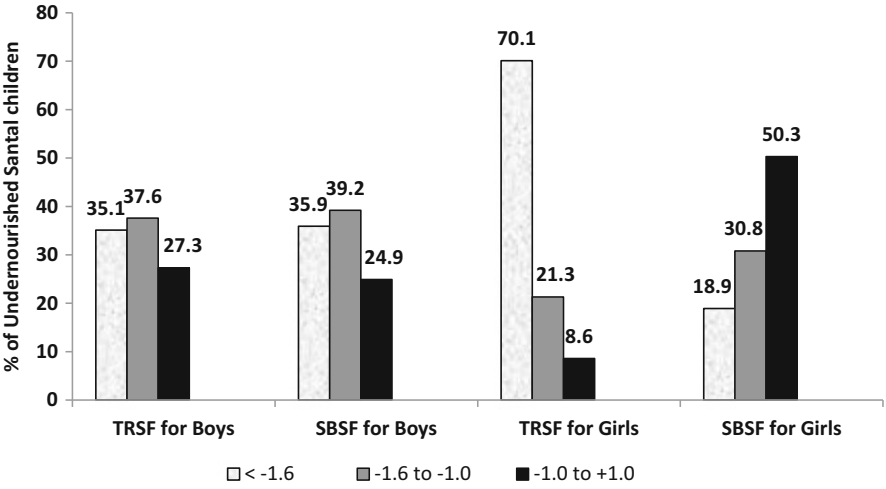

Fig. 6 Distribution of percentage of Santal boys in different nutritional categories (in terms of z-scores) developed by Frisancho (1990) for TRSF and SBSF in boys and girls

noted here that TAZ (61.5% boys and 90.8% girls) appears to be better indicator of undernutrition than that of HAZ (52.9% boys and 62.6% girls) and WAZ (57.5% boys and 63.6% girls) in this population (Chowdhury and Ghosh 2013).

Skinfold Thickness and Socioeconomic Status in Santal Children

Santal children belong to the lower and upper-lower socioeconomic status (Chowdhury et al. 2008). Previously, it has been found that undernutrition (as measured by height-for-age, weight-for-age, and weight-for-height parameters) in Santal children was directly associated with their poor socioeconomic status (Chowdhury et al. 2008). A significant association between socioeconomic factors and fat area in Santal children was found in further study (Chowdhury et al. 2009). Similarly, the significant relationship between socioeconomic factors with skinfold thickness was also identified in Santal children (Chowdhury and Ghosh 2013). A strong relationship between TRSF and socioeconomic status was also observed when Santal children were distributed in different socioeconomic classes according to the nutritional categories measured by TRSF (Fig. 7). A higher percent of undernourished (<-1 z-score of TRSF) boys (42.5%) and girls (53.3%) was observed in lower socioeconomic class which was found to be much higher than undernourished children present in upper-lower (26.4% boys and 35.4% girls) and lower-middle (3.8% boys and 2.7% girls) socioeconomic classes. Results showed that children in lower socioeconomic class suffered in undernutrition more than that of comparatively higher socioeconomic classes. These findings are in agreement with the observation of Ryan et al. (1990) and Garn and Ryan (1981) who reported

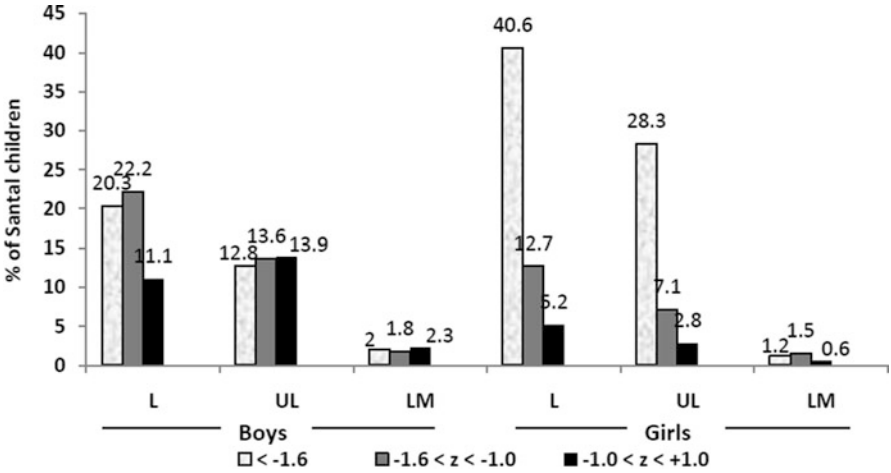

Fig. 7 Distribution of percentage of Santal children of different socioeconomic classes in different nutritional categories of TRSF developed by Frisancho (1990)

that children of higher-income group families had greater skinfold thickness than children of lower-income group families. A positive association between skinfold thickness and socioeconomic characteristics has also been reported in British children (Tauleria et al. 1995). It may be inferred that socioeconomic status is another significant factor, in addition to the nutritional status, that affects skinfold thickness of Santal children.

The question may arise whether the height, weight, and skinfold thickness of undernourished children from other communities, especially from the tribal community, will be affected similar to that of Santals. Some studies were carried out on the height, weight, and selected skinfolds in tribals such as Sugali and Bharia. The location of height-for-age and weight-for-age growth curves of Sugalis (below 5th percentile) in the reference curve of WHO indicated undernutrition in these children. But the thickness for TRSF and SBSF of these Sugali boys and girls (around 25th percentile of NHANES reference) appears to be less affected (Reddy and Rao 2000). Bharia, like the Sugali, is another primitive tribe of Madhya Pradesh. Children of this tribe also suffered from undernutrition as it is evident from the location of their height and weight curves (around 5th percentile) relative to the WHO reference (Tiwari et al. 2007). Again, the thickness of TRSF and SBSF of Bharia tribal children (around 25th percentile for both boys and girls in NHANES reference) is less affected than that of Santal children. However, the skinfold thickness in well-nourished children of Sugalis and Bharia are not available, and the magnitude of the effect of undernutrition on the skinfold of these children cannot be ascertained. Probably the skinfold thickness varies in the children of different tribal communities having similar nutritional status. Manshande et al. (1985) suggested that the environmental conditions can play an adaptive role in accumulation of subcutaneous fat. Thus it appears the

skinfold thickness in well-nourished and undernourished children of other tribal and non-tribal communities should be investigated to get information on the influence of undernutrition on the skinfold thickness of the different locations of the children.

Conclusion

Thus it appears that the growth curves and velocity of growth curves of skinfolds of different locations are changed in undernourished Santal Children. SBSF and TRSF are sensitive parameters and probably good markers for undernutrition in Santal children (from growth, velocity of growth, and association).

Policies and Protocols

Policies

The Government has accorded high priority to the issue of undernutrition and is implementing several programs (such as Integrated Child Development Scheme ICDS, Indira Gandhi Matritva Sahyog Yojana IGMSY, National Rural Health Mission NRHM, etc.) of different Ministries/Departments through State Government/UT Administration, which have the potential to improve the current nutritional situation in India. For better access to nutrition and health services, frontline workers (Anganwadi Workers AWW, Accredited Social Health Activist ASHA, Auxiliary Nurse Midwife ANM) have been appointed by health department and ICDS; these functionaries are an interface between the community and the Government systems. In spite of such endeavor, undernutrition probably exists in observed population, and additional preventive program should be undertaken by policy maker.

Protocols

Measurement and Calculation of BMI-for-Age z-Score

Height and weight of each child were measured by standard technique (Lee and Nieman 2007). BMI was calculated from height (m) and weight (kg; weight/height2). Sex-specific BMI-for-age z-score values based on World Health Organization reference z-score values for each child were used (WHO; World Health Organization 2007).

Measurement of Skinfold Thickness

Skinfolds were measured using the Harpenden skinfold caliper with a constant spring pressure of 10 g/mm^2. All sites were measured on the right side of the body. Mean of the three measurements was taken in every skinfold location.

- Triceps skinfold (TRSF)
  The subject's arm hanged loosely at the side, with the palm of the hand facing anteriorly to properly determine the posterior midline. The skinfold site was marked along the posterior midline of the right upper arm, over the triceps muscle, midway between the lateral projection of the acromion process of the scapula and the inferior margin of the olecranon process of the ulna. The measurer stood behind the subject and grasped the skinfold with the thumb and index finger of the measurer's left hand about 1 cm proximal to the skinfold site; the caliper was kept perpendicular to the long axis of the skinfold and recorded.
- Subscapular skinfold (SBSF)
  The subscapular site of each subject was marked 1 cm below the lowest angle of the scapula. The site was located by gently feeling for the inferior angle of the scapula. The skinfold was measured with the subject standing with arms relaxed to the sides. The skin was grasped 1 cm, above and medial to the site along the axis.
- Suprailiac skinfold (SPSF)
  This skinfold was measured just above the iliac crest at the midaxillary line. The subject stood erect with feet together and arms hanging by the sides. The measurer grasped the skinfold about 1 cm posterior to the midaxillary line, and the reading was recorded.
- Medial calf skinfold (MCS)
  The measurement was taken with the subject sitting; the right leg was flexed about 90° at the knee with the sole of the foot flat on the floor. The point of the maximum calf circumference was marked at the medial aspect of the calf. A vertical skinfold grasped about 1 cm proximal to the marked site, and the reading was recorded.
- Biceps skinfold (BCSF)
  The subjects faced the measurer with the arm held relaxed at the side, and the palm was facing forward. The skinfold was picked up over the belly of the biceps and 1 cm above the line marked for MUAC and TRSF on a vertical line, joining the center of the antecubital fossa to the head of the humerus. The caliper jaws were applied at the marked level, and the skinfold reading was recorded.

Assessment of Socioeconomic Status

Socioeconomic status (SES) of Santal children was assessed using the updated Kuppusswami scale (Kumar et al. 2007). The scale is based on monthly family income, parental education, and parental occupation. Structured questionnaires were used to collect information on socioeconomic characteristics of subjects' families from their parents and/or school authorities.

Measurement of Nutritional Status

Nutritional status was calculated by z-scores using the age- and sex-specific skinfold thickness reference values of World Health Organization (2007). Nutritional categories were assessed according to the classification referred by Frisancho (1990). Children with a z-score below −1 of any skinfold-for-age were considered to be

undernourished, children with a z-score between −1 and + 1 of any skinfold -for-age were considered to be nutritionally normal, and children with a z-score below −1.6 were considered to be severely undernourished.

Statistical Analyses

Descriptive statistics were used to compute the mean and standard error of mean (SEM) for different parameters by age and sex. Stepwise forward regression was employed to find out the association of nutritional and socioeconomic status with different skinfold thickness parameters. The statistical analyses were performed using statistical package for social science (SPSS software, Version 20.0.).

Dictionary of Terms

- **Undernutrition** – A poor nutritional status developed due to inadequate consumption or malabsorption of one or more nutrients.
- **Skinfold** – The skin over a particular location is folded and measured by skinfold caliper to assess the nutritional status. The skinfold thickness is a measure of the underlying subcutaneous fat.
- **Santal** – A primitive tribe of India inhabiting mostly in the state of West Bengal, Bihar, Jharkhand, and Orissa. Santal is the third largest tribe of India.
- **Growth curve** – A graphical representation of the changes of any physical dimension with time in developing subjects.
- **Stunting** – A reduced growth rate in terms of height for a particular age group in human development.
- **Underweight** – A type of undernutrition where the body weight of a subject is low considering his/her age.

Summary Points

- Different anthropometric parameters are affected by the poor nutritional status in growing children.
- Height-for-age, weight-for-age, BMI, and MUAC are used to evaluate the grade of undernutrition in children.
- Santals, the third largest tribe of India, live in remote places of different states and is characterized by poverty, illiteracy, and nutritional problems.
- A large number of Santal children suffer from stunting, underweight, and wasting.
- Skinfold thickness parameters are sensitive makers and good indicators to assess nutritional status in children.
- The growth curves of skinfold-for-age from different locations (triceps, biceps, subscapular, suprailiac, and calf) may be used to assess the nutritional status of Santal children.

- Undernutrition does not affect the skinfold thickness in different locations uniformly, and the sensitivity of skinfold thickness of some locations are good markers (TRSF, SBSF, and SPSF), while others may not be changed in undernutrition (biceps skinfold, BCSF, and calf skinfold, CSF).
- Subscapular skinfold (SBSF) was strongly associated with the nutritional status (BMIAZ and HAZ) in Santal boys, whereas in Santal girls, TRSF appeared to be strong predictor to assess nutritional status.
- Triceps skinfold (TRSF) is a sensitive indicator of undernutrition in both the boys and girls, and SBSF is less sensitive to identify undernutrition in girls but not in boys.
- The triceps-for-age z-score (TAZ) appears to be better indicator of undernutrition than that of height-for-age z-score (HAZ) or weight-for-age z-score (WAZ) in Santal children.
- The growth of different skinfold thickness in well-nourished Santal children was found to be different than that of undernourished Santal boys and girls.
- In well-nourished Santal boys, four skinfold thicknesses (TRSF, BCSF, SBSF, and SPSF) showed significant correlation with each other but not with CSF, whereas in undernourished boys, significant correlation was found between BCSF and SPSF/SBSF and between SBSF and CSF.
- In well-nourished Santal girls, all the five skinfold parameters (TRSF, BCSF, SBSF, SPSF, and CSF) showed significant correlation with each other but, in undernourished girls, significant correlation was found between TRSF and BCSF/SPS/SBS/CSF and between BCSF and SPSF/SBSF/CSF.
- The socioeconomic status is another significant factor, in addition to the nutritional status, that affects skinfold thickness of Santal children.

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Assessment of Dysphagia and Sarcopenia for Nutritional Applications: Practical Implications for Malnourished Older Patients Who Require Rehabilitation

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Abstract

Dysphagia is caused by various diseases and results in malnutrition, dehydration, and death. Dysphagia screening tools, a method for classifying severity, and a diagnostic test are necessary to identify and treat dysphagia in older adults with disabilities. The Eating Assessment Tool-10, water swallowing test, the repetitive saliva swallowing test, pulse oximetry, and cervical auscultation are used commonly in clinical practice to screen for dysphagia. Assessing dysphagia severity using the functional oral intake scale and the Food Intake LEVEL Scale are recommended for classifying patients' swallowing function and deciding therapeutic strategies. Videofluorography and fiber-optic endoscopic evaluation of swallowing are considered to be the gold standard for definite dysphagia. Sarcopenia potentially causes dysphagia, namely sarcopenic dysphagia, in which it is difficult to swallow due to sarcopenia of the generalized and swallowing muscles. Both rehabilitation and nutritional care should be used to treat sarcopenic dysphagia, based on the concept of rehabilitation nutrition. Rehabilitation nutrition includes comprehensive evaluation and intervention using rehabilitation and nutritional care of people with disabilities. It is necessary to first screen for dysphagia in older adults and then classify its severity and/or make a dysphagia diagnosis. Sarcopenia should be classified using consensus-based criteria such as the European Working Group on Sarcopenia in Older People or the Asian Working Group for Sarcopenia. Malnutrition can cause dysphagia or delayed recovery of swallowing function. Validated nutritional screening tools are useful. It is important to recognize that older adults with malnutrition require evaluation of dysphagia and sarcopenia in clinical practice.

Keywords

Sarcopenia · Dysphagia · Sarcopenic dysphagia · Dysphagia screening · Dysphagia classification · Rehabilitation nutrition · Malnutrition · Older adults · Validity · Reliability

List of Abbreviations

ADL	Activities of daily living
AWGS	Asian Working Group for Sarcopenia
DSS	Dysphagia Severity Scale
EAT-10	Eating Assessment Tool-10
EWGSOP	European Working Group on Sarcopenia in Older People
FEES	Fiber-optic endoscopic evaluation of swallowing
FILS	Food Intake LEVEL Scale
FOIS	Functional oral intake scale
ICF	International Classification of Functioning, Disability and Health
MNA	Mini Nutritional Assessment
MUST	Malnutrition Universal Screening Tool
MWST	Modified water swallowing test
NRS2002	Nutritional Risk Screening
QOL	Quality of life

RSST	Repetitive saliva swallowing test
SGA	Subjective Global Assessment
VF	Videofluorography
WST	Water swallowing test

Introduction

Dysphagia is caused by various conditions such as stroke and neurodegenerative diseases and results in malnutrition, dehydration, choking, and ultimately death. The prevalence of dysphagia in older adults is high. For example, 48% of community-dwelling subjects aged 70 years or older and 73% of institutional residents were reported to have the disorder (Cabre et al. 2009). The reason for this high prevalence of dysphagia in older adults is the concomitant increase in the prevalence of diseases causing dysphagia and reduced swallowing function associated with aging *per sé* (Wirth et al. 2016). Furthermore, older adults with dysphagia are often asymptomatic and may be easily overlooked by healthcare professionals. Unidentified dysphagia may be recognized from avoidance of food and/or liquids which trigger choking or aspiration. Notwithstanding the presence of symptoms, dysphagia is an apparent risk factor for malnutrition and dehydration as it reduces dietary and liquid intake (Foley et al. 2009; Carrion et al. 2015).

Interestingly, it has been reported that both sarcopenia and malnutrition can cause dysphagia or delayed recovery of swallowing function (Maeda and Akagi 2016; Nishioka et al. 2017a; Wakabayashi 2014). Evaluating nutritional status and/or sarcopenia using swallowing function in older adults may improve not only nutritional status but also functions and activities including swallowing capacity. Based on these findings, we have proposed a concept of rehabilitation nutrition that incorporates simultaneous intervention of rehabilitation and nutrition (Wakabayashi and Sakuma 2014). This chapter focuses on (1) screening tools and classification of dysphagia, (2) concept, characteristics, and diagnostic diagram of dysphagia caused by sarcopenia (sarcopenic dysphagia), and (3) the concept and clinical implication of rehabilitation nutrition, a method used to comprehensively evaluate swallowing function and sarcopenia.

Screening, Classification and Diagnosis of Dysphagia

Nurses and other medical professionals often perform dysphagia screening in clinical practice. The main purpose of dysphagia screening is to identify patients at risk of dysphagia who are likely to require detailed assessment offered by speech and language therapists, rehabilitation doctors, etc. Screening tools should therefore be simple, have high sensitivity against actual dysphagia, and be able to be performed at the bedside (Table 1). Classification of severity and a detailed examination of swallowing function are required to assess dysphagia

Table 1 Validity of common screening tools for dysphagia

Tools	Sensitivity	Specificity	Reference
EAT-10(Japanese ver.)[a]	0.758 0.522	0.749 0.897	DSS $\leq$ 4 (Suspected aspiration) DSS $\leq$ 6 (Suspected dysphagia)
WST(3 oz)[b]	0.76	0.59	Videofluoroscopic modified barium swallow examination (observation of swallow)
MWST (3 ml)[c]	0.553	0.808	Videofluorography (aspiration)
RSST[d]	0.981	0.658	Videofluorography (aspiration of any volume of barium)
Pulse oximetry[e]	0.56–0.87	0.39–0.97	$\geq$2% desaturation
Pulse oximetry + swallow test[e]	0.73—0.94	0.63–0.76	Coughing, choking, voice change, subjective assessment of aspiration, or $\geq$2% desaturation
Cervical auscultation[f]	0.23–0.94	0.50–0.74	Dysphagia, penetration, aspiration, or pharyngeal residuals

[a]Wakabayashi and Kayashita (2014)
[b]DePippo et al. (1992)
[c]Osawa et al. (2013)
[d]Oguchi et al. (2000)
[e]Bours et al. (2009)
[f]Lagarde et al. (2016)

severity and make decisions on dysphagia treatment. The following section describes screening tools for dysphagia, a method for classifying severity, and diagnostic examination methods.

Screening Tools

EAT-10

The EAT-10 is a 10-item questionnaire for dysphagia screening developed by Belafsky et al. (2008). Each lower order item of the EAT-10 is a subjective evaluation of dysphagia, with scores ranging from 0 points (no problem) to 4 points (severe problem). The 10 components of the EAT-10 are as follows: (1) my swallowing problem has caused me to lose weight; (2) my swallowing problem interferes with my ability to go out for meals; (3) swallowing liquids takes extra effort; (4) swallowing solids takes extra effort; (5) swallowing pills takes extra effort; (6) swallowing is painful; (7) the pleasure of eating is affected by my swallowing; (8) when I swallow food it sticks in my throat; (9) I cough when I eat; and (10) swallowing is stressful. If the total score is 3 points or more, the subject is suspected of having dysphagia. Cronbach's alpha coefficient for the test has been reported to be 0.960, indicating high internal consistency, while a high correlation coefficient calculated using the test-retest method (0.72–0.91) indicated good reproducibility.

Burgos and colleagues examined the validity of the Spanish version of EAT-10 and showed Cronbach's coefficient alpha was 0.87 (Burgos et al. 2012). In Japan, the authors developed a Japanese version of EAT-10 and verified its reliability and

validity (Wakabayashi and Kayashita 2014). This showed that Cronbach's coefficient alpha was high (0.946), while a validity test showed a significant negative correlation between EAT-10 and DSS as the standard reference of dysphagia severity. In cases with an EAT-10 score of three or more, the sensitivity to aspiration was 0.758 and the specificity was 0.749. Interestingly, the inability to reply to the EAT-10 *per sé* had a high specificity for dysphagia (sensitivity 0.489, specificity 0.951), which may be effective for defining dysphagia. In older adults who require long-term care, the EAT-10 showed a significant association with nutritional status and ADL (Wakabayashi and Matsushima 2016). However, an evaluation of the psychological characteristics of the EAT-10 using Rasch analysis showed weak structural validity and internal consistency, item redundancy, and a lack of easy and difficult items (Cordier et al. 2016).

WST/MWST

The WST estimates the possibility of aspiration by evaluating cough and changes in voice or respiratory status after swallowing 3 oz of water. In 1992, DePippo et al. developed the WST as a screening tool for aspiration (DePippo et al. 1992). The test results were related to aspiration confirmed by VF, with a sensitivity and specificity for aspiration of 76% and 59%, respectively. Another version of the WST which uses a smaller volume of water (30 mL) was proposed in Japan by Kubota et al. (1982). Later, Saito and his colleagues modified Kubota's test as the MWST that used only 3 ml of water (Saito 2000). In the MWST, the assessor pours 3 ml of cold water to the floor of the patients' oral cavity and instructs the patient to drink it. The assessor then evaluates the presence of swallowing, cough, and change in voice and respiratory status and classifies the findings into five categories (Table 2). If the grade is 4 or more, patients repeat the test again and describe the worse categories. With a cut-off value of grade 3, the sensitivity to discriminate aspiration was reported to be 0.553 and the specificity 0.808 (Osawa et al. 2013). When combined with the food test, the sensitivity was high (0.90), whereas the specificity was rather low (0.56) (Tohara et al. 2003).

RSST

The RSST is a simple screening tool that involves touching the skin surface of the hyoid bone and thyroid cartilage with the forefinger and middle finger, respectively, and repeat saliva swallowing in 30 s (Oguchi et al. 2000). If the hyoid bone and thyroid cartilage completely overcomes each finger, this is counted as 1 by the

Table 2 Scoring method of MWST

Score of 1	Inability to swallow
Score of 2	Dyspnea after water swallowing
Score of 3	Cough or dysphonia after water swallowing
Score of 4	Subject was able to swallow the water but unable to perform either of two dry (saliva) swallows within 30 s
Score of 5	Subjects was able to complete swallowing the water and both dry swallows

assessor. If the count is less than three times in 30 s, the patient is suspected as having a swallowing problem. The RSST has a high correlation with the findings of VF. The sensitivity of the test has been reported to be high and ranged between 0.80 and 0.98 especially for discriminating aspiration, whereas the specificity was 0.54–0.66 (Oguchi et al. 2000). If the patients can understand the methodology of the RSST, it is a useful screening test that anyone can perform without the risk of choking.

Pulse Oximetry

Pulse oximetry is a simple, noninvasive screening method that involves attaching a small probe to a fingertip and estimating oxygen saturation in the arterial blood based on the difference in absorbance of red and infrared light. Aspiration may occur when oxygen saturation decreases after ingestion of food and drinking water. The sensitivity of the method ranges between 73% and 87% with a specificity of 39–87% for aspiration confirmed by VF or FEES using the change in oxygen saturation before and after swallowing. Lower validity has been reported in older adults and patients with respiratory disease (Ramsey et al. 2003). A combination of the water test and oxygen de-saturation showed a sensitivity of 73–98% and specificity of 63–76% for coughing, choking, and voice changes, indicating the method has good validity as a screening test for dysphagia (Bours et al. 2009).

Cervical Auscultation

Cervical auscultation is a clinical examination for estimating transfer of food and liquid through the pharynx and/or the presence of residual food by listening to swallowing sounds. A stethoscope is commonly used for cervical auscultation, while other devices have been used in some cases such as microphones, accelerometers, and Doppler sonars. A systematic review showed that the sensitivity and specificity of cervical auscultation for dysphagia ranged from 23% to 94% and 50–74%, respectively. This review concluded that the method should not be used alone to identify dysphagia (Lagarde et al. 2016).

Classification of Dysphagia Severity

FOIS

The FOIS is a method for classifying oral intake ability and is based on the actual condition of the patients' nutritional intake (Table 3). Severity is classified into seven levels according to the combination of oral intake and alternative nutrition, from stage 1 (nothing by mouth) to stage seven (total oral diet with no restrictions). The method has high reproducibility with a kappa coefficient of 0.86–0.91, indicating a degree of interexaminer coincidence. The correlation coefficient between the FOIS and dysphagia severity based on VF was reported to be 0.54 (Crary et al. 2005).

FILS

The FILS is a classification method for dysphagia severity developed in Japan. The investigator classifies severity into 10 levels based on observations of the

Table 3 Grading methods of FOIS

Level	Types of intake
1	Nothing by mouth.
2	Tube dependent with minimal attempts of food or liquid.
3	Tube dependent with consistent oral intake of food or liquid.
4	Total oral diet of a single consistency.
5	Total oral diet with multiple consistencies but requiring special preparation or compensations.
6	Total oral diet with multiple consistencies without special preparation, but with specific food limitations.
7	Total oral diet with no restrictions.

patient's eating: (1) "No swallowing training is performed except for oral care"; (2) "Swallowing training not using food is performed"; (3) "Swallowing training using a small quantity of food is performed"; (4) "Easy-to-swallow food less than the quantity of a meal (enjoyment level) is ingested orally"; (5) "Easy-to-swallow food is orally ingested in one to two meals, but alternative nutrition is also given"; (6) "The patient is supported primarily by ingestion of easy-to-swallow food in three meals, but alternative nutrition is used as a complement"; (7) "Easy-to-swallow food is ingested orally in three meals. No alternative nutrition is given"; (8) "The patient eats three meals by excluding food that is particularly difficult to swallow"; (9) "There is no dietary restriction, and the patient ingests three meals orally, but medical considerations are given"; and (10) "There is no dietary restriction and the patient ingests three meals orally (normal)." The method has high sensitivity to changes in the state of ingestion, with reliability between evaluators being 0.70 to 0.90, reliability within evaluators 0.83–0.90, and a strong correlation coefficient between the FILS and FOIS of 0.96–0.99 (Kunieda et al. 2013).

Diagnostic Tests

VF

VF is an examination that involves feeding modified food containing barium to a patient, taking X-rays from the side or front and recording fluoroscopic images (Fig. 1). The advantage of VF is that it directly evaluates how patients masticate and form an alimentary bolus for transfer from the oral cavity to the stomach. However, the limited testing time required to avoid excessive radiation exposure and problems conducting inspections of patients who find it difficult to maintain the same position in a wheelchair are well-known limitations. The unpalatable and uncomfortable texture of barium may also influence the test results (Jaffer et al. 2015). VF can evaluate functional abnormalities such as nasopharyngeal closure dysfunction, delayed swallowing reflex, morphologic abnormalities of organs associated with swallowing, pharyngeal residues, aspiration, and gastroesophageal reflux. The test can be used not only for decision-making of favorable food texture or patient' posture during eating, but also as a gold standard for diagnosing dysphagia. As a consequence, it is one of the most common clinical examinations in rehabilitation practice.

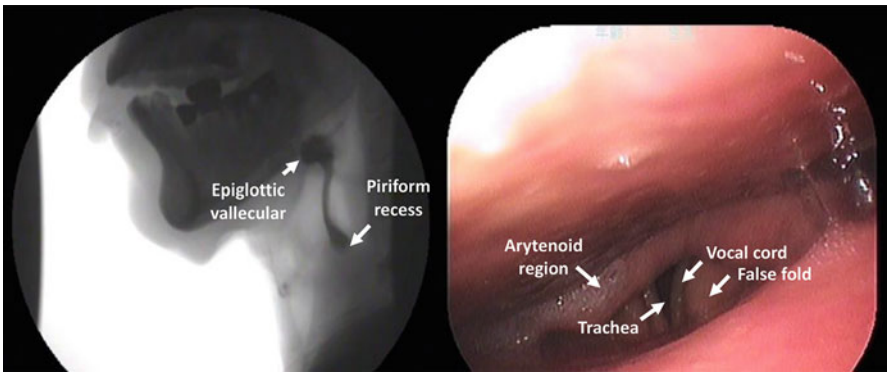

Fig. 1 VF and FEES. (*Left*) VF showing barium in the epiglottic vallecular passing through the esophagus during normal swallowing. (*Right*) FEES demonstrated in the arytenoid region, vocal cords, and false folds beyond the walls of the oropharynx

FEES

FEES is another well-known diagnostic test in which a fiberscope is inserted from the nasal cavity to directly observe the pharynx (Fig. 1). FEES is less invasive, can be evaluated safely and conveniently, and is effective for diagnosing patients with dysphagia. Unlike VF, there is no radiation exposure. When the patient undertakes the compensation method of swallowing, the clinician can directly see the swallow and is able to provide feedback and instructions on effective swallowing on a screen (Jaffer et al. 2015). On the other hand, FEES cannot evaluate esophageal function, the upper esophageal sphincter, or determine the moment of swallowing.

Sarcopenic Dysphagia

As proposed by Rosenberg in 1988, sarcopenia initially represents an age-related loss of muscle mass and function (Rosenberg 1997). Skeletal muscle mass decreases significantly with age with lean body mass being 9% lower between 65 and 74 years and 12% lower at ages >75 years, compared with that measured between 35 and 44 years (Atlantis et al. 2008). Although the original concept of sarcopenia focused on decreasing skeletal muscle mass, recent consensus has changed to also include a decline in muscular strength and physical function. The cause of sarcopenia has also been changed to include not only aging but also nutritional deficit, inactivity, and disease (Chen et al. 2014; Cruz-Jentoft et al. 2010). Nutritional deficiency plays an important role in all causes of sarcopenia. In fact, 76% of older hospitalized patients with disuse and sarcopenia were shown to have malnutrition (Sanchez-Rodriguez et al. 2016). Healthcare professionals should therefore carry out nutritional assessment of older adults with sarcopenia.

Dysphagia is often caused by various diseases, especially in subjects older than 70 years (Cabre et al. 2009). Although the major cause of overt dysphagia in older adults

is central nervous system disease such as stroke, aging itself can also cause a functional decline in swallowing (presbyphagia) (Wirth et al. 2016; Wakabayashi 2014). A nutritional deficit and/or sarcopenia may also evoke dysphagia and delayed recovery of swallowing function. In 1996, Veldee et al. showed that protein energy malnutrition may cause dysphagia through immunological deterioration, fatigue, decline of respiratory muscle strength, and decreased swallowing muscle mass. (Veldee and Peth 1992). Tongue pressure was reported to be lower in community-dwelling older adults with aspiration compared to that measured in those without aspiration (Butler et al. 2011). This suggested that declining swallowing muscle strength may exacerbate swallowing function. We also found that stroke rehabilitation patients undergoing tube feeding with severe malnutrition risk, which is related closely with sarcopenia, had the worst recovery to achieving full oral intake (Nishioka et al. 2017a). Indeed, in daily clinical practice, we sometimes found that older adults with sarcopenia showed dysphagia in spite of no apparent causable disease such as stroke. These cases raised the possibility that dysphagia may be caused by sarcopenia of the swallowing muscles, namely sarcopenic dysphagia.

Sarcopenic dysphagia occurs when it is difficult to swallow due to sarcopenia of the generalized and swallowing muscles (Wakabayashi 2011, 2014). The swallowing muscles include the intrinsic muscle of the tongue and the mimic, masticatory, suprahyoid, infrahyoid, palatal, pharyngeal, and esophageal muscles. The mechanism of sarcopenic dysphagia is considered to be a two-step process. Older adults with presbyphagia and age-related sarcopenia causing aspiration pneumonia may subsequently have their muscle decline aggravated by invasion, disuse, or nutritional deficiency during treatment of pneumonia, resulting in complete sarcopenic dysphagia (Fig. 2). Confirmation of this concept was reported by Kuroda and colleagues who demonstrated an association between feeding swallowing function and arm muscle circumference, suggesting the possibility of sarcopenic dysphagia (Kuroda and Kuroda 2012). Similarly, sarcopenia was shown to be an independent explanatory factor for swallowing disorders in older hospitalized patients (Maeda et al., 2016a), with low skeletal muscle mass being a risk factor for the onset of dysphagia 60 days after hospital admission (Maeda and Akagi 2016). These findings indicate sarcopenia may cause dysphagia in older subjects.

Rehabilitation and nutritional care should both be provided for this type of dysphagia (Wakabayashi and Sakuma 2014; Clave and Shaker 2015). At this time, although there are no unified definitions and diagnostic criteria for sarcopenic dysphagia, a provisional diagnostic algorithm has been proposed by the working group on sarcopenic dysphagia (WGSD). We are currently verifying the validity and reliability of this algorithm (Fig. 3).

Concept and Assessment Method of Rehabilitation Nutrition

Dysphagic patients often have malnutrition with its prevalence being 2.4 times higher than in subjects with normal swallowing function. This relationship is not seen in the acute phase but often is seen during the rehabilitation stage (Foley et al.

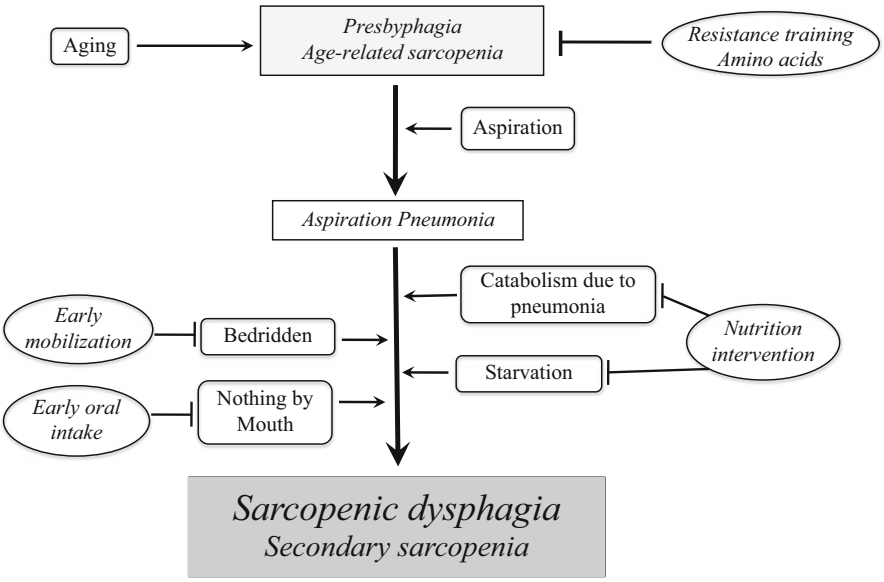

Fig. 2 Underlying cause and progression of sarcopenic dysphagia in older adults. Sarcopenic dysphagia may develop in frail older adults with presbyphagia and age-related sarcopenia. When these people develop aspiration pneumonia there is a rapid exacerbate sarcopenia of generalized- and swallowing muscle. The underlying cause of sarcopenia development may be disuse during acute care (sometimes unnecessarily), inappropriate nutritional care, or muscle catabolism due to stress responses to pneumonia and comorbidity. As a result, muscle weakness deteriorates (secondary sarcopenia) and sarcopenic dysphagia becomes obvious

2009). It has been reported that 17% of older adults with dysphagia had malnutrition, while 34% were at risk of malnutrition (Carrion et al. 2017). When malnutrition and dysphagia co-exist, the 1-year mortality reached 65% (Carrion et al. 2015). In other words, older people with dysphagia, especially those who are receiving long-term swallowing rehabilitation, are more likely to develop malnutrition and have a poor prognosis. It is therefore necessary to evaluate the presence and cause of malnutrition and sarcopenia as well as swallowing function in these patients.

We advocate rehabilitation nutrition as a concept that includes comprehensive evaluation and rehabilitation and nutritional care intervention for people with disabilities and/or older adults (Wakabayashi and Sakuma 2014). Rehabilitation nutrition for people with a disability and frail older people is defined as (1) holistic evaluation by ICF of the presence and causes of nutritional disorders, sarcopenia, and excess or deficiency of nutrient intake, (2) rehabilitation nutrition diagnosis and rehabilitation and nutrition goal setting, and (3) achieving the highest body functions, activities, participation, and QOL by improving nutritional status, sarcopenia, and frailty using "nutrition care management that takes into account rehabilitation" and "rehabilitation that considers nutrition." Rehabilitation nutrition is a process that includes: (1) rehabilitation nutrition assessment and diagnostic reasoning, (2) rehabilitation nutrition diagnosis, (3) rehabilitation nutrition goal setting, (4) rehabilitation nutrition intervention, and (5) rehabilitation nutrition

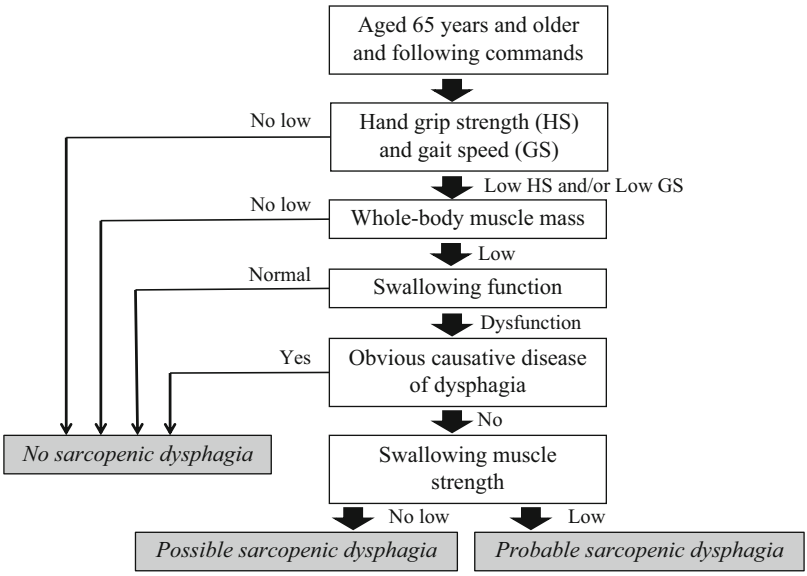

Fig. 3 Proposed diagnostic algorithm for sarcopenic dysphagia. This algorithm is for use in adults aged 65 years or older who can follow an instruction. Sarcopenic dysphagia is suspected if the individual has low handgrip strength or low gait speed, low general muscle mass, swallowing dysfunction, and no causative disease of dysphagia. Subjects with low swallowing muscle strength (using tongue pressure) are classified as possible sarcopenic dysphagia, while those without this finding are classified as probable sarcopenic dysphagia

monitoring. Using this concept enables comprehensive evaluation of older adults with dysphagia and sarcopenia undergoing rehabilitation.

To screen for dysphagia, it is desirable to use the screening tool described above that can be performed simply and noninvasively at the bedside such as the EAT-10, MWST, or RSST. Nutrition screening should be conducted on all older subjects who receive rehabilitation using validated tools such as the NRS 2002 (inpatient), MUST (home), MNA, or its short form version (older adults) or SGA (Kondrup et al. 2003). If the patients cannot answer the questions in these nutritional screening tools due to aphasia, post cerebral aphasia, or cognitive impairment, they can obtain help to complete the screening from family or a caregiver. Calf circumference in the nonparalyzed side (male $\leq$ 31 cm, female $\leq$ 30 cm) is effective for nutritional screening in poststroke patients where body weight measurement is difficult due to contracture or paralysis (Nishioka et al. 2017b).

Sarcopenia should be classified using consensus-based criteria such as EWGSOP (Cruz-Jentoft et al. 2010) or AWGS for Asian populations (Chen et al. 2014). However, it is often difficult to measure body composition, grip strength, and walking speed in patients with cognitive impairment or hemiplegia. For measuring muscle mass, calf circumference can be substituted for patients with decreased muscle mass as $<$34 cm for males and $<$33 cm for female (Kawakami et al. 2015). More than 90% of patients in whom it was difficult to measure walking

speed were shown to have sarcopenia using only measurements of grip strength and muscle mass (Maeda et al. 2016b). It is therefore possible to identify sarcopenia with a high probability using only muscle mass and strength in patients who require assistance to mobilize.

Policies and Protocols

When dysphagia is suspected in elderly people requiring rehabilitation, we first evaluate the possibility of a swallowing disorder using a simple and validated questionnaire such as the EAT-10. If it is possible that the patient has a swallowing disorder, the WST/MWST or the RSST is performed to increase the likelihood of the presence of dysphagia. Accurate diagnostic methods such as VF and VE are carried out to diagnose patients suspected of having a swallowing disorder or to determine the posture and food type required to avoid aspiration. In order to determine treatment policy, it is useful to classify the severity using methods such as the FOIS or the FILS. Because dysphagia in older adults is often accompanied by sarcopenia and malnutrition, potential causes of dysphagia, we suggest that it is necessary to carry out concurrent assessment of sarcopenia and nutritional status. Consensus-based criteria proposed by the EWGSOP or the AWGS (for Asian population) should be used to define sarcopenia. Validated tools such as the NRS2002, MNA, MUST, or SGA are recommended to screen for malnourished patients. The concept of rehabilitation nutrition is useful for comprehensive assessment of older adults with disabilities. The rehabilitation nutrition care process includes rehabilitation nutrition assessment and diagnostic reasoning, rehabilitation nutrition diagnosis, rehabilitation nutrition goal setting, rehabilitation nutrition intervention, and rehabilitation nutrition monitoring.

Dictionary of Terms

- **Sensitivity** – The proportion of the subjects with positive test results among those who actually have a certain disease or symptom. A test with high sensitivity is useful for rule out.
- **Specificity** – The proportion of the subjects with negative test results among those who do not have a certain disease or symptom. A test with high specificity is useful for obtaining a definitive diagnosis.
- **Cronbach's coefficient alpha** – Cronbach's coefficient alpha is an index of the correlation between the scores of individual items. If the coefficient is ≥ 0.80, the correlation is considered excellent, while a coefficient < 0.50 correlation is considered low.
- **Sarcopenia** – A syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength caused by aging, disuse, diseases, and/or nutritional deficit and associated with increased risk of adverse outcomes such as physical disability, poor quality of life, and death.

- **ICF** – The unified framework for comprehensive assessment of people with disabilities proposed by the World Health Organization. The framework has three layers that include body functions and structure, activities and participation that influence health, and environmental and personal factors.

Summary Points

- Dysphagia is observed in 48–73% of institutional or hospitalized older patients.
- Dysphagia in older adults is caused by diseases and/or aging itself (presbyphagia).
- Dysphagia screening tools should be simple, noninvasive, and able to be carried out at the bedside.
- Using validated screening tools such as EAT-10, WST/MWST, and RSST is recommended in the clinical setting.
- Classification methods of dysphagia severity determine the degree of the condition in greater detail than screening tools and are fundamental for decision making on dysphagia treatment.
- Validated classification methods include the FOIS and FILS.
- VF and FEES are used as the gold standard for diagnosing dysphagia.
- Sarcopenic dysphagia is a new concept as a cause of dysphagia.
- Sarcopenic dysphagia is defined as difficulty swallowing caused by sarcopenia of the generalized and swallowing muscles.
- A consensus-based diagnostic flowchart for sarcopenic dysphagia has been proposed; its prerequisite is the presence of dysphagia, presence of sarcopenia in the whole body and swallowing muscles, and no significant causative disease of dysphagia.
- The combination of rehabilitation and nutritional assessment and intervention based on rehabilitation nutrition is effective in older adults with sarcopenia and dysphagia.
- Rehabilitation nutrition is a new concept for comprehensive evaluation and intervention based on ICF that has the aim of improving nutritional status, sarcopenia, and frailty in older adults with disabilities.

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Comparing Characteristics of Malnutrition, Starvation, Sarcopenia, and Cachexia in Older Adults

42

Skye Marshall and Ekta Agarwal

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Abstract

Wasting syndromes such as malnutrition, starvation, sarcopenia, and cachexia have been increasingly recognized in health care and are the subject of significant research endeavors internationally. It is the purpose of this chapter to compare the characteristics (definitions and diagnosis, etiology, prevalence and outcomes) of each of these conditions in older adults. Protein-energy malnutrition is a syndrome caused by the inadequate bioavailability of energy and/or protein over time, leading to the catabolism of lean tissues with or without loss of fat mass, occurring most frequently in older adults. Malnutrition prevalence ranges from 10% to 50% depending on the setting. Simple starvation is a physiological state referring to the metabolic alterations, such as hypophosphatemia and the production of ketones as the primary energy source, which are activated in a state of food and nutrient deprivation, but when it causes physiological consequences it is clinically referred to as malnutrition. Starvation-related malnutrition is only diagnosed when protein-energy malnutrition is caused by food and nutrient deprivation over a long period of time in the absence of disease processes and inflammation. Sarcopenia is the loss of muscle mass, strength, and function that occurs due to age-related changes in physiology and ranges in prevalence from 10% to 50% depending on age group. Cachexia is a complex syndrome which presents with the loss of body weight, predominately skeletal muscle, as a result of metabolic abnormalities related to disease processes. Cachexia may be considered as a type of disease-related malnutrition and ranges in prevalence from 10% to 50% depending on the underlying disease. Malnutrition, starvation, sarcopenia, and cachexia result in the catabolism and subsequent dysfunction of multiple organ systems and skeletal muscle, leading to poor patient outcomes such as physical dysfunction, hospitalizations, poor quality of life, and increased risk of death.

Keywords

Elderly · Aged · Malnutrition · Starvation · Sarcopenia · Cachexia · Prevalence · Undernutrition · Nutrition assessment · Diagnosis

List of Abbreviations

AIDS	Acquired immune deficiency syndrome
AND	Academy of Nutrition and Dietetics
ASPEN	American Society for Parenteral and Enteral Nutrition
BIA	Bioimpedance analysis

CT	Computed tomography
DEXA	Dual-Energy X-Ray
ESPEN	European Society of Clinical Nutrition & Metabolism
EWGSOP	European Working Group on Sarcopenia in Older People
HMB	β-Hydroxy-β-methylbutyrate
HPHE	High protein-high energy
ICD	International Statistical Classification of Diseases and Health Related Problems
IL	Interleukin
IWGS	International Working Group on Sarcopenia
MNA	Mini nutritional assessment
MRI	Magnetic resonance imaging
ONS	Oral nutrition support
PEM	Protein energy malnutrition
PG-SGA	Patient-generated subjective global assessment
SGA	Subjective global assessment
TNF	Tumor necrosis factor
UK	United Kingdom

Introduction

In1974 Dr Charles Edwin Butterworth Jr put the first spotlight on wasting syndromes in older adults when he published "The Skeleton in the Hospital Closet" (Butterworth 1974). Since then, wasting syndromes such as malnutrition, sarcopenia, and cachexia have been increasingly recognized in health care and are the subject of significant research endeavors internationally (Krumdieck 1998). However, despite over 40 years of research aimed at improving the nutritional status of older adults; malnutrition, sarcopenia, and cachexia remain highly prevalent worldwide in the community, hospitals, and residential aged care (Watterson et al. 2009). In addition, there is significant confusion in the literature and in clinical practice between malnutrition, starvation, sarcopenia, and cachexia (Tzankoff and Norris 1978). Therefore, it is the purpose of this chapter to explore the current evidence regarding the definition and diagnosis, etiology, prevalence and outcomes of each of these conditions in older adults.

Malnutrition in Older Adults

Definition and Diagnosis

The term "malnutrition" traditionally refers to any state of an individual resulting from the inadequate and/or excessive intake of nutrients. Each nutrient has a range of intake that is required for optimal health, and an intake below or above this is associated with impaired physical function (2005). However, over time the term "malnutrition" has come to be frequently used to refer to "protein-energy malnutrition" (PEM), a

syndrome caused by the inadequate bioavailability of energy and/or protein, leading to the catabolism of lean tissues with or without loss of fat mass, occurring most frequently in older adults (2008). Other nutrients may also be deficient; however, the lack of sufficient energy and/or protein is the defining etiological characteristic of PEM (2015). However, there is no international consensus on the definition of PEM, nor even its name, as it is also referred to as "protein-energy undernutrition" or "protein-calorie malnutrition." The loss of lean tissues (comprising 35–50% of total body weight in healthy adults) with or without fat mass presents clinically as decreased body weight, evidence of muscle wasting, and compromised homeostasis in organ function, blood cells, and immune cells (Pleuss 2005).

Reflecting this variable clinical presentation, there is no single measure able to diagnose PEM. Instead, a diagnosis is reached through a global examination by a highly-trained health professional of an individual's medical status, anthropometry, biochemistry, dietary intake, nutrition-related symptoms, and a physical examination. To support this process, health professionals frequently use a "nutrition assessment tool" to gather the required information and to guide interpretation. Although not all clinicians and researchers utilize a nutrition assessment tool to make a diagnosis of PEM, it is considered best-practice to do so and is increasingly required in health care facilities (Watterson et al. 2009). There are three nutrition assessment tools which are validated for use in older adults; these are the Mini Nutritional Assessment (MNA) (Guigoz and Vellas 1997), the Subjective Global Assessment (SGA) (Detsky et al. 1987), and Scored Patient-Generated Subjective Global Assessment (PG-SGA) (Marshall et al. 2016b).

Protein-energy malnutrition may also be defined and diagnosed by its severity and cause. Reflecting the amount of body weight lost and/or severity of muscle wasting in a certain time-frame, an individual may be categorized as "mildly malnourished," "moderately malnourished," or "severely malnourished." However, there is also no consensus as to how each of these categories is defined and usually is based upon the nutrition assessment tool utilized or the amount of weight lost in a certain period.

In 2012, the Academy of Nutrition & Dietetics (AND) and the American Society for Parenteral and Enteral Nutrition (ASPEN) reached a consensus on the set of etiology-based diagnostic characteristics for PEM (White et al. 2012). There were three etiology-based diagnostic criterion agreed upon, which are outlined in Fig. 1. Beyond these three categories, the consensus definition also categorizes PEM as severe or nonsevere (moderate), based on the degree of inadequate energy intake, weight loss, fat loss, muscle wasting, fluid accumulation, and grip strength (White et al. 2012).

Etiology

Protein-energy malnutrition is a condition that develops over time, with a latency period ranging from days to years depending on the initial nutritional state of the individual and the severity of malnutrition-causing symptoms and environments. Inadequate energy and protein can be caused by: (1) not consuming sufficient dietary

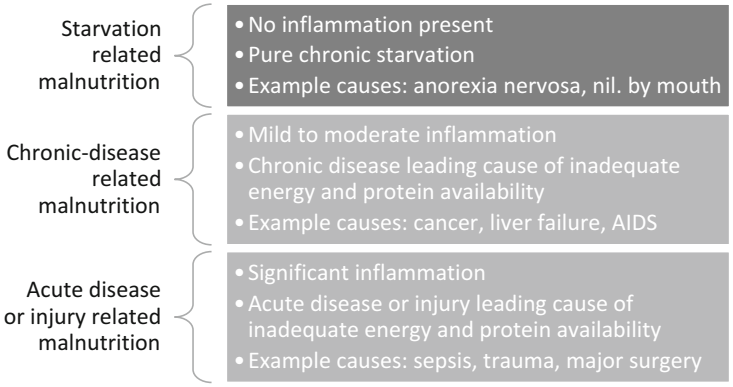

Fig. 1 Consesus etiology-based protein-energy malnutrition definitions (Adapted from White et al. 2012). The characteristics of the three subtypes of protein-energy malnutrition, which differ primarily on the cause of malnutrition. AIDS, acquired immune deficiency syndrome

sources of energy and protein to meet an individual's nutrient requirements, which is exacerbated in states of hypermetabolism or (2) not absorbing the energy or protein consumed. Additionally, it should be highlighted that in any one person, there may be one or many contributing causes.

There are innumerable causes of inadequate energy and protein dietary intake, examples of which are shown in Fig. 2. When hypermetabolism is present, the body's demand for energy and protein increases significantly and may in some cases double (Marshall 2017). This is often confounded by a low level of nutrition knowledge, where older adults may fail to recognize increased nutrient requirements or the types of foods required to prevent malnutrition (Marshall et al. 2016a). Similarly, not absorbing the consumed energy and protein also has diverse causes, including:

1. *Diseases related to maldigestion and malabsorption*. Examples are short bowel syndrome, gastrointestinal catabolism as a result of malnutrition, gastritis, inflammatory bowel syndrome, cystic fibrosis, pancreatic and liver diseases, and bariatric surgery.
2. *Diseases related to excessive nutrient losses*. Examples are diarrhea, steatorrhea, vomiting, protein losing enteropathy, surgical drains, stomas, fistulae, hemodialysis, and blood loss.

Prevalence

The prevalence of PEM varies widely according to the setting and the geographical location but also varies according to the age group of a sample as well as the diagnostic tool used. Cereda and associates recently published a meta-analysis of PEM prevalence, diagnosed by the MNA, in older adults (>60 years) according to

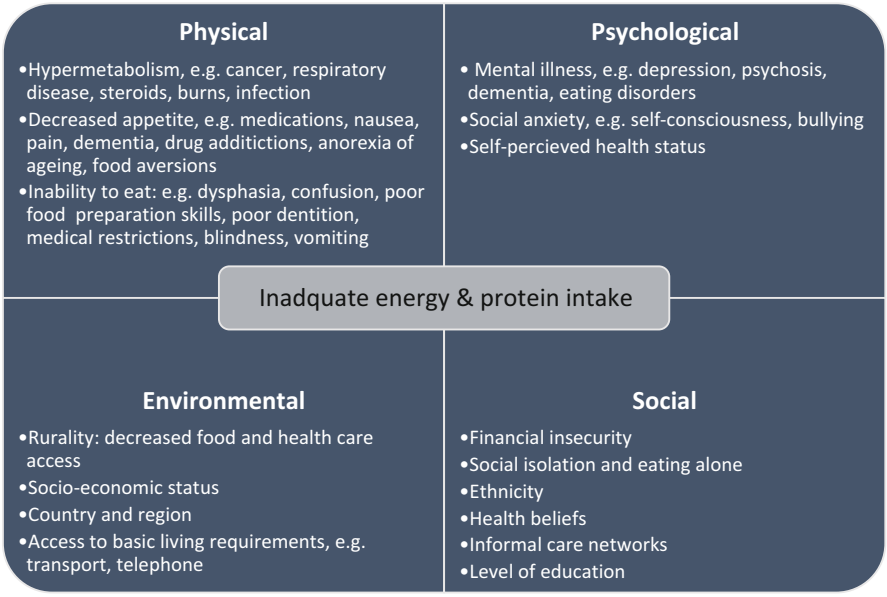

Fig. 2 Social, environmental, physical, and psychological factors which decrease a person's ability to consume adequate energy and protein (Marshall 2017). These are diverse and overlapping risk factors which increase the chances that an older adult will consume less energy and protein in their food, which in turn increases the chances they will become malnourished

health care setting (Cereda et al. 2016). The highest prevalence was found in long-term care (29%; $n = 23$ studies) and rehabilitation and subacute care (29%, $n = 15$ studies), followed by acute care (22%, $n = 66$ studies) and nursing homes (18%, $n = 44$ studies), then home care services (9%, $n = 15$ studies), out-patients (6%, $n = 37$ studies) and the community (3%, $n = 58$ studies) (Cereda et al. 2016). Additionally, this meta-analysis found that the prevalence of PEM, according to the MNA, was consistently and significantly higher in European countries as opposed to Asian countries across all settings, although other geographical locations were not described for comparison (Cereda et al. 2016).

However, reviews and diagnostic studies have found that the MNA considers fewer patients as malnourished than other tools including the SGA, PG-SGA, and ICD, and therefore true PEM prevalence may be higher (Marshall 2016; Marshall et al. 2016b).

The impact of rurality on the prevalence of malnutrition has not been evaluated in a meta-analysis; however, a review in the rehabilitation and subacute setting found that the highest prevalence was found to be in rural settings (53–65% in rural Australia, 32–49% in urban Australia, all measured using the SGA) (Marshall 2016). There are many social, cultural, economic, and physical changes that occur in ageing, which increases the risk of protein-energy malnutrition; where many of the risk factors outlined in Fig. 2 are more likely to occur in older adults. Research has consistently found age to be a risk factor for increasing risk of malnutrition

(Agarwal et al. 2012). In Europe ($n = 15{,}043$ participants, $n = 325$ hospitals, $n = 25$ countries), every 10 years of life was found to increase risk of PEM by 14% (OR: 1.14 [95% CI: 1.09–1.19]; $P < 0.0001$).

Outcomes

The physiological and psychosocial consequences of malnutrition are significant and diverse (Agarwal et al. 2016). Confusion, fatigue, and weakness, common symptoms of malnutrition, are often attributed to other conditions leading to frequent misdiagnosis and under-recognition of malnutrition (Wellman and Kamp 2008). Malnutrition causes systemic catabolism of fat mass and lean tissues, including vital organs of the liver, heart, respiratory system, and skeletal muscle mass (Figs. 3, 4, and 5). Catabolism of these lean tissues cause a disruption in homeostasis in nearly all physiological systems, leading to diverse health outcomes (Figs. 3, 4, and 5). In addition to those shown in Figs. 3, 4, and 5, malnutrition also causes fluid and electrolyte imbalances which may further affect kidney and cardiac function. The combination of decreasing function in multiple organ systems as a result of malnutrition increases risk of mortality. Malnutrition has been found to be an independent risk factor for death, with studies finding the risk of death doubled in hospital (RR: 2.63 [95% CI: 1.55–5.27] $P < 0.05$) (Correia and

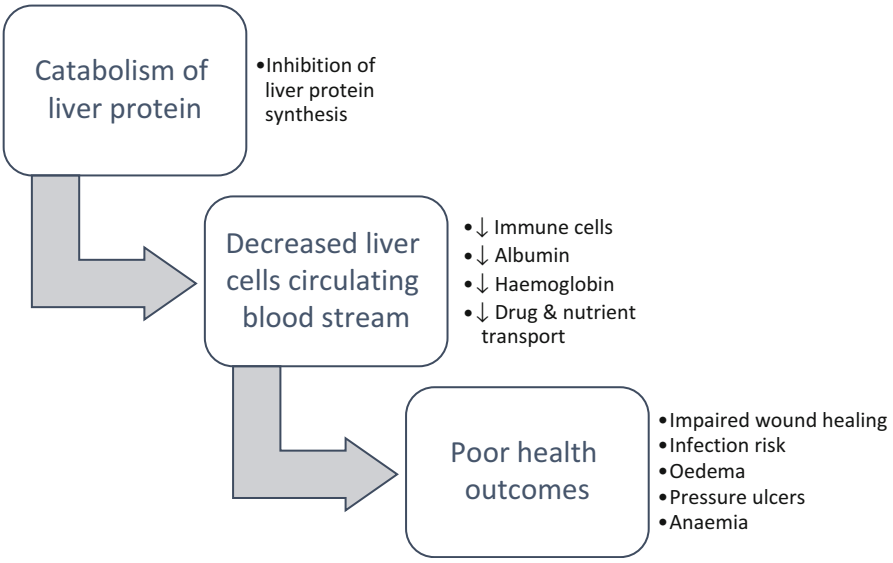

Fig. 3 The physiological process and health outcomes related to liver catabolism as a consequence of protein-energy malnutrition (2006; Stratton et al. 2003; Pleuss 2005; Ferreira et al. 2011; Martyn et al. 1998). This figure describes how the breakdown of liver mass and function which occurs in malnutrition will have increased risk of impaired wound healing, infection, swelling, pressure sores, and anemia

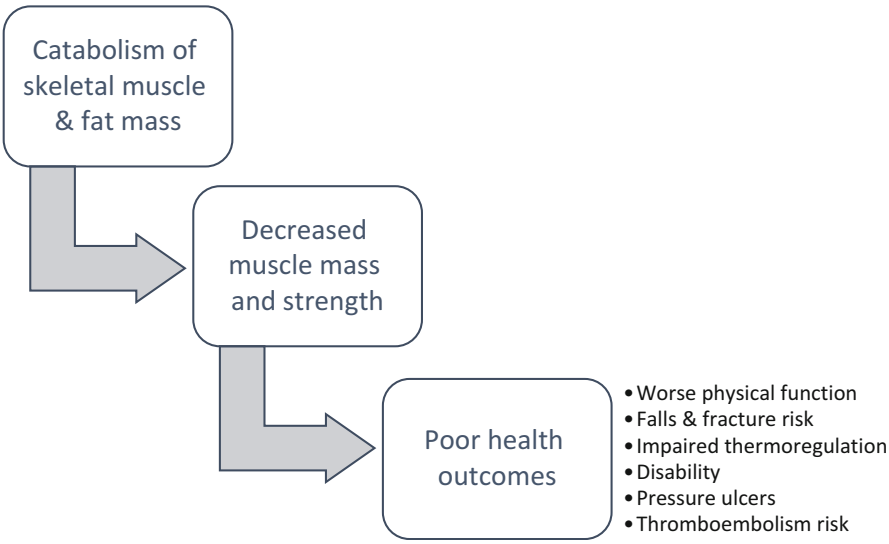

Fig. 4 The physiological process and health outcomes related to skeletal muscle and fat mass catabolism as a consequence of protein-energy malnutrition (2006; Stratton et al. 2003; Pleuss 2005; Ferreira et al. 2011; Martyn et al. 1998). This figure describes how the breakdown of muscles and loss of fat which occurs in malnutrition will have increased risk of impaired physical function, falls and fractures, body temperature regulation, pressure sores, and obstruction of blood flow

Waitzberg 2003) (OR: 1.92 [95% CI: 1.09–3.34] $P = 0.023$) (Agarwal et al. 2013) and more than tripled in the year following discharge (HR: 3.41 [95% CI: 1.07–10.87] $P = 0.038$) (Charlton et al. 2012).

As would be expected with such significant poor health outcomes, individuals who are malnourished may experience a lower quality of life, apathy, depression, self-neglect, hypochondriasis, poor self-efficacy, poor body image, confusion, decreased interest in food, loss of libido, and engage less frequently in social activities (2006; Bottone et al. 2012; Marshall et al. 2014). Additionally, these poor health outcomes caused by malnutrition contribute a significant economic burden, where the cost of treating a patient with malnutrition in the UK is more than double the cost of treating a well-nourished patient due to the increased use of health-related resources (Guest et al. 2011). Using data on health care utilization by people with malnutrition, it was recently estimated that the cost of treating the health problems associated with malnutrition in the UK is in excess of £13 billion per year (Elia and Stratton 2005). In the USA, disease-related malnutrition in the community setting was found to have an annual burden of USD$156.7 billion (£120.2 billion) to society, the large majority of which was due to medical complications associated with malnutrition (Snider et al. 2014). In order to offset the great patient and economic burden, it is essential that the condition is addressed by a multidisciplinary team with the support of the health care system, the community, and society in general.

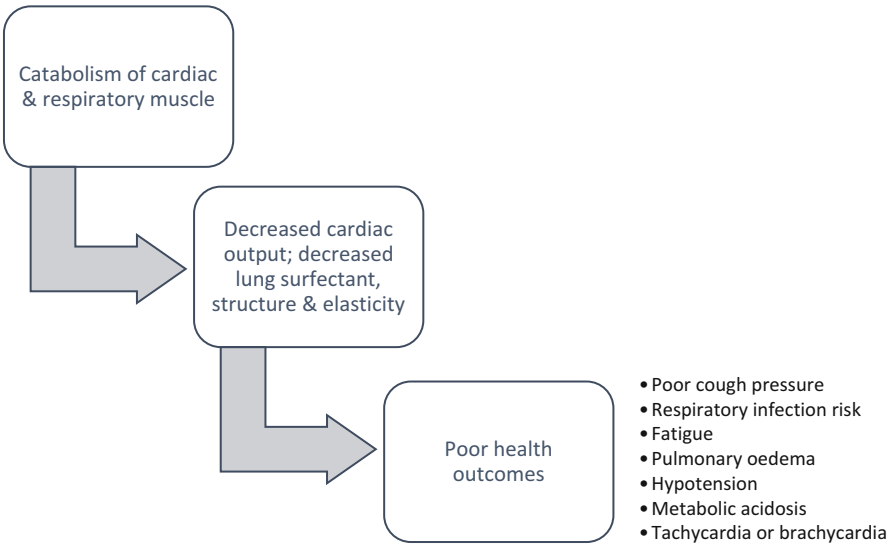

Fig. 5 The physiological process and health outcomes related to respiratory and cardiac muscle catabolism as a consequence of protein-energy malnutrition (2006; Stratton et al. 2003; Pleuss 2005; Ferreira et al. 2011; Martyn et al. 1998). This figure describes how the breakdown of the heart and lungs which occurs in malnutrition will have increased risk of lung infections and water retention, fatigue, low blood pressure, poor coughing, and other heart problems

Starvation in Older Adults

Definition and Diagnosis

Simple starvation is a physiological state referring to the metabolic alterations, such as hypophosphatemia and the production of ketones as the primary energy source, which are activated in a state of food and nutrient deprivation, but when it causes physiological consequences it is clinically referred to as malnutrition (Gallagher 2008; Litchford 2008). Some definitions of starvation are analogous to PEM, that is, a chronic inadequate intake of protein and energy, causing a loss of both fat-mass and fat-free mass (Thomas 2007); which is reflected in starvation being one of the etiology-based definitions of PEM (Fig. 1). Starvation-related malnutrition is only diagnosed when PEM is caused by food and nutrient deprivation over a long period of time in the absence of disease processes and inflammation (White et al. 2012).

Extended periods of starvation may also lead to marasmus, another form of malnutrition also called adapted starvation defined as uncomplicated starvation without inflammation (Gallagher 2008; Litchford 2008). Again, this definition of marasmus, or adapted starvation, is analogous to starvation-related malnutrition. Starvation may also refer to the deprivation of particular nutrients, for example, the deprivation of protein when carbohydrate is regularly consumed may lead to

kwashiorkor, which is also a form of malnutrition characterized by hypoalbuminemia (Gallagher 2008). Overall, starvation may be an important component of malnutrition in some clinical situations, but starvation-related malnutrition should be used as a clinical diagnosis to enhance consistency in terminology across health care facilities and disciplines (White et al. 2012).

Etiology

Starvation-related malnutrition (Fig. 1) is due to pure chronic starvation or anorexia nervosa (White et al. 2012). The social, environmental, physical, and psychological factors which cause PEM (Fig. 2) may also be the cause of starvation-related malnutrition, excepting disease states accompanied by inflammation. One of these physiological causes that occurs in older adults is the "anorexia of ageing," which refers to little to no dietary intake related to the decreased appetite which occurs as part of normal ageing (Visvanathan 2003). Appetite is controlled by interactions between the cortex, limbic system and midbrain, as well as peripheral inputs from the gut, adipose tissue, and endocrine system (Visvanathan 2003). These processes may work less efficiently with increasing age leading to consumption of a less varied and lower quality diet (Visvanathan 2003). However, poor appetite leading to starvation and subsequently weight loss and/or malnutrition is not part of the normal ageing process and is preventable (Huffman 2002).

Prevalence

There is no data regarding the prevalence of starvation-related malnutrition as opposed to PEM in general. However, starvation-related malnutrition may be more likely to be the type of PEM found in older adults living in the community, as malnutrition diagnosed in health care facilities is likely to be acute, disease or injury-related, associated with the reasons for admission.

Outcomes

After several days of starvation, the gastrointestinal tract begins to atrophy, reducing its absorptive capacity; however, with recommencement of nutrient intake, absorptive capacity is returned within days (Beyer 2008). During extended starvation, several organ systems modify their metabolism. Liver gluconeogenesis decreases the production of glucose, and kidney production of glucose increases. The substrates used in liver gluconeogenesis originate from skeletal muscle, which releases glycogenic amino acids, pyruvate, and lactate to be used for gluconeogenesis via the Cori cycle (Gallagher 2008). In the renal gluconeogenesis, the glutamine released by skeletal muscle is deaminated to alpha-ketoglutarate which is used to produce glucose (Gallagher 2008).

Apart from catabolism of lean tissues such as skeletal muscle, fat loss also occurs in starvation-related malnutrition which provides a more stable energy source than glucose produced by gluconeogenesis (Gallagher 2008). During starvation, low insulin levels allow fatty acids to be released from adipocytes and transported to the liver, where they form ketones. Ketones then enter the blood stream and are able to be used as an energy source (Gallagher 2008).

These processes reveal the varying clinical presentation in starvation-related malnutrition as opposed to forms caused by injury or disease; where starvation-related malnutrition more typically presents with low fat and muscle mass, other forms of malnutrition may occur with a the loss of lean tissues only (White et al. 2012).

In terms of poor health outcomes, these are the same for starvation-related malnutrition and PEM in general, excepting outcomes related to low fat mass such as increased fracture risk, may be more common (Stratton et al. 2003).

Sarcopenia in Older Adults

Definition and Diagnosis

The term "sarcopenia," derived from the Greek words "sarx" (flesh) and "penia" (loss), was coined by Professor Irwin Rosenberg in 1989 to describe age-related loss of muscle mass (Baumgartner et al. 1998). Eventually, with improved understanding of sarcopenia, loss of muscle quality, strength, and function or "dynapenia" were also linked with loss of muscle mass (Morley et al. 2014). Under the auspices of four international working groups, namely, European Working Group on Sarcopenia in Older People (EWGSOP), the European Society for Clinical Nutrition and Metabolism Special Interest Groups (ESPEN-SIG), the International Working Group on Sarcopenia (IWGS), and the Society of Sarcopenia, Cachexia and Wasting Disorders, consensus definitions of sarcopenia were released (Table 1) (Cruz-Jentoft et al. 2010; Fielding et al. 2011; Morley et al. 2011; Muscaritoli et al. 2010). These consensus definitions led to sarcopenia being recently recognized as a disease entity in the International Classification of Diseases with its own unique ICD-10 code (Anker et al. 2016).

All four definitions call for the quantification of muscle mass and muscle strength along with measuring deficits in muscle function (Scharf and Heineke 2012). Currently, several parameters are available to measure age-related sarcopenia. A summary of available diagnostic methods along with advantages and limitations is shown in Table 2. With the development of its own unique disease classification code, it is extremely important for identifying the most suitable biomarker for correctly diagnosing, monitoring, and treating sarcopenia (Scharf and Heineke 2012).

Etiology

The etiology of age-related sarcopenia is multifactorial. Declines in nutritional intake, malabsorption of nutrients, reduced physical activity levels, progressive and irreversible loss of motor neurons, although normal in ageing, result in loss of

Table 1 Consensus definitions of sarcopenia. There is no perfect or completely accepted definition of sarcopenia. These are the different recognized definitions put forth by various organizations. EWGSOP, European Working Group on Sarcopenia in Older People; ESPEN-SIG, European Society for Clinical Nutrition and Metabolism Special Interest Groups; IWGS, International Working Group on Sarcopenia

Working group	Definition
European Working Group on Sarcopenia in Older People (EWGSOP) (Cruz-Jentoft et al. 2010)	The presence of: Low skeletal muscle mass AND Low muscle strength (e.g., handgrip) OR Low muscle performance assessed by walking speed or muscle power The presence of all three conditions indicates "severe" sarcopenia
International Working Group on Sarcopenia (IWGS) (Fielding et al. 2011)	The presence of: Low skeletal muscle mass AND Low muscle function assessed by walking speed AND/OR Increased fat mass
Society of Sarcopenia, Cachexia and Wasting Disorders (Morley et al. 2011)	The presence of: Loss of muscle mass AND Limited mobility in the absence of specific diseases of muscle, peripheral vascular disease, disorders of the central and peripheral nervous system, or cachexia
European Society for Clinical Nutrition and Metabolism Special Interest Groups (ESPEN-SIG) (Muscaritoli et al. 2010)	The presence of: Low skeletal muscle mass AND Low muscle strength assessed by walking speed

muscle mass and function (Cruz-Jentoft et al. 2010; Malafarina et al. 2012). Older adults confined to bed due to illness or trauma are highly prone to loss of muscle mass (Malafarina et al. 2012). Age-related loss of immunity causes an increase in inflammatory cytokines and is associated with reduction in muscle mass and strength in older adults (Malafarina et al. 2012). Deterioration in levels of hormones such as estrogen, testosterone, and insulin-like growth factors also contribute to the development of sarcopenia (Malafarina et al. 2012). Coexisting conditions such as osteoporosis, obesity, and type 2 diabetes are also associated with sarcopenia in older adults (Cruz-Jentoft et al. 2010; Malafarina et al. 2012).

Prevalence

Ageing is associated with a 1% decline in muscle mass per decade from 30 years of age, accelerating to approximately 8% loss of muscle mass along with a 10–15% loss of leg strength per decade from the age of 40–70 years (Kim and Choi 2013). After the age of 70 years, this escalates to 15% loss in muscle mass and 25–40% loss in leg strength per decade (Kim and Choi 2013). Although age-related loss of muscle mass is greater in

Table 2 Diagnosis of sarcopenia (Adapted from Scharf and Heineke 2012; Yu et al. 2016). There are various methods available to test if a patient has sarcopenia. This table outlines these and summarizes their benefits and limitations. BIA, Bioelectrical impedance analysis; CT, Computed tomography; DEXA, Dual-energy X-ray absorptiometry; EWGSOP, European Working Group on Sarcopenia in Older People; MRI, Magnetic resonance imaging. SARC-F has no full non-abbreviated title

	Method	Description	Advantages	Limitation
Imaging-related quantification of muscle mass	Magnetic resonance imaging (MRI)	Calculates segmental and total muscle mass; assesses muscle quality through assessing fat infiltration in muscle; quantifies edema and visceral organs	High quality image resolution; accurately quantifies and determines quality of whole-body and regional adipose tissue and skeletal muscle tissue; safe	Expensive; requires specialized setting, trained staff, and specific software; time-intensive; non-portable
	Computed tomography (CT)	Reconstructs image when x-ray attenuation through tissues is detected; identifies adipose tissue, skeletal muscle, bone, visceral organs, and brain tissue	High quality image resolution; accurately quantifies and determines quality of body composition at tissue-organ level; identifies total and regional adipose tissue and skeletal muscle tissue	Expensive; requires specialized setting, trained staff,and specific software; time-intensive; non-portable; considerable radiation
	Dual-energy X-ray absorptiometry (DEXA)	Using low radiation x-rays can differentiate between bone and soft tissue allowing for fat mass to be estimated from soft tissue; assesses bone mineral density	Determines fat, lean and bone tissue for whole body and specific regions; non-invasive, fast; accurate	Requires specialized setting, trained staff, and specific software; hydration and thickness of tissue can influence measurements
	Bioelectrical impedance analysis (BIA)	Measures total body water to estimate fat- and fat-free mass	Portable, simple to use, inexpensive, no radiation exposure, quick	Hydration status and presence of edema can distort results; limited use in those with BMI > 34 kg/m^2 as likely to underestimate fat mass

(*continued*)

Table 2 (continued)

	Method	Description	Advantages	Limitation
Functional tests for muscle performance	EWGSOP algorithm	Measures gait speed and handgrip strength	Low cost; standardized test; simple to use	Results confounded by comorbidities (degenerative and inflammatory conditions); lacks validation studies; not useful in patients with arthritis in hand
	SARC-F questionnaire	Assesses 5 domains: strength, independence, rising from chair, climbing stairs and falls history	Rapid; cost-effective; no measurements required	Low sensitivity

males, sarcopenia-related loss of function and increased disability is greater in women at threefold versus males at twofold (Kim and Choi 2013). Sarcopenia has been identified in 1–30% of community-dwelling older adults, 14–33% of those living in aged care facilities, 10% of those in acute care, and in 50% of those aged over 80 years (Cruz-Jentoft et al. 2014). Prevalence of sarcopenia is also noticeably high in conditions such as stroke (53%) and hip fractures (71%) due to rapid muscle loss associated with denervation and inflammation, respectively (Morley et al. 2014).

Outcomes

Almost 40% of the total body mass and 75% of the body's cell mass is comprised of skeletal muscle, which has a pivotal role in mobility and function (Lang et al. 2010). Age-associated sarcopenia has major ramifications on health-related outcomes in older adults (Fig. 6).

Cachexia in Older Adults

Definition and Diagnosis

Cachexia is a complex syndrome which presents with the loss of body weight, predominately skeletal muscle, as a result of metabolic abnormalities related to disease processes (Evans et al. 2008). The 2008 consensus definition of cachexia is:

Fig. 6 Consequences of sarcopenia (Developed from Lang et al. 2010 and Rolland et al. 2008). These are the poor health outcomes which can be caused by having the condition of sarcopenia

> Cachexia is a complex metabolic syndrome associated with underlying illness and characterized by loss of muscle with or without loss of fat mass. The prominent clinical feature of cachexia is weight loss in adults (corrected for fluid retention) or growth failure in children (excluding endocrine disorders). Anorexia, inflammation, insulin resistance and increased muscle protein breakdown are frequently associated with cachexia. Cachexia is distinct from starvation, age-related loss of muscle mass, primary depression, malabsorption and hyperthyroidism and is associated with increased morbidity. (Evans et al. 2008)

Cachexia is further defined by consensus in the form of "cancer cachexia," the ongoing loss of skeletal muscle mass (with or without fat loss) which cannot be fully reversed by nutrition support and leads to progressive functional impairment. The loss of lean tissues in cancer cachexia is caused by inadequate dietary intake combined with abnormal metabolism (Fearon et al. 2011). Cachexia has been further staged into cachexia and pre-cachexia. The consensus diagnostic criteria and definition of pre-cachexia according to the 2015 ESPEN consensus paper (Muscaritoli et al. 2010) is shown in Fig. 6. However, this ESPEN consensus paper did not provide

Pre-cachexia (all criteria must be met)	Cachexia (weight loss and 3 of the 5 other criteria must be met)
• Underlying chronic disease • Unintentional weight loss ≤5% usual body weight during past 6-monhts • Chronic or recurrent systemic inflammatory response • Anorexia or anorexia-related symptoms	• Weight loss of ≥5% in 12-months (or BMI <20kg/m^2) • Decreased muscle strength • Fatigue • Anorexia • Low fat-free mass index • Abnormal biochemistry (increased inflammatory marekrs, anaemia or hypoalbuminurea)

Fig. 7 The consensus definitions and diagnostic criteria of pre-cachexia according to Muscaritoli et al. (2010) and cachexia according to Evans et al. (2008). These are the criteria which help define if a person has pre-cachexia or cachexia

diagnostic criteria for cachexia; therefore, the diagnostic criteria and definition of cachexia by a 2008 consensus paper is also shown in Fig. 7 (Evans et al. 2008).

There is disagreement in the literature as to whether cachexia is a type of malnutrition (i.e., disease-related malnutrition) or is a separate condition. Some consensus publications state expert agreement that they are separate conditions, where not all malnourished patients are cachectic, but all cachectic patients are malnourished (Muscaritoli et al. 2010). However, this statement will still hold true with the acceptance that cachexia is the same condition as disease-related malnutrition, as both are caused by disease in the presence of inflammation. This is reflected in a more recent consensus statement by ESPEN, which considers disease-related malnutrition and cachexia to be essentially the same condition due to the similarities in presentation and etiology. However, it should be noted that not all persons with chronic-disease related malnutrition may be diagnosed as having cachexia, and further clinical examination should be undertaken to evaluate the level of inflammation in the individual (Cederholm et al. 2015).

Etiology

Conditions which predispose to cachexia and disease-related malnutrition are those that present with systemic inflammation including cancer, chronic infection, chronic kidney disease, chronic obstructive pulmonary disease, AIDS, rheumatoid arthritis, chronic heart failure, and liver failure (Evans et al. 2008; Muscaritoli et al. 2010). The loss of skeletal muscle in cachexia is a result of increased resting energy expenditure mediated by elevated levels of proinflammatory cytokines (e.g., TNF-α, IL-1, IL-6) and decreased anti-inflammatory cytokines (e.g., IL-4, IL-12, IL-15) (Bauer et al. 2006; Muscaritoli et al. 2010). This systemic inflammation causes metabolic abnormalities including a prolonged acute phase protein response, increased muscle proteolysis, and impaired lipid, carbohydrate, and protein metabolism (Bauer et al.

2006; Muscaritoli et al. 2010). Therefore, cachexia is purported to not respond to typical dietary intervention which aims to provide a high protein-high energy diet, and instead other treatment approaches are being explored such as omega-3 fatty acids and mixtures of elemental amino acids (Dewey et al. 2007; Eley et al. 2007), and states of malnutrition and sarcopenia have been described as a "pre-cachectic state," where nutritional intervention may have the most benefit (Evans et al. 2008). However, some research has shown that nutrition intervention may impact upon the pathogenesis of cachexia, although nutrition intervention alone is insufficient to treat the condition (Wilson and Morley 2003; Evans et al. 2008; Isenring and Teleni 2013).

Prevalence

The global prevalence of cachexia is estimated to be 1% of the patient population, which equates to approximately nine million people (von Haehling and Anker 2014). However, the prevalence of cachexia is complicated by the lack of an overall accepted definition and diagnostic criteria, as well as most criteria being difficult to assess, such as the need for measurement of overall lean body mass (e.g., via DXA scans) and assessment of biochemistry (Tan and Fearon 2008). Additionally, the prevalence of cachexia is expected to vastly differ according to the underlying disease process (e.g., cancer versus cardiac failure or pancreatic cancer versus lung cancer) and population characteristics. Following a brief review of the literature, the prevalence of cachexia as reported in various studies is 10–50% (Table 3).

Outcomes

The loss of lean tissues in cachexia results in the dysfunction of multiple organ systems seen in malnutrition (Figs. 4, 5, and 6). However, the weight loss in cachexia differs from the weight loss in malnutrition; although both are characterized by the loss of lean tissues, the loss of lean tissues compared to fat mass is accelerated in cachexia (Bauer et al. 2006). Cachexia also significantly increases the risk of death beyond the rate seen in standard protein-energy malnutrition and is one of the primary causes of death in cancer (Bauer et al. 2006). The increased risk of death in cachexia may be due to the detrimental effect that decreased lean tissues has on the efficacy of medical treatment, such as increased risk of complications, and lower doses and duration of chemotherapy (Donohoe et al. 2011).

Policies and Protocols

National and state-based health services in many countries have a nutrition care policy which aims to address geriatric wasting syndromes such as malnutrition, sarcopenia, and cachexia. Policies for health services typically address nutrition

Table 3 The prevalence of cachexia in different disease states and populations. This table reports the prevalence of cachexia, as a percentage of various populations with other diseases as specified

Underlying disease	Study	Population sample	Reported prevalence (%)
Cancer, all types	Von Haehling and Stefan, 2014 (von Haehling and Anker 2014)	Estimated for entire European population	30
Unresectable pancreatic cancer	Tan and Fearon, 2008 (Tan and Fearon 2008)	Unclear	20
Resectable pancreatic cancer	Bachmann et al. 2008 (Bachmann et al. 2008)	$N = 277$, mean age 64–65 years, 35% female. Germany	41
Colorectal cancer	Thoresen et. al. 2013 (Thoresen et al. 2013)	$N = 77$, mean age 63 years, 47% female. Norway & Canada	28
Chronic obstructive pulmonary disease	Von Haehling and Stefan, 2014 (von Haehling and Anker 2014)	Estimated for entire European population	35
Chronic heart failure	Von Haehling and Stefan, 2014 (von Haehling and Anker 2014)	Estimated for entire European population	10
Chronic heart failure	Christensen et al. 2013	$N = 238$. Not further described	11
Chronic heart failure	Anker et. al. 1997 (Anker et al. 1997)	$N = 171$, mean age 60 years, 11% female. United Kingdom	16
Severe rheumatoid arthritis	Von Haehling and Stefan, 2014 (von Haehling and Anker 2014)	Estimated for entire European population	10
End stage chronic kidney disease	Von Haehling and Stefan, 2014 (von Haehling and Anker 2014)	Estimated for entire European population	50

screening and assessment but may also address the provision of food and monitoring systems (2011). In recognition of the high burden of malnutrition, the United States of America has mandated compulsory nutrition screening upon admission to any health care facility (2017). In the United Kingdom, although individual health services may have policies to manage malnutrition, there is no policy at the national level, and instead there is a guidance document (2015). Nutrition policies may also be supported by malnutrition pathways or nutrition care protocols, which guide clinicians to implementing best-practice, for example, the Malnutrition Action Flowchart in Australia (Banks).

Outside of the health service, there is generally poor development and implementation of malnutrition policies in community and residential aged care settings, as each organization is independent and managing malnutrition may not be part of accreditation standards. However, there are some facilities which are proactive in

addressing this problem and many non-government organizations have arisen to support implementation of malnutrition policies and pathways, such as Managing Adult Malnutrition in the Community (Brotherton et al. 2012) and the UK and Canadian Malnutrition Task Forces (2017a, c).

Dictionary of Terms

- **Acute disease or injury related malnutrition** – Protein energy malnutrition caused by factors related to acute disease or an injury in the presence of a high degree of inflammation.
- **Cachexia** – A complex syndrome which presents with the loss of body weight, predominately skeletal muscle, as a result of metabolic abnormalities related to disease processes.
- **Disease related malnutrition** – Protein energy malnutrition caused by disease-related processes in the presences of a mild to moderate degree of inflammation.
- **Protein energy malnutrition** – A syndrome caused by the inadequate bioavailability of energy and/or protein over time, leading to the catabolism of lean tissues with or without loss of fat mass, occurring most frequently in older adults.
- **Sarcopenia** – The loss of muscle mass, strength, and function that occurs in ageing.
- **Starvation-related malnutrition** – Protein energy malnutrition due to pure chronic starvation or anorexia nervosa but is also affected by social, environmental, physical, and psychological risk factors.

Summary Points

- Protein-energy malnutrition, a syndrome caused by the inadequate bioavailability of energy and/or protein over time, leading to the catabolism of lean tissues with or without loss of fat mass, occurring most frequently in older adults.
- Inadequate energy and protein intake in malnutrition can be caused by not consuming sufficient dietary sources of energy and protein to meet an individual's nutrient requirements, which is exacerbated in states of hypermetabolism, and/or not absorbing the energy or protein consumed. This is further impacted by psychological, environmental, social, and economic risk factors.
- Malnutrition causes systemic catabolism of fat mass and lean tissues, including vital organs of the liver, heart, respiratory system, and skeletal muscle mass.
- Simple starvation is a physiological state referring to the metabolic alterations, such as hypophosphatemia and the production of ketones as the primary energy source, which are activated in a state of food and nutrient deprivation, but when it causes physiological consequences it is clinically referred to as malnutrition.
- Starvation-related malnutrition is due to pure chronic starvation or anorexia nervosa but is also affected by social, environmental, physical, and psychological risk factors.

- During extended starvation, several organ systems modify their metabolism. Liver gluconeogenesis decreases the production of glucose and kidney production of glucose increases. Fat loss also occurs in starvation-related malnutrition which provides a more stable energy source than glucose produced by gluconeogenesis.
- Sarcopenia is the loss of muscle mass, strength and function that occurs in ageing.
- The etiology of age-related sarcopenia is multifactorial. Declines in nutritional intake, malabsorption of nutrients, reduced physical activity levels, progressive and irreversible loss of motor neurons, although normal in ageing, result in loss of muscle mass and function.
- Sarcopenia has a significant impact on the independence of older adults and is also known to increase the risk of depression and type II diabetes.
- Cachexia is a complex syndrome which presents with the loss of body weight, predominately skeletal muscle, as a result of metabolic abnormalities related to disease processes.
- Conditions which predispose to cachexia and disease-related malnutrition are those that present with systemic inflammation including cancer, chronic infection, chronic kidney disease, chronic obstructive pulmonary disease, acquired immune deficiency syndrome, rheumatoid arthritis, chronic heart failure, and liver failure.
- Cachexia also significantly increases the risk of death beyond the rate seen in standard protein-energy malnutrition and is one of the primary causes of death in cancer.

Conclusion

There is ample evidence showing that weight and muscle loss seen in PEM, starvation, sarcopenia, and cachexia are associated with frailty, loss of independence, poor prognosis, and increased mortality. Although these conditions present with similar characteristics, differential diagnosis is important in the clinical setting so that the patient may be linked with the most appropriate treatment and that new methods of management may be developed.

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Bioelectrical Impedance Analysis and Malnutrition in Cancer

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Teresa Małecka-Massalska, Tomasz Powrózek, and Radosław Mlak

Contents

Abstract

Significant weight loss caused by malnutrition seems to be one of the leading symptoms associated with cancer development. Presence of malnutrition in oncologic patients is most frequently a result of insufficient nutrition and overactivation of inflammatory response. It leads to progressive wasting of the body as a consequence of the increased body catabolism and changes in body composition, and even may develop cancer cachexia – a systemic wasting that cannot be completely compensated by conventional nutrition support. In numerous cancer patients, presence of malnutrition is commonly a first indicator and symptom of the tumor development.

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The nutrition status of such patients is significantly associated with risk of the disease complication during therapy and may result in extension of patients' hospitalization. Moreover, in some group of cancer individuals, the progressive malnutrition leading to cachexia is a main cause of death and not underlying disease. Based on above facts early malnutrition assessment is required for cancer nutritional support. There are various tools used in nutritional status evaluation in the form of scales, questionnaires or risk assessments, anthropometric examinations, and biochemical tests. Despite that they provide a valuable information about patient's nutrition status, also require arrangement various method of their evaluation. Currently, the particular attention is drawn into imaging tools which could provide as a single method the assessment of a body composition. Among these tools the bioelectrical impedance analysis serves as an objective, reliable, and noninvasive method of malnutrition assessment. Bioelectrical impedance analysis measures body compositions electronically, and parameters obtained during examination refer to body vitality at a cellular level, e.g., fluid distribution in the body, fat mass or fat free mass. Also, other parameters derived from bioelectrical impedance analysis demonstrate high clinical utility in malnutrition assessment in cancer patients.

Appropriate evaluation of body composition in cancer patients may provide diagnostic or prognostic information, which seems more valuable than weight loss or body mass index change. In present study the literature review of bioelectrical impedance analysis usefulness in cancer malnutrition assessment is described.

Keywords

Body cell mass · Cachexia · Capacitance of membrane · Extracellular water · Fat free mass · Hydration status · Impedance · Intracellular water · Lean body mass · Malnutrition · Nutrition status · Phase angle · Reactance · Resistance · Total body water

List of Abbreviations

BCM	Body cell mass
BF	Body fat
BIA	Bioelectrical impedance analysis
BMI	Body mass index
Cm	Capacitance of membrane
CRC	Colorectal cancer
ECM	Extracellular mass
ECW	Extracellular water
FFM	Fat free mass
FM	Fat mass
HCC	Hepatocellular carcinoma
HNC	Head and neck cancer
ICW	Intracellular water
LBM	Lean body mass
NSCLC	Non–small cell lung cancer
PA	Phase angle

R	Resistance
SGA	Subjective global assessment
TBW	Total body water
Xc	Reactance
Z	Impedance

Definition of Malnutrition

Malnutrition due to starvation, disease, or aging can be defined as a "state resulting from lack of uptake or intake of nutrition leading to altered body composition (decreased fat free mass) and body cell mass leading to diminished physical and mental functions and impaired clinical outcome from disease" (Sobotka 2012).

Patients who are identified at risk of malnutrition have two options of the diagnostic criteria which one of them should be fulfilled: the first requires a body mass index (BMI) <18.5 kg/m^2. The alternative way includes weight loss (unintentional) $> 10\%$ indefinite of time, or $>5\%$ over the last 3 months combined with either:

- BMI < 20 kg/m^2 if <70 years of age, or <22 kg/m^2 if ≥ 70 years of age or
- fat free mass index (FFMI) <15 and 17 kg/m^2 in women and men, respectively (Cederholm et al. 2016)

According to the etiology, malnutrition is divided into three main areas:

1. Disease-related malnutrition (DRM) with inflammation
2. Disease-related malnutrition without inflammation (acute disease or injury-related and chronic DRM with inflammation)
3. Malnutrition without disease (socioeconomic-, hunger-, or psychologic-related) (Cederholm et al. 2016).

The second etiology area includes cancer cachexia and other disease-specific cachexia.

Nutrition Assessment Tools

The nutrition assessment methods with various scope of usage have been described below.

Nutrition Interview

The basic way of assessing the malnutrition status is taking precise patient history. It facilitates the determination of the risk of nutrition disorders or the evaluation of the degree of the process severity. The assessment of daily food intake provides key information.

There are various tools used in nutritional status evaluation in the form of scales, questionnaires, or risk assessments. Below, we have listed the ones with the greatest potential for the evaluation of the nutritional status in cancer patients that are recommended by ESPEN:

- MUST scale *(Malnutrition Universal Screening Tool)*
- NRS-2002 (*Nutritional Risk Screening*)
- MNA-SF *(Mini Nutritional Assessment – short form)*
- SGA (*Subjective Global Assessment*)
- PG-SGA (*Patient-Generated Subjective Global Assessment*)

Physical Examination

The patient history is supplemented with physical examination. The most important element of the examination is body weight assessment and the measurement of involuntary body weight loss in time. However, it should be noted that the factor of time is, in this case, dependent on the treatment phase.

In 2015, according to ESPEN consensus the following body weight loss cutoff points have been established:

1. Involuntary body weight loss $>$ 5% within 3 months or
2. Involuntary body weight loss $>$ 10% in undefined time (Cederholm et al. 2015)

Studies confirmed that body weight loss is an important prognostic factor (Jagielska 2011). They demonstrated a correlation between body weight loss and response to treatment and patient survival time (Jagielska 2011).

Anthropometric Examinations

The anthropometric examinations that are the most important and most frequently used in the nutritional status assessment are (http://polspen.pl/assets/files):

- BMI (*body mass index*)
- WHR (*waist to hip ratio*)
- Measurement o skinfolds in appropriate sites, most frequently over the biceps, triceps, under the shoulder blade, and over the hip
- Arm circumference
- Fat tissue percentage using anthropometric formulas

Functional Tests (http://polspen.pl/assets/files)

The most important functional tests are:

- Direct stimulation of muscles (most frequently electric stimulation of adductor pollicis muscle)

- Respiratory resistance measurement
- Hand grip

Biochemical Tests

The most frequently used laboratory examinations evaluating the nutritional status are:

- Albumin level
- Hemoglobin level
- Transferrin level
- Prealbumin level
- Retinol binding protein level
- Fibronectin level
- CRP level
- IL-6 level
- Number of lymphocytes in peripheral blood

Image and Other Tools

The most frequently used imaging examinations evaluating the nutritional status are:

- CT (computed tomography)
- MRI (magnetic resonance imagery)
- Adipometry (ultrasound of adipose tissues)
- BIA (bioelectrical impedance analysis)

Introduction to Bioelectrical Impedance Analysis

Impedance constitutes a measurement parameter of the bioelectrical impedance analysis. Impedance is the total resistance of the body to alternating current. In the cylindrical structure it depends on the cylinder length L and the cross section area A, according to formula $Z = \rho L/A$, where ρ is the specific resistance of a material (Fig. 1).

The cylinder volume is calculated by multiplying the length by the cross section area A:

$$V = A \times L$$

Formula $Z = \rho L/A$ can also be expressed as $A = \rho L/Z$ and introduced to the above formula, which results in the formula:

$$V = \rho L^2/Z$$

It is assumed for the purpose of measurement that human body consists of five cylinders (upper limbs, trunk, lower limbs), which are connected in serious electrically (Fig. 2).

Impedance consists of two elements:

1. **Resistance (R)**, which is the resistance (measured in Ohms) of the whole body water and electrolytes dissolved in it
2. **Reactance (Xc),** which is the capacitive resistance of body cells acting like condensers

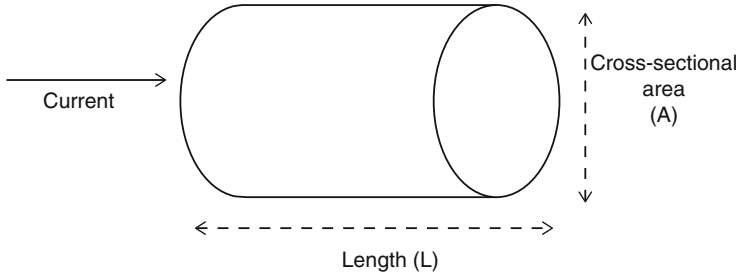

Fig. 1 Cylindrical model for the correlation between impedance and geometry (Source: own preparation based on: (Kyle et al. 2004))

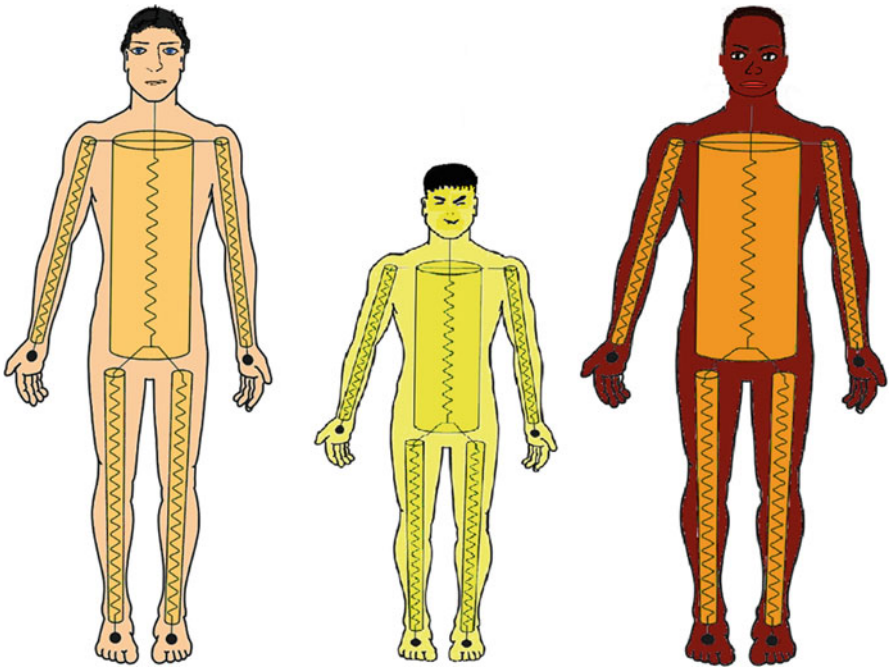

Fig. 2 Human body as a conductor consisting of five cylinders, in three ethnic groups (Source: own preparation based on: (Kushner 1992))

Ad. 1. Resistance (R) (resistivity, electric resistance, active resistance) is the resistance of a conductor towards alternating current, which means that it is inversely proportional to the total amount of water in the organism. Due to significant amount of water and electrolytes, the lean body mass is a good electric current conductor, while the fat tissue is characterized with high resistance.

Resistance is a perfect measure of the amount of body water in healthy people with normal body weight. About 95% of resistance is generated in the limbs and thus it depends on changes of the water content in the extremities. If the measured resistance exceeds normal values, which can occur in case of low water content in limbs (low temperature, high pressure), when using this method, the water content calculation will produce results that are too low, and thus, the fat tissue content will look too high. When blood circulation in the limbs increases or is partly blocked, the resistance values fall and the water and lean body mass content will be too high while the calculated fat tissue mass will be too low.

Ad. 2. Reactance (Xc) (capacitative or passive electric resistance) is the resistance of a capacitator towards alternating current. All cell membranes in the body act as mini-capacitators due to the content of protein-lipid layers. Thus, reactance is the measure of the body cell mass.

The living (live) cells of the body, tissues, organs, and the entire living organisms demonstrate active-capacitative character. Resistance is nonlinear and changes with the frequency of the conducted current (decreases when the frequency increases). Capacitative reactance (capacitance) also demonstrates curvilinear correlation with the frequency (Fig. 3).

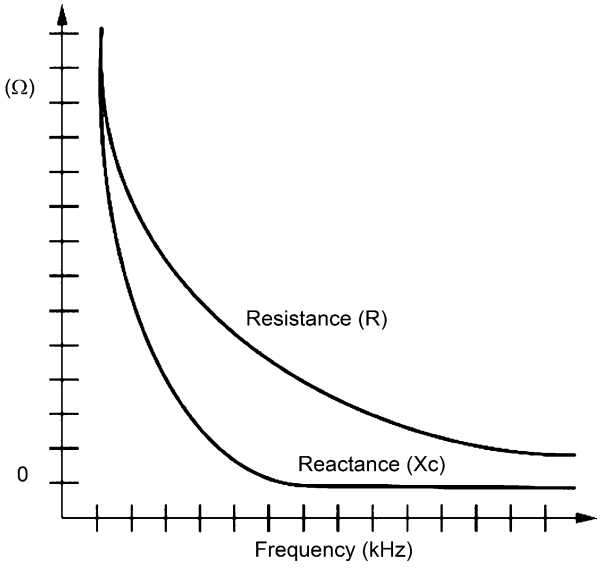

Fig. 3 The correlation between capacitative resistance (Xc) and inductive resistance (R) at defined alternating current frequency (Source: own preparation based on: (Kushner 1992))

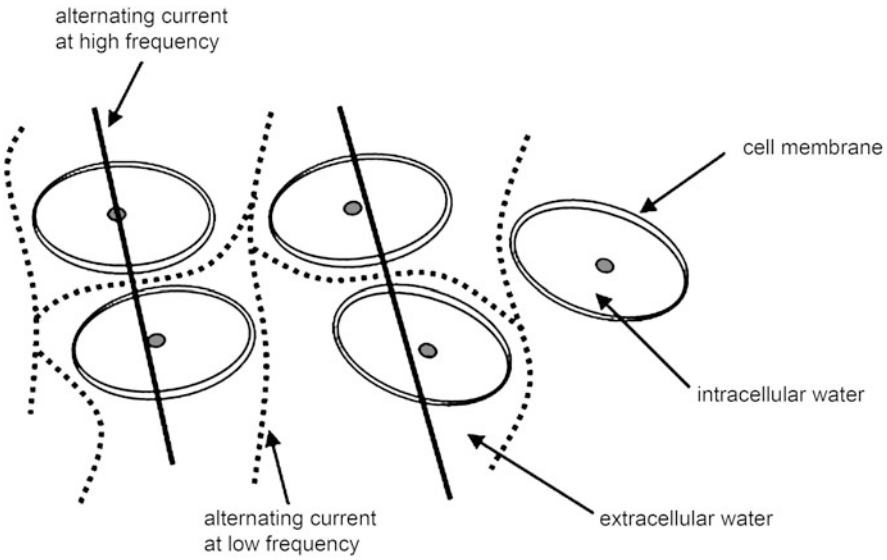

Fig. 4 A graph illustrating the alternating current distribution in relation to the frequency spectrum (Source: own preparation based on: (Cole 1972))

At zero (or low) frequency, electric current does not penetrate the cell membrane, which acts as an insulator and therefore the current only flows through the extracellular fluid which is then responsible for the measurement of the R element of the whole body resistance.

At indefinite (or very high) frequency, the capacitator acts like a perfect (or almost perfect) capacitator and thus the total R – resistance of the organism – reflects the combined resistance of extra- and intracellular fluid (Fig. 4).

Thanks to bioelectrical impedance analysis we obtain direct values (raw data) and indirect values (calculated data) presented below (Table 1). The difference between them results from the fact that the latter (indirect values) do not constitute direct BIA measurement results but can be calculated only after applying appropriate formulas.

The correlation between reactance and resistance is reflected in a parameter obtained using bioelectrical impedance analysis in the form of phase angle. Phase angle is calculated on the basis of resistance and reactance according to phase angle formula = arc tangent Xc/R. Biological significance of the phase angle is not fully understood but it is considered to be an indicator of cellular health. Phase angle value is closely correlated with body cell mass (BCM) (Gonzalez et al. 2013). Higher phase angle value is correlated with better cell function. It is an indicator of cell membrane integrity. Its value is influenced by the difference between the potential on the internal and external side of cell membranes. In case of well-nourished cells, their reactance is high, which implicates high phase angle value. In contrast, cells in bad condition lose membrane integrity, which is reflected by low phase angle value. Thus, it depends on the condition of integral components of cells responsible for biochemical and energetic activity (Selberg and Selberg 2002; De Oliveira et al.

Table 1 Division of parameters obtained from bioelectrical impedance measurements

Raw data	Calculated data
Cm – capacitance of membrane	FFM – fat free mass
Z – impedance	LBM – lean body mass
R – resistance	BCM – body cell mass
Xc – reactance	FM – fat mass; BF – body fat
PA – phase angle	ECM – extracellular mass
	TBW – total body water
	ECW – extracellular water
	ICW – intracellular water

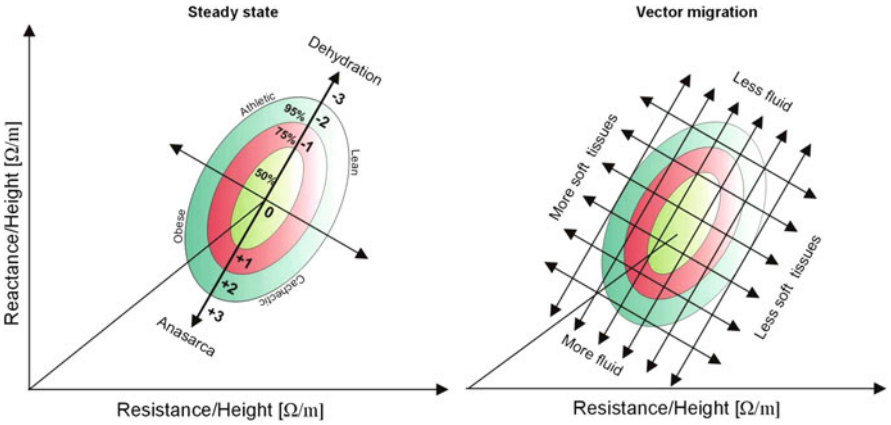

Fig. 5 Bioelectrical impedance vector analysis (BIVA) method (Source: own preparation based on: (Piccoli 2002))

2012). In this aspect, the value of this parameter reflects the efficiency of energy processes and proteolysis, indicating direct relationship with patient health status (De Oliveira et al. 2012).

The resistance and reactance values can be presented as a graphic illustration of human body resistance on the coordinate system (bioelectrical impedance vector analysis) (BIVA acc. to Piccoli) (Piccoli et al. 1994, 2002). In order to do that, the capacitative resistance of body cells (Xc, reactance) is marked on the Y axis, and the water and its electrolyte content resistance (R, resistance) on the X axis. Both values are related to body height (Xc/height and R/height), i.e., standardized for the length of the conductor. Placing the measured values of Xc/height and R/height on the coordinate system allows for determination of an individual vector that looks like an arrow (Fig. 5). This vector picture allows to examine both values (R and Xc) in a patient (Toso et al. 2000, 2003). Because the equation only includes body conductivity characteristics, the analysis is not independent from patient's body weight. The content of fat tissue that acts as electrical insulator is not included in the analysis either.

Due to the fact that the BIA vector position also depends on factors influencing physiology (sex, age, BMI) separate evaluation of pathological changes in the body content (overhydration, dehydration, low BMI, cachexia) may be conducted by comparing the measurement results with tolerance ellipses for the same sex and comparable age group and BMI.

Literature Review of BIA Usefulness in Malnutrition Assessment

BIA is a reliable, noninvasive, and, above all, an objective tool to assess body composition. It can be used both in healthy subjects as well as in various groups of patients (Barbosa-Silva and Barros 2005). It has been widely used in patients affected by kidney disease (hemo- or peritoneal-dialyzed patients), COPD, AIDS, cirrhosis, and heart failure. Recently, most researchers suggest beneficial effect of using BIA to assess body composition (as a tool for screening for malnutrition or cachexia) in different sets of cancer patients (Mulasi et al. 2015; Grundmann et al. 2015). Depending on the type of tumor, weight loss occurs in 30–90% of patients. At the time of diagnosis, in approx. 60% of patients with lung cancer and approx. 80% of patients with cancer of the upper gastrointestinal tract, significant decreases in body weight are recorded. Weight loss is an unfavorable prognostic factor for patients with most types of cancer. Mortality of patients with cachexia syndrome was reported to be as high as 80%. Death usually occurs when weight loss reaches 30–40%. In approx. 20% of patients the direct cause of death is cancer cachexia. Interestingly, signs of malnutrition precede the diagnosis of cancer by several months (Argilés et al. 2013; Dewys et al. 1980; Fearon et al. 2011). Therefore, appropriate body composition evaluation in cancer patients may provide diagnostic or prognostic information, which seems more valuable than weight loss or BMI change.

In cancer patients, BIA parameters are used for an objective evaluation of nutrition status (to distinguish between patients with normal nutrition, malnutrition, and cachexia) and usually compared with subjective scales (SGA, NRS) or as a prognostic factor (assuming that adverse changes in body composition serve as a marker of disease progression).

Phase Angle

The phase angle is used as a general measure of the membrane integrity of the cells. It provides information about the state of a cell and the overall condition of a patient's body. To date, in cancer patients, only few parameters have been evaluated widely, of which PA was assessed most frequently. It was evaluated in solid (HNC, hepatocellular carcinoma (HCC), nonsmall cell lung cancer (NSCLC), pancreatic cancer, breast cancer, colorectal cancer (CRC), and hematological tumors. BIA usefulness as a tool for assessing the nutritional status of cancer patients has been

confirmed in many studies. In the study by Gupta et al. (2008b) in advanced colorectal cancer, PA cutoff of 5.2 was 51.7% sensitive and 79.5% specific whereas a cutoff of 6.0 was 82.8% sensitive and 54.5% specific in detecting malnutrition. Büntzel et al. (2012) showed that in HNC patients PA value of 4.0–5.0 was assessed as high risk of malnutrition development. Moreover they noticed stabilized PA in survivors (4.7 to 5.2) whereas patients who died exhibited a significantly lower phase angle (4.6 to 3.7). Also Małecka-Massalska et al. (2016a) demonstrated that well-nourished HNC patients had a statistically significantly higher PA score (5.25) as compared to those who were malnourished (4.73). A PA cutoff of 4.73 was 80% sensitive and 56.7% specific in detecting malnutrition diagnosed by SGA in these populations. However, Stegel et al. (2016) noted that PA does not allow to distinguish malnourished HNC patients from cachectic ones. Whereas several researchers suggest the possibility of using the PA as a predictive factor for the applied therapy (Schwenk et al. 2000). Burden et al. (2010) in a study concerning colorectal cancer suggest that nutritional screening would be beneficial preoperatively to identify weight-losing patients at an early stage in the care pathway when they initially enter the secondary care system. Małecka-Massalska et al. (2014a) showed that PA measured at 50 kHz was found to be significantly lower in patients after surgery than before treatment (4.22 vs. 4.69 respectively). Moreover, numerous studies confirm that PA could be used as a prognostic factor in different sets of cancer patients. Gupta et al. (2004) showed that advanced colorectal patients with a PA $\leq$ 5.57 had a significantly shorter median survival (8.6 months) compared to those with a PA $>$ 5.57 (40.4 months). In the next study (Gupta et al. 2008a), they demonstrated similar results in breast cancer patients in which PA $\leq$ 5.6 had significantly shorter survival (23.1 months), than those with a PA $>$ 5.6 (49.9 months). The same authors (Gupta et al. 2009) also confirmed above findings in another set of cancer patients (advanced NSCLC). In this study, patients with PA $\leq$ 5.3 showed poor survival (7.6 months) when compared to other patients with PA $>$ 5.3 (12.4 months). These results were confirmed later by other authors (Sánchez-Lara et al. 2012). Lower PA (cutoff 5.8) correlates with worse patient survival (11 months) when compared to higher PA $\geq$ 5.8 (17 months), and this parameter was an independent prognostic factor of NSCLC patient survival. Similar results were obtained by Norman et al. (2010) in several solid and hematological tumors. They concluded that standardized PA is an independent predictor for impaired functional and nutritional status and a better indicator of 6-months' mortality than are malnutrition and disease severity in cancer. In the study of Władysiuk et al. (2016) performed in patients with advanced HNC, lower PA value ($<$4.733) was associated with significantly higher risk of overall survival (OS) shortening (19.6 vs. 40 months respectively). Such results were also demonstrated in hepatocellular carcinoma patients. In this study, Schütte et al. (2015) found that PA values (cutoff 4.8) affect patient survival. Lower PA ($<$4.8) was associated with shorter OS (298 days) when compared with higher PA value ($\geq$4.8) (399 days). Similar results were also obtained by Lee et al. (2014) in a set of terminal patients (advanced stages of solid and hematologic cancers) previously treated with surgery, CTH and RTH. In this case, PA presents positive correlation with patient survival. Lower PA score was

related with poor disease prognosis; survival was shorter than 25 days when PA was <4.4 and longer than 50 days when PA was above >4.4. Moreover, a PA analysis can be used to determine nutritional risk in cancer patients undergoing RTH. In a study by Souza et al. (2015) conducted in pre-radiotherapy patients (with advanced stages of solid and hematologic cancers), lower PA (cutoff 5.2) was established as a criterion for identifying patients with higher risk of malnutrition. Also Castanho et al. (2013), in their study in non–small cell lung cancer (NSCLC) advanced patients (stage IB-IIIB), concluded that PA may be used as an assessment tool for evaluation of needs for nutritional treatment in those patients because it informs about disease severity. They demonstrated that PA is closely related with tumor mass volume, which may provide important information about early nutritional intervention requirements in NSCLC patients. In another study (Richter et al. 2012) conducted in pancreatic cancer, the authors show that parenteral nutrition (supported with sufficient level of calories) started in appropriate moment led to improvement of nutritional status of patients. Moreover, Jager-Wittenaar et al. (2014) showed that PA and other BIA parameters are appropriate for FFM evaluation and can be used as a part of a comprehensive malnutrition evaluation in HNC patients.

Capacitance of Membrane

To date, only one paper is available describing a raw data derived parameter, other than PA, useful in evaluation of nutritional status of cancer patients. In a study by Małecka-Massalska et al. (2016b) conducted in advanced (stage III and IV) HNC patients, higher Cm values in well-nourished patients, compared to malnourished ones, were observed (1.41 vs. 1.01 respectively). Cutoff value of 0.743 (established by ROC analysis) was characterized by very high specificity (98%) and medium-low sensitivity (37%) in the detection of malnutrition. Moreover, in patients with Cm values below cutoff, shorter OS was noted when compared to other patients (12.1 and 43.4 months, respectively).

Calculated Parameters

Raw data are used for estimation of numerous calculated parameters including: total body water (TBW), intracellular body water (ICW), extracellular body water (ECW), body cell mass (BCM) and, thus, body fat mass (FM), fat-free body mass (FFM), lean body mass (LBM), extracellular mass (ECM), ECM/BCM index, body fat (BF), and other. These parameters, also characterizing nutritional status of a patient, usually play supporting role in malnutrition assessment (Piccoli et al. 1997; Schwenk et al. 1998).

In a study by Małecka-Massalska et al. (2014b) conducted in patients with HNC (in operable stages), the well-nourished patients had significantly lower ECM/BCM ratio (1.11 vs. 1.28) when compared to malnourished patients. Cutoff value established at 1.194 (according to ROC analysis) was 76% sensitive and

63% specific in detection of malnutrition condition. In another study (Castanho et al. 2013), ECM/BCM ratio was evaluated as malnutrition indicator of NSCLC (stage IB-IIIB) patients. The values >1.22 were assessed as high risk indicator of malnutrition development in studied subjects. Moreover, authors considered that the ECM/BCM ratio is a valuable marker of patients' nutritional status and also could be used as a potential marker of hypermetabolic tumors. Regarding patients' survival, the study participants with the highest ECM/BCM ratio (>1.5) had shorter survival time compared to patients with lower value of this parameter. In recent German study (Schütte et al. 2015), the positive correlation of BCM with body weight, BMI, and MUAC (circumference of thigh and mid-upper arm circumference) as well as negative correlation of this parameter with age was found in HCC patients. Moreover, authors reported that differences in BCM percentage among studied subjects had no impact on patients' survival probability. Interestingly, study results demonstrated a significant correlation between BIA screening (BCM, ECW%TBW, PA) and NRS (The Nutritional Risk Score) in malnutrition assessment of HCC patients, whereas such correlation was not observed between BIA and MNA (Mini Nutritional Assessment) screening tool. Trabelsi et al. (2006) considered that also parameters such as FMI (fat mass index) and FFMI (free-fat mass index) can provide reliable assessment of malnutrition in patients suffering from various cancers. According to study results, BIA analysis of FMI and FFMI can be more objective screening tool for malnutrition detection than BMI because some patients demonstrate relative high BMI despite the presence of malnutrition. The high utility of BIA for reliable assessment of FFM was also proved in group of HNC patients (Jager-Wittenaar et al. 2014). In a study by Lopes et al. (2013), both anthropometric and BIA methods were used to assess malnutrition status of CRC patients in two periods, before and after surgery. Parameters including FFM, TBW, and BFM demonstrated high utility to measure changes of body composition in patients who underwent surgery. Considering this, the mentioned parameters are especially important to estimate nutritional status of surgically treated CRC patients as well as to detect early symptoms of developing malnutrition, which could facilitate personalized nutritional intervention in future. Usefulness of FFM, BCM, and TBW was also evaluated in malnutrition screening of preoperative CRC patients (Burden et al. 2010). Measurements of body composition using BIA demonstrated that malnourished CRC patients showed less FFM in contrast to not malnourished subjects. The significant differences in FFM between patients who had lost <10% of their body weight and those who had lost >10% of their body weight were reported. These patients could potentially benefit from BIA nutritional screening, because it allows identification of weight-losing patients at an early stage. The significant correlation between FFM and BMI was found in patients with various types of advanced cancer (Slaviero et al. 2003). Furthermore, MNA and weight change demonstrated no significant relationships with results of BMI and FFM in study population. It may suggest that analysis of FFM could improve assessment of malnutrition performed by only MNA and BMI measurement (Gupta et al. 2006; Muramatsu et al. 2015) (Table 2).

Table 2 Overview of research using BIA parameters in the assessment of malnutrition in cancer patients

Study (country)	Year	Cancer (stage)	Study design	Study group (M-male, F-female)	Age (years ±SD)	Malnutrition assessment	BIA parameters
Władysiuk et al. (Poland)	2016	Head and neck cancer (stage III and IV)	Prospective	75 patients (M = 67, F = 8) therapy naive	Mean: 56.9 ± 8.2	PG-SGA, BIA	PA (Mean: 5.04 ± 0.88)
Małecka-Massalska et al. (Poland)	2016b	Head and neck cancer (stage III and IV)	Prospective	75 patients (M = 67, F = 8) therapy naive	Mean: 56.9 ± 8.2	PG-SGA, BIA	Cm (Mean: 1.75 ± 0.55)
Małecka-Massalska et al. (Poland)	2016a	Head and neck cancer (stage III and IV)	Prospective	75 patients (M = 67, F = 8) therapy naive	Mean: 56.9 ± 8.2	PG-SGA, BIA	PA (Median: 5.04 ± 0.88)
Stegel et al. (Slovakia)	2016	Head and neck cancer	Prospective	55 patients analyzed pre- and post-RCTH	Unknown	SGA	PA (before and after treatment)
Schütte et al. (Germany)	2015	Hepatocellular carcinoma (HCC)	Prospective	51 patients (M = 44, F = 7)	Mean: 66.2 ± 9.82	Anthropometric, BIA	PA (Mean: 4.77 ± 0.89), TBW, ECW, ICW, FM, BCM
Souza et al. (Brazil)	2015	Solid and hematologic tumors	Prospective	93 patients (M = 67, F = 26) pre-RTHC	Median: 62 ± 12.74	Anthropometric, PG-SGA, BIA	PA (Median: 5.95 ± 1.0)
Muramatsu et al. (Japan)	2015	Pancreatobiliary tract and urothelial cancer	Retrospective	37 patients (M = 23, F = 14) treated with gemcitabine-based CTH	Mean: 68.3	BIA	BFR, SMR, BMI, basal metabolism

Małecka-Massalska et al. (Poland)	2014a	Head and neck cancer (operable stages)	Prospective	31 patients (M = 28, F = 3) analyzed pre- and post-surgically	Mean: 57.9 ± 8	SGA, BIA	PA (Mean: 4.69 ± 0.71)
Małecka-Massalska et al. (Poland)	2014b	Head and neck cancer (operable stages)	Prospective	75 patients (M = 67, F = 8) preoperative	Mean: 56.9 ± 8.2	SGA, BIA	ECM/BCM
Jager-Wittenaar et al. (Netherlands)	2014	Head and neck cancer	Prospective	24 patients (M = 20, F = 4) analyzed pre- and twice post-therapy	Mean: 60.4 ± 8.3	BMI, BIA	PA, ECW/ICW, FFM
Lee et al. (South Korea)	2014	Terminal cancers – solid and hematologic (advanced stages)	Prospective (preliminary)	28 patients (M = 13, F = 15) previously treated with surgery or CTH and RTH	Range from 30 to >70	Unknown	PA (Median: 4.50)
Castanho et al. (Brazil)	2013	Nonsmall cell lung cancer (NSCLC) (stage IB-IIIB)	Cross-sectional study	30 male patients, therapy naive	Mean: 65.6 ± 9.3	Anthropometric, BIA	PA (Mean: 5.66 ± 0.90) ECM/BCM (1.28 ± 0.14)
Lopes et al. (Portugal)	2013	Colorectal cancer (operable stages)	Prospective	50 patients (M = 33, F = 17) analyzed pre- and post-surgically	Mean: 66 ± 12	Anthropometric, BIA, PG-SGA	BFM, FFM, TBW, SLM
Büntzel et al. (Germany)	2012	Head and neck cancer	Retrospective	66 patients (M = 50, F = 16) adjunctive or primary RTH	Mean: 67.7	BIA	PA

(*continued*)

Table 2 (continued)

Study (country)	Year	Cancer (stage)	Study design	Study group (M-male, F-female)	Age (years ±SD)	Malnutrition assessment	BIA parameters
Richter et al. (Germany)	2012	Pancreatic cancer	Retrospective	17 patients (M = 11, F = 6) parenteral nutrition support, CTH	Mean: 64 Median: 66	SGA	PA, BCM, ECM, ECM/BCM
Sánchez-Lara et al. (Mexico)	2012	NSCLC (stage IIIB and IV)	Prospective	119 patients (M = 64, F = 55) treated with paclitaxel-based CTH	Mean: 60.5 ± 12.5	SGA	PA (Mean: 5.8 ± 1.8)
Norman et al. (Germany)	2010	Solid and hematologic tumors	Prospective	399 patients (M = 208, F = 191) therapy naive	Mean: 63.0 ± 11.8	SGA	PA (Mean: 4.59 ± 1.12)
Burden et al. (United Kingdom)	2010	Colorectal cancer (operable stages)	Prospective	87 patients (M = 54, F = 33) preoperative	Mean: 64.5	SGA, MUST	Fat (kg, %), FFM (%), DLW (kg), BCM, TBW (%)
Gupta et al. (USA)	2009	NSCLC (stage IIIB and IV)	Retrospective	165 patients (M = 93, F = 72) newly diagnosed and treated – not specified	Median: 56 ± 9.1	BIA	PA (Median: 5.3)
Gupta et al. (USA)	2008a	Breast cancer (stage I–IV)	Retrospective	259 female patients newly diagnosed and treated – not specified	Median: 49	SGA	PA (Median: 5.6)

Gupta et al. (USA)	2008b	Colorectal cancer (stage III and IV)	Retrospective	73 patients (M = 38; F = 35)	Mean: 56 ± 11.4	SGA	PA (Mean: 5.7 ± 1.3)
Trabelsi et al. (Tunisia)	2006	Different cancers	Retrospective	83 patients (M = 18, F = 65) surgically treated	Unknown	BMI	FFMI, FMI
Gupta et al. (USA)	2006	Colorectal cancer (stage III and IV)	Retrospective	58 patients (M = 35, F = 23) newly diagnosed and previously treated – surgery and CTH	Mean: 58 ± 10.6	SGA	PA (Mean: 5.7 ± 1.4)
Gupta et al. (USA)	2004	Colorectal cancer (stage IV)	Retrospective	52 patients (M = 30, F = 22) newly diagnosed and previously treated	Mean: 55.8 ± 10.8	SGA	PA (Mean: 5.6 ± 1.5), LBM, FM, BCM
Slaviero et al. (Australia)	2003	Lung, breast, and prostate cancer (locally advanced and/or metastatic)	Retrospective	73 patients (M = 40, F = 33) treated with palliative CTH	Mean: 61 Median: 63	Mini Nutritional Assessment	TBW, FFM, FFM (%), FM

Dictionary of Terms

- **Body cell mass (BCM)** – In contrast to extracellular mass (ECM), it reflects all the metabolically active tissues of the body, such as organ, muscle, and blood cells. Body cell mass also includes water inside the living cells, called intracellular water (ICW).
- **Bioelectrical impedance (BIA)** – Imaging examination evaluating the nutritional status by electrical measurement of body composition. BIA is a reliable, noninvasive, and an objective tool to assess body composition which can be used both in healthy subjects and in various group of patients, e.g., cancer, chronic diseases.
- **Cachexia** – Marked state of weight loss and malnutrition usually associated with a chronic disease or cancer that cannot be compensated by nutritional support.
- **Extracellular mass (ECM)** – Reflects all the metabolically inactive tissues of the body, such as blood plasma or bone minerals. Extracellular mass also includes extracellular water (ECW).
- **Extracellular water (ECW)** – Reflects the total amount of body water contained outside cells, together with intracellular water (ICW) is a component of total body water (TBW).
- **Intracellular water (ICW)** – Reflects the total amount of body water contained within cells, together with extracellular water (ECW) is a component of total body water (TBW).
- **Fat free mass (FFM)** – (Lean or nonfat components) BIA calculated parameter that refers total amount of all body components except fat.
- **Fat free mass index (FFMI)** – Is an indicator of body condition alternative to BMI and is used to calculate amount of muscle mass with following formula: value from the percentage ratio of body fat percentage, the height in meters, and weight in kg.
- **Malnutrition** – State characterized by faulty nutrition because of inadequate uptake or intake of nutrients leading to altered body composition resulting in diminished physical and mental functions and impaired clinical outcome from disease.
- **Nutritional status** – Reflects the index of nutrients intake, digestion, and absorption influenced by a diet as well as ability of assimilated nutrients level to maintain normal metabolic integrity.
- **Phase angle (PA)** – Is a BIA parameter calculated on the basis of resistance and reactance according to following formula = arc tangent Xc/R. Phase angle is usually used as a general measure of the membrane integrity of the cells.
- **Reactance (Xc)** – (Resistance of a capacitator towards alternating current) raw parameter obtained from BIA measurement. Resistance is the capacitive resistance of body cells acting like condensers.
- **Resistance (R)** – (Resistance of a conductor towards alternating current) raw parameter obtained from BIA measurement. Resistance is the resistance (measured in Ohms) of the whole body water and electrolytes dissolved in it.

- **Total body water (TBW)** – Parameter reflecting water contained in lean body mass. Total body water consists of two compartments: extracellular water (ECW) and intracellular water (ICW). Total body water is calculated parameter of BIA referring basic hydration status of the body.

Summary Points

- Weight loss occurs in 30–90% of cancer patients depending on the type of tumor.
- Bioelectrical impedance analysis is one of the objective methods for the nutrition assessment.
- Parameters obtained by bioelectrical impedance analysis are two types: direct (impedance, resistance, reactance, capacitance of membrane, phase angle) and indirect (fat free mass, lean body mass, body cell mass, fat mass, extracellular mass, total body water, extracellular water, intracellular water). The difference between them results that the latter (indirect values) do not constitute direct bioelectrical impedance measurement results but can be calculated only after applying appropriate formulas.
- Either direct or indirect parameters are used in the nutrition assessment.
- Impedance measured by bioelectrical impedance analysis is a sum of resistance and reactance.
- Resistance is inversely proportional to the total amount of water in the organism, and reactance is the measure of the body cell mass.
- The correlation ratio reactance and resistance is reflected in a parameter named phase angle. Phase angle is calculated according to the formula: phase angle = arc tangent reactance/resistance.
- Phase angle is considered to be an indicator of cellular health and cell membrane integrity. The value is closely correlated with body cell mass. Higher phase angle value is correlated with better cell function.
- Phase angle utility in nutrition assessment among cancer patients is well established.
- Certain phase angle cutoff was assessed in different types of cancer as predictive in detecting malnutrition. Numerous studies confirm usefulness of phase angle as a prognostic factor in different sets of cancer patients.

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Part VII

Medical Causes of Malnutrition, Prevalence, and Impact

Arthritis-Induced Anorexia and Muscle Wasting

44

Ana Isabel Martín and Asunción López-Calderón

Contents

Abstract

Rheumatoid arthritis is a systemic inflammatory disease that destroys articular cartilage and bone and leads to joint damage. Cachexia is common in rheumatoid arthritis patients and is called rheumatoid cachexia, characterized by a reduction in muscle mass with body fat gain and stable or slightly increased body weight. Loss of muscle mass in rheumatoid cachexia leads to weakness, loss of independence, and increased risk of death. Muscle atrophy seems to be secondary to an increase in muscle proteolysis, but not to a decrease in myogenesis. A variety of factors are involved in the pathogenesis of rheumatoid cachexia, including excessive cytokine production, physical inactivity, hormone deficit modifications, and glucocorticoid treatment. In this chapter, we summarize the mediators

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involved in the increase in fat mass and skeletal muscle wasting observed in rheumatoid arthritis patients.

Keywords
Rheumatoid arthritis · Cachexia · Inflammation · Anorexia · Skeletal muscle · Muscle wasting · Proteolysis · Ubiquitin-proteasome system · Autophagy · Cytokines · TNFα · NF-kB · COX-2 · IGF-I · Glucocorticoid

List of Abbreviations

AgRP	Agouti-related peptide
Akt	Protein kinase b
Atrogin-1	Muscle atrophy F-box or MAFbx
BMI	Body mass index
COX-2	Cyclooxigenase-2
FFM	Fat-free mass
IGF-I	Insulin-like growth factor-I
IGFBPs	IGF binding proteins
IL	Interleukin
LC3b	Microtubule-associated protein light chain 3
MuRF1	Muscle ring finger 1
NF-kB	Nuclear factor kappa B
NPY	Neuropeptide Y
RA	Rheumatoid arthritis
TNFα	Tumor necrosis factor α

Introduction

Rheumatoid arthritis (RA) is the most frequent form of inflammatory arthritis. RA is an autoimmune disease characterized by autoantibody production and chronic and systemic inflammation that can affect many tissues, mainly synovial tissue (Fig. 1). These clinical features cause joint destruction and functional disability with a substantial economic burden on patients, their families, and society in terms of cost and lost productivity. Joint destruction from synovitis primary affects small diarthrodial joints symmetrically (wrists, fingers, feet, knees, and ankles).

Although the etiology remains unknown, several genes and environmental factors (mainly tobacco smoke), hormones, infections, and gut macrobiota act together to cause pathological events (Fig. 2). The disease can occur at any age, but it is most common within the fourth and sixth decade of life, and its incidence increases with age. The prevalence of RA in the world population is approximately 0.5–1%, with a frequency in women three times higher than in men (Scott et al. 2010). Ovarian steroids seem to play a protective role, since RA often develops at times when sex steroid hormone levels are changing, such as in the postpartum and perimenopausal period. Furthermore, oral contraceptive and postmenopausal estrogen therapy reduce the risk of RA (D'Elia et al. 2003). It is also more likely to occur in urban versus rural

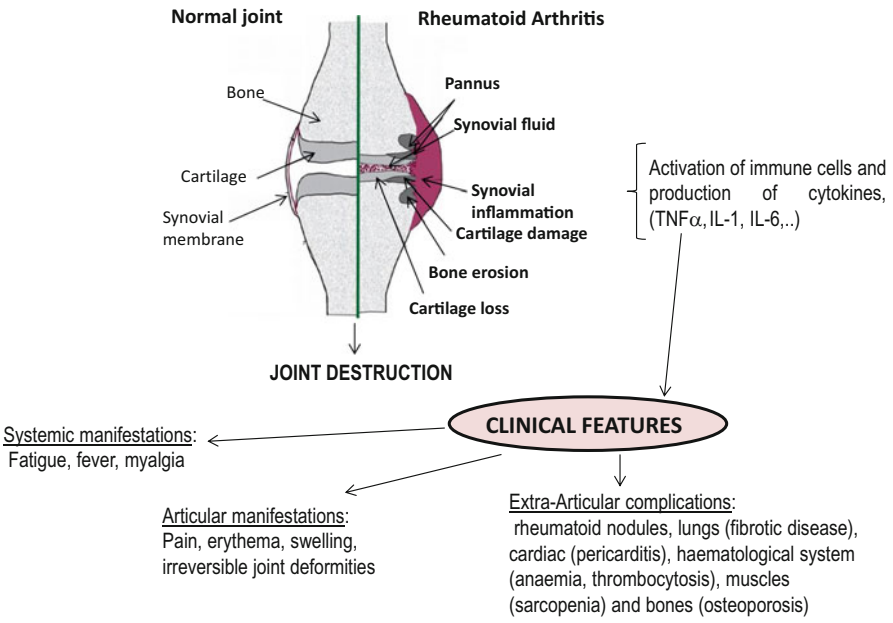

Fig. 1 Schematic view of a normal and an affected joint by rheumatoid arthritis (RA). RA increases inflammation and cellular activity in affected joint. The pathobiology of RA involves immune cells and the complex interaction of many pro-inflammatory cytokines, including TNFα and IL-6. These cytokines cause local and systemic symptoms associated with this disease. *IL* interleukin, *TNFα* tumor necrosis α

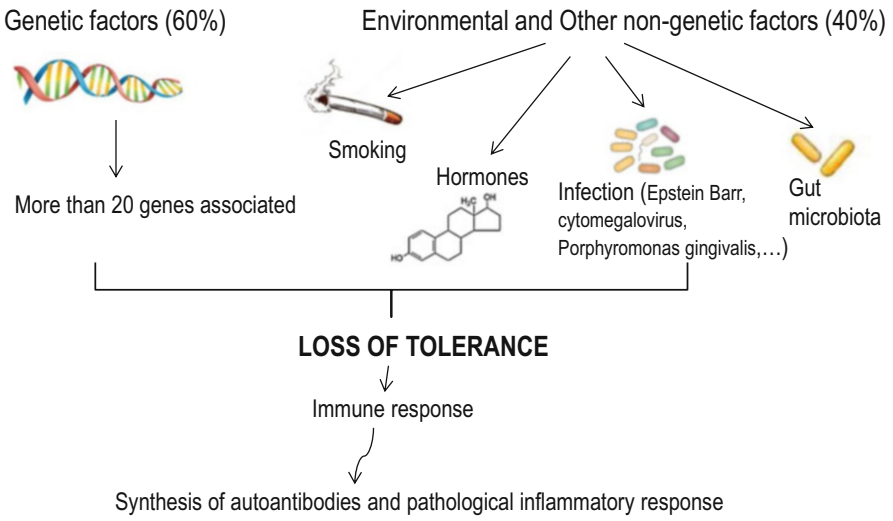

Fig. 2 Etiology of rheumatoid arthritis (RA). Causes of RA remain yet unknown, but there seems to be a combination of genetic and environmental factors

populations. The geographic distribution of RA is worldwide, with the highest prevalence found in specific tribes of Native Americans and Alaska Natives (>1.2%), and the lowest prevalence described in countries such as South Africa, Pakistan, China, Nigeria, Indonesia, the Philippines, and Argentina.

That rate of mortality is two to three times higher in the population with RA versus the population without RA of similar age and sex. Life expectancy of patients with RA decreases by 20 years, which can vary depending on the therapy. This reduction is associated with an increased prevalence of several comorbidities. Comorbidities most frequently seen in RA include cardiovascular disease (mainly coronary disease), osteoporosis, lung disease, malignancies, and neuropsychiatric disease. The average patient with RA has two or more comorbid disorders (Dougados et al. 2014).

Rheumatoid Cachexia

The term "cachexia" originates from the Greek terms *kakos* and *hexis*, which mean "bad condition." It generally connotes a state of advanced malnutrition and wasting, with a decrease in body cell mass and muscle atrophy. To date, accurate criteria used to define cachexia by different research groups vary. The prevalence of cachexia depends on the clinical criteria considered for the diagnosis and the measurement method used. A group of scientists and clinicians reached a consensus on the clinical definition of cachexia: *a complex metabolic syndrome associated with underlying illness and characterized by loss of muscle with or without loss of fat mass. The prominent clinical feature of cachexia is weight loss in adults (corrected for fluid retention) or growth failure in children (excluding endocrine disorders). Cachexia is distinct from starvation, age-related loss of muscle mass, primary depression, malabsorption, and hyperthyroidism and is associated with increased morbidity.*" (Evans et al. 2008). Using this definition, the number of people in Europe, USA, and Japan with cachexia and severe forms of RA was 190,000, a prevalence estimated at approximately 0.8% of the population (von Haehling et al. 2016).

The first description of altered body composition in RA patients was reported in 1873 (Paget 1873). Roubenoff et al. (1994) introduced the term "rheumatoid cachexia" to describe the special body composition that often characterizes patients with RA. Cachexia associated with illnesses such as cancer and heart failure is characterized by severe muscle and fat loss. However, in RA patients with cachexia, the decrease in fat-free mass, predominantly skeletal mass, is often associated with an increase in fat mass. In this case, body mass index (BMI) values are within the normal range. This condition has also been termed "rheumatoid cachexia" or "rheumatoid cachectic obesity." Stavropoulos-Kalinoglou et al. (2011) reported that RA patients have a decreased BMI, which is approximately 2 kg/m^2 lower than that of the general population for a given body fat content. Therefore, for the general population where individuals with a BMI of >30 kg/m^2 are classified as obese and those with a BMI of 25–30 kg/m^2 are characterized as overweight, it was proposed that in patients with RA, these values be reduced for overweight and obesity to 23 and to 28 kg/m^2,

respectively. Patients with RA have 13–14% less body cell mass than their age-, sex-, race-, and BMI-matched controls (Walsmith and Roubenoff 2002). If a loss greater than 40% of baseline body cell mass is associated with death, the 13–14% decrease observed in RA patients, although not directly fatal, severely compromises muscle strength, balance, and functional mobility and reduces quality of life. In addition, rheumatoid cachexia has been reported to worsen disease outcomes and to increase the prevalence of metabolic syndrome and hypertension, which may contribute to the increased mortality in RA. This is because the main store of body protein is the muscle, and depletion of body protein weakens adaptation to metabolic stress and the capacity of patients to overcome secondary infection and concomitant illness.

In RA patients, several studies have shown deficits in muscle cross-sectional area, muscle density, and muscle strength compared with controls. These deficits seem to be significantly greater among those patients with less adiposity and greater inflammatory disease (Baker et al. 2015a, b). Decreased muscle density, measured by peripheral quantitative computed tomography, indicates intramuscular fat infiltration strongly associated with limitation in physical performance and disability. In the general population, obesity is related to the major complications also found in RA patients: cardiovascular mortality and disability. However, there is no conclusive evidence about the relationship between high BMI values and cardiovascular risk in RA patients. There is evidence that patients with low BMI (<18.5) have the highest cardiovascular risk, while obese patients may be partially protected (Escalante et al. 2005). This finding is the opposite of the pattern found in the general population, in which obesity is clearly linked to an increase in cardiovascular mortality. In contrast, other authors have reported strong positive associations between obesity and worse RA disease outcomes, adverse cardiovascular risk factors, and a higher prevalence of comorbidities (Ajeganova et al. 2013). This controversy may be the result of the potentially important confounders in RA patients (i.e., disease activity, antirheumatic and/or cardiovascular disease medication, and smoking). Nevertheless, low BMI in RA is associated with the highest rates of erosive disease and disability, whereas an increased BMI protects the joints (Kaufmann et al. 2003). The protective effect of high BMI seems to be present in overweight or obese RA patients before the diagnosis of RA. This effect continues during the first few years of RA, with a greater reduction in radiographic joint damage in obese than in normal-weight RA patients (van der Helm-van Mil et al. 2008). Serum adiponectin in RA seems to be the link between high adiposity and less progressive and erosive disease. Taking into account that this hormone has a negative effect on the bone metabolism promoting bone resorption (Wang et al. 2014), and that obesity decreases adiponectin, decreased adiponectin levels in RA patients with high BMI may explain the less erosive course of the disease (Giles et al. 2011). In summary, even if BMI poorly reflects body composition, low BMI in RA indicates severe cachexia. RA patients with intermediate BMI probably have good disease control, but they can be cachectic. Obese RA patients may have a decreased risk of joint damage progression, whereas there is no clear association with the risk of cardiovascular death (Challal et al. 2016).

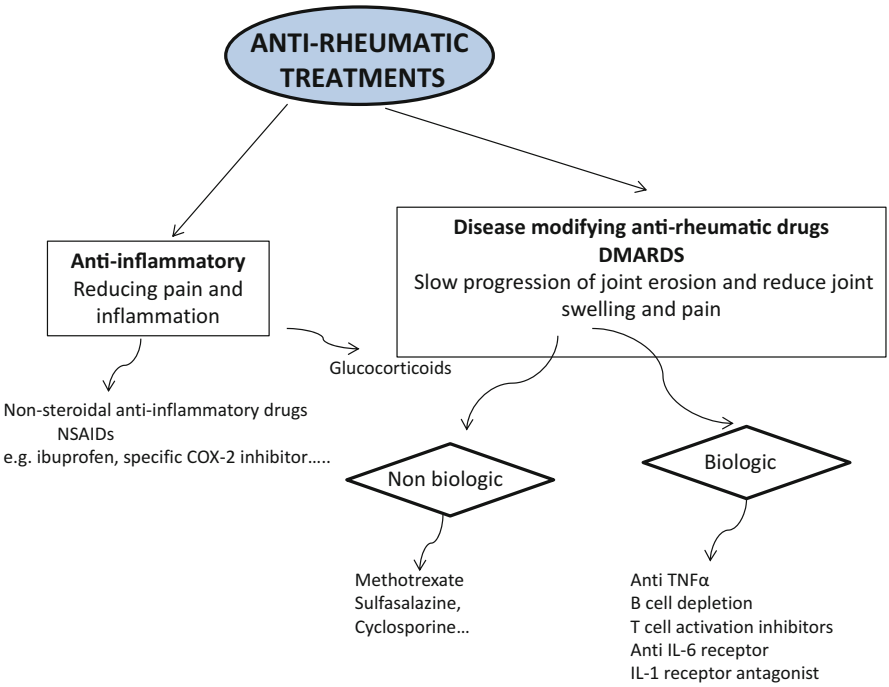

Fig. 3 Antirheumatic therapies in rheumatoid arthritis (RA). Two classes of drugs are used to treat RA. The first class of drugs, anti-inflammatory drugs, simply alleviate pain and swelling. The second, disease-modifying antirheumatic drugs (DMARDs) are able slow disease and prevent joint deformity. The treatment of RA usually involves the use of combination therapy

There are sex differences in body composition phenotypes of RA. Men with RA tend to have greater muscle wasting than women with RA. In addition, men with RA have higher fat mass. These data could indicate that disease-related body composition alterations overall are greater in men than in women with RA (Baker et al. 2015a). Furthermore, there are also sex differences in treatment responses, with men responding better in early RA (Jawaheer et al. 2012).

Antirheumatic Treatments

When studying rheumatoid cachexia, antirheumatic treatments can interfere with the effects of RA on food intake and cachexia. Obviously, systemic inflammation is decreased by antirheumatic treatments, and many of the side effects of inflammation can be modified by these treatments. Among the most common RA treatments (see Fig. 3), methotrexate has been reported to decrease whole-body proteolysis in RA patients (Rall et al. 1996). In addition to muscle proteolysis, treatment with methotrexate decreases anorexia and body weight loss in an experimental model of RA (Jurcovicova et al. 2009). Similarly, inhibition of COX-2 by administering a

nonsteroidal anti-inflammatory agent (NSAID) increases food intake and body weight gain, whereas it decreases skeletal muscle atrophy in arthritic rats (Granado et al. 2007). Anti-TNFα, the *second-line* of medical treatment for RA, has important actions on food intake as well as on fat and skeletal muscle. TNFα appears to play an important role in reducing adipose tissue mass. Accordingly, an increase in fat mass after 2 years of treatment with an anti-TNFα antibody has been reported in RA patients (Engvall et al. 2010). Glucocorticoids, on the contrary to the aforementioned, have an inhibitory effect on muscle mass, and chronic elevation of their levels induces muscle atrophy, both indirectly and directly on a muscular level (Bodine and Furlow 2015). Therefore, glucocorticoid therapy can be an important factor in rheumatoid cachexia, since it modifies body composition, increasing fat mass and decreasing skeletal muscle mass. In summary, antirheumatic treatments not only decrease inflammation but can also both increase and decrease muscle proteolysis and increase food intake and fat mass. Therefore, some of the modifications in body composition found in RA patients, such as an increase in fat mass, can be due to the antirheumatic treatments rather than to the disease itself.

Mechanisms of Rheumatoid Cachexia

The exact pathophysiological mechanisms of rheumatoid cachexia are not fully elucidated. The etiology is probably multifactorial and it is likely that the severity of cachexia in RA patients will depend on a combination of intensity, duration, and activity of inflammatory disease. A variety of factors are involved in the pathogenesis of RA cachexia, including malnutrition, excessive cytokine production, physical inactivity, and hormones.

Malnutrition and Food Intake

Systemic inflammation is associated with anorexia, low nutrient intake, and accelerated loss of skeletal muscle mass. However, RA is not associated with anorexia and is refractory to dietary intervention alone. There is great disparity between studies on the prevalence of malnutrition in RA patients, ranging from 26% to 71% (Elkan et al. 2009). However, in most studies, RA patients were treated with methotrexate, nonsteroid anti-inflammatory drugs, TNFα, and prednisolone, and all these antirheumatic treatments have been demonstrated to increase appetite.

In rats with adjuvant-induced arthritis, when arthritis is developing, body weight loss and muscle wasting are associated with a decrease in food intake. However, body weight loss and muscle atrophy are not only due to anorexia, since body and skeletal muscle weight loss in arthritic animals is higher than in pair-fed animals. Furthermore, pair-fed animals lose fat mass (Martín et al. 2008), but skeletal muscle is similar to that of controls with free access to food (Castillero et al. 2009). Experimental arthritis increases COX-2 levels in the hypothalamus (Gómez-SanMiguel et al. 2013), whereas administration of a COX-2 inhibitor, which

decreases prostaglandin levels, increases food intake in arthritic animals, even to levels higher than those observed in control animals (Granado et al. 2007). These data are agreement with those showing that increased release of prostaglandins, by COX-2 induction, plays an important role in inflammation-induced anorexia (Johnson et al. 2002).

When the external signs of the illness have reached their maximum value, arthritic rats have not anorexia, but their skeletal muscle wasting continues. Therefore, as reported in RA patients (Roubenoff et al. 1994), muscle atrophy in rodents with experimental arthritis seems to be mainly due to inflammation rather than to anorexia. Similarly, monkeys with collagen-induced arthritis have a decrease in body weight together with a decrease in food intake only during the early phase of the illness, and cachexia in these monkeys was not the consequence of a decrease in caloric intake (Horai et al. 2013). All these data suggest that the decrease in fat-free mass observed in RA patients during a flare is not only due to a decrease in food intake, but rather to an increase in resting energy expenditure. Although in some studies absolute resting energy expenditure in RA patients is not different to that of controls, after adjustment (for body cell mass or free-fat mass) resting energy expenditure was increased in RA patients (Roubenoff et al. 1994; Binymin et al. 2011).

Muscle Wasting

In RA patients with rheumatoid cachexia, muscle wasting is associated with a decrease in muscle cross-sectional area and strength (Beenakker et al. 2010). Arthritis-induced skeletal muscle atrophy is predominantly found in type II muscle fibers in RA patients (Edström and Nordemar 1974) and in experimental animals (Castillero et al. 2011; Horai et al. 2013). The decrease in muscle mass can be secondary to an increase in muscle proteolysis and/or a decrease in protein biosynthesis and muscle formation. Gibson et al. (1991) reported that RA-induced muscle atrophy was secondary to an increase in muscle proteolysis, whereas muscle protein synthesis in patients with RA (that had never received corticosteroids) was similar to controls. It has recently been reported that muscles of RA patients are able to increase protein synthesis after several different types of stimuli, such as acute resistant physical exercise and protein intake, in a way similar to that of controls (Mikkelsen et al. 2015).

A decrease in muscle remodeling and regeneration has not been reported in RA patients or in experimental models of arthritis. On the contrary, it is known that inflammation in skeletal muscle triggers satellite cell proliferation and muscle regeneration. Wróblewski and Nordemar (1975) reported that satellite cells in muscles are more common in RA patients and are regarded as an index of muscle regeneration. In addition, RA does not affect in vitro regenerative potential of human satellite cells compared to controls (Duijnisveld et al. 2011). Local macrophage-derived IGF-I (insulin-like growth factor-I) has been reported as a key factor in inflammation resolution and during muscle regeneration (Tonkin et al. 2015). These data are in agreement with those showing that there is an increase in both IGF-I and IGFBP-5 in skeletal muscle, together with the myogenic marker MyoD in

experimental arthritis (Castillero et al. 2009). All these data indicate that muscle atrophy in RA is primarily due to an increase in the rate of muscle protein breakdown rather than to a decrease in muscle formation.

Skeletal Muscle Proteolysis

Muscle wasting can occur through different proteolytic systems: autophagy, calcium-activated proteases, such as calpains and caspases, and the ubiquitin-proteasome system. Most of the muscle atrophy reported in experimental models of inflammatory diseases seems to be due to activation of the ATP-dependent ubiquitin-proteasome proteolytic pathway.

The ubiquitin–proteasome pathway mediates the degradation of the most abundant contractile proteins. The degradation of proteins occurs in the proteasome, which is a complex consisting of one or three large enzymes. Proteins destined for degradation by proteasome should be labeled with ubiquitin monomers. Conjugation of ubiquitin to proteins occurs in a series of steps involving three distinct enzymatic components: an E1 ubiquitin-activating enzyme, an E2 ubiquitin-conjugating enzyme, and an E3 ubiquitin ligating enzyme. The key enzymes in this process are two E3 ubiquitin ligases, muscle ring finger 1 (MuRF-1) and muscle atrophy F-box (MAFbx) or atrogin-1, which confer substrate specificity. In muscle wasting induced by different illnesses, MuRF1 and atrogin-1 are upregulated (Lecker et al. 2004). Therefore, these E3 ubiquitin-ligating enzymes are called atrogenes and serve as muscle atrophy markers. Arthritis in experimental animals decreases muscle protein content and increases the expression of both atrogenes atrogin-1 and MuRF1 in skeletal, but not in cardiac, muscle (Granado et al. 2005; Teixeira et al. 2013). The ubiquitin-proteasome pathway has not been analyzed in the skeletal muscle of RA patients without antirheumatic treatments. Mikkelsen et al. (2015) were unable to find differences in atrogin-1 and MuRF1 expression between RA patients and controls. However, their RA patients were treated with methotrexate and, as mentioned above, methotrexate has been reported to decrease proteolysis. At a cellular level, atrogene expression can be upregulated by activation of the two pathways: nuclear factor kappa B (NF-κB) and p38/MAP kinase. Between these two pathways, our group has reported NF-kB activation in skeletal muscle of arthritic rats (Gómez-SanMiguel et al. 2016), whereas the possible role of the second pathway in arthritis-induced muscle atrophy has not yet been studied. NF-kB is an important transcription factor that mediates many effects of the inflammatory response, including the expression of the two atrogenes: atrogin-1 and MuRF1 (Fig. 3).

Autophagy is another proteolytic system that is involved in the pathogenesis of muscle wasting under different catabolic conditions. An increase in autophagic activity in the atrophic muscle has been observed in experimental models of cancer and sepsis, as well as in cancer patients with cachexia (Aversa et al. 2016). Although studies in RA patients assessing the role of autophagy in rheumatoid cachexia are still lacking, it can play a role in muscle wasting, since autophagy plays a key role in the pathogenesis of rheumatoid arthritis. It is increased in the synovial tissues of

patients with active RA and the autophagy markers such as LC3II are correlated with the disease activity and severity (Zhu et al. 2017). Arthritis-induced by adjuvant injection in rats increases the autophagy marker genes such as LC3b, as well as the conversion of LC3b I to LC3b II by lipidation in skeletal muscle. Furthermore, treatments that decrease muscle wasting downregulate atrogene and autophagy in this experimental model of rheumatoid arthritis (Gómez-SanMiguel et al. 2016). All these data suggest that, as it has been reported in sepsis, arthritis-induced muscle proteolysis is mainly due to an increase in the activity of ubiquitin-proteasome pathways and autophagy.

Mediators of Muscle Proteolysis

Muscle proteolysis and wasting in RA can be the result of inflammation, low physical activity, decreased secretion of IGF-I, and glucocorticoid therapy.

Inflammation. In rheumatoid cachexia, relative excess of pro-inflammatory cytokines seems to be the central feature. The pro-inflammatory cytokine tumor necrosis factor-α (TNFα) and interleukin 1ß (IL-1ß) not only have a central role in the pathogenesis of joint inflammation and destruction in RA (Fig. 1), but also exert a powerful influence on whole-body protein and energy metabolism (Fig. 4). TNFα and IL-1β levels are increased in RA patients during flares, but their levels are undetectable during the quiescent phases of disease (Morley et al. 2006). These cytokines, together with IL-6, and IFN-γ are also known as sarcoactive "muscle-active" cytokines and have been proposed as inducers of muscle wasting (Rall et al. 1996). Anti-TNFα biologic agents are still the therapy of choice for rheumatoid arthritis. It has been postulated that TNFα can contribute to muscle wasting directly, increasing skeletal muscle proteolysis (Zhao et al. 2015), and indirectly decreasing anabolic hormones and growth factors that regulate muscle mass, such as IGF-I (Granado et al. 2006a). Therefore, it is logical to think that blocking TNFα would attenuate cachexia in RA patients. However, only 5 out of 14 studies with anti-TNFα treatment reported significant increases in BMI and/or body weight (Peluso and Palmery 2016). Most studies show that anti-TNFα treatments reduce disease activity and increase body weight, but this increase is mainly due to an increase in fat mass in RA patients (Engvall et al. 2010). Similarly, TNFα blockade in arthritic rats increases food intake, serum IGF-I, body weight, and fat mass, but is unable to prevent muscle proteolysis and atrophy (Granado et al. 2006a, b).

IGF-I deficiency. Circulating IGF-I plays an important role in the control of skeletal muscle mass; it decreases proteolysis and increases protein synthesis and myogenesis (Fig. 5). Rheumatoid arthritis is associated with a decrease in circulating IGF-I or its bioavailability (Engvall et al. 2008). Similarly, experimental arthritis also decreases circulating and liver IGF-I (Castillero et al. 2009). In addition, a dysfunction of IGF binding proteins (IGF-BPs) in serum and skeletal muscle has been reported in humans and experimental animals with arthritis (Engvall et al. 2008; Castillero et al. 2009). Decreased circulating IGF-I levels in

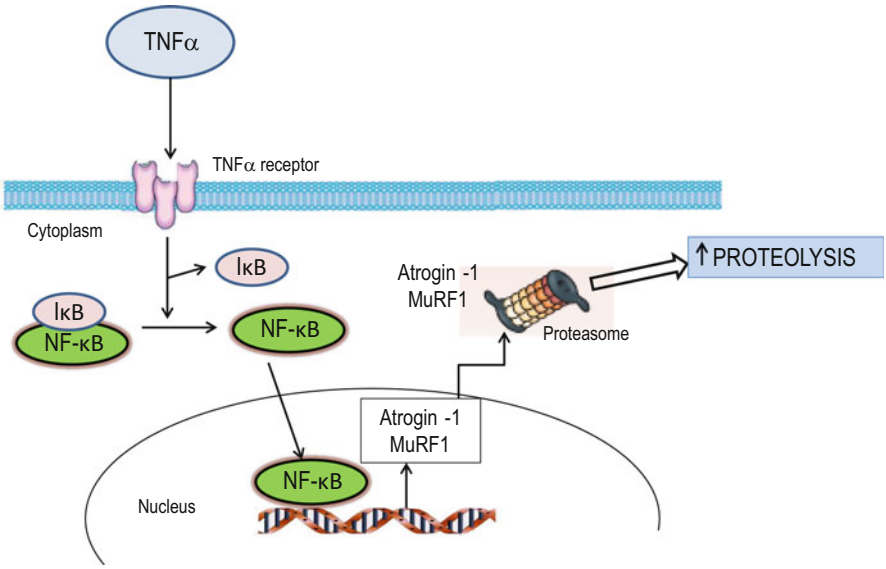

Fig. 4 TNFα receptor signaling pathway. Signaling initiates with the binding of TNFα to its receptor. TNFα induces proteolysis via activation of the NF-κB pathway. NFκB is in the inactive state when it is sequestered in cytoplasm by a family of inhibitory proteins called IκB. In response to TNFα, IκB is degraded. This leads to nuclear translocation of NFκB and activation of NFκB-mediated gene transcription, resulting in an increased transcription of atrogin-1 and MURF1, which are involved in the proteolysis of myofibrillar proteins via the proteasome system. *IkB* I-kappa-B-beta, *MURF1* muscle RING finger-containing protein 1, *NFκB* nuclear factor kappa B, *TNFα* tumor necrosis factor α

RA are correlated with both disease severity (activity, joint destruction, and disability) and with muscle atrophy (Baker et al. 2015b). Taking into account that circulating, but not muscle IGF-I, is decreased in arthritis and that systemic administration of IGF-I ameliorates arthritis-induced skeletal muscle atrophy (López-Menduiña et al. 2010), the decrease in circulating IGF-I during inflammation can be one of the causes of muscle wasting.

Low physical activity. It has been postulated that muscle wasting and the reduction in circulating IGF-I observed in RA patients is more related to their sedentary lifestyle than to inflammation (Lemmey et al. 2001). However, patients with quadriceps atrophy caused by forced leg immobilization, after tibial fracture, have a decrease in the rate of muscle protein synthesis (Gibson et al. 1987), whereas as mentioned above, muscle protein biosynthesis was unaffected by RA. Recently, Teixeira et al. (2013) demonstrated in mice that arthritis and immobilization activate skeletal muscle atrophy, but the pathways activated in the two experimental models were different. In addition, arthritis, as in other inflammatory diseases, mainly induces atrophy of the type II myofibers (Edström and Nordemar 1974; Castillero et al. 2011). On the contrary, disuse-induced muscle atrophy mainly affects type I

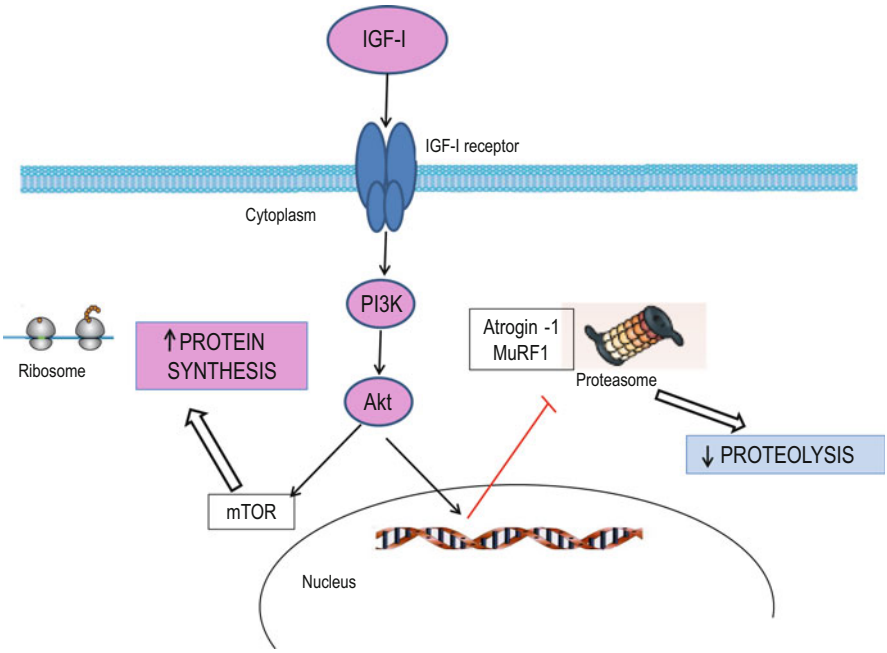

Fig. 5 Schematic illustration of IGF-I signaling pathway. Lines (in *red*) ending with a perpendicular segment represent inhibitory pathways. The binding of IGF-I to IGF-I receptor activates PI3K, which induces Akt activation. When activated, Akt stimulates protein synthesis through mTOR. Akt also inhibits protein degradation by activating the expression of MuRF1 and atrogin1. *Akt* protein kinase B, *IGF-I* insulin-like growth factor-1, *mTOR* mammalian target of rapamycin, *MURF1* muscle RING finger-containing protein 1, *PI3K* phosphatidylinositol-3 kinase

myofibers (Wang and Pessin 2013). Therefore, we can assume that muscle atrophy in arthritis is not mainly due to decreased mobility.

Glucocorticoid therapy. When used in excess, glucocorticoids have a catabolic effect on skeletal muscle that is opposite to that of IGF-I and results in muscle atrophy (Fig. 6). Endogenous glucocorticoids often increase in acute inflammatory diseases, but RA patients have normal or decreased cortisol levels compared to healthy controls (Eijsbouts et al. 2005). Nevertheless, exogenous glucocorticoids are widely used to treat inflammation in RA. In spite of the stimulatory effect of glucocorticoids on food intake in humans, chronic elevation of their levels induces muscle atrophy and weakness (Bodine and Furlow 2015). In women with juvenile idiopathic arthritis, glucocorticoid treatment has a negative effect on muscle and bone mass, whereas it has a positive effect on fat mass (Brabnikova Maresova et al. 2014). Glucocorticoid treatment decreases muscle protein biosynthesis in RA patients (Gibson et al. 1991), and when glucocorticoids are chronically elevated, they also increase muscle proteolysis (Bodine and Furlow 2015). In addition to their effects on muscle cells, glucocorticoids inhibit plasma concentrations of IGF-I, an anabolic hormone that increases muscle mass.

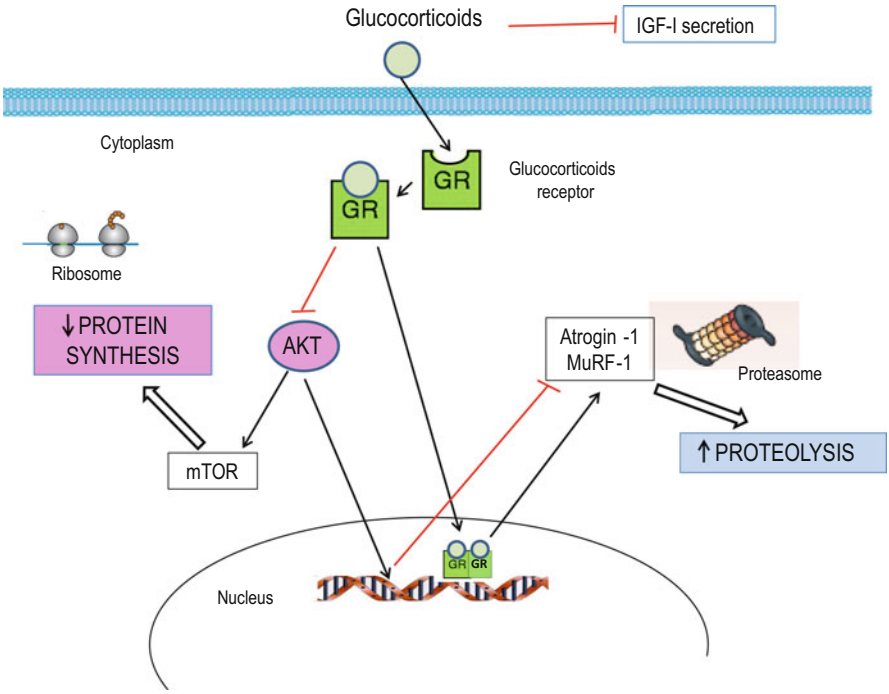

Fig. 6 Mechanism of action of glucocorticoids. Lines (in *red*) ending with a perpendicular segment represent inhibitory pathways. Glucocorticoids interact with cytosolic glucocorticoid receptor. The majority of the effects occur via glucocorticoid receptor dimerization, nuclear translocation to nucleus, and transcription activation. Nuclear GR increases MuRF1 and atrogin-1 expression, which induces proteolysis by the proteasome system. A reduction in protein synthesis occurs via cytosolic ligand bound GR monomers that inhibit Akt activation and mTOR system. *Akt* protein kinase B, *GR* glucocorticoid receptor, *IGF-I* insulin-like growth factor-1, *mTOR* mammalian target of rapamycin, *MURF1* muscle RING finger-containing protein 1, *PI3K* phosphatidylinositol-3 kinase

Policies and Protocols

Rheumatoid arthritis is a systemic inflammatory disease that often induces skeletal muscle proteolysis and atrophy. This chapter points out the importance of rheumatoid cachexia found in rheumatoid arthritis patients as a consequence of the state of chronic inflammation. The decrease in skeletal muscle mass in these patients increases their morbidity and mortality and contributes to the decline in their quality of life. Skeletal muscle health is an important contributor to overall health and wellbeing, and muscle wasting limits mobility and exacerbates the impact of other long-term conditions leading to premature death. Rheumatoid cachexia is often associated with no modifications in body weight, since the decrease in skeletal

muscles mass is associated with an increase in fat mass. This altered body composition is associated with the development of several comorbidities, primarily cardiovascular disease, and obesity that have a negative impact on functional ability, pain, and global health in the patients.

Body composition is generally not assessed in rheumatoid arthritis patients. Therefore, specific policies aimed to detect rheumatoid cachexia. Assessing both disease activity and body composition, by measuring both muscle mass and fat mass, should be considered in order to prevent the development of rheumatoid cachexia and improving outcomes in rheumatoid arthritis.

Dictionary of Terms

- **Atrogenes** – Enzymes that conjugate ubiquitin to muscle proteins. There are two ubiquitin ligases specifically involved in myofibrillar protein degradation: mouse atrophy gene-1 (atrogin-1) also described as rat muscle atrophy F-box (MAFbx) and rat muscle RING finger 1 (MuRF1). The polyubiquitin-tagged protein is recognized by the proteasome and is degraded to small peptides.
- **Body mass index (BMI)** – Weight divided by height squared.
- **Ubiquitin-proteasome proteolytic system** – It is a complex multicatalytic enzyme responsible for intracellular protein degradation. This system consists of three sequential processes: recognition of the protein for degradation, addition of several copies of the small protein ubiquitin onto proteins to mark them, and proteolysis of the protein inside the proteasome system.
- **Resting energy expenditure** – Represents the energy expended at rest, although not necessarily basal conditions, by a fasted individual in a thermo-neutral environment. There are many individual factors that contribute to a variation in this parameter: age, gender, body size, body composition, muscle mass, ethnicity, hormonal status, and genetic and environmental influences.
- **Rheumatoid cachexia** – A decrease in skeletal muscle mass that can be associated with an increase in fat mass. In this condition, there is no body weight loss and body mass index is within the normal values.

Summary points

- Skeletal muscle atrophy has been reported in two-thirds of people with rheumatoid arthritis, where muscle type II is more affected than type I.
- Rheumatoid arthritis patients can have normal body mass index values, since their muscle mass is decreased and their fat mass is increased.
- Muscle skeletal wasting in rheumatoid arthritis patients seems to be secondary to an increase in muscle proteolysis rather to a decrease in muscle generation.
- The proteolytic pathways ubiquitin-proteasome and autophagy have been found elevated in experimental models of rheumatoid arthritis.

- Systemic inflammation and insulin-like growth factor-1 deficiency contribute to skeletal muscle atrophy in rheumatoid arthritis patients.
- Chronic elevation of glucocorticoid levels by glucocorticoid therapy can increase the loss of muscle mass and function in rheumatoid arthritis RA patients.

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Malnutrition in Older Adults in the United States

45

Angela M. Fraser

Contents

Abstract

The scientific literature clearly establishes the relationship between good nutrition and healthy aging. Unfortunately though, a large number of older Americans are at risk for poor nutrition not only because of the aging process but because of poverty, rurality, and/or lack of access to good quality food. Poor nutrition can easily translate into malnutrition, a leading cause of morbidity and mortality among older Americans. Malnutrition is not an inevitable side effect of aging, it can be prevented or at least managed. Medical conditions and lifestyle, social and psychological factors all play a role in increasing one's risk for malnutrition. Prevention of or treatment for malnutrition must be improved to reverse its deleterious effects among older Americans. Despite the availability of evidence that demonstrates the benefits of good nutrition for healing and recovery, there is no consensus for implementing optimal nutrition care with gaps associated with

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nutrition screening, assessment, intervention, monitoring, and overall care for malnourished and at-risk hospitalized older adults. Moreover, the US government spends $51.3 billion each year to prevent malnutrition in community-dwelling older adults. In 1965, the US Congress passed the Older Americans Act (OAA), which aims to provide support (including nutrition) services for older Americans. Title III of the OAA (of the seven titles) specifically addresses the delivery of nutrition services, including nutrition education, to older adults. To date, few evidence-based nutrition education programs target this population making it clear that thoroughly tested curricula are needed, especially programs tailored to the culture and context of the older adult and their environment.

Keywords

Older adults · Malnutrition · Nutrition screening · Nutrition education · Lifestyle factors · Social factors · Medical conditions · Nutrition education · Poverty · Rurality

List of Abbreviations

AAA	Area Agency on Aging
AARP	American Association of Retired Persons
AoA	Administration on Aging
eCOMS	Electronic clinical quality measures
ENP	Elderly Nutrition Program
MNA	Mini nutritional assessment
MNA-SF	Mini nutritional assessment, short form
OAA	Older Americans Act
SNAP	Supplemental Nutrition Assistance Program
SPM	Supplemental poverty measure
US	United States

Malnutrition in Older Adults in the United States

The United States is rapidly becoming an "older" nation. In 2014, 42 million Americans (1 in 7) were age 65 or older. By 2030, this number is projected to increase to 72 million (or 1 in 5) (Federal Interagency Forum on Aging-Related Statistics 2016). In addition to the US population growing older, Americans in general are becoming less healthy. Compared to 16 other developed countries, the United States ranks last in terms of the health status of its population (Institute of Medicine 2013).

Historically, the most common causes of death among Americans were infectious disease or acute illness (Centers for Disease Control and Prevention 2009). Today, the most common causes of death are chronic diseases – heart disease, diabetes, cancer, and stroke – of which many are associated with poor nutrition (Kochanek et al. 2016). One recent study of older Americans illustrates the magnitude of the situation. Nearly all study respondents (92%) reported having at least one chronic

disease and 77% at least two (National Council on Aging 2015a). The high rates of obesity among older Americans, as well as the general population, are further compromising the health of Americans. Obesity is a well-recognized risk factor for many chronic diseases, including diabetes, heart diseases, and some cancers. In 2011–2014, about 35% of older Americans were obese, compared to 22% in 1988–1994 (Federal Interagency Forum on Aging-Related Statistics 2016).

The scientific literature clearly establishes that good nutrition is the foundation of healthy aging as many chronic diseases are treated by consumption of a healthful diet. Unfortunately though, a large number of older Americans are at risk for poor nutrition not only because of the aging process but because of poverty, rurality, and/or lack of access to good quality food. These three conditions alone or in combination can impact one's nutritional status and hence one's overall health and ability to live independently longer. Helping older Americans to live independently is vital because aside from personal reasons it costs less than institutionalized care. Older adults who maintain a good nutritional status could have decreased Medicare expenditures. Medicare, a social health insurance program available to Americans who are age 65 or older and to the disabled, is used by 55 million people and constitutes about 15% of all federal spending in the United States (Centers for Medicare and Medicaid Services 2015). Moreover, and most importantly, almost 90% of older Americans want to live in their home and community as long as possible, according to a report by the AARP Public Policy Institute and the National Conference of State Legislatures (Farber et al. 2011). The bottom line is that it costs a lot less for someone to age in their home than to be admitted into a long-term care facility (Clark 2013).

Malnutrition

Poor nutrition can easily translate into malnutrition, a leading cause of morbidity and mortality among older Americans, if it is not identified quickly then properly managed. Malnutrition is defined as the inadequate intake of nutrients, particularly protein, which contributes to the development of acute and chronic disease conditions. Malnutrition and weight loss can also contribute to sarcopenia, the age-associated loss of skeletal muscle mass and function, which directly effects recovery, mobility, and the ability to live independently. Lastly, malnourished older adults also have reduced homeostatic reserves limiting their ability to recover from an acute illness or injury making them more vulnerable to medical complications and an increased likelihood of admission to a long-term care facility (Starr et al. 2015).

Published evidence suggests that 20–50% of all US hospital patients are at risk for or are malnourished at the time of hospital admission (Barker et al. 2011) and up to 20% of malnourished patients and 38% of well-nourished patients experience nutritional decline during a single hospital stay (Braunschweig et al. 2000). It is estimated that as many as 65% of older Americans may be malnourished upon hospital admission (Sullivan et al. 1995). To further exacerbate this problem,

hospitalized patients are more vulnerable to nutritional decline because of dietary restrictions, treatments, and medical conditions, as well as, poor appetite and gastrointestinal problems they may experience as an outcome of hospitalization. In one study, Sullivan et al. (1999) noted that 20% of hospitalized older patients had an average nutrient intake of less than 50% of their calculated maintenance energy requirements. In addition, patients (across all age groups) who were malnourished while hospitalized had a greater risk of complications, readmissions, and length of stay, which is estimated to increase healthcare costs by 300% (Correia and Waitzberg 2003). Nutritional status is another important factor in "posthospital syndrome," which can result from the stress of hospitalization (Krumholz 2013).

In addition, disease-associated malnutrition has also been identified as a prevalent condition, particularly among older Americans (Goates et al. 2016). Malnutrition not only intensifies the effect of existing disease conditions but can also lead to increased incidents of infection, including flu, pneumonia, and foodborne disease. To illustrate, older adults are ten times more likely than the general population to develop complications or die from foodborne disease presumably because of their age and nutritional status (Buzby 2002).

Because there are no national prevalence rates for malnutrition among older Americans, much of what is known is derived from research studies conducted in healthcare settings (Mogensen and DiMaria-Ghalili 2015). Current estimates of the prevalence of adult malnutrition range from 15% to 60% depending on the patient population and criteria used to identify its occurrence. According to the National Resource Center on Nutrition, Physical Activity and Aging, it is believed that between 35% and 50% of older residents in long-term care facilities are malnourished. To illustrate the magnitude (and cost) of this problem, in 2014, about 67,000 paid, regulated long-term care providers served about nine million people in the United States. Excluding Medicare spending on home health and skilled nursing facilities, total long-term care services spending was US$241.7 billion in 2011. Less is even known about the rate of malnutrition in community-dwelling older Americans. DiMaria-Ghalili et al. (2013) estimated the prevalence of malnutrition using a probability sample of 3209 community-dwelling older adults (aged 60 years and older) to be 56.3% at risk of malnutrition and 5.9% malnourished. However, these statistics need to be used with caution as the sample was limited to one geographic region in the United States.

Risk Factors for Malnutrition

Malnutrition is not an inevitable side effect of aging, it can be prevented or at the very least managed. Medical conditions and life-style/social, and psychological factors all play a role in increasing one's risk for malnutrition (Table 1). Medical conditions, of which there are many, can lead to decreased appetite or difficulty eating (Shils and Shike 2006). In 2014, 22% of older Americans reported having a disability, such as limitation in vision, hearing, mobility, communication, cognition, and self-care, with women more likely to report a disability than men (24% vs. 19%)

Table 1 Risk factors for malnutrition

Medical conditions
• Poor appetite
• Poor dentition, other oral problems and dysphagia
• Loss of taste and smell
• Respiratory disorders
• Gastrointestinal disorders
• Endocrine disorders
• Infections
• Physical disability
• Drug interactions
Lifestyle and social factors
• Lack of knowledge about food, cooking, and nutrition
• Isolation/loneliness
• Poverty
• Inability to shop or prepare food
Psychological factors
• Confusion
• Dementia
• Depression
• Bereavement
• Anxiety

(Federal Interagency Forum on Aging-Related Statistics 2016). All of these disabilities can directly affect one's ability to care for oneself as well as shop for groceries, prepare meals, and feed oneself – all essential to maintaining good nutritional status (Getty et al. 2016). Long-term health illnesses, such as dementia and stroke, can also affect one's ability to prepare and eat healthy meals as well as directly impact one's appetite (National Institute on Aging 2015).

Dental problems can also affect the taste of food and make chewing nearly impossible (Moynihan 1995). Older Americans with the worst oral health are, not surprisingly, those who are economically disadvantaged, lack insurance, and are members of racial/ethnic minorities. Many older Americans do not have dental insurance because they often lose dental benefits when they retire and Medicare does not cover dental costs. The situation may be worse for older women, who generally have lower incomes and may never have had dental insurance. About 25% of adults age 60 or older no longer have any natural teeth. Missing teeth can affect what one eats as individuals without teeth tend to prefer soft, easily chewed foods. Moreover, because dentures are not as efficient for chewing food as natural teeth, denture wearers also may eat soft foods and avoid fresh fruits and vegetables known to be essential to good health. In addition, older Americans continue to experience dental decay having new tooth decay at higher rates than children. Severity of periodontal disease increases with age – about 23% of 65- to 74-year-olds are classified as having severe disease with men more likely than women to have

more severe disease. At all ages, people at the lowest socioeconomic level are reported to experience the most severe periodontal disease.

Dry mouth, a side effect of some prescription drugs, and diseases like Parkinson's can interfere with swallowing, hence decrease food consumption (Turner and Ship 2007). Moreover, most older Americans take both prescription and over-the-counter drugs (Centers for Disease Control and Prevention 2006) with residents of long-term care facilities – about 5% of all older Americans – taking an average of eight drugs each day. Over 400 commonly used medications can be the cause of a dry mouth. In addition, painful conditions that affect the facial nerves are more common among the elderly and can be debilitating. These conditions can affect mood, sleep, and oral-motor functions, such as chewing and swallowing. Neurological diseases, such as Parkinson's disease, Alzheimer's disease, Huntington's disease, and stroke also negatively impact oral sensory and motor functions (Centers for Disease Control and Prevention 2006). In addition, some prescription drugs commonly prescribed for older adults can suppress the appetite or affect the way food tastes, causing nausea and vomiting. This particular problem is often compounded because many older Americans take multiple medications.

Taste and smell also diminish as one ages making it difficult to distinguish off-flavors related to spoiled (and potentially unsafe) foods or to even enjoy the taste of food resulting in less food consumed (Boyce and Shone 2006). Additionally, older adults are more likely than any other group to have dietary restrictions, including limits on sodium, fat, protein, and sugars, which can result in bland and unappealing food choices so much so that older adults stop eating (Zeanandin et al. 2012). Lastly, vision also weakens as one ages. The ability to distinguish between colors decreases as one ages, especially between blues and greens, making food preparation and detecting spoiled food more difficult.

In addition to medical conditions, life-style/social and psychological factors also increase the risk for malnutrition among older Americans (Shils and Shike 2006). In 1966, 29% of older Americans lived below the poverty threshold but by 2014, this number decreased to over 4.5 million (or 10% of the total population). However, this figure might not accurately capture the extent of poverty among older Americans. The US Census Bureau uses monetary income thresholds that vary by family size and composition to determine who is living in poverty (U.S. Census Bureau 2016). However, in 2011, the US Census Bureau released a Supplemental Poverty Measure (SPM), which takes into account regional variations in cost of living as well as noncash benefits received, such as food assistance programs (Supplemental Nutrition Assistance Program, National School Lunch Program, Women, Infants, and Children Program), rent subsidies, and energy assistance (Short 2015) and non-discretionary expenditures. The SPM indicates that 14% of older Americans (not 10% as determined using the standard poverty measure) are living in poverty. This difference is mainly attributed to out-of-pocket expenses, such as increased healthcare and prescription drug costs. As health care, prescription drug, and utility costs increase, many older Americans cut back on their food budget hence leading to decrease food consumption (DiMaria-Ghalili 2014). As a share of total expenditures, in 2014 healthcare costs increased with age (Health Care Cost Institute 2015).

Across all age groups, healthcare expenditures were reported to be substantially higher for those who are age 85 or older compared to those in the younger age groups. This is important to note as by 2030, it is predicted that the fastest growing age group in the United States will be those who are age 85 or older, exceeding 8.9 million people (United States Census Bureau 2014). For example, the share of healthcare expenditures for those who are age 75 or older was 16% or more than double that for those who are younger (ages 45–54) (7%) (Federal Interagency Forum on Aging-Related Statistics 2016). This problem is amplified among those who are classified as poor/near poor. Moreover, these expenditures are expected to continue to increase. From 1977 to 2013 the percentage of household income older Americans allocated to out-of-pocket spending for healthcare services increased from 12% to 17% (Federal Interagency Forum on Aging-Related Statistics 2016).

Many older Americans, particularly those who live in rural areas and some urban neighborhoods do not have access to grocery stories that have a variety of good quality food. Instead those living in these areas must rely upon small convenience-type stores that typically sell highly processed foods, high fat and carbohydrate snacks, and few fresh foods (Inagami et al. 2006). These food deserts, a term defined by the US Department of Agriculture, are often found in impoverished urban areas and rural areas (Fig. 1).

For older Americans who live far from a supermarket or grocery store, not having access to a private vehicle makes it difficult to shop so they must rely on public or alternative means of transportation, which is often not available in rural areas in the United States. However, even when grocery stores are nearby, older Americans might not drive. One study suggests that more than 600,000 people age 70 or older stop driving each year and become dependent on others to meet their transportation needs (Foley et al. 2002). Poor vision, memory impairment, and an inability to care for oneself were common reasons cited by older Americans for why they stopped driving.

Another risk factor for malnutrition is a solitary life. Many older adults (29%; 13.3 million) live alone with almost half of women age 75+ (46%) living alone. The possible negative effects of living alone can be worsened as the proportion of leisure time older Americans spend socializing and communicating – visiting friends or attending or hosting social events – declines with age. The time spent socializing and communicating was about 11% for those ages 55–64 and 9% for those age 75 and over (Federal Interagency Forum on Aging-Related Statistics 2016). Social contact can also have a positive effect on eating well and can increase morale and well-being, factors that can contribute to lack of appetite (Higgs and Thomas 2016).

Although frequently unrecognized and untreated in older Americans, depression affects an estimated six million older Americans, with change in food intake a common symptom (National Alliance on Mental Illness 2009). Depression has been found to predict poor quality of life (Goldney et al. 2000), cognitive decline (Comijs et al. 2001), functional decline (Bruce et al. 1994; Penninx et al. 1998), mortality (Barefoot and Schroll 1996; Blazer et al. 2001), and resource use (Beekman et al. 2002). The proportion of older Americans is higher in rural areas than in urban areas, with these numbers expected to increase in the next decade

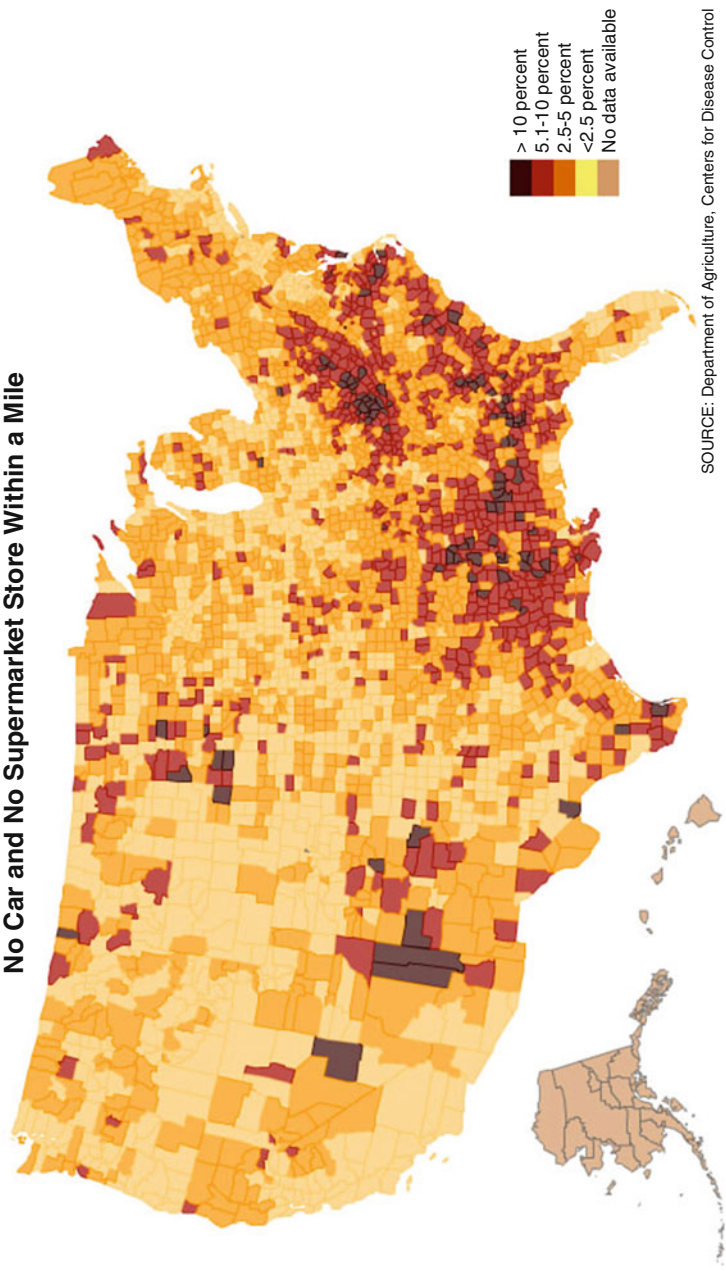

Fig. 1 : Food deserts in the United States

(Baernholdt et al. 2012). The potential negative effects of rural life on depression include long distances to social activities and services that could limit opportunities for social interaction and engagement. One study of community-dwelling older adults according to geographic location showed lower scores on social functioning in rural areas than urban areas suggesting that rural older adults may be socially isolated (Baernholdt et al. 2012).

Food insecurity defined as *"the inability to acquire or consume an adequate quality or sufficient quantity of food appropriate for one's health in socially acceptable ways, or the uncertainty that one will be able to do so"* (Wolfe et al. 2003) also contributes to malnutrition. The overall rate of food insecurity among Americans as a whole remains above 18% – higher than the 2007 – with those at greatest risk – the poor/near poor, minorities, the unemployed and disabled, and those residing in the southeastern United States. Even when one has food, the quality of that food may not meet one's nutritional needs, sometimes called overnutrition, which is another form of malnutrition. Whites have the lowest rate of food insecurity (15%) followed by Hispanics (30.4%) and Black (32.4%). Currently married and never married respondents have the lowest rates of food insecurity, whereas the widowed, divorced and separated have the highest. Older Americans who reported food insufficiency were more likely to have lower mean intakes of several nutrients; lower intake of the vegetable and meat groups; lower dietary variety; lower mean serum levels of certain nutrients; higher risk of being underweight, and in poor or fair health.

Screening for Malnutrition

Prevention of or treatment for malnutrition must be improved to reverse its effects among older Americans. However, a review of nationally representative data on cost and utilization indicated that, in 2010, only 3.2% of patients had a diagnosis of malnutrition, suggesting malnutrition may be under-recognized and underdiagnosed in hospitals (Corkins et al. 2014). Despite the availability of high-quality evidence that demonstrates the benefits of nutrition for healing and recovery, there is no consensus among healthcare professionals for implementing optimal nutrition care with gaps associated with nutrition screening, assessment, intervention, monitoring, and overall care for malnourished and at-risk hospitalized older adults. Nutrition screening can trigger a nutrition assessment for patients found to be at risk. The nutrition assessment is the basis upon which diagnosis, care plans, and treatments are made. For instance, a national survey of hospital-based health professionals in the United States showed that 36.7% reported they conduct nutrition screening at admission, 50.8% did so within 24 h, and only 69% documented the findings in the patient's medical record (Patel et al. 2014). In addition, no national benchmarking of malnutrition in acute-care hospitals exists in the United States because malnutrition screening and assessment are not standardized as to date there is no universal definition of malnutrition. In 2012, the Academy of Nutrition and Dietetics and the American Society for Parenteral and Enteral Nutrition collaborated to develop clear characteristics to define three broad categories of malnutrition

Table 2 Summary of malnutrition characteristics established by the Academy of Nutrition and Dietetics and the American Society for Parenteral and Enteral Nutrition in 2012

Type of malnutrition	Acute illness or injury		Chronic illness		Social and environmental circumstances	
Degree of malnutrition	Nonsevere (moderate) malnutrition	Severe malnutrition	Nonsevere (moderate) malnutrition	Severe malnutrition	Nonsevere (moderate) malnutrition	Severe malnutrition
Energy intake	<75% of estimated need for >7 days	≤50% of estimated needs for ≥5 days	<75% of estimated need for ≥1 month	<75% of estimated need for ≥1 month	<75% of estimated need for ≥3 months	≤50% of estimated needs for ≥1 month
Weight loss	1% to 2% in 1 week	>2% in 1 week	5% in 1 month	>5% in 1 month	5% in 1 month	>5% in 1 month
	5% in 1 month	>5% in 1 month	7.5% in 3 months	>7.5% in 3 months	7.5% in 3 months	>7.5% in 3 months
	7.5% in 3 months	>7.5% in 3 months	10% in 6 months	>10% in 6 months	10% in 6 months	>10% in 6 months
			20% in 1 year	>20% in 1 year	20% in 1 year	>20% in 1 year
Body fat wasting	Mild	Moderate	Mild	Severe	Mild	Severe
Muscle wasting	Mild	Moderate	Mild	Severe	Mild	Severe
Presence of edema	Mild	Moderate to severe	Mild	Severe	Mild	Severe
Grip strength	Not applicable	Measurably reduced	Not applicable	Measurably reduced	Not applicable	Measurably reduced

(White et al. 2012). These characteristics include the etiology of malnutrition, recognizing the influence of illness and inflammation on nutritional status. These types of malnutrition have subcategories of moderate (or nonsevere) or severe malnutrition, depending on specific parameters and described in Table 2.

Addressing malnutrition is also aligned with the Triple Aim and National Quality Strategy priorities (Agency for Healthcare Research and Quality 2017). Clinical consensus recommendations highlight the need for early identification and nutrition care in managing malnutrition in healthcare settings (Tappenden et al. 2013). Two studies showed that implementation of optimal nutrition care from admission to discharge improved identification of high-risk patients and decreased time to nutrition consult, length of hospital stay, and 30-day readmission rate (Brugler et al. 1999; Somanchi et al. 2011).

The Joint Commission, a US-based nonprofit tax-exempt organization that accredits more than 21,000 health care organizations and programs in the United States, requires nutrition screening upon hospital admission. But, even if a patient is determined to be at risk for malnutrition or is malnourished, this might not be properly coded in the medical record. Another problem is that screening is routinely not performed at discharge. A nationally representative sample of US hospital discharges from the 2010 Healthcare Cost and Utilization Project reported that

Table 3 Determine checklist of risk factors for malnutrition

Disease
• Chronic diseases impairing appetite or ability to eat
• Mood and cognitive disorders
Eating poorly
• Inadequate food or poor food quality/caloric intake
• Skipping meals; drinking too much alcohol
Tooth loss or mouth pain
• Missing or rotting teeth
• Missing or poorly fitting dentures
Economic hardship
• Inadequate income to buy food
Reduced social contact
• Single, widowed
• No family or community supports
Multiple medicines
• Causing nausea, taste alterations, constipation, or anorexia
• Includes dietary supplements, over-the-counter medications, and herbal products
Involuntary weight loss or gain
• Due to undiagnosed medical conditions such as cancer, malabsorption syndromes, metabolism-altering states
Needs
• Assistance with self-care
• Problems with transportation for shopping
• Problems with ambulation or cooking
Elder years
• Older than age 80

only 3.2% of patients were diagnosed with malnutrition even though other studies suggest a much higher prevalence of malnutrition (Corkins et al. 2014). Many studies report a direct relationship between the degree of malnutrition and increased length of hospital stay, treatment costs, and readmission to hospital rates. In 2010, 38.7% of hospital discharges and 44.8% of days of hospital care were attributed to older adults (Centers for Disease Control and Prevention 2012).

The Academy of Nutrition and Dietetics has identified clinical quality measures that could result in early detection and prevention of malnutrition. These electronic clinical quality measures (eCQMs) could improve patient outcomes and reduce cost. The four eCQMs focus on malnutrition care in patients' ages 65+ years in the hospital setting (Table 3). They include: (1) completion of a malnutrition screening within 24 hours of admission; (2) completion of a nutrition assessment for patients identified as at-risk for malnutrition within 24 hours of a malnutrition screening; (3) nutrition care plan for patients identified as malnourished after a completed nutrition assessment; and (4) appropriate documentation of a malnutrition diagnosis. The eMeasures are just one component of a broader initiative called Malnutrition

Quality Improvement Initiative, which aims to advance evidence-based, high-quality, and patient-driven care for hospitalized older adults who are malnourished or at-risk for malnutrition (Academy of Nutrition and Dietetics 2016).

Older adults may also become more vulnerable to becoming malnourished during recovery, especially if they have a weakened state, are unable to take care of themselves and live alone. Nutrition screening must be administered after an older patient has been discharged from the hospital or a skilled care or rehabilitation facility. It must also become part of routine geriatric assessments. The mini nutritional assessment (MNA) tool, developed specifically for older adults, has two validated forms available in 27 languages. The short form (MNA-SF) consists of seven items that assess changes in food intake, weight, mobility status, current disease or psychological stress, presence of neuropsychological problems, and body mass index. The long form MNA consists of 18 items – all 7 items on the MNA-SF and anthropometric measurements (Nestle Nutrition Institution 2009).

Preventing Malnutrition

The US government spends approximately $51.3 billion each year to prevent malnutrition in community-dwelling older adults as cost of treating it and the treatment of associated chronic diseases is high. In 1965, the US Congress passed the Older Americans Act (OAA), which aims to provide support (including nutrition) services for older adults. The legislative intent of OAA is to make community-based services available to older adults who may be at risk of losing their independence with a focus on those in social or economic need or who are classified as low-income minorities (National Committee to Preserve Social Security and Medicare 2016). Title III of the Older Americans Act (of the seven titles) specifically addresses the delivery of nutrition services to older adults through regional Area Agencies on Aging (AAAs). OAA nutrition programs, provided through the US Department of Health and Human Services, include congregate meals, which are meals served at senior centers and in other group settings, and home-delivered meals to more than 2.4 million older adults each year. The Administration on Aging (AoA) Elderly Nutrition Program (ENP), which is charged with executing the OAA, provides grants to states to support nutrition services to older Americans throughout the United States.

The goal of the ENP is to improve the nutritional status of older Americans and to offer them opportunities to socialize and create a social network. The meals and other nutrition services provided through ENP are provided in a variety of group settings, such as senior centers, faith-based settings, schools, as well as delivered to the homes of those who are home bound. Meals served under the ENP must provide at least one-third of the recommended dietary allowances established by the Food and Nutrition Board of the Institute of Medicine of the National Academy of Sciences, as well as the Dietary Guidelines for Americans (Administration on Aging 2014). In practice, participants typically receive more – approximately 40–50% of required nutrients. The ENP also provides a range of

related services through the aging network's estimated 4000 nutrition service providers, such as nutrition screening, assessment, education, and counseling. In fact, one older American can be fed a home-delivered meal for an entire year for about the cost of 1 day in a hospital (Meals on Wheels America 2015). In addition to providing nutrition and nutrition-related services, the ENP provides an important link to other needed supportive in-home and community-based services, such as home health aide services, transportation, physical activity programs, and even home repair and home modification programs. Participants must be age 60 or older. While there is no income means test for participation, services target older Americans who have the greatest economic or social need, with attention given to low-income racial/ethnic minorities and those who live in rural areas. In addition, others may also receive service – a spouse of any age; disabled persons under age 60 who reside in housing facilities occupied primarily by the elderly where congregate meals are served; disabled persons who reside at home and accompany older persons to meals; and nutrition service volunteers. As Native Americans tend to have lower life expectancies and higher rates of illness at younger ages than the general US population, Tribal Organizations, funded under Title VI, Grants for Native Americans, can set the age at which older people can participate in ENP.

Nutrition programs funded by the Older Americans Act are also required to provide nutrition counseling to identify persons at high risk for malnutrition. The Nutrition Screening Initiative was developed to address the prevalence of malnutrition among older adults. This initiative was spearheaded by the American Academy of Family Physicians and the American Dietetic Association to promote the integration of nutrition screening and intervention into healthcare for older adults. The DETERMINE Your Nutritional Status instrument, designed by the American Academy of Family Physicians, the National Council on the Aging, and others as part of the National Screening Initiative, aims to assess the risk for poor nutritional status or malnutrition among older Americans (Berdanier et al. 2013). The DETERMINE checklist has also been used to measure an individual's change in level of nutritional risk over time. Individuals at high nutritional risk are defined as individuals who score six or higher (Table 3). The checklist is based on the following symptoms of poor nutrition – chronic disease, eating poorly, tooth loss/mouth pain, economic hardship, reduced social contact, multiple medicines, involuntary weight loss/gain, need for assistance in self-care, and age 80 or older. The checklist must be completed annually and recorded for all older adults who receive congregate meals, home delivered meals, or nutrition counseling.

Choices for Independence is an integrated set of strategies and tactics designed to strengthen the Older Americans Act, particularly as it relates to preventing malnutrition among older Americans (U.S. Department of Health and Human Services 2007). This national initiative focuses on the development of health promotion interventions targeting older adults so they can live independently longer. Choices are supported by scientific evidence that shows that improving quality of life and decreasing health care costs can be achieved through low-cost health promotion programs that target older adults (Wacker and Roberto 2008).

Other programs offered through the US Department of Agriculture also serve older adults. The Supplemental Nutrition Assistance Program (SNAP) serves over four million older adults each year, with only 42% of eligible older adults enroll in SNAP due to participation barriers (National Council on Aging 2015b). The Commodity Supplemental Food Program provides 600,000 older adults each year with healthy food boxes to supplement their diets (United States Department of Agriculture 2015). The USDA also administers the Senior Farmers' Market Nutrition Program (800,000 participating older adults) and the Child and Adult Care Food Program (120,000 participating older adults). The Senior Farmers Market Nutrition Program awards grants to all 50 states, the District of Columbia, US territories (American Samoa, Guam, the Northern Mariana Islands, Puerto Rico, and the US Virgin Islands), and the 566 federally recognized Indian tribal governments to provide low-income seniors with coupons that can be exchanged for eligible foods (fruits, vegetables, honey, and fresh-cut herbs) at farmers' markets, roadside stands, and community-supported agriculture programs (National Conference of State Legislatures 2016).

Nutrition Education

If older Americans do not know how to practice good nutrition, their risk for nutrition- related health problems will continue to increase underscoring the need for effective nutrition education. People aged 50 or older were first recognized as a specific population group needing special consideration in the 2005 Dietary Guidelines for Americans (U.S. Department of Health and Human Services 2005). Per the OAA, one service each site that provides meals must provide nutrition education once per month. Unfortunately, delivery of these classes is occurring in a haphazard manner across the United States without consideration for the appropriateness of the information presented and the effectiveness of the delivery strategy, making behavior change unlikely.

To date, there are few evidence-based programs targeting this population making it clear that thoroughly tested curricula are needed, especially those that are tailored to the culture and context of the older adult and their environment (Partnership for Livable Communities, 2010). Older Americans who attend congregate meal sites experience a higher rate of food insecurity (29.1%) than those who do not (Duerr 2006) so for this reason alone may be at risk for malnutrition. Older adults living in rural America are also at higher risk for food insecurity (Pheley et al. 2002), which is contrary to the assumption that these older adults may still be gardening or receiving farm produce. Food choices and food preparation practices are a culturally based phenomenon that relies heavily on ritual and context (Aselage and Amella 2010; Fjellstrom 2004). To assist in the elimination of health disparities, one goal of *Healthy People 2020*, culturally, contextually, and environmentally appropriate education interventions targeting older adults must be created (Centers for Disease Control and Prevention 2011).

Little evidence is available to show the effect of OAA-required nutrition education on older adults. One systematic literature review to determine the effect of nutrition education interventions for community-dwelling older adults yielded only three randomized control trials of interventions provided as part of the OAA Nutrition Program (Bandayrel and Wong 2011).Because each study examined the effect of a unique and specific nutrition education intervention and sample sizes differed greatly (with 44, 104, and 703 participants, respectively), results were difficult to compare (Kupka-Schutt and Mitchell 1992; Mayeda and Anderson 1993; Mitchell et al. 2006). Only one study (with the largest sample size) yielded significant, positive change in dietary behaviors, specifically in use of dietary supplements (Mitchell et al. 2006).

Conclusions

As the US population continues to grow older, more attention must be given to addressing the nutritional needs of older Americans. Malnutrition is a problem but the magnitude of the problem is unknown because of the lack of data. To effectively address this problem, we need to collect national-level data on the prevalence of malnutrition among older Americas in both healthcare settings (i.e., hospitals and long-term care facilities) as well as in community-dwelling older Americans. These data will allow for informed interventions to be directed in high need areas. But, to collect such data there is a need for universal accepted procedures for screening for malnutrition not only upon hospital admission but during other healthcare visits. Preventing malnutrition, which is grounded in good nutrition, is essential to helping older Americans live independently.

Procedures

Malnutrition is not an inevitable side effect of aging, it can be prevented or at least managed if properly diagnosed. As of 2017, the extent of malnutrition among older Americans is unknown because of the lack of national-level data. To prevent malnutrition, at a minimum we need to:

- Establish a universal, measurable definition of malnutrition that is accepted and used across the healthcare spectrum.
- Develop universal procedures to screen for malnutrition not only upon hospital admission but during other healthcare visits.
- Collect national-level data on the prevalence of malnutrition among older Americans in both healthcare settings (i.e., hospitals and long-term care facilities) and among community-dwelling older adults.

National-level data will help inform the development and delivery of nutrition interventions for use by the government, educators, and healthcare professionals.

Moreover, such a data set could also result in the more efficient use of the US$51.3 billion the US government spends each year to prevent malnutrition in community-dwelling older adults as these data can be used to direct the delivery of nutrition interventions to high-need areas.

Dictionary of Terms

- ***Alzheimer's disease*** – A degenerative brain disease of unknown cause that is the most common form of dementia, that usually starts in late middle age or in old age, that results in progressive memory loss, impaired thinking, disorientation, and changes in personality and mood.
- ***Cognitive decline*** – Decreased memory and thinking skills.
- ***Dementia*** – A progressive condition (such as Alzheimer's disease) marked by the development of memory impairment, loss of the ability to understand or express speech, and the inability to plan and initiate complex behavior.
- ***Functional decline*** – The decrease in physical and/or cognitive functioning resulting in one not being able to engage in activities of daily living, such as bathing, cooking meals, and shopping for food.
- ***Homeostatic reserves*** – The redundancy of the body's ability to overcome acute and chronic health insults.
- ***Huntington's disease*** – A hereditary brain disorder that is a progressive, neurodegenerative condition marked by depression, irritability, mood swings, deficits in memory and concentration, dementia, difficulty in swallowing, jerky movements, and loss of coordination.
- ***Parkinson's disease*** – A chronic progressive neurological disease chiefly of later life that is marked especially by tremor of resting muscles, rigidity, slowness of movement, impaired balance, and a shuffling gait.
- ***Periodontal disease*** – Any disease affecting the supporting structures of the teeth.
- ***Pneumonia*** – An acute disease that is marked by inflammation of lung tissue that is characterized by fever, chills, cough, difficulty in breathing, fatigue, chest pain, and reduced lung expansion, and is typically caused by an infectious agent (such as a bacterium, virus, or fungus).
- ***Sarcopenia*** – The reduction in skeletal muscle mass due to aging.

Summary Points

- The United States is rapidly becoming an "older" and less healthy nation.
- Malnutrition is a leading cause of morbidity and mortality among older Americans primarily because of poverty, rurality, and/or lack of access to good quality food.
- Medical conditions (chronic diseases and those associated with growing older) and lifestyle, social, and psychological factors all play a role in increasing one's risk for malnutrition.

- A review of nationally representative data suggest that malnutrition may be under-recognized and underdiagnosed in hospitals.
- To date, there is no consensus among healthcare professionals for implementing optimal nutrition care for malnourished and at-risk hospitalized older adults.
- Malnutrition screening and assessment are also not standardized as to date there is no universal definition of malnutrition.
- In 1965, the US Congress passed the Older Americans Act, which provides support for nutrition services to community-dwelling older adults through the Elderly Nutrition Program.
- The US government spends US$51.3 billion each year on the Elderly Nutrition Program.
- The meals and other nutrition services provided through the Elderly Nutrition Program are provided in a variety of group settings, such as senior centers, faith-based settings, schools, as well as delivered to the homes of those who are home bound.
- Each participating site must provide nutrition education once per month, with current delivery occurring in a haphazard manner across the United States.
- To date, few evidence-based nutrition education programs target older adults making it clear that thoroughly tested curricula are needed, especially programs tailored to the culture and context of the older adult and their environment.

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Nutritional Status in Malnourished Older Diabetics

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Alejandro Sanz-París and Beatriz Lardiés-Sánchez

Contents

Abstract

Diabetes mellitus is especially prevalent in the elderly. It has a different presentation, management, and complication rate in this population. Diabetes in the older people increases the risk of suboptimal nutrition, being malnutrition highly prevalent in elderly diabetic patients. Malnutrition should be screened for in these patients and, when present, should prompt a revision in diet and drug therapy. In malnourished diabetic patients, diabetes-specific enteral nutritional formulas have been specially developed as effective alternatives for providing nutrients, improving glycemic control, and reducing the incidence of complications.

Keywords

Diabetes · Malnutrition · Older population · Nutritional status

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Introduction

Diabetes mellitus is one of the most prevalent endocrine disorders in the general population, being especially prevalent in the elderly. Around 20% of the western society population over 65 years of age have diabetes, and several studies have shown that abnormal glucose tolerance is present in more than 60% of adults older than 60 years of age, as a result of decreased insulin sensitivity and impairment of pancreatic beta-cell function with age (Sanz-París et al. 2013). Diabetes is associated with an increased risk of suffering malnutrition and other geriatric syndromes and chronic health conditions, placing an important burden on healthcare systems.

Malnutrition is also more common and increasing in older population; currently 16% of those >65 years and 2% of those >85 years are classified as malnourished. The aging process involves a deterioration in some functions that can result in reduced appetite, difficulty in chewing, inflammation of the gums, and a poor quality diet, which can negatively impact nutritional status (WHO 2015). Studies in developed countries found that up to 15% of community-dwelling and home-bound elderly, 23–62% of hospitalized patients, and up to 85% of nursing home residents suffer from malnutrition (Ahmed and Haboubi 2010).

Diabetes in the elderly increases the risk of suboptimal nutrition, being malnutrition highly prevalent in elderly diabetic patients. Malnutrition should be screened for in these patients and, when present, should prompt a revision in diet and drug therapy. In particular, the possibility of reducing unnecessary drug therapy should be considered (Vischer et al. 2010).

Diabetes in Elderly People

There is increasing evidence suggesting that type 2 diabetes mellitus has a different presentation, management (including dietary therapy), and complication rate in older population.

There are several factors affecting glucose metabolism with age: genetic and ethnic influence, resistance to insulin-mediated glucose uptake, progressive reduction of insulin secretion from the pancreas, changes in body composition (relative increase adipose tissue in relation to muscle mass), changes in food intake, impaired mobility and physical activity, psychological factors (such as stress, isolation), use of medications that impair insulin sensitivity, and finally lifestyle modifications (efforts at weight loss and physical activity), which lead to improved insulin sensitivity (Rizvi 2009) (see Fig. 1).

Diabetes in the elderly increases the risk of suboptimal nutrition, hospitalizations, nursing home admissions, and physical disability that substantially impairs quality of life. Diabetes is linked to higher mortality, reduced functional status, and increased risk of institutionalization in elderly frail population (see Table 1). Older people with diabetes is a heterogeneous population that includes people residing independently in communities, in assisted care facilities, or in nursing homes. These patients usually present common geriatric syndromes such as cognitive impairment,

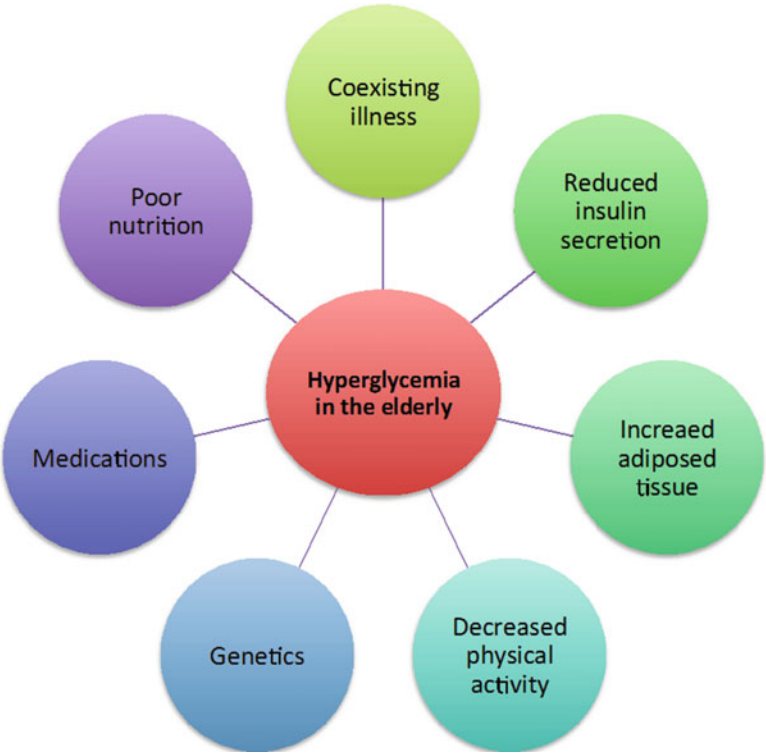

Fig. 1 Pathogenesis of diabetes mellitus in the elderly

Table 1 Negative consequences of diabetes mellitus in the elderly

Diabetes mellitus
Suboptimal nutrition
Hospitalizations
Higher mortality
Reduced functional status
Increased risk of institutionalization
Impaired quality of life

depression, urinary incontinence, and falls, and there is a high risk for polypharmacy. This implies that management of diabetes in older adults should be individualized, taking into account all these variables (Kirkman et al. 2012).

Older adults with diabetes are at risk of the same microvascular and macrovascular complications as those at younger ages, although absolute cardiovascular risk is much greater at a more advanced age (Kennedy 2016). Moreover, they are more likely to have coexistent chronic conditions like hypertension, dyslipidemia, and cardiovascular disease that may impact their nutritional requirements (Leon-Sanz et al. 2015).

The overall goals for glycemic control, as well as risk factor management, should be individualized considering several factors such as individual's overall health, level of patient engagement, risk for hyperglycemia and other adverse reactions, duration of diabetes, comorbidities, vascular complications, and life expectancy, since the risk of complications is duration-dependent (Kennedy 2016). Amelioration of symptoms is foremost, although reducing morbidity from complications is a worthy long-term goal. Older adults who are functional, are cognitively intact, and have significant life expectancy should receive diabetes care using goals developed for younger adults (Kirkman et al. 2012).

Factors that affect the patients' quality of life and their ability to manage their condition should be assessed. The importance of diet, blood glucose monitoring, and adherence to prescribed medications are important aspects of care for the frail elderly with diabetes.

The vulnerability to hypoglycemia is substantially increased in older adults. Thus, avoidance of hypoglycemia is an important consideration in establishing goals and choosing therapeutic agents in the elderly.

On the other hand, older patients with diabetes should receive individualized counseling regarding lifestyle modification, including a medical nutrition evaluation. The nutrition prescription is tailored based upon medical, lifestyle, and personal factors. Appropriate diet, loss of excess weight, and regular aerobic and resistance exercise continue to be beneficial in the majority of this population. Individualization of advice in these areas is important, with due consideration given to comorbidities, family, and social support (Kennedy 2016).

Malnutrition in Older Adults

Nutrition is an important element of health in the older population and affects the aging process (Ahmed and Haboubi 2010). Older people are at an increased risk of inadequate diet and malnutrition, which is defined as a state with a deficiency, excess, or imbalance of energy, protein, and other nutrients that causes adverse effects on body form, function, and clinical outcome (Stratton et al. 2003). A poor nutritional status is associated with a decline in functional status, decreased bone mass, impaired muscle function, immune dysfunction, anemia, reduced cognitive function, poor wound healing, delay in recovering from surgery, and higher hospital readmission rates and mortality (Ahmed and Haboubi 2010; Lim et al. 2012) (see Fig. 2).

The prevalence of malnutrition in the elderly is increasing and its etiology is multifactorial (Chapman 2006). Aging is associated with a decline in number of physiological functions that can affect nutritional status. Older people often have reduced appetite and energy expenditure, coupled with a decline in biological and physiological functions such as reduced lean body mass, changes in cytokine and hormonal level, and changes in fluid electrolyte regulation, delayed gastric emptying, and diminished senses of smell and taste (Ramos Martínez et al. 2004). Healthy older people are less hungry and are fuller before meals, consume smaller meals, eat

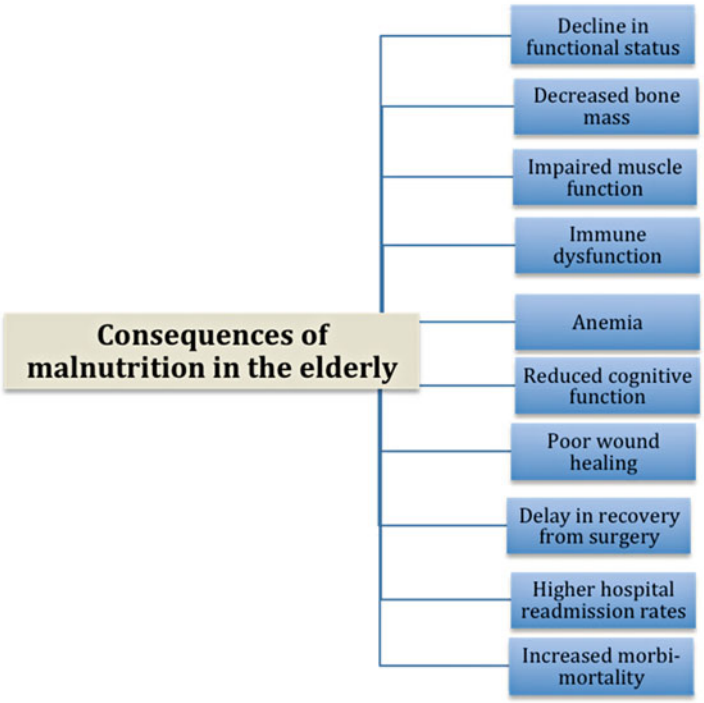

Fig. 2 Consequences of malnutrition in the elderly

more slowly, have fewer snacks between meals, and become satiated after meals more rapidly after eating a standard meal than younger people. Moreover, several factors such as physical conditions (e.g., poor dentition, dysphagia, altered taste and smell, swallowing difficulties), psychosocial conditions (low income, limited knowledge of diet and cooking skills, alcohol or drug abuse, poverty, isolation, inability to shop, functional impairments leading to difficulties in preparing or consuming food), and medical conditions (e.g., infection, stroke, drugs, anorexia, palatability, dietary restrictions, eating disorders, altered bowel conditions, cancer, dementia, depression, diabetes, etc.) all play a role in the complex etiology of malnutrition in older people (Kirkman et al. 2012). Moreover, basal metabolic rate decline with age and higher prevalence of obesity is difficult to reconcile with sharply lowered energy intakes (Drewnowski and Warren-Mears 2001) (see Fig. 3).

Assessment of Nutritional Status in Older Adults

Nutritional assessment is important to identify and treat patients at risk. Several tools have been used for the evaluation of nutritional status in the elderly. To quantify nutritional intake, different methods can be used. Twenty-four hour recall is

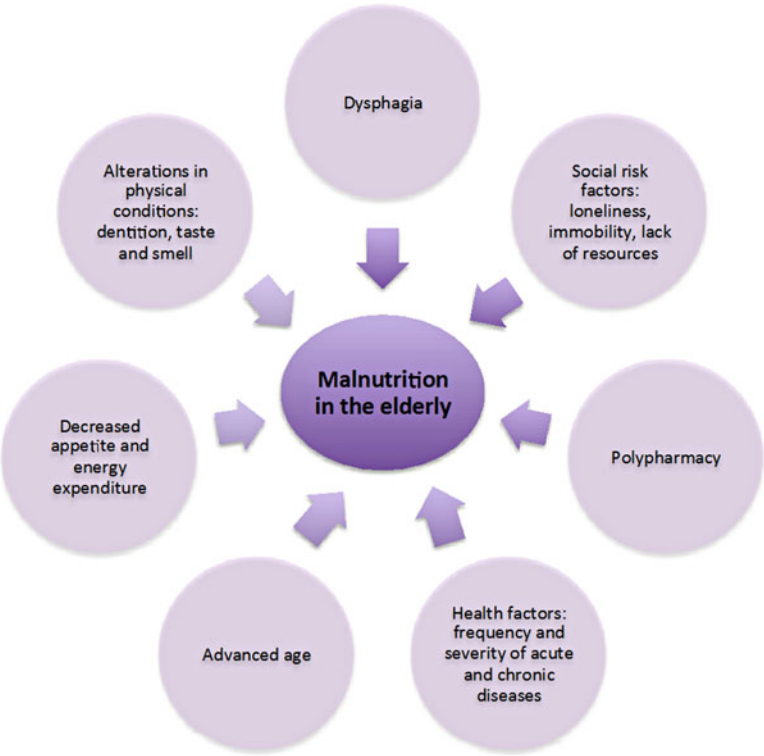

Fig. 3 Risk factors of malnutrition in the elderly

commonly used and is based on an interview in which the patients recall all food consumed in the previous 24 h, and also food records for 7 days for all food and drink consumed can be used. A food frequency multiquestion questionnaire can also be used to explore dietary intake over a period of time (Pirlich and Lochs 2001).

Screening of malnutrition is vital in identifying and monitoring patients, and it should assess body mass index (BMI) and percentage of unintentional weight loss (which is one of the best predictors of worst clinical outcome) and should also consider the time over which nutrient intake has been unintentionally reduced and/or the likelihood of future impaired nutrient intake. Structured screening tools may identify nutrition-related issues that warrant evidence-based interventions (Cena et al. 2008).

The European Society for Clinical Nutrition and Metabolism (ESPEN) recommends the use of the Mini Nutritional Assessment (MNA) tool, specifically designed for older adults, because the predictive validity has been evaluated by demonstrating its association with adverse health outcome in fragile elderly patients. This tool also takes into account relevant physical and social aspects, besides the dietary habits. It has been evaluated in acute care facilities showing an inverse relationship between its final score and mortality. Moreover it is simple to perform and may help

determine whether referral to a registered dietitian for medical nutrition therapy is needed. The MNA test includes 18 items in four sections: anthropometric assessment (four questions concerning weight, height, and body circumferences), global evaluation (six questions concerning lifestyle, medication, and mobility), dietetic assessment (six questions concerning number of meals, food and fluid intakes, and autonomy of feeding), and subjective assessment (two questions concerning self-perceptions of health and nutrition). The score obtained (maximum, 30) classifies the elderly in three categories: malnourished (<17), at risk of malnutrition (17–23.5), and well nourished (>24) (Guigoz 2006).

The Malnutrition Universal Screening Tool (MUST) may also be used (Donini et al. 2016). The MUST score is calculated using three steps: body mass index (BMI), percentage weight loss in the past 6 months, and disease effect. Each resulting score has its own set of recommended interventions. Higher scores represent a greater risk of malnutrition.

As the population ages, it is more important than ever that the implications of malnutrition are recognized and addressed (Willis 2017).

Nutritional Status and Malnutrition in Elderly Diabetic Patients

Disease-related malnutrition is common in diabetic patients. Older adults with diabetes appear to be at risk of nutritional impairment compared with nondiabetic people (Hamdy et al. 2014), and in fact previous studies have shown that diabetic patients are at a higher risk of malnutrition than nondiabetic patients (Alvarez-Hernandez et al. 2012). Residents with diabetes may have numerous underlying risk factors for poor nutritional status including multiple medications affecting gastrointestinal function and appetite, medical comorbidities, disabilities affecting the ability to eat and drink safely, low mood, and poor cognition.

Diabetes mellitus is characterized by hypermetabolism. Metabolic and nutritional factors play a role in the development of DM and complicate its course. These patients have a high metabolic rate, especially in patients with DM and renal failure. Despite a high metabolic rate, patients with type 2 DM are prone to be obese (currently the main cause of this type of diabetes). Even though type 2 DM patients are obese, they have reduced lean body weight, compared with nondiabetic obese individuals, and an exaggeration of the normal loss of muscle mass with age (Park et al. 2007).

Patients with type 2 DM suffer mainly from macronutrient overnutrition (an important etiological factor in causing the disease), and those with type 1 DM tend to be undernourished at the time of diagnosis. In fact, a common presentation of patients with type 1 diabetes is weight loss, included marked loss of lean mass. Micronutrient deficiencies are frequent in both type 1 and type 2 DM.

Type 2 diabetes mellitus usually occurs in the context of obesity and is associated with insulin resistance, so it is expected to find diabetic elderly patients, though suffering some level of malnutrition, with higher body mass index than those without diabetes.

Elderly people with diabetes share nutritional problems of older people with chronic diseases, but they have also specific nutritional deficiencies which are specific of this disease. An increased prevalence of malnutrition has been described especially in patients with nephropathy and diabetic foot ulcers (Turnbull and Sinclair 2002).

Current treatment recommendations are based on lifestyle modifications and incremental drug therapy. However, this approach could lead to inappropriate priorities upon aging, when diabetes may be compounded by malnutrition and reduced insulin resistance. The overlap of malnutrition and glucose intolerance in older people is common but frequently overlooked. The prevalence increases with frailty, physical infirmity, and institutionalization.

In hospitalized elderly diabetic patients, nutritional assessment is of outmost importance not only because it is the first step to correct malnutrition but also because it can prompt a reduction of unnecessary anti-glycemic therapy, thus preventing hypoglycemic episodes.

In the study of Liu et al. (2017), the aims were to explore the prevalence of malnutrition among elderly diabetic patients admitted to the hospital and to explore the relationships between malnutrition and geriatric syndromes, diabetic complications, and clinical outcomes. Of 302 participants, the prevalence of malnutrition, risk of malnutrition, and normal nutrition was 18.5%, 33.1%, and 48.3%, respectively. Moreover, diabetic microvascular complications and activities of daily living dependence were independently associated with malnutrition. Malnourished patients had longer hospital stays and higher mortality rates than patients either at risk of malnutrition or with a normal nutritional status. Multivariate analysis also showed that malnutrition was independently associated with an increased risk of death.

Recently, in a multicenter study (Sanz-París 2016a), the prevalence of malnutrition according to the new ESPEN definition in elder hospitalized diabetic patients was assessed. In the study was evaluated whether this new diagnosis of malnutrition predicted clinical outcomes in these patients. 1014 hospitalized diabetic patients ($\geq$65 years) from 35 hospitals were screened being at risk of malnutrition using the short version of the MNA. The new ESPEN definition, with MNA-SF as initial screening, identified 68 malnourished geriatric individuals with diabetes (6.73% of the cohort). Additionally, malnutrition lengthened the hospital stay, increased 2.7 times the odds of dying in the hospital, and decreased to one third the odds of being discharged home.

In the study of Solóirzano-Pineda et al. (2012), the incidence of malnutrition was assessed in diabetic and nondiabetic patients in a surgery department, assessing the nutritional status by means of VSG, CONUT, and MNA. The conclusion was that the incidence of malnutrition in surgical diabetic patients is twofold higher than in nondiabetic patients.

Malnutrition is widely prevalent in patients with chronic kidney disease. In the study of Khan (Khan et al. 2009), the primary objective was to assess the utility of subjective global assessment (SGA) as an indicator of malnutrition in patients with diabetic nephropathy, in combination with anthropometric and biochemical parameters. The SGA method was performed to evaluate nutritional status in 40 patients

Table 2 Points to carry out in elderly diabetic patients

Screen for malnutrition using a validated screening tool
Agree glycemic targets and blood glucose monitoring
Identify those patients at risk of hypoglycemia and formulate clear care plans to minimize risk
If risk of malnutrition is identified, give first-line dietary advice including enriching food
Ensure everyone involved has a clear understanding of the goals and targets of dietary intervention
If episode of risk of malnutrition resolves, reinforce previous dietary recommendations

with diabetic nephropathy. Malnutrition was identified in 63% of patients, mild to moderate in 48%, and severe in 15%. The study confirmed that a high degree of malnutrition was prevalent in patients with diabetic nephropathy, as shown by anthropometric and biochemical variables.

Moreover, in diabetic patients, metabolic disturbances of poorly controlled DM may account for the higher resting energy expenditure observed in the chronic renal failure diabetes group (Aversani et al. 2001).

Malnutrition can present a significant clinical and public health problem among older diabetic patients, implying an increased risk of morbidity, hospitalization, and mortality. Therefore, early detection and intervention is important (Mann et al. 2004).

Identifying patients at risk via screening is the first step to providing effective intervention, followed by appropriate management strategies that are implemented once undernutrition or the risk of undernutrition has been identified (Flanagan et al. 2012), (Casimiro et al. 2001). Overly restrictive eating patterns, either self-imposed or provider-directed, may contribute additional risk for older adults with diabetes (see Table 2).

A few studies have evaluated the prevalence of malnutrition in hospitalized elderly diabetic patients by means of the Mini Nutritional Assessment (MNA®) tool. The study of Leon-Sanz et al. (2015) is the first study that evaluates the prevalence of malnutrition and its costs in adult hospitalized patients with diabetes using the NRS®-2002 screening tool. In this study, a third of diabetic patients admitted at Spanish hospitals are malnourished, and these patients have longer hospitalizations and higher costs than those at no nutritional risk. This study also suggests that nutrition support is a cost-effective strategy in malnourished patients as it helps reduce the length of hospital stay and the incidence of complications.

People with diabetes are already at risk of poor healing and poor health outcomes because of the complications of the disease. Despite the increased risk in this vulnerable group, malnutrition often remains unrecognized and untreated.

Dietary intervention may be required for varying lengths of time, depending on the underlying contributing factors and the patient's individual requirements.

In patients with diabetes and malnutrition, immediate measures to consider include glycemic control and, in older patients with type 2 diabetes, a review of diabetes medication. Rapid weight loss, as 5–10% body weight, may require an immediate reduction of diabetes medication to prevent hypoglycemia. Moreover,

many diabetic individuals complain of dry mouth (xerostomia), a condition that can affect oral health, nutritional status, and diet selection and edentulous.

On the other hand, older people with diabetes are more susceptible to develop problems with fluid and electrolyte balance due to physiological renal impairment and changes in thirst perception (Drewnowski and Warren-Mears 2001).

Nutrition is an integral part of diabetes care for all ages, but there are additional considerations for older adults and several points to take into account. There is much literature published about the role of dietary therapy in the management of people with diabetes, but most studies focus on the middle-aged patients, and current diet and lifestyle recommendations mainly focus on young and middle-aged diabetic patients. Diabetic patients develop malnutrition and lose lean body weight rapidly when stressed by intercurrent illness. Nutritional support should not be delayed in these patients.

Diabetic patients are at risk of both malnutrition and hospital readmissions, often requiring nutritional support (Elia et al. 2005). Reduced intake due to medical, social, and physiological factors should be addressed (Ahmed and Haboubi 2010). VIDA study is the first Spanish multicenter study describing nutritional status of a large sample of elderly inpatients with diabetes mellitus. 21.22% of the 1098 patients were malnourished. This result depends on age and sex and can increase mortality rate (Gómez-Candela et al. 2016).

Older people in isolation should have social services assistance and "meals on wheels" to help improve food intake. When nutrition needs are not being met with usual intake, oral nutrition support in older malnourished diabetic patients includes any of the following advice to improve nutritional intake: fortified food with protein, carbohydrate, and/or fat, plus minerals and vitamins, snacks, altered meal patterns, and the provision of dietary advice. Additional interventions may include encouraging smaller more frequent meals, fortifying usual foods, changing food texture, or adding liquid nutrition supplements (either regular or diabetes-specific formulas) between meals. Oral supplements or enteral feeding should be considered in patients at high risk or in patients unable to meet daily requirements. Supplements have been shown to improve clinical and functional outcomes and reduce mortality rate. See Table 3.

Postprandial hyperglycemia significantly contributes to overall glycemic control in type 2 diabetes patients. Epidemiological studies have shown a strong and independent relationship between postprandial blood glucose excursions and cardiovascular comorbidities in type 2 diabetes patients. Diets with a low glycemic load are recommended in controlling postprandial plasma glucose levels. The amount of carbohydrate eaten is important to control blood glucose levels. All varieties are fine but those that are more slowly absorbed (lower glycemic index) will not affect blood glucose levels as much.

For diabetic patients in need of nutritional support, diabetes-specific enteral nutritional formulas have been specially developed as effective alternatives for nutritional treatment in diabetic subjects, which aim to result in lower (postprandial) glucose levels than standard formulas (McMahon et al. 2013). Traditionally, these formulas contained less carbohydrates (35–40%) and typically more fat with a large

Table 3 Key points to take into consideration with regard to nutrition in the elderly patient with diabetes

The prevalence of diabetes increases with age and is likely to coexist with other chronic health conditions
Appetite changes, palatability, dietary restrictions, and psychosocial issues become increasingly important factors in the elderly
Nutritional support should be guided by glycemic control, long-term risk of vascular complications, and the patient's preferences and quality of life
Awareness of nutritional guidelines and tailoring them to the elderly diabetic patient is a prudent approach

contribution from monounsaturated fatty acids, generally more than 60% of total fat content (Elia et al. 2005). However, nutritional guidelines for diabetic patients published by the Diabetes Nutrition Study Group of the European Association for the Study of Diabetes recommend that the fat content of a diet should not exceed 35%, and carbohydrate intake should range between 45% and 60%. They have shown to improve glycemic control and reduce the incidence of complications (Gosmanov and Umpierrez 2012). They are an effective alternative for providing nutrients and maintaining glycemic control due to their content of slowly digested and absorbed carbohydrates and monounsaturated fats (Sanz-Paris 2016b; Alish et al. 2010; Ojo and Brooke 2014; Lansink et al. 2011; Vaisman et al. 2009) (see Table 4).

Enteral feeding is indicated if a patient is severely malnourished or if food cannot be taken orally due to medical illness, e.g., stroke. Patients with difficulty swallowing, e.g., stroke patients, need speech and language therapy and possibly percutaneous endoscopic gastrostomy (PEG) feeding in the long term. In the short term, a nasogastric tube can be used.

On the other hand, reduced intake and unbalanced diet predispose older people to vitamin and mineral deficiencies. In patients with proven deficiencies of micronutrients, supplementation should be given. The major function of vitamin D is to maintain calcium and phosphorus homeostasis and promote bone mineralization. Vitamin D deficiency leads to reduced bone density and increased risk of falls and cardiovascular disease. Dietary requirements on older people are higher due to decreased exposure to sunlight, thinning of the skin, and reduced skin production. Calcium and vitamin D supplementation have been shown to reduce the incidence of hip fractures (Ahmed and Haboubi 2010).

In a review of vitamin D and diabetes (Song et al. 2013), it was suggested that vitamin D and calcium may play a role in the development of type 2 DM by influencing inflammation and the destruction of pancreatic beta cells. Low calcium and vitamin D intake are indeed associated with development of DM. Vitamin D appears to increase the insulin response to glucose stimulation while not influencing basal insulinemia. Low vitamin D levels were associated with more severe insulin resistance, development of albuminuria, cardiovascular disease, and higher HbA1c levels.

Table 4 Advantages and disadvantages of diabetes-specific formulas in diabetic patients

Advantages	Disadvantages
Designed specifically for patients with DM and/or hyperglycemia They contain less carbohydrates, more fiber, and more fat than standard solutions, while the protein content and micronutrient composition are similar It results in lower glycemic, lower HbA1c, and lower glycemic variability They reduce the requirements of regular insulin An increase in fiber content helps glycemic control, improves insulin sensitivity, and decreases the glycemic index	The high content of fat and fiber delay more gastric emptying, which may be negative in patients with diabetic gastroparesis or delayed gastric emptying (due to neuropathy, medical treatment, or hemodynamic compromise) No clear reduction in complications or mortality

Policies or Protocols

Medical nutrition therapy is the process by which the nutrition prescription is tailored for people with diabetes based upon medical, lifestyle, and personal factors and is an integral component of diabetes management and diabetes self-management education. It has proven to be beneficial in older adults with diabetes. Unique challenges with aging, such as altered taste perception, coexisting illnesses and dietary restrictions, compromised dentition, altered gastrointestinal function, impaired food shopping, preparation capabilities, and memory decline leading to skipped meals, should be considered before developing meal plans. In general, it is best to avoid complex dietary and treatment regimen in this elderly population.

Nutritional management requires a holistic approach, and underlying causes such as chronic illness, depression, medication, and social isolation must be treated. Recommendations should take into account the patient's culture, preferences, and personal goals and abilities.

Nutrition education should be individually tailored and should incorporate patience, kindness, humor, understanding, and above all a respect for the differences that make each older person an individual.

The elderly diabetic population stands to benefit enormously from streamlining and optimizing diet planning in order to enhance longevity, minimize complications, and improve quality of life. There is a paucity of definitive, long-term studies examining the part that nutrition plays in the overall health and metabolism of older people. In order to meet these challenges, it is imperative to understand the role of dietary factors in the genesis and progression of glucose intolerance and diabetes in the older individual and implement evidence-based recommendations tailored to the specific circumstances (Abatecola and Paolisso 2009; Canadian Diabetes Association Clinical Practice Guidelines Expert Committee et al. 2013; Miller et al. 2004).

On the other hand, though energy needs decline with age, macronutrient needs are similar throughout adulthood. Meeting micronutrient needs with lower caloric intake is

challenging; therefore, older adults with diabetes are at high risk for deficiencies (Kirkman et al. 2012).

The main goal should be to help to improve oral food intake. Optimal nutrition promotes both functional health status and mental well-being. Dietary diversity and variety promote enjoyment and satisfaction with the diet, and modifying the dining environment in care homes has been shown to improve dietary intake.

The issue of attainment and maintenance of an optimal body weight in elderly diabetic persons may not be as straightforward as in other age groups, and the risk-benefit ratio may be different as well.

Although increased prevalence of overweight and obesity in the elderly contributes to insulin resistance and hyperglycemia, older inhabitants of long-term care facilities who suffer from diabetes tend to be underweight. Both may signify inadequate nutritional status and lead to increased morbidity and mortality. The attendant problems of appetite changes, palatability of food, dietary restrictions, loneliness, and depression may affect the type and quantity of food consumed by elderly persons. Although glucose control and health concerns are important factors in diet modification in the older population, other considerations include quality of life and individual preferences. Customizing of nutritional guidelines to the needs of the older diabetic patient makes sense.

Dictionary of Terms

- **Malnutrition** – lack of proper nutrition, caused by not having enough to eat and by inadequate or unbalanced intake of nutrients or their impaired assimilation or utilization.
- **Nutritional screening** – is a rapid, simple, and general procedure to identify subjects who may be at nutritional risk or potentially at risk and who may benefit from appropriate nutritional interventions.
- **Diabetes mellitus** – a chronic disease associated with abnormally high levels of the sugar glucose in the blood.
- **Hypoglycemia** – is when blood sugar decreases to below normal levels. In people with diabetes, levels below 3.9 mmol/L (70 mg/dL) are diagnostic.
- **Percutaneous endoscopic gastrostomy (PEG)** – is a procedure that allows nutritional support for patients who cannot take food orally. It involves placement of a tube through the abdominal wall and into the stomach through which nutritional liquids can be infused.

Summary Points

- Diabetes mellitus is one of the most prevalent endocrine disorders in the general population, being especially prevalent in the elderly.
- Diabetes in older people increases the risk of suboptimal nutrition, hospitalizations, nursing home admissions, and physical disability that substantially impairs quality of life.

- A poor nutritional status is associated with a decline in functional status, decreased bone mass, impaired muscle function, immune dysfunction, delay in recovering from surgery, and higher mortality.
- Malnutrition is highly prevalent in elderly diabetic patients, so it should be screened for in this population.
- Elderly people with diabetes share nutritional problems of older people with chronic diseases, but they have also specific nutritional deficiencies which are specific of this disease.
- Nutritional support should be guided by glycemic control, long-term risk of vascular complications, patient's preference, and quality of life.
- In malnourished diabetic patients, diabetes-specific enteral nutritional formulas have shown to improve glycemic control and reduce the incidence of complications.

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Malnutrition in Tuberculosis

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Contents

Abstract

Malnutrition poses a serious and long-lasting impact on developmental, economic, social, and medical background for communities on a global scale. Tuberculosis on the other hand is ranked among the top ten causes of mortality worldwide. The two are prominently coexistent with evidence suggesting TB leading to malnutrition and vice versa giving rise to a vicious cycle benefiting each

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other. Various modulations in immune responses complicate this bi-directional relationship. In this chapter, we have aimed to bring together the vast data on malnutrition and tuberculosis, about what affects what and how, for a better understanding of this relationship. The chapter also talks about micronutrient malnutrition as an integral part of malnutrition. Micronutrient malnutrition too, just like protein malnutrition, negatively affects the state of tuberculosis in TB patients. The various studies done can give an idea on using these data in targeting tuberculosis by overcoming the nutritional deficiencies that worsen the state of tuberculosis in both children and adults.

Keywords

Tuberculosis · Latent tuberculosis infection · Macrophages · Phagocytosis · Malnutrition · Cell-mediated immunity · Protein-energy malnutrition · Kwashiorkor · Secondary immune deficiencies · Anthropometric defects · Severe acute malnutrition · BCG vaccination · Micronutrient malnutrition · Nutritional supplement

List of Abbreviations

Ab	Antibody
Ag	Antigen
BCG	Bacillus Calmette-Guérin
CMI	Cell-mediated immunity
CYP27B1	Cytochrome P450, family 27, subfamily B, polypeptide 1
DN/DCs	Dendritic cells
GALT	Gut-associated lymphoid tissue
IFN	Interferon
IL	Interleukin
Mtb	*Mycobacterium tuberculosis*
NK	Natural killer cells
PPD	Purified protein derivative
ROS/RNS	Reactive oxygen species/reactive nitrogen species
SAM	Severe acute malnutrition
TB	Tuberculosis
TGF	Transforming growth factor
TNF	Tumor necrosis factor
VDR	Vitamin D receptor

Introduction

Nutrition is the most vital element for a healthy growth and development. Various studies have stated the role of malnutrition in developing an immune-deficient state in an individual, thereby making the host susceptible to various opportunistic infections. The relationship between the two was precisely explained for the first time by Scrimshaw et al. (1959). Scrimshaw et al. (1959, 1968) showed how

malnutrition and infection generate what is known as a "vicious cycle" wherein both the units interact and benefit from one another. They proposed that malnutrition increased susceptibility to infection, which by nature caused a furthermore deterioration of the nutritional status perpetuating a cycle that eventually led to extreme protein-energy malnutrition (PEM) or kwashiorkor, usually fatal. These interactions were observed to be usually synergistic with both malnutrition and infection together causing a more pronounced effect (Scrimshaw 2003).

Nutrition and the Immune System: The Critical Interlink

Host resistance to infections is a combined effect of various components of the immune system such as the CMI by T cells, antibody production by B cells, the complement system, barrier defense such as the skin and mucosa, interferons, etc. For a healthy life, it is very important that all these components work fine. The work published by Scrimshaw et al. (1968) showed that the cyclical interaction between nutrition and infection had a critical yet unknown connection to the host's immune system. How nutrition affected the immune system resulting in an infection-malnutrition cycle was the unanswered question. With developing technologies however, various animal and human analysis studies were able to give an insight to the subject leading to a detailed knowledge on effects of nutrition on the immune system.

Tuberculosis: An Introduction

Tuberculosis (TB), one of the top ten leading causes of mortality caused 1.4 million deaths and resulted in an estimated 10.4 million new cases in 2015 (World Health Organization 2016). About one-third of the world's population might have either active/latent TB which makes the disease a major public health priority (Lawn and Zumla 2011). The causative agent of TB, *Mycobacterium tuberculosis*, resides in humans causing either an asymptomatic, noncontagious, latent infection or an activated TB disease with the former being more prevalent than the latter. In a healthy individual, the first exposure to the bacterium is contained by the immune system resulting in latent infection. An immune deficiency caused by various factors such as HIV infection, diabetes, or undernutrition can also result in a progress from the latent to active form of the disease.

Tuberculosis: Pathogenesis and Immunology

The interactions between bacterial virulence and host resistance are a key factor in the pathogenesis of tuberculosis (Cooper 2009). *M. tuberculosis* is spread by airborne droplet nuclei, transmitted when an infected individual coughs and disperses these droplets, which can then be inhaled into the airways and alveoli of a new host. Experimental models have shown that the early host response to *M. tuberculosis*

infection is characterized by an influx of phagocytic cells including primarily resident alveolar macrophages and recruited neutrophils. Following the establishment of *M. tuberculosis* infection in the airways and lung parenchyma, the bacilli are believed to be phagocytosed by the alveolar macrophages and are taken up by neutrophils and dendritic cells (DCs). Macrophages and neutrophils may constitute a first line of defense by, for example, expression of antimicrobial peptides that may function in the early immune response. After infection of the host with *M. tuberculosis*, macrophages and neutrophils and the context of their activation may influence the subsequent immune response toward potential clearance or containment of the pathogen, resulting in persistent latent infection or the development of active disease (Ernst 2012). Clearance of resident bacteria by alveolar macrophages is dependent on the presence of lymphocytes as well as activation by IFN-γ, released by Th1 cells and other cells of the immune response such as NK cells and $CD8^+$ T cells that migrate to the site of infection in response to chemotactic signals generated by infected macrophages. The efficient interaction between infected macrophages and antigen-specific T cells in the host plays a vital role in the successful elimination of the bacterium. Immunity against tuberculosis is thus predominantly a Th1-type immunity involving the production of pro-inflammatory cytokines, specifically IFN-γ and IL-12. Interferon gamma (IFN-γ) plays an important role in activating macrophages, thus initiating phagocytosis, which aids in phagosome maturation, production of reactive nitrogen intermediates (RNI) and antigen presentation, all having important roles in immune response against TB (O'Garra et al. 2013).

Malnutrition: An Introduction

Malnutrition is a condition caused by an improper diet due to either an inadequate food supply (undernutrition) or too much dietary intake (overnutrition). Undernutrition can be either acute (too thin for their height – wasting) or chronic (too short for their age – stunted). Overnutrition is caused by the intake of excessive unhealthy food and not enough exercise leading to overweight (BMI of 25–30) and/or obesity (BMI > 30). Both under- and overnutrition lead to various health disorders with undernutrition leading to an increased risk to more severe forms of infectious diseases such as measles, malaria, tuberculosis, and pneumonia in comparison to obesity that leads to conditions such as diabetes, hypertension, stroke and other such heart disorders, cancer, etc. Nevertheless, both the forms of malnutrition are to be seriously taken with respect to the growing health issues of them in the developing world. According to the Economic and Social Development Department of the Food and Agriculture Organization of the United Nations (FAO-IFAD-WFP 2015), there were around 795 million undernourished people in 2014, of which about 13% of people were undernourished in the developing world with India having the highest rates especially in child/pediatric malnutrition (30% of children in India are estimated to be malnourished). There is also another form of malnutrition which is caused due to the deficiencies in appropriate micronutrients in a person's diet leading to a poor or improper development in health especially in children and pregnant

women. Micronutrients although needed in micro amounts can lead to severe health conditions if absent in the diet as they play an important role in proper production of enzymes and hormones. To summarize, malnutrition can be caused due to the improper uptake of an improper diet but can be prevented simply by taking the right amount of food with the right nutrients and thereby nullifying, to an extent, the risk to various infections.

Malnutrition and Tuberculosis

Malnutrition, specifically undernutrition, and tuberculosis are both widely spread conditions in the world until the current date. What is worse is the fact that the two are closely interlinked and fortunately or unfortunately share a bi-directional relationship, i.e., tuberculosis may lead to wasting and/or malnutrition, whereas malnutrition has a tendency to predispose the person to tuberculosis (Macallan 1999). Fortunately because treating one might reduce the effects of the other and unfortunately because the worsening of either can negatively influence the other and thereby lead to detrimental effects in unison. Moreover, it is almost impossible to determine whether malnutrition led to TB or vice versa since it is difficult to know the nutritional status of an individual with active TB before the onset of the disease.

Malnutrition → Tuberculosis: Does Malnutrition Play a Role in the Onset of Tuberculosis?

PEM is known to negatively affect CMI which is the major host immune response against tuberculosis thereby acting as an important risk factor (Cegielski and McMurray 2004). A number of researchers have looked at the rate at which malnutrition can become a risk factor in developing tuberculosis both on experimental animals and facts from human case and cohort studies. One systematic review by Lonnroth et al. (2010) compared various studies that related TB with malnutrition and came up with a conclusion that BMI and TB incidence shared a log-linear inverse dose relationship specifically in people between the BMI range of 18.5–30 kg/m^2.

Protein malnutrition in guinea pigs interfered with interactions between the macrophages and T lymphocytes in addition to mycobactericidal/static activity of macrophages (Dai and McMurray 1998). Protein malnutrition-mediated increase in TGF-β (transforming growth factor β) by live Mtb H37Rv-infected macrophages was also observed, showing a role in mediating immunosuppression and immunopathogenesis in tuberculosis. Protein-malnourished guinea pigs showed weak responses to PPD skin tests and an impaired lymphocyte proliferation (Mainali and McMurray 1998). Chronic protein deficiency in BCG-vaccinated guinea pigs caused a decrease in PPD-stimulated IL-2 production required for initiating and amplifying immune responses (McMurray et al. 1989) as well as significant decrease in the IFN-γ and TNF-α production (McMurray et al. 1986). However, on nutrient supplementation to the vaccinated malnourished guinea pigs, the PPD skin reactivity and the

vaccine-mediated control of bacterial loads were reversed to normal and were in fact indistinguishable from the vaccinated animals that have never been protein deficient (McMurray et al. 1986).

PCM in mice revealed a compromised CMI to tuberculosis leading to huge burdens of bacillary loads and a marked increase in the bacterial dissemination along with a poor granulomatous response and T-cell defects, which reversed on re-feeding with the appropriate diet (Chan et al. 1996), similar to guinea pigs. The loss of resistance to TB by the malnourished mice model was due to an IFN-γ defect that causes a diminished nitric oxide (NO) production, typically responsible for containment of infection, thus leading to virulence (Chan et al. 1992). A murine study (Ishikawa et al. 2009) showed how an undernourished mouse is devoid of a particular antibody that is known to normally respond to the TB vaccine antigen introduced. They concluded that undernourishment not only affects the CMI but also humoral immunity. In a more recent study (Hoang et al. 2015), the effects of PEM on the mycobacterial-specific immunity conferred by vaccination was assessed. PEM during the infection in an already vaccinated mouse resulted in a complete blockade of protection given by the vaccine with a loss in the IL-2+ memory CD4 T cells. PEM during the vaccination phase resulted a decrease in the vaccine efficacy characterized by an overall reduction in all T-cell subsets (with the exception of the TNF-α^{+} cells). However, the interesting part was that an improved diet replenishing the required adequate nutrients resulted in the reconstitution of the T-cell response supported by an improved pathogen clearance.

A diet deficient in protein, vitamin, and minerals resulted in an increase in the number of TB cases in Denmark. With the supplementation of the same, the rate of TB incidence drastically reduced. In another study, there was a considerable difference in TB rate in British POW supplemented with a proper diet (1.2%), in comparison to the Russian POW (15–19%). Both the groups shared the same living and working conditions and were exposed to the similar chance of infection, and yet the Russians saw rapid onset and increased severity of TB with poor granuloma formation, thus supporting the idea of a deficit CMI in malnourished individuals. In fact, the nature of dietary eating too seems to play a rather important role in developing a risk; the lifelong vegetarian Indians had a threefold higher risk of TB in comparison to the omnivore Indians and assumed cobalamin deficiency to be the causal agent. In London, a decrease in the frequency of meat/fish consumption was accompanied with an increase in the risk to TB (Strachan et al. 1995). All these studies outlined the concept of immunodeficiency caused by nutrition, i.e., nutritional immunodeficiency. A study in Peru (Pelly et al. 2005) somewhat gives evidence to this concept. The Peruvian data shows how subjects with low protein in the body were less likely to show a positive tuberculin skin test thereby suggesting an insufficient immune response to infection. A study by Anuradha et al. (2016) recently showed that coexistent malnutrition, defined by low BMI levels, in latent TB individuals is associated with diminished circulatory/systemic levels of protective and pro-inflammatory cytokine response (Type 1-IFN-γ and TNF-α; Type 17-IL-22; IL-1 family-IL-1α, IL-1β, and IL-6) and a heightened regulatory cytokine response (IL-10 and TGF-β). To the best of our knowledge, this was the first study to examine the relationship between cytokine responses and BMI in LTBI in humans.

The study confirms the results from animal studies that characterized malnutrition with a decreased IFN-γ and TNF-α response and an increased TGF-β response. The study not only measured the systemic levels but also the TB antigen-stimulated levels and found that a low BMI (LBMI) is associated with diminished levels of TB antigen-stimulated pro-inflammatory cytokines. Also, showing positive and negative correlations to two of the known protective cytokines (TNF-α and IL-1β) and regulatory cytokines (IL-10 and TGF-β), respectively, the study indicated the former as very strong positive predictors and the latter as very strong negative predictors for the effect of nutrition on TB infection.

Hughes et al. (2009) studied the function of DCs in children with severe acute malnutrition (SAM) in Zambia. On tuberculin stimulation, they observed a defect in the numbers of mDCs accompanied by lower spontaneous DC IL-12 production and fewer IFN-γ-producing T cells in the severely malnourished children. Around 17% of the study patients also witnessed a DC maturation failure measured by the downregulation of the HLA-DR instead of upregulation. These DCs called as the "anergic" DCs were associated with low T-cell proliferation and had a strong correlation with the IL-10 generation. However, on replenishing the nutritional supplements, the numbers increased and the maturation defects restored although not guaranteeing their survival, i.e., survival largely depended on the DC numbers in the infants at the time of admission. Thus, malnutrition appears to exhibit many negative effects on the immune system of the host against infection by *Mycobacterium tuberculosis*.

Malnutrition in Tuberculosis: Effect of Tuberculosis on the Nutritional State

Tuberculosis is a disease that is often known to "consume" an individual and thereby gained popularity as a "consumption" disease particularly in the eighteenth and nineteenth century (Macallan 1999). Tuberculosis is known to be the most frequent underlying cause of wasting worldwide, with the mechanism of the same being unknown (Schwenk and Macallan 2000). In fact, the nutrition status of a patient is a principal determinant of both morbidity and mortality from tuberculosis as the disease is known to affect the metabolism of important nutrients such as protein along with some micronutrients such as zinc, selenium, etc. (Kakkar 2014). Even in the coinfection of TB and HIV, a condition most common in the developing world, the major cofactor of wasting is tuberculosis, drawing even more attention to questioning the mechanism by which the disease causes such a condition and is thus often referred to as the "slimming disease" owing to the wasting effects it causes (Lucas et al. 1994). Several studies reported that patients with active pulmonary TB are malnourished, as indicated by reductions in the level of visceral proteins, anthropometric indexes, and micronutrient status (Onwubalili 1988; Saha and Rao 1989). Several studies have shown how tuberculosis leads to nutritional depletion with reduction in the BMI (Onwubalili 1988; Harries et al. 1988) and people with lower BMI have as much as fivefold higher risk to tuberculosis (Tverdal 1986). Apart from BMI, there were visible

reduction in the skinfold thickness and a great reduction in the MUAC (mid-upper arm circumference), a major representative of wasting.

Tuberculosis leads to wasting presumably due to the production of pro-inflammatory cytokines (Matthys and Billiau 1997) since utilization of amino acids and protein synthesis may be inhibited due to the presence of pro-inflammatory cytokines. Although the mechanism of wasting in TB is unknown, factors such as reduction in the appetite, nutrient and micronutrient malabsorption, altered metabolism (Macallan et al. 1998), loss of fat and lean tissues, as well as anorexia (Miller et al. 2000), all represent the major contributing factors for wasting in individuals affected with tuberculosis disease. The association of TB with cachexia or wasting syndrome is further supported by the prevalence of low serum concentrations of leptin, a satiety-regulating hormone, in patients with PEM (Schaible and Kaufmann 2007). Serum albumin concentrations, another marker of PEM, were found to be significantly lower in the untreated pulmonary TB patients when compared to the healthy controls (van Lettow et al. 2003).

Both malnutrition and tuberculosis are individually associated with high mortality rates especially in the developing world. This brings out the question as to how coexistence of malnutrition in tuberculosis affects the mortality rate of such individuals. Research has shown an increased risk of mortality in patients with malnutrition along with tuberculosis, with the risk increasing superlatively with the degree of malnutrition, according to few studies. A study in the Thyolo District in Malawi (Zachariah et al. 2002) showed that the 35% of patients suffering from moderate to severe malnutrition experienced death earlier than the rest, thereby making moderate (BMI = 16.0–16.9 kg/m^2) to severe (BMI < 16.0 kg/m^2) malnutrition an important risk factor for early mortality rates. A review paper by Schaible and Kaufmann (2007) also estimated that malnutrition is responsible for a nearly twofold increase in the risk of death in TB patients. A recent study done in Central India showed the associated risk of mortality with nutritional status of adult patients having pulmonary tuberculosis. They also saw that severe undernutrition at diagnosis was associated with a twofold higher risk of death (Bhargava et al. 2013).

Malnutrition and its association with tuberculosis have been widely studied in adults in comparison to children. Although the known association between the two, very few studies have been done on the same in children. However, extrapolating data from the various studies done on the adults, BCG-vaccinated children and the LTB-infected children throw some insights on malnutrition in children playing a crucial role in increasing the risk of TB (Cegielski and McMurray 2004). In a study by Satyanarayana et al. (1980) wherein they studied the effect of nutrition on tuberculin sensitivity after BCG vaccination, they observed an association of severe malnutrition with a reduced rate of skin test positivity in children. This association suggests an impaired cellular immune function, thereby leading to an increase in the risk of developing active TB disease. In yet another study, transmission of the active disease to children in household contacts with adults having PTB was assessed. It was observed that the children that were severely underweight were at more risk to acquire the active disease (Singh et al. 2005). However, there have been no studies that directly address malnutrition as a risk factor for TB in children.

Micronutrients and How They Play a Role in TB Immunity and Malnutrition

Malnutrition is a composite syndrome of multiple nutrient deficiencies, and single nutrient deficiencies rarely occur. Micronutrients like iron, zinc, and vitamin A are exceptions that can also occur alone but can complicate PEM (Chandra 1997). The above discussion mainly concerned PEM and on how proteins play a rather important role as a macronutrient in providing immunity against tuberculosis and to keep a check on malnutrition. Micronutrients, however, also play a vital role in the same and are important for the "complete diet" of an individual. Micronutrients are trace elements that although required only in small amounts are important for the proper growth and development of an individual and play a role in the key metabolic pathways along with immune cell function. Chandra (1990) gave a detailed outlook on the concepts of how micronutrients affect the immune system and paralyze its functions. They came up with five concepts: (i) immune responses are altered early during the initial reductions in the micronutrient intake; (ii) the degree of immune impairment relies on the person's age, the nutrient that is deficient, the level of its deficiency, its interactions with the other nutrients, and the presence of any other concomitant infections; (iii) the corresponding abnormalities in the immune functions help us to know the risks associated with the micronutrient deficiency; (iv) too much of the nutrient intake can also cause detrimental effects on the immune system; and (v) determining the lower and upper limit along with the minimum intake required for the individual can be determined by tests on immunocompetence.

Micronutrients include vitamins such as vitamins A, B, C, D, E, etc. and minerals such as zinc, iron, copper, selenium, etc. Each has a different role in providing host immunity. The following data has been collected from various review papers (Chandra 1979; Keusch 1990; Bhaskaram 2002; Bourke et al. 2016; Ibrahim et al. 2017) that have efficiently compiled the data on how the presence and absence of various micronutrients (both as a single nutrient deficiency and in combinations) lead to a state of malnutrition that affects the immune responses in the host against infections such as TB.

Iron, an important micronutrient for both innate and adaptive immunity, is known to have a role in various pathways of the immune response. Iron deficiency leading to anemia is a major public health problem, and anemia is common in tuberculosis patients. However, too much iron has detrimental effects to host defense. It impairs the macrophage suppression of invading microorganisms. Moreover, it is also known to be essential for the growth of various pathogens when available in the free form. This makes it tough to determine the effect of iron deficiency in increasing the susceptibility to an infection. There are studies in support of both, viz., studies that show iron deficiency increased the risk to infection and studies that show that iron supplementation increased the susceptibility to infections. When an infection occurs, the reticuloendothelial cells seize iron from the blood and phagocytes by releasing lactoferrin which binds to iron more efficiently than the siderophores of the bacteria thereby depriving the pathogen from iron resulting in an impaired bacterial replication along with an impaired killing of the bacteria by the phagocytes. These

studies demand more research in order to establish optimal levels of iron and on how we can use iron repletion effectively by maximizing the host defense and minimizing the pathogen multiplication.

Zinc, a cofactor for various enzymatic reactions, is an important micronutrient necessary for regulating various immune responses of the body. Its functions include promoting Th1 cell differentiation and cell responses by increasing IL-2, IFN-γ, and IL-12Rβ2 expression levels and the deficiency leading to lymphopenia with reduced CD4/CD8 ratio. It also promotes the activity of the thymic hormone with the deficiency leading to thymic atrophy with a reduced number of circulating T cells and a delayed or reduced PPD sensitivity. Another important function is regulating the release of pro-inflammatory cytokines. The deficiency is accompanied by a reduced number of the Th1 cytokines along with an impaired NK cells and macrophage phagocytosis functions. The decreased phagocytosis thereby affects macrophage-mediated in vitro cellular killing, proved to be restored after zinc supplementation. These functions make zinc deficiency a serious trouble, and unfortunately one-fifth of the world's population is affected by zinc deficiency. Also, around half a million child deaths (under the age of 5 years) each year are due to deficiency of zinc in the nutrition (Krebs et al. 2014), and this often accompanies childhood PEM. Zinc deficiency has profound effects on cellular function, and its presence is necessary for proper childhood growth and sexual maturation. In another study, plasma zinc levels were measured before and after the antituberculosis treatment (ATT), and the levels were shown to improve post-treatment, thus suggesting to be used as a marker for treatment response as well as for disease severity.

Vitamin A deficiency is a global health problem that affects 100 million to 140 million children and is the leading cause of childhood blindness worldwide. The only source of retinol (vitamin A) is through diet; it is absorbed by the enterocytes and stored in the liver. PEM leads to inadequate amino acids necessary for the synthesis of vitamin A transport proteins in the liver, thus complicating the state of vitamin A deficiency. Retinoic acid (RA) plays a role in the proper differentiation of epithelial cells, the lack of which causes pathogenic bacterial and viral invasion easier. It also has key roles in regulating both innate and adaptive immunities. Vitamin A deficiency seems to have an association with a reduction in the ILC3s that in turn led to reduced expression of IL-17 and IL-22 rendering increased susceptibility to acute enteric bacterial infection. The same vitamin A-deficient mice also exhibited an increase in the IL-13-producing ILC2 population with consequent increases in the amount of intestinal mucus, goblet cell hyperplasia, and resistance to intestinal helminths as a result of retinoic acid receptor (RARα)-dependent signaling, thus regulating the balance between the two subsets. Studies on the effect of vitamin A on tuberculosis have not yet been extensively done. The few ones that are done seem to show that the levels of it are lower in tuberculosis infection, but the levels seem to come back to normal even in the absence of supplementation. RA therapy for tuberculosis significantly increased the numbers

of $CD4^+$ and $CD8^+$ T cells, NK cells, and $CD\text{-}163^+$ macrophages in the infected lung tissues in rats with tuberculosis. There was also a significant difference in the severity of tuberculosis histopathology between control and RA-treated rats having tuberculosis thus showing that administration of RA seems to curtail the growth of Mtb in vivo. A combined micronutrient supplementation that includes vitamin A and zinc with other micronutrients might be helpful for TB patients since zinc plays a role in vitamin A metabolism and its deficiency leads to a secondary vitamin A deficiency. A recent study in 2017 on Moroccan patients with tuberculosis confirmed the association of low plasma vitamin A concentration to the TB disease, thus suggesting that vitamin A deficiency could be considered as a risk factor for infection with Mtb, but the role of vitamin A in enhancing immunity against Mtb is to be further assessed. Finally, vitamin A deficiency has been shown to increase the risk of TB ten - fold, making it one of the most important risk factor for active TB (Aibano et al. 2017).

Vitamin D deficiency is estimated to affect one billion people worldwide and is common in children and young adults. Vitamin D synthesis in the skin by means of UV light exposure (present in sunlight) is of critical importance due to the absence of vitamin D in dietary food. Most of the deficiency occurs due to lack of exposure of the skin to sunlight. Serum 25-hydroxyvitamin D [$25(OH)VD_3$] level is used to categorize a person as vitamin D deficient or insufficient, i.e., vitamin D deficient when the serum $25(OH)VD_3$ level is below 25 nmol/l and vitamin D insufficient when the serum $25(OH)VD_3$ level is 50–75 nmol/l. It exhibits roles in both pro-inflammatory antimicrobial effector function and suppression of anti-inflammatory activity. CYP27B1 is a gene that provides instructions for making the enzyme 1-alpha-hydroxylase (1α-hydroxylase), a vitamin D-activating enzyme that converts vitamin D to its active form. Recently several evidences suggest that this expression is under the control of several immune signaling pathways and has a role in exhibiting innate immune responses to bacterial infection. Mtb infection enhances TLR2-mediated induction of CYP27B1 and VDR in monocytes. Various studies showed that TLR4/NFκB and IFN-γ receptor (IFNγR)/STAT1 pathways also induced the expression of CYP27B1 and VDR with the IFN-γ receptor (IFNγR)/STAT1 pathway leading to an antibacterial effect by inducing autophagy as well as generation of the antimicrobial peptides cathelicidin (LL37) and β-defensin-2 that restrict the growth of Mtb in the macrophages in the presence of sufficient vitamin D, thus suggesting importance of vitamin D in rendering protection. 1,25-dihydroxyvitamin D enhanced the Mtb-induced expression of IL-1β that increased antimycobacterial activity via the NLRP3/caspase-1 inflammasome. VDR signaling also enhances the production of cathelicidin (LL37) and β-defensin-2.

Studies that show the effect of vitamin D on tuberculosis infection and treatment have resulted in promising results. A lot of studies are done in adults in the support that individuals with vitamin D deficiency are at an increased risk to get active TB pertaining to the VDR polymorphisms. Moreover, vitamin D deficiency enhanced relapse after ATT in a study done on HIV-TB-coinfected

individuals in both HIV-uninfected and HIV-coinfected patients. In some studies, the sputum smear conversion rates in support of treatment accelerated on vitamin D supplementation and also brought back the serum inflammatory cytokines and chemokines to normal level. In a randomized, placebo-controlled trial on smear-positive PTB patients in London where three doses of 2.5 mg each of vitamin D3 (cholecalciferol) were given as an adjunct with ATT, a significantly improved sputum conversion time was observed specifically in the subjects that had the tt genotype of the TaqI vitamin D receptor polymorphism. Surprisingly, a lower dose (100,000 IU) of oral cholecalciferol at three time points after ATT did not give positive results. Similarly, two doses of 600,000 IU of intramuscular vitamin D3 after ATT gave better results than the placebo. Even with respect to extra-pulmonary TB, adjunctive vitamin D with ATT showed improved results clinically. In 2015, it was shown in two articles that vitamin D levels and the incidence of active tuberculosis disease among contacts of patients with pulmonary tuberculosis are inversely associated. Moreover, the incidence of latent tuberculosis infection (LTBI) in the contacts of patients with PTB is reduced by vitamin D supplementation. All of these studies suggest that the presence or absence of vitamin D leads to immunomodulation however not explaining any direct beneficial effect in any of the clinical trials.

Selenium plays a critical role in mycobacterium clearance reportedly by reducing oxidative stress and enhancing the antioxidant status in the TB-infected individual via selenoproteins.

Cholesterol helps in enhancing the cytotoxicity of human lymphocytes, and its deficiency in TB affects the cellular immunity further, thereby proving to have negative effects. Infections are known to be accompanied by increased energy expenditure, tissue breakdown, and a decrease in the circulating levels of trace elements such as zinc and iron, thereby leading to an increased requirement of both macro- and micronutrients in infectious diseases like tuberculosis (Semba et al. 2010).

Nutrition Supplementation as Adjunctive Antituberculosis Therapy (ATT): Can Nutrition Supplementation Improve or Enhance ATT?

Tuberculosis leads to wasting which comprises of losses both in the fat mass and lean/protein mass, and a nutritional replenishment requires approximately an equal increase in both fat and fat-free/lean tissue masses. Antituberculosis treatment or therapy by itself is seen to increase the weight of the individual on therapy. The nutritional repletion occurs on treatment provided the intake is more than the body needs for energy expenditure required for tissue regeneration. However, studies have shown that this increase is more in the fat mass and not the lean mass suggesting an ongoing anabolic block even during treatment. Hence, for the complete nutritional replenishment, protein intake provided in the everyday diet as a nutritional supplement might be helpful in achieving a balanced increase in both the masses. Also, the

replenishment by treatment is a slow process with one study showing a significantly lesser increase in the MUAC (mid-upper arm circumference) and serum albumin levels even at the end of 1 year of therapy (Onwubalili 1988). Also, the nutritional status was better in the ones having a longer stay in the hospitals owing to the patient care and better food supply in hospitals questioning if the unavailability of proper food resources is limiting the recovery of TB patients undergoing chemotherapy (Kennedy et al. 1996). Vitamins and minerals also can be given as adjuvants in ATT and have proven to enhance the lymphocyte proliferation.

However, a more elaborate understanding of the pathophysiology of wasting is necessary, but an additional nutritional supplementation definitely aids in the recovery from tuberculosis by improving the ability to fight the disease and improving the treatment response, thereby reducing mortality, but the evidence for the same is unfortunately limited. The nutritional requirements may differ for individuals and can be assessed based on the nutritional status at the time of diagnosis by measuring the BMI. A good nutritional status can in fact be a preventative measure against TB and many such infections. Thus, nutritional supplementation is a novel approach recommended to improve TB outcome and also might help in the fast recovery of tuberculosis patients.

Policies and Protocols

Policies

Our chapter attempts to describe the importance of nutrition in developing a robust defense mechanism in the host against a pathogen (in our case *Mycobacterium tuberculosis*). It is important that the patient and his caretaker are both well informed about the effects that nutrition can have in the treatment of the disease to ensure that proper nutrition is provided. Also, along with the drugs for treatment, a proper diet schedule that includes the necessary nutrients (either in the food or as additional supplements) can possibly enhance the treatment. One can also find which nutrient deficiency they have so as to focus on eradicating that particular deficiency for better treatment results. Proper supervised diet and environmental conditions can have major effects on the rate of treatment of an individual.

Protocols

TB: Signs and Symptoms

- Latent TB infection (LTBI) – asymptomatic and noncontagious in nature.
- Active TB disease – long-lasting cough, often with blood or mucus, loss of appetite and thereby loss of weight, fatigue, fever, weakness, night sweats, chills, and chest pain in case of pulmonary TB. In case of extrapulmonary TB, the symptoms reflect respective to the organ infected.

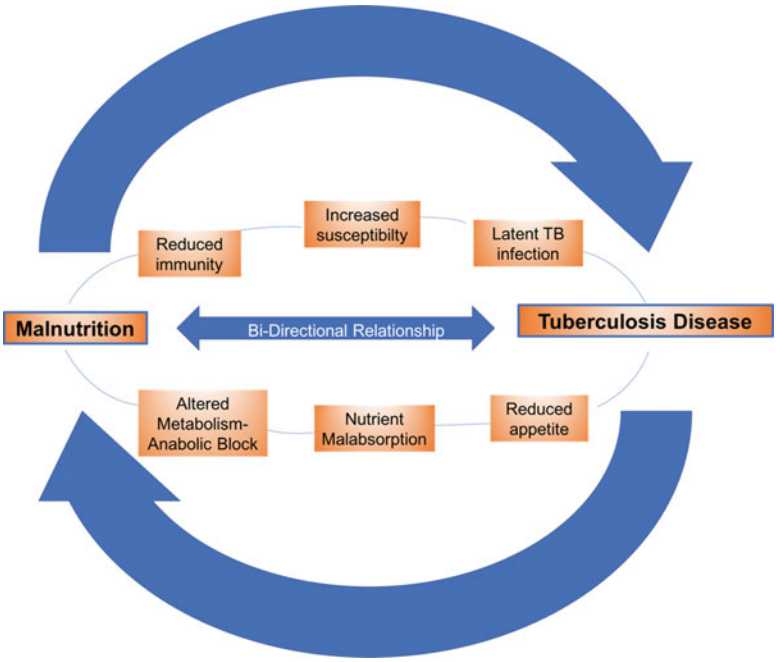

Fig. 1 Tuberculosis-malnutrition: a bi-directional relationship. The figure represents the vicious cycle of malnutrition and tuberculosis where one worsens the state of the other. Given also are the various parameters that result in this cycle

TB: Diagnosis

Various tests are used to diagnose TB in humans. These include PPD skin test (less sensitivity), QuantiFERON-TB Gold In-Tube test and T-SPOT, chest X-ray, sputum smear microscopy (SSM), culture test, and the recently added GeneXpert test. The PPD skin test and QuantiFERON test are used for LTB diagnosis, whereas the chest X-ray, SSM, and culture tests are specific for active pulmonary TB.

Dictionary of Terms

- **Adoptive transfer** – The transfer of cells, usually immune cells, from a healthy individual or patient to another patient. It is gaining popularity as an immunotherapy for certain ailments.
- **All-cause mortality** – The deaths that occur within a specific time period without accounting for the exact cause of the disease.
- **Anthropometric indicators** – Indicators that are used to assess the size, shape, and composition of the human body, to interpret the type and stage of malnutrition.

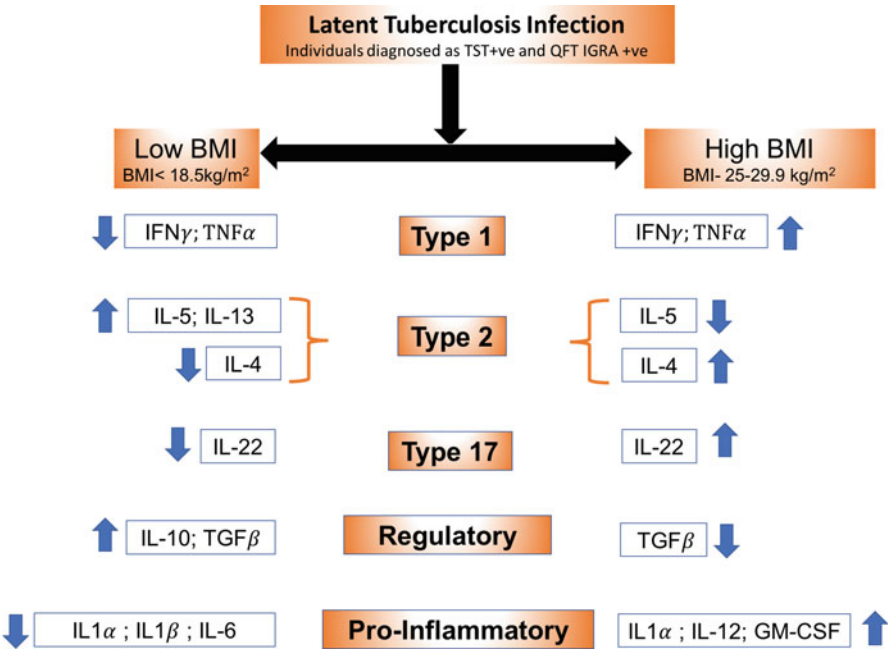

Fig. 2 BMI levels modulate the immune responses in LTBI individuals. The figure shows the upregulation and/or downregulation of the various Type 1, Type 2, Type 17, regulatory, and pro-inflammatory cytokines in LTBI-infected individuals with low and high BMI levels. LTBI, latent tuberculosis infection; BMI, body mass index

- **BMI** – **Body mass index** of an individual is calculated as his weight to square of the height ratio. It basically gives an idea about what is an ideal weight for a person's respective height and assesses malnutrition in adults.
- **MUAC** – Mid-upper arm circumference is one of the anthropometric measurements of the upper arm to assess malnutrition in children with age ranging between 6 and 59 months.

Summary Points

- The chapter aims at looking into the cause-effect relationship shared by malnutrition and tuberculosis.
- Malnutrition and occurrence of infection have long been linked with each other, with one affecting the other.
- Evidences suggest that tuberculosis is no exception.
- Malnutrition predisposes the person to secondary immune deficiencies predisposing them to tuberculosis.
- Tuberculosis itself leads to wasting thereby infamously also known as "slimming disease."

- Coexistence of the two seems to be extremely detrimental.
- Pediatric TB and malnutrition although widely prevalent have not been studied as coexistent infection extensively.
- Our chapter also looks into how micronutrient malnutrition complicates the tuberculosis disease.
- Finally, the chapter also throws light upon nutritional replenishment affecting the antituberculosis therapy (Figs. 1 and 2; Table 1).

Table 1 Studies on effects of nutritional supplementation on tuberculosis treatment. The table lists various studies done globally to witness the effect of micro- and macronutrient supplementation on antituberculosis treatment. The table also shows the respective conclusions obtained along with the journals in which the results were published

S. no.	Author and year	Result	Journal
1	Karyadi et al. (2002)	Vitamin A and zinc supplementation resulted in • An increased TB treatment efficacy after 2 months of the ATT • An earlier sputum smear conversion	Am J Clin Nutr
2	Das et al. (2003)	Iron supplementation accelerated the hematopoiesis in TB patients with mild or moderate anemia during the initial treatment phase	Br J Nutr
3	Range et al. (2006)	Multivitamin/mineral (MVM) with zinc supplementation during the ATT reduced mortality rates in HIV-TB co-infected individuals	Br J Nutr
4	Villamor et al. (2008)	Micronutrient supplementation • Reduced the risk of early TB recurrence in HIV-TB co-infected patients • Increased T cell numbers with reduced complications in the HIV uninfected individuals	J Infect Dis
5	Schon et al. (2011)	Supplementation of a diet rich in arginine showed • No overall clinical effect in TB patients • Increased cure rate in HIV-TB co-infected sub group • Poor clinical outcome in the co-infected sub group having low initial levels of eNO (nitric oxide in exhaled air)	Tuberculosis (Edinb)
6	Isanaka et al. (2012)	Iron overload and deficiency both showed association with poor treatment outcomes and increased mortality in TB patients	PLoS One
7	PrayGod et al. (2012)	Protein energy supplementation given to the individuals co-infected with pulmonary TB & HIV showed no overall changes on the body weight and composition however showed an increase in the handgrip strength	Br J Nutr
8	Jeremiah et al. (2014)	Nutritional supplementation leaded to a higher rifampin exposure in HIV-TB co-infected group reducing the negative effects of HIV on the drug levels during co-infection	Antimicrob Agents Chemother

TB tuberculosis, *ATT* antituberculosis treatment, *HIV* human immunodeficiency virus, *MVM* multivitamin/mineral

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Malnutrition in Hepatitis C Virus (HCV) Disease

48

Faisal Waseem Ismail and Ehsun Naeem

Contents

Abstract

Hepatitis C is a viral infection of the hepatocytes that commonly presents as a chronic progressive liver disease. In its chronic form, HCV is often associated

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with nutritional disturbances secondary to the impaired metabolic function of the liver. In fact, the prevalence of malnutrition is believed to be as high as 65–90% in patients with chronic liver disease.

Malnutrition in Chronic HCV may present as protein-calorie malnutrition, manifesting as Sarcopenia or as micronutrient deficiencies. In addition to the signs and symptoms of the underlying liver disease, malnourished patients often present with weight loss, anorexia, nausea, skin and vision changes, as well neurological deficits. In chronic liver disease, nutritional impairment stems from a combination of disturbances in oral intake, absorption of macro- and micronutrients, and regulation of the physiological and biochemical processes of the body.

Subjective Global assessment is often favored as a screening tool for easy identification of malnutrition in HCV patients as it can be used in all settings. More Specialized tools like bioelectric impedance analysis and Dual-energy X-ray absorptiometry are not widely available and only indicated in certain patients such as liver transplant candidates. Additional laboratory investigations such as Serum levels of proteins like albumin may also provide useful clues regarding the nutritional status of HCV patients, but only in the absence of decompensated liver disease.

Management of malnutrition in chronic HCV patients resolves primarily around restoring muscle health. A significant aspect of management is to ensure a balanced diet that contains an adequate amount of calories and limits the amount of fat and simple sugars like fructose that have been implicated in the progression of liver disease.

Keywords

Hepatitis C · Chronic liver disease · cirrhosis · malnutrition · Sarcopenia · Insulin resistance · Subjective Global Assessment · Bioelectric impedance analysis · Dual-Energy X-ray absorptiometry · anthropometry · albumin · dietary supplementation · physical activity

List of Abbreviations

BCAA	Branched-Chain Amino Acids
CLD	Chronic Liver Disease
HCV	Hepatitis C Virus
INR	International Normalized Ratio
MELD	Model for End-Stage Liver disease
PCM	Protein-Calorie Malnutrition
PEG tube	Percutaneous Endoscopic Gastrostomy tube
SFT	Skin fold thickness
TIPS	Transjugular Intra-hepatic Porto-systemic shunt

Introduction

Hepatitis C is a viral infection of the hepatocytes spanning a spectrum from acute infection to chronic progressive liver disease and decompensated cirrhosis in advanced cases. Acute HCV is usually asymptomatic, very rarely leading to hepatic failure. More commonly though, Hepatitis C presents as gradually progressive chronic liver disease with up to 85% of patients going on to develop cirrhosis over the next 20–30 years (Chen and Morgan 2006) (Fig. 1).

As the primary metabolic organ in the body, liver holds a central role in the regulation of biochemical processes with respect to nutrient intake. Minimal hepatocyte injury in the earlier stages of HCV infection manifests only as subtle nutritional deficits. Only when a significant proportion of hepatocytes have been destroyed, as often seen in chronic liver disease in HCV patients, does the liver function begin to be affected. These alterations in liver function tend to result in poor nutritional intake as well as malabsorption of consumed nutrients and may have significant implications on the body's nutritional status. Studies have indicated the prevalence of malnutrition to be as high as 60–90% among patients suffering from chronic liver disease (Lautz et al. 1992; DiCecco et al. 1989)

Malnutrition in HCV is a serious complication that negatively impacts survival, quality of life, and response to stressors like infections and surgery (Merli et al. 1996). In cirrhotic patients, it manifests not just in the form of Protein-Calorie Malnutrition (PCM) but also Micronutrient deficiencies, particularly in case of an advanced liver disease. Both PCM and micronutrient deficiencies have been linked with a poorer outcome in patients with compensated as well as decompensated

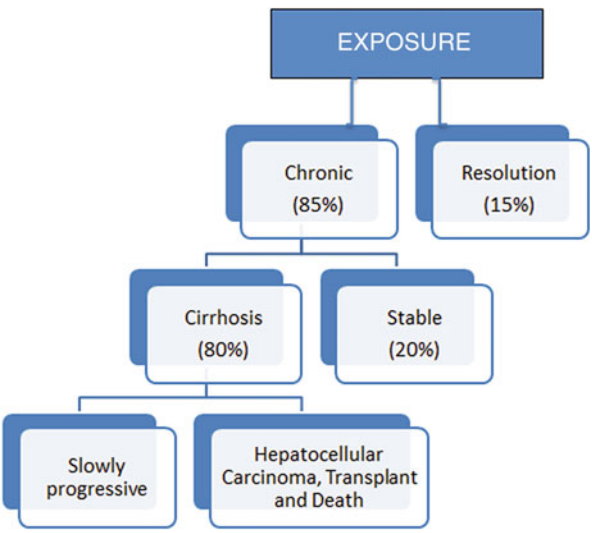

Fig. 1 Natural history of hepatitis C virus infection, depicting the relative frequency of each possible outcome (Cabré and Gassull 2000; Møller et al. 1994, Plauth et al. 1997)

cirrhosis. On the other hand, obesity correlates equally well with a poor prognosis in HCV patients and has been linked to high viral loads and liver damage in patients, particularly the elderly (Petta et al. 2010).

PCM in chronic HCV has been linked to an increase in mortality as well as morbidity by predisposing patients to complications like variceal bleeding and ascites (Cabré and Gassull 2000; Møller et al. 1994; Plauth et al. 1997). It manifests as Sarcopenia or loss of lean muscle mass which is regarded as the most objective feature of malnutrition in patients with chronic liver disease.

Deficiency of micronutrients like vitamins A, C, K, B complexes contributes equally to morbidity by causing skin and vision changes, coagulopathies, and neurological symptoms.

HCV patients commonly co-present with obesity and fatty liver (Menta et al. 2015), which are thought to potentiate the pro-inflammatory state observed in these patients, as evidenced by the higher circulating and hepatic CRP levels in obese patients with HCV (Jonsson et al. 2008). In addition, Visceral adiposity is also thought to enhance HCV-induced whole-body Insulin resistance due primarily to the role of free fatty acids released by visceral adipocytes. Furthermore, studies have demonstrated increased levels of ALT in overweight patients, suggesting an increased risk for liver disease (Ruhl and Everhart 2003).

Chronic liver diseases like hepatitis C that cause cirrhosis are among the most common indications for liver transplantation worldwide (Mazurak et al. 2017). The Model for End-Stage Liver Disease or MELD score used by most liver transplant centers for prioritization of organ allocation, despite its innumerable benefits, is limited by its failure to adequately include nutritional status in the evaluation of patients (Wiesner et al. 2003). For this reason, other tools like the Child-Pugh classification that use objective measures of nutritional status such as the International Normalized Ratio (INR) and Serum Albumin levels are deemed superior to the MELD score, further highlighting the importance of nutritional status as a prognostic indicator in patients with chronic HCV.

Etiology

Malnutrition in chronic liver disease has a multifactorial etiology. Combined disturbances in dietary intake, absorptive capacity, and carbohydrate metabolism, as well as a negative energy balance, predispose patients to nutritional impairment in chronic liver diseases, like HCV (Fig. 2).

Food aversion and intolerance in hepatitis C secondary to signs and symptoms of CLD such as nausea, anorexia, gastritis, and encephalopathy lead to a reduced dietary intake. Additionally, most patients with hepatitis C treated with interferons and direct acting antivirals tend to experience loss of appetite and metallic taste as adverse effects. Deficiencies in vitamin A and zinc lead to an altered sense of taste

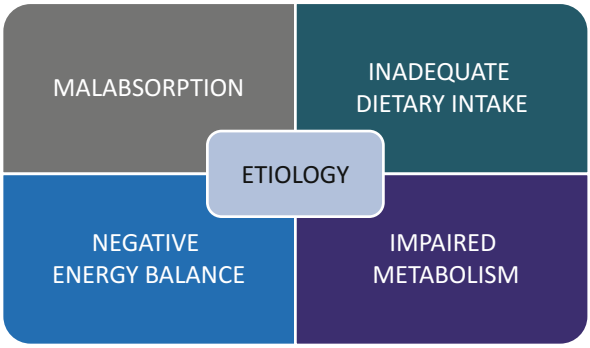

Fig. 2 Etiological factors of malnutrition in HCV and liver disease patients

for food, already low in Sodium, further compounding the problem of reduced oral intake (Madden et al. 1997).

Bile salt deficiency due to reduced entero-hepatic recycling in patients with impaired liver function has been proposed as the primary mechanism of Malabsorption in cirrhotic patients (Vlahcevic et al. 1971; Romiti et al. 1990). Other contributing factors include altered intestinal motility and mucosal injury that may be secondary to portal hypertension or bacterial overgrowth, which in itself impairs absorption of nutrients via small intestinal mucosal surface (Bode 2003, 1980; Dinda et al. 1988; Sarfeh et al. 1986).

In addition to depletion of glycogen stores, hepatocyte injury, in conjunction with insulin resistance, impairs glucose regulatory processes like gluconeogenesis further increasing reliance on alternative energy fuels such as lipids and fats, for the metabolic needs of the body. In addition to the depletion of vital macromolecules, preferential oxidation of lipids further reduces the respiratory quotient in CLD patients relative to those with normal liver function (Merli et al. 1990)

A combination of reduced urea hepatic protein synthesis, impaired intestinal protein absorption, and increased urinary nitrogen excretion predispose cirrhotic patients to sarcopenia and also lead to a lowered ratio of branched-chain amino acids to aromatic acids in the blood.

CLD patients often exhibit an increase in resting energy expenditure, as measured by indirect calorimetry. Hypermetabolism thought to be due to an increase in beta-adrenergic activity has been found to correlate well with a reduction in survival in CLD patients by some studies (Müller et al. 1999). The increased frequency of hypermetabolism in patients with complications like ascites and its negative impact on survival highlight the need to identify and address these complications earlier on in the disease course.

Obesity is often associated with elevated circulating levels of cytokines like leptin released by adipocytes (Schmidt et al. 2015). These adipocytokines with pro-fibrogenic properties are thought to play an important role in inducing a state of low-grade inflammation and hepatic fibrosis due to their pro-fibrogenic properties (Saxena and Anania 2015), as shown in Fig. 3. Free fatty acids secreted by visceral

Fig. 3 Role of obesity in progression of liver cirrhosis in HCV patients

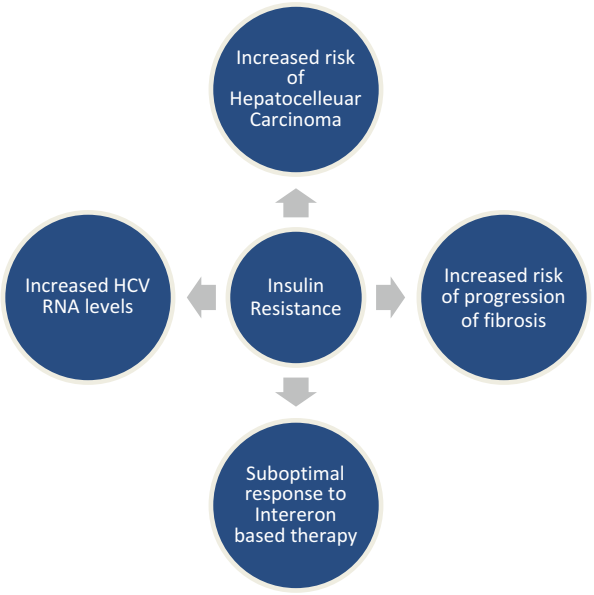

Fig. 4 Impact of insulin resistance in HCV infected patients

adipocytes also impair insulin signaling in the skeletal muscle and liver by activating the lipid-activated protein kinase C family, contributing to insulin resistance in HCV patients (Kawaguchi and Mizuta 2014) (Fig. 4).

Presentation

Nutritional assessment of patients with hepatitis C allows for identification of nutritional risks that may contribute further to morbidity and mortality. Recognition of macro- and micronutrient deficiencies is important as their correction with supplemental nutrition can significantly reduce the risk of infections as well as in-hospital morbidity and improve liver function parameters of such patients. This assessment needs to be much more detailed in patients presenting with decompensated cirrhosis as they are more likely to be at risk for nutrient deprivation. In general, adequate assessment of the nutritional status requires a thorough history, detailed physical examination, and appropriate laboratory investigations.

History

Pertinent findings in patient history may include changes in weight, which can be quantified as the percentage of weight lost involuntarily over the preceding 6 months. Generally, weight loss $> 10\%$ is taken as severe. It is important to note however that a patient with decompensated cirrhosis, presenting with salt and water retention, may not be able to report an accurate history of weight changes.

Dietary intake may be assessed using the 24-h dietary recall to identify incorrect dietary practices commonly observed in patients with CLD, who are often advised to consume a diet low in protein by ill-informed attendants as well as healthcare practitioners.

Other symptoms to inquire about include gastrointestinal disturbances such as diarrhea, vomiting, or constipation that may indicate underlying malabsorption (Quigley 1996).

The severity of the underlying liver disease can be assessed by inquiring about complications like ascites and encephalopathy that may manifest as increasing abdominal girth, respiratory difficulties, and altered mental status or disturbances in sleep wake cycle.

Micronutrient deficiencies may present in advanced cases of liver disease with symptoms like night blindness or photophobia (vitamin A), burning of the mouth or tongue (vitamin B12, folate), easy bruising (vitamin C, K), paresthesias (thiamine, pyridoxine), or even skin lesions (zinc, vitamin A, niacin).

Physical Examination

A general physical examination should be performed on all patients at risk for nutritional deficiencies with particular attention to the presence of peripheral edema and ascites, muscle wasting, and signs of vitamin/mineral deficiencies such as pallor (iron deficiency), hyperkeratosis (vitamin A), dermatitis (vitamin A), bruising (vitamin C, vitamin K), glossitis (vitamin B12, folate, niacin), angular stomatitis (vitamin B12), and reduced lower extremity deep tendon reflexes (vitamins B12, B1).

An accurate assessment of the patient's weight and height needs to be performed and recorded for reference in future as it may facilitate tracking of the patient's physical parameters.

Screening

To date, several nutritional assessment tools have been developed, and although many of these remain in use in various parts of the world, no one tool has been accepted as the Gold standard yet.

To be effective, a screening tool needs to be simple, easy to use, and patient-friendly, and for that reason, subjective global assessment is one of the most

Table 1 Guidelines for subjective global assessment categories

Stage A	Stage B	Stage C
Well nourished	>5% eight loss within a few weeks	Obvious signs of malnutrition[a]
OR		
Recent non fluid weight gain And/Or Improvement in dietary intake or patient reported symptoms	No weight stabilization or weight gain Definite increase in Intake Mild subcutaneous tissue loss	Clear and convincing evidence of weight loss

[a]Severe loss of subcutaneous tissue and/or peripheral edema

commonly employed scales to identify patients suffering from nutritional deprivation.

It combines multiple elements of nutritional assessment to classify the severity of malnutrition from mild to severe. These components include recent weight loss, changes in dietary intake, gastrointestinal symptoms, functional capacity, signs of muscle wasting, and the presence of pre-sacral or pedal edema. On the basis of these assessments, patients are classified as well nourished (grade A), moderately malnourished (grade B), or severely malnourished (grade C)

The SGA is a simple bedside method, proven to be adequate for the purpose of identification of malnutrition among patients with HCV. It is an excellent tool to assess nutritional status that can be applied on the bedside as it uses elements that can all be derived from a focused history and physical examination, rendering expensive, and time-consuming laboratory tests unnecessary (Hasse et al. 1993).

SGA, however, has not been found to be as reliable as handgrip strength, in predicting the complications of cirrhosis, as indicated by some studies (Alvares-da-Silva and Reverbel da Silveira 2005) (Table 1)

Laboratory Evaluation

Several laboratory and radiologic investigations may provide objective evidence of malnutrition in patients with HCV. Of these, specialized investigations like bioelectrical impedance analysis and dual-energy X-ray absorptiometry are not performed routinely but may be indicated in certain cases of an advanced liver disease, cirrhosis, or patients being evaluated for liver transplant.

Serum levels of proteins including albumin, prealbumin, transferrin, and coagulation factors, which are generally regarded as useful indicators of nutritional status in the general population, are not as reliable in patients with decompensated cirrhosis due to hepatic synthetic dysfunction, limiting their use in HCV to patients with acute infection and early cirrhosis. Of these proteins, changes in levels of prealbumin are more reflective of acute changes in nutritional status owing to its relatively short

half-life of two to three days, as compared to albumin which has a half-life of approximately 20 days.

Reduced hepatic synthesis, decreased muscle mass, and increased tubular secretion are all responsible for decreased serum levels of creatinine, another marker of lean muscle mass, in cirrhosis.

Serum levels of fat-soluble vitamins may be normal in HCV patients in the absence of advanced liver disease when fat malabsorption due to impaired enterohepatic recirculation of bile salts leads to deficiencies of these vitamins. Plasma levels of vitamins A, D, and E, as well as INR, may be deranged in such patients.

Levels of water-soluble vitamins such as folate and zinc may also be deranged. Serum vitamin B12 levels may be elevated in chronic HCV and cirrhosis due to hepatic cytolysis and release of stored vitamins and minerals. Patients with a history of concurrent alcohol abuse may also show decreased levels of thiamine. Deficiencies of other B complexes may present with decreased levels of hemoglobin and deranged mean corpuscular volume of RBC's.

Ancillary Tests

Anthropometry

Among anthropometric measures used to identify malnutrition in HCV patients, mid-arm muscle circumference (MAMC) and hand-grip strength have been found to be the best indicators of protein-calorie malnutrition. Based on studies, a mid-arm muscle circumference of <23 cm in combination with a handgrip strength of <30 kg is thought to have a sensitivity of 94% and negative predictive value of 97% for diagnosing PCM (Figueiredo et al. 2000).

Meanwhile, body fat reserves have found to correlate best with skin-fold thickness, which has produced similar results as DEXA scans when used in studies on patients without ascites. For this purpose, the SFT over triceps may easily be measured at the midpoint between the acromion and olecranon using a caliper. A value lower than the 5th centile suggests severe malnutrition.

Miscellaneous Tests

Several other specialized tools for nutritional assessment can be used in certain cases of chronic liver disease, but are not routinely employed primarily due to their cost and limited availability.

Bioelectrical impedance analysis (BIA) uses impedance which measures body water to estimate the fat content of the body. One of the limitations to its use is that even small variations in electrode placement tend to result in relatively large errors in measured impedance.

Dual-energy X-ray absorptiometry (DEXA) scan can be used to accurately measure fat mass in patients with chronic liver disease but is unable to estimate

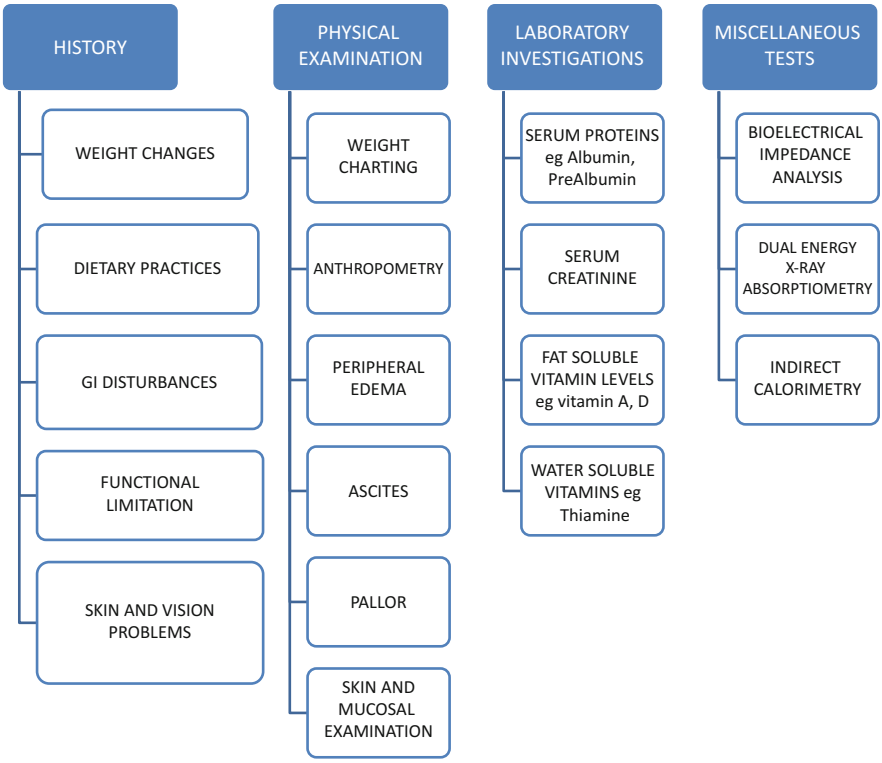

Fig. 5 Clinical, laboratory, and radiological tools for assessment of malnutrition in HCV patients

the lean body mass due to increases in extracellular water in patients with decompensated liver disease (McCullough et al. 1991).

An accurate assessment of the nutritional needs using resting energy expenditure can significantly help in management of patients with chronic HCV as well as cirrhosis secondary to other causes. This can be done either using the Harris Benedict equation (HBE) that relies on factors like weight, age, gender, and height or using Indirect Calorimetry which is considered to be the gold standard assessment (Plevak et al. 1994; Peng et al. 2007). Because it is not widely available though, its use has been limited to patients unable to meet their nutritional requirements despite adequate therapy (Fig. 5).

Management

Ensuring adequate nutrition can significantly improve quality of life in HCV patients. Good nutrition results in improved compliance with pharmacologic therapy and has also been found to hinder disease progression by preventing the development of HCV complications like cirrhosis and hepatic encephalopathy.

In the absence of decompensated cirrhosis or comorbid conditions like diabetes mellitus, HCV patients usually do not require special diets. However, it is imperative that they consume a balanced diet, containing an adequate amount of calories and proteins to fight the infection as well as antioxidants to combat free radicals responsible for hepatocyte damage. In addition, frequent small meals have been found to improve nitrogen and substrate use, diminish fat and protein oxidation, and prevent depletion of glycogen stores.

Major principles in management of malnutrition in chronic HCV revolve around restoring muscle health. In accordance with this, most interventions are focused on providing a diet that is adequate in proteins and calories and integrate physical activity into the patient's lifestyle. Because management of malnutrition in such patients is directed at addressing the underlying etiology, it is important to obtain a good understanding of the nutritional intake patterns and barriers to healthy eating. The best way to achieve that is through a detailed history and physical exam at each clinic visit, as well as counseling sessions with a dietician, who may be able to further draw up an individualized plan for each patient based on his/her need (Tandon et al. 2017). Because a dietitian may not be available in many clinical settings, physicians may need to familiarize themselves with guideline recommendations for both diet and activity.

HCV patients who are unable to meet their caloric requirements due to a diminished nutrient intake and early satiety should be advised to take frequent small volume meals up to 4–6 times/day (Amodio et al. 2013). Patients presenting with refractory ascites with a low MELD score and those suffering from portal hypertensive enteropathy may be offered TIPS in order to improve nutrient absorption. Replacement of trace elements such as zinc, magnesium, and vitamin A may be considered in patients presenting with food aversion due to micronutrient deficiencies, as per their symptoms and serum levels.

Enteral feeding via a feeding tube is only recommended for patients who are unable to consume food orally despite the above interventions, as tolerance is often an important issue with a significant number of patients prematurely removing their feeding tubes themselves. Therefore, an oral route should be preferred as the first line intervention, even in the presence of complications like esophageal varices. Due to the high risk of complications, PEG tube placement is discouraged as well.

Patients with cirrhosis are prone to fluctuating sugar levels and are thus advised to avoid fasting longer than 6 h as it may trigger a hypoglycemic episode and lead to additional complications. Cirrhotic patients are also advised to take small frequent meals throughout the day as well as an evening snack of complex carbohydrates to decrease lipid oxidation and reduce skeletal muscle proteolysis.

It is generally recommended that HCV patients limit fat to 25% of the total calories in their diet. The general recommendation for diet composition is to limit carbohydrates to 50%, mostly in the form of complex sugars, protein to 20%, and total caloric intake to 30–40 kcal/Kg/day of the desirable body weight. It is also recommended that the consumption of processed sugars like fructose be avoided given its association with increases in the severity of liver fibrosis in HCV patients with genotype 1 (Petta et al. 2013). Substances like alcohol that can significantly enhance the rate of disease progression should also be avoided.

Dietary Supplementation

For patients with advanced liver disease presenting with steatorrhea due to bile acid deficiency, fat-soluble vitamins and medium chain triglycerides that do not need bile for absorption may be supplemented in their diet. These are often available as nutritional drinks and can be easily taken even by patients with reduced tolerance to oral intake.

Consuming vegetable-based proteins that contain fewer aromatic amino acids and supplementing the diet with branched chain amino acids should also be considered in patients at risk for hepatic encephalopathy, which is often accompanied by an impaired balance of these amino acids. Some studies have also implicated treatment with interferon and ribavirin in inducing this imbalance in the early stage of therapy, which further suggests the importance of correcting this imbalance early in the course of the disease.

Physical Activity

Moderate physical activity is recommended for all HCV patients and has shown improvements in muscle mass, exercise capacity, and quality of life in general (Román et al. 2014; Zenith et al. 2014; Berzigotti et al. 2017). Exercise will not affect the course of infection, but it can help relieve fatigue, stress, and depression as well as improve appetite and strengthen immunity. HCV patients can also benefit from physical activity due to its effect on obesity. For patients with decompensated cirrhosis, however, physical activity needs to be carefully prescribed and individualized to each patient's exercise tolerance and presence of complications like esophageal varices. Deconditioning and fatigue are major issues for patients with cirrhosis that represent significant barriers to physical activity (Ney et al. 2017). Formal guidelines for physical activity in cirrhosis are not yet available. Therefore, it is important that all patients undergo screening for varices and the required variceal prophylaxis before an exercise regimen is initiated (García Pagan et al. 1996; Bandi et al. 1998) (Fig. 6).

Policies and protocols

Policies

Role of Health Care Providers and Local Government in Ensuring Food Security for All HCV Patients

In this chapter, we have described some screening tools available for the identification of malnutrition as well as its management in HCV patients. Below, we talk about some policies that could be adopted to ensure adequate nutrition for patients with chronic liver disease, and the role of health care providers, as well as the local government in the implementation of these policies.

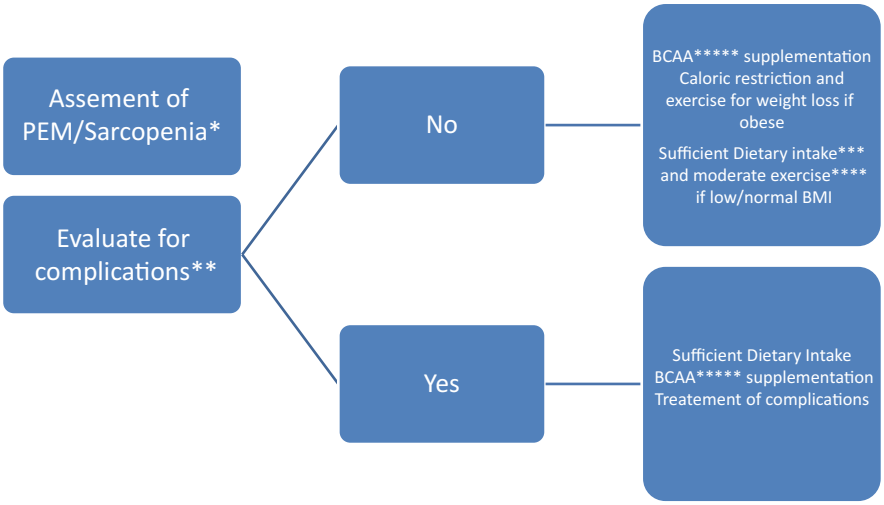

Fig. 6 Overview of management of HCV patients with complicated and decompensate liver disease. (*Based on anthropometry, radiological investigations, and indirect calorimetry, **Complications may include ascites, hepatic encephalopathy, portal hypertension, or hepatopulmonary syndrome, ***Sufficient dietary intake: 30–40 Kcal/kg of body weight per day, ****Walking 5000 or more steps/day or 3 sessions/week of 1 h exercise at an intensity of 60–70% of maximal heart rate, *****BCAA: Branched Chain Amino Acids)

HCV patients can be categorized as those with acute HCV, compensated cirrhosis, and decompensated cirrhosis. We recommend screening all HCV patients with a simple screening tool like SGA to identify patients suffering from nutritional impairment, and limiting the use of more specialized diagnostic measures, such as BIA and DEXA scans, to patients with an advanced form of liver disease, such as those with decompensated liver disease, awaiting liver transplantation.

HCV patients should be offered counseling sessions with a dietician as well as a physical therapist, preferably at each clinical visit so that they may be screened for under-nutrition and managed appropriately, if at risk. In settings where a limitation of resources may present a barrier, physicians should take it upon themselves to familiarize themselves with the dietary needs of HCV patients, as well as the protocols in place, for dietary intervention in malnourished individuals. They should also take advantage of the clinic visits to obtain a dietary history from all patients to ensure an adequate provision of nutrients. It falls to the health divisions of local governments to ensure screening and management of under-nutrition at all healthcare facilities in that area. This can be achieved through regular audits by the concerned authorities as well as appointments of trained professionals by the government at each of these facilities.

In addition to provision of antiviral medication, as is common practice in many third-world countries like Pakistan, local government should take also develop policies to provide aid, in the form of food packets, to HCV patients who may be unable to meet their nutritional needs on their own, due to financial constraints and

the stress of their sickness. For the purpose of identification of such disadvantaged patients, the local government may rely on feedback from healthcare professionals as well as nongovernmental organizations, familiar with the socio-economic circumstances of these patients.

Protocols

ESPEN Guidelines for Screening and Management of HCV Patients with Cirrhosis and Liver Transplant Candidates

Use SGA to identify malnutrition in patients with cirrhosis and bio-electric impedance analysis to quantify undernutrition in such patients.

As per ESPEN guidelines, ensure energy intake of 35–40 kcal/kg/day, protein intake of 1.2–1.5 g/kg/day. For patients unable to meet these caloric requirements with normal daily food intake, supplementary nutrition may be provided, preferably via the enteral route. Tube feeding is only indicated in patients who are unable to maintain or tolerate oral intake. PEG tube is associated with adverse outcomes and should not be used until other options have been exhausted (Plauth et al. 2006).

For nutritional supplementation, whole protein formulas are generally recommended. In patients presenting with cirrhosis complicated with ascites, concentrated high energy formulas should generally be preferred. BCAA-enriched formulas have been found to improve outcomes in patients with encephalopathy and should, therefore, be supplemented in the diet of cirrhotic patients with concomitant encephalopathy (Plauth et al. 2006).

HCV is the most indication for liver transplantation as patients frequently progress to decompensated cirrhosis. For liver transplant candidates, in addition to the usual protocol for Cirrhotic patients, it is recommended that normal food/enteral nutrition be initiated within 12–24 h following liver transplantation. A nasogastric tube or catheter jejunostomy may be employed for early enteral nutrition posttransplant.

Dictionary of Terms

- **Ascites** – Presence of excess fluid in the peritoneal cavity.
- **Compensated liver disease** – Absence of signs of cirrhosis in patients with chronic liver disease.
- **Decompensated liver disease** – Development of complications like jaundice, encephalopathy, ascites or variceal hemorrhage in the setting of chronic liver disease.
- **Hepatic encephalopathy** – Altered mental status in the setting of chronic liver disease, secondary to metabolic derangements.
- **Bio-electric impedance** – Opposition to the flow of current in a circuit.
- **Indirect calorimetry** – A method to determine energy consumption of the body that relies on quantification of oxygen consumed and carbon dioxide produced.

- **Protein-calorie malnutrition** – Deficient intake of proteins and calories in diet.
- **Respiratory quotient** – Ratio of oxygen consumed to carbon dioxide produced.
- **Sarcopenia** – Loss of skeletal muscle.
- **Visceral adiposity** – Fat collection around the body organs.

Summary

- Hepatitis C is a viral infection of the liver.
- Chronic hepatitis C is often accompanied by nutritional deficits.
- Nutritional impairment can have significant implications on the long-term prognosis of Hepatitis C patients.
- Nutritional deficits in chronic liver disease may present as protein-calorie malnutrition or micronutrient deficiencies.
- Obesity correlates with a poor prognosis in patients with hepatitis C.
- Hepatitis C patients may develop insulin resistance that impairs carbohydrate metabolism.
- Sarcopenia is the most objective feature of malnutrition in chronic liver disease
- Subjective global assessment is the most commonly used screening tool to identify malnutrition in chronic liver disease.
- Dietary supplementation is indicated only in patients with decompensated liver disease.
- Symptomatic patients should be evaluated for micronutrient deficiencies with laboratory assays.
- Enteral feeding is only recommended for patients who are unable to meet caloric needs via oral intake.
- Cirrhotic patients are advised to take frequent small volume meals at least 4-6 times a day.
- Moderate physical activity may help relieve fatigue, stress, and depression in patients with hepatitis C.
- Physical therapy must be carefully prescribed for patients with decompensated cirrhosis.

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Malnutrition in Dialysis

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Abstract

Malnutrition continues to be a problem in chronic kidney disease (CKD) patients on hemodialysis (HD), resulting in increased morbidity and mortality. Factors that reduce food intake and increase hypercatabolism play important role in malnutrition seen in dialysis patients. Various factors contributing to malnutrition syndrome in dialysis are anorexia due to uremia with electrolyte and hormone imbalances, metabolic abnormalities, gastrointestinal disorders, psychological factors, severe dietary restriction, social problems, increased inflammation, hypercatabolism, and nutrient losses during hemodialysis and peritoneal dialysis. Detecting malnutrition in CKD patients is very challenging. Dialysis patients commonly show protein depletion and energy deficit. The term protein-energy wasting (PEW) syndrome describes the loss of body protein mass and reduction in calorie reserves in dialysis patients. Some dialysis patients can also get overweight or obese and do not appear to have energy deficit but may have protein malnutrition. PEW syndrome is associated with a poor prognosis. The pathogenesis of PEW is incompletely understood and has multifactorial etiopathogenesis. Comorbidities and underdialysis also contribute to anorexia and malnutrition in dialysis. Intra-dialysis hyperalimentation may be provided for patients who continue to lose weight despite oral supplementation (at least 50% or more of the prescribed caloric intake).

Keywords

Chronic kidney disease · Dialysis · Malnutrition syndrome · Protein-energy wasting · Malnutrition management

List of Abbreviations

BMI	Body mass index
CKD	Chronic kidney disease
ESRD	End-stage renal disease
GFR	Glomerular filtration rate
IDPN	Intradialytic Parenteral Nutrition
MHD	Maintenance hemodialysis
PEW	Protein-energy wasting
PNA	Protein nitrogen appearance

Introduction

The main function of kidneys is to facilitate waste product (produced from protein metabolism) excretion, to maintain and regulate fluid, electrolyte, and hormonal balance. A progressive decline in kidney function is represented by decrease in GFR.

This leads to accumulation of waste products in the blood like urea and creatinine. This causes uremic toxicity which is a primary indication for renal replacement therapy (dialysis or kidney transplantation).

Chronic kidney disease (CKD) is defined as the presence of kidney damage or a glomerular filtration rate (GFR) of < 60 ml/min/1.73 m^2 for 3 months or more. Markers of kidney damage include the presence of proteinuria or albuminuria, hematuria (after exclusion of other causes), or structural abnormalities confirmed on renal imaging. A progressive decline in kidney function is represented by decrease in GFR. Therefore patients are classified according to GFR, ranging from stages 1 and 2 where there are persistent urinary abnormalities but preserved renal function to stages 3, 4, and 5 that represent advancing CKD leading to end-stage renal disease (ESRD) (GFR <15 ml/min/1.73 m^2). As the kidney function declines to stage 5, the patients of CKD need to undergo renal replacement therapy.

Risk factors for the high mortality rate of individuals with ESRD include comorbid conditions such as diabetes and cardiovascular disease; inflammation; dialysis inadequacy; catabolic effects such as acidosis, hyperparathyroidism, and hyperphosphatemia (Mehrotra and Rishishwar 2015); infections; and insulin resistance. Malnutrition in dialysis patients is often characterized by hypoalbuminemia, decreased prealbumin, hypocholesterolemia, decreased serum creatinine (due to reduced muscle mass), decreased transferrin, and overall dry weight loss including lean body mass and fat mass. Causes of malnutrition in dialysis patients also include inadequate protein and calorie intake due to reduced appetite, dietary restrictions, impaired gastrointestinal motility, social constraints, or physical incapacity. In addition, nutrient losses during dialysis, alterations in protein metabolism, and inflammation may contribute to malnutrition. Malnutrition is highly prevalent in the ESRD population and is a strong predictor of morbidity and mortality. The components of protein-energy wasting are strongly correlated with 100- to 200-fold higher mortality risk, in patients with end-stage renal disease than in the general population. There are multiple issues that may lead to malnutrition in ESRD patients on dialysis and need to be addressed (Chen et al. 2017; Cano et al. 2007).

Chronic Kidney Disease: A Hypercatabolic State

Some CKD patients on dialysis are in hypercatabolic state. Resting energy expenditure (REE) in CKD patients (15 with progressive CKD not on dialysis, 15 on hemodialysis, and 10 on peritoneal dialysis) was found to be 10 to 20% higher than predicted values derived in normal and overweight populations. REE was higher among both peritoneal and hemodialysis patients compared with non-dialysis patients. The net energy expenditure is the total of REE and energy expenditure with physical activity. Many dialysis patients are inactive, so they are in decreased physical activity disbursement. The BMI in some hemodialysis patients shows an increasing trend despite increased resting energy disbursement rate (Anderton et al. 2015; Beddhu et al. 2015; Neyra et al. 2003).

Protein Metabolism Alterations in CKD

Metabolic acidosis, a result of uremia, has been shown to stimulate amino acid and protein catabolism, and also decrease albumin synthesis, and stimulate muscle protein catabolism. Dialysis patients also experience impaired small intestinal protein digestion and absorption as compared to healthy controls. Several gastrointestinal abnormalities are reported in dialysis patients including gastrointestinal motility disorders and small bowel bacterial overgrowth. These could potentially disrupt protein digestion and absorption (Garibotto et al. 2015; Wang and Mitch 2014).

Nutrient Losses During Dialysis

Both hemodialysis and peritoneal dialysis and reuse procedure for hemodialysis result in increased losses of protein into dialysate. Protein loss as high as 20 grams in one hemodialysis session has been reported with polysulfone dialyzers which are reused with bleach. Dialysis procedure also can contribute to amino acid losses of 5 to 8 grams per hemodialysis session, and this increases with high efficiency dialyzers. Albumin losses are seen when dialyzers are reprocessed, specifically after the sixth reuse (Ikizler et al. 1994). Loss of protein-rich blood due to gastrointestinal bleeding, frequent blood collection for laboratory testing, and blood left in the dialyzer may all contribute to malnutrition in dialysis patients. Water-soluble vitamins and other micronutrients are also lost into dialysate during hemodialysis (Carrero et al. 2010; Kalantar-Zadeh et al. 2004).

Protein Depleted and Reduced Calorie Intake and Loss of Appetite in Dialysis Patients

Significant anorexia was reported in 35–50% of ESRD patients (Bossola et al. 2006). Patients on dialysis should have a daily calorie and protein intake of 30–35 kcal/kg body weight per day and 1.2 g/kg/day individually. Calorie and protein requirements are thus greater in dialysis patients than in healthy individuals. However, actual protein and calorie intake are often below the recommendations for dialysis patients. In a cohort of 53,933 MHD patients, low daily protein intake over time was associated with increased risk for death. In a study on hemodialysis patients, protein intake <1.2 g/kg/day was predictive of higher mortality. A longitudinal study of CKD patients on MHD over a 9-year period showed that the dietary intake reduced over time. This study concluded that long-term hemodialysis failed to correct under nutrition in dialysis patients (Shinaberger et al. 2006; Antunes et al. 2010; Mekki et al. 2012).

Most dialysis patients follow potassium-, phosphorus-, and sodium-restricted diets and thus have limited food choices. Comorbid conditions like heart disease, diabetes, gastrointestinal dysfunction, increased infections, inflammatory state due to chronic kidney disease and dialysis, hyperparathyroidism, emotional and

psychological disorders, and inadequate dietary support may all contribute both to reduced nutrient-protein-energy intake and catabolism (Duenhas et al. 2003).

The Dialysis Procedure and Catabolism

In reduced protein synthesis with loss of amino acids in dialysate, we can conclude that dialysis procedure is catabolic. Amino acid losses into dialysate can range from 4 to 8 g/day both with dialysis procedure. The use of bio-incompatible membrane in hemodialysis patients can lose more amino acid. In patients on maintenance hemodialysis where their dialyzer may be recycled can increase the losses of protein and amino acids from dialysate. Increasing adequacy of dialysis with frequent dialysis does not seem to degrade nutritional indices, but results in improvement in malnutrition (Ikizler et al. 2002; Ikizler 2005).

Adequacy of Dialysis and Malnutrition

Evidences suggest that accumulated toxins that result from kidney disease suppress appetite and contribute to the decline of nutritional status in dialysis patients. Wang et al. showed that improved dialysis adequacy is associated with better dietary intake and improved nutrition (Kaysen et al. 2012; Wang et al. 2003). The dialysis adequacy is often reported in terms of Kt/V (where K stands for dialyzer clearance of urea, t for dialysis time, and V for volume of distribution of urea, approximately equal to patient's total body water) which is a dimensionless number. The Kt/V target in hemodialysis is $\geq$1.3, to make sure that the delivered dose of dialysis is at least 1.2. In a study on 44 adult dialysis patients, it was found that serum albumin levels were significantly higher in patients with a Kt/V greater than 1.2 compared to those with a Kt/V less than 1.2 (Terrier et al. 2008). Another study on 140 MHD patients revealed that adequate hemodialysis quality (Kt/V $\geq$ 1.2) was significantly associated with higher values of anthropometric parameters, dry weight, and body protein status (Stolic et al. 2010).

Dietary Restrictions and Medications in Dialysis Patients

Food becomes less palatable when there are strict dietary restrictions, and it also results in reduced protein and calorie intake along with reduced intake of trace elements, vitamin, and micronutrients. The intradialytic weight gain can minimize restriction in fluid and can decrease in caloric intake. Many beverages contain a substantial amount of calories, and some solid food supplements have higher fluid content (Kalantar-Zadeh et al. 2015). Nutritional interventions and dietary restrictions are essential part of dietary prescription for dialysis patients. Medications, such as phosphate binders, can bind and impair nutrient absorption. They can also reduce

appetite and cause constipation (Kalantar-Zadeh et al. 2013). Patient education can be very helpful in getting patient's cooperation for control of mineral bone disorders.

Causes of Malnutrition in MHD Patients

- Loss of appetite
- Diabetic status with gastroparesis and diarrhea, which could cause PEW syndrome
- Inadequate dietary recommendations
- Comorbidity
- Depression
- Inadequate dialysis and its complications (nausea, hypotension)
- Socioeconomic factors (social deprivation)
- Mechanical compression of stomach and intestine in polycystic kidney disease
- Immobility and reduced ability to purchase food
- Chronic inflammation also results from inadequate dental hygiene status
- Dialysis-associated loss of amino acids and proteins
- Metabolic acidosis

Malnutrition, Inflammation, and Atherosclerosis (MIA Syndrome)

ESRD is a multifactorial disease leading to complications like malnutrition, inflammation, and atherosclerosis (MIA syndrome) which is associated with high mortality. Many studies have reported malnutrition in as many as 23–76% of hemodialysis patients. Malnutrition is independently associated with an increased risk of hospitalizations and mortality in patients with ESRD. Factors associated with dialysis that are associated with malnutrition and inflammation are bio-incompatibility of dialysis membrane, loss of residual renal function, presence of infections in the vascular access, dialysis hypotension, impure dialysate, volume overload, and nutrient losses during dialysis. Retention of uremic toxins in the body results in decreased appetite and inflammation. This triggers catabolic pathways in the body, leading to loss of body fat and muscle causing malnutrition (Kirushnan et al. 2017).

Common Causes of Malnutrition in MIA Syndrome

1. Accumulation of anorectic factors
2. Elevated serum leptins
3. Inflammation and/or infection: increased inflammatory and catabolic cytokines (IL-6, TNF-α) and acute phase proteins
4. Gastropathy/enteropathy: blood loss due to GI bleeding
5. Loss of metabolic processes: reduced synthesis of amino acids, glucose, and fatty acids

6. Physiological factors: medication, depression, poverty, and alcohol/drug abuse
7. Hemodialysis-related factors: inadequate Kt/V, post-dialysis fatigue, cardiovascular instability, nausea, and vomiting

Management of MIA Syndrome

Adequate dialysis, using ultrapure dialysate fluid, use of biocompatible dialysis membrane, administration of erythropoietin (EPO) to improve anemia, control of hypertension and hyperlipidemia, and lifestyle modifications (exercise, smoking cessation) all play important role in management of MIA syndrome.

How to Prevent and Treat Malnutrition in Hemodialysis Patients

Malnutrition is strongly related to inflammation and mortality in dialysis patients.

Several approaches to treat and/or prevent malnutrition have been attempted. Nutritional screening, dietary counseling, and improving dialysis adequacy may be effective in malnutrition prevention, depending how early they are implemented. In addition, pharmacological intervention, oral nutritional supplements, IDPN, or artificial nutrition have also been tried with variable success both for prevention and treatment of malnutrition.

Dietary Counseling to Prevent Protein-Energy Wasting

The role of nutritional supplements to treat PEW is also not clear because the recommendations for increased protein intake do not suggest or recommend use of oral protein supplements, unless patients have specific defined clinical indications. The recommended protein intake can be estimated from dry weight. Many obese dialysis patients receive adequate caloric intake despite reduced dietary protein intake (Zha and Qian 2017; National Kidney Foundation 2000).

If a high protein diet is recommended to dialysis patients, then it is important to monitor their metabolic parameters especially blood urea. Sometime very high protein intake could be toxic for dialysis patients as it could worsen uremia, metabolic acidosis, and hyperphosphatemia. Hemodialysis patients should get regular metabolic parameters checked every month. These interventions can improve nutritional status in patients with PEW, when they have history of progressive decrease in protein intake, reduced protein nitrogen appearance (PNA, which is a marker for protein intake in dialysis patients), decrease in serum albumin, and decrease in dry weight. Patients on dialysis should also be carefully evaluated for causes of anorexia and other possibilities associated with PEW. The progressive decrease in BMI, albumin, and PNA should be carefully assessed in all patients who have the history of decreased protein intake. Decrease in appetite may also be caused by a subclinical occult infection. Diabetic patients should be carefully evaluated for

symptoms of gastroparesis and diarrhea, both which are common manifestations of autonomic neuropathy and could cause and aggravate PEW syndrome (Mitch and Remuzzi 2004; Marzocco et al. 2013; Streja et al. 2013).

Oral Nutritional Supplements for Treatment of PEW

For patients selected for treatment of malnutrition with various supplements, it is recommended that oral dietary supplements, which are useful and effective, should be used first. Oral nutritional supplements specifically formulated for dialysis patients are available as a combination of energy and protein, energy only, or protein only. Supplements may be in the form of solid food, powder, or liquid formulation.

For patients who have history of hyperkalemia or volume overload on dialysis, an oral "renal failure" supplement, which has double the calorie and protein and 30–35% less potassium and phosphorus content per mL of supplement, has been formulated. A different approach is required in patients with severe anorexia who are unable to increase their oral intake. Overnight supplementation by nasoenteral feeding tube may be an alternative and more effective in patients with severe anorexia (Cohen et al. 2007).

The Role of Inflammation and Comorbidities in Malnutrition

The nutritional status affects the acute, chronic, or occult systemic illness. In PEW syndrome chronic lung disease, congestive heart failure, and malignancy also contribute both to the production and acceleration of PEW. Poor nutritional status also occurs during acute illness. Serum albumin and nutritional indices are known to worsen after hospitalization. Anorexia and gastroparesis (slowing of gastric emptying) also worsen PEW. PEW is more pronounced in the presence of inflammation (related to increased energy expenditure). PEW is also related to increase in pro-inflammatory cytokine levels and increased oxidative stress (imbalance between the productions of free radicals).

Serum albumin (marker of nutrition) is indirectly related to the inflammatory markers such as C-reactive protein (CRP). Systemic inflammation can also lead to protein wasting. It is not clear how inflammation leads to energy and protein wasting in dialysis patients. Despite comorbidities, high levels of CRP, low levels of plasma albumin, and lower muscle mass, some dialysis patients have tendency to have higher BMI (with evidence of obesity) in comparison to the national norms. The higher CRP levels in CKD and dialysis patients appear to be due to increase in adipose tissue (major source of inflammatory cytokines), associated with obesity as compared to underweight dialysis patients. Some studies have shown negative correlation between nutritional status and inflammation. Inflammation can coexist in ESRD patients who have malnutrition due to associated comorbidities. Malnutrition and inflammation may also be associated with immunodeficiency, nutrient malabsorption, hypercatabolism, and weight loss (PEW) (Kramer et al. 2006; Ramkumar et al. 2004; Axelsson et al. 2005).

The Role of Insulin Resistance in Malnutrition

Some reports in literature have suggested that there is role of insulin resistance in malnutrition which may result in muscle protein breakdown and catabolism in hemodialysis patients (both with and without type 2 diabetes). Improvement in insulin action with oral hypoglycemic agents (rosiglitazone) is reported to ameliorate proteolysis in animal models of diabetes. Insulin resistance seen more in obese dialysis could lead to protein wasting and could result in muscle wasting (Siew et al. 2007; Wang et al. 2006).

The Role of Metabolic Acidosis in Malnutrition

Metabolic acidosis in malnutrition can contribute to proteolysis, and its correction has been reported to provide benefit in CKD dialysis patients in some clinical studies and experimental animal models.

In a randomized trial, oral bicarbonate supplementation was compared to standard of care (no bicarbonate supplementation) in adult CKD patients with reduced creatinine clearance (15 to 30 mL/min/1.73 m2) with metabolic acidosis (serum bicarbonate 16 to 20 mmol/L). At 2 years, nutritional parameters showed improvement with bicarbonate supplementation compared with standard care group who were not given oral bicarbonate (de Brito-Ashurst et al. 2009). In patients on hemodialysis, serum bicarbonate levels inversely correlate with serum creatinine. But serum bicarbonate levels are already elevated in dialysis patients with decreased muscle mass and PEW (Kaysen et al. 2003). The roles of bicarbonate supplementation for improving muscle mass in such dialysis patients who have PEW and alkalosis are not clear.

Role of Various Drugs Which Stimulate Appetite

Megace (megestrol acetate) is an oral derivative of the steroid progesterone. Nutritional benefits of megestrol acetate include increased oral intake, weight gain, and increased serum albumin. However, these drugs when used in dialysis patients have potential side effects that include fluid retention, diarrhea, encephalopathy, depression, hypervolemia, and irregular menses (Bossola et al. 2005).

Role of Intradialytic Parenteral Nutrition (IDPN)

Patients who lose weight continuously and have extremely reduced serum albumin levels below 3.2 g/dL despite oral supplementation and in those who have severe gastroparesis and are unable to tolerate oral supplementation are candidates for IDPN. IDPN, which can be given during dialysis, is convenient to use and can

benefit a select group of such dialysis patients. But IDPN is a costly and less efficient nutritional supplementation than oral supplement. IDPN costs about twice as much as dialysis cost. Thirty percent of the nutrients of IDPN are not delivered to the patient and lost into the dialysate during dialysis. IDPN also fails to provide adequate protein and calories for treating PEW syndrome. IDPN is only used for a hemodialysis patient who can consume at least 50% of the prescribed caloric intake orally. Amino acids, dextrose, and lipids can be infused at the end of dialysis. Adequate clinical and biochemical assessment and monitoring is required during IDPN in dialysis patients to prevent and minimize the risks of complications due to IDPN (Alp Ikizler et al. 2013; Pupim et al. 2002; Kopple 1999).

Growth Hormone Therapy in Dialysis Patients

Administration of recombinant human growth hormone can reduce muscle wasting and catabolism. Growth hormone therapy can improve nutritional status and lower the blood urea nitrogen (BUN) levels in hemodialysis patients. The benefit from recombinant human growth hormone is hypothesized to be mediated by an increase in free insulin-like growth factor-1 (IGF-1) levels (Garibotto et al. 1997; Iglesias et al. 1998).

Growth hormone has been used in children on dialysis with favorable results on growth. But patients can develop growth hormone resistance which can accelerate protein catabolism. This can result in more severe malnutrition in dialysis patients. In a multicenter trial, significant increase in serum high-density lipoprotein, cholesterol, and transferrin levels along with decrease in C-reactive protein and homocysteine levels was observed in hypoalbuminemic MHD patients given growth hormone therapy (Kopple et al. 2011).

Intradialytic Fat Emulsion Use During Dialysis

Fat emulsions have been shown to be an efficient source of energy in malnourished dialysis patients. The use of fat emulsions as a supply of energy is supported by various reports: (a) fat stores in the postabsorptive state are preferentially oxidized in dialysis patients; (b) essential fatty acid deficiency has been reported in dialysis patients; (c) fat emulsions have a high energy to volume ratio and are iso-osmolar, thus allowing tolerable intravenous peripheral infusion. But the dose of fat emulsions should not exceed ~1 g/kg body weight/day. Patients on intradialytic fat emulsion therapy should have regular monitoring of plasma triglycerides.

Recombinant human growth hormone, androgenic anabolic steroids, anti-inflammatory drugs, and zinc have all been studied in dialysis patients with PEW. But their role is still experimental, and these agents should not be used for routine clinical care and interventions (Avery-Lynch 2006; Cano et al. 1994; Druml and Kierdorf 2009).

Androgenic Anabolic Steroids

Limited data on androgenic anabolic steroid use in dialysis patients is available. There are reports of increase in body weight, muscle mass, and serum albumin with the use of androgenic anabolic steroids, but their long-term efficacy and the risk for adverse effects are unclear (Gascón et al. 1999; Johansen et al. 1999; Barton Pai et al. 2002).

Role of Zinc and Various Anti-inflammatory Agents

Dialysis patients often have decreased taste acuity, which has been reported to be associated with zinc deficiency. But role of zinc deficiency in malnutrition has not been established (Atkin-Thor et al. 1978).

Anti-inflammatory agents have been used in patients with malnutrition-inflammation syndrome complex. But their role is also not very definite (Kalantar-Zadeh et al. 2003).

Role of Vitamin D Supplementation Use During Dialysis

Vitamin D deficiency is very common in patients with CKD and in health population, also associated with muscle protein imbalance. In CKD patients during dialysis, bone mineral disorder (BMD) is common, resulting from vitamin D deficiency, increased fibroblast growth factor-23, and also secondary hyperparathyroidism (Mehrotra et al. 2016). Vitamin D deficiency also contributes to muscle degradation, and vitamin D supplementation (both calcirol D2 and 1,25-OH2 vitamin D) has been shown to improve muscle size, strength, and metabolism and also results in improvement in serum albumin levels (Gordon et al. 2007).

Enhanced activity of catabolic hormones, acidemia, inflammation, and decreased nutritional intake in dialysis patients can cause PEW with loss of body mass and muscle wasting (Tripathi et al. 2010).

Association of various demographic, anthropometric, inflammatory and appetite regulating markers has been investigated with malnutrition-inflammation syndrome in ESRD patients. Genetic aspect of the inflammation and appetite regulation has also been evaluated. The results obtained from demographic and anthropometric data in our study revealed that CKD patients have mixed type of malnutrition. Almost 69.3% patients had malnutrition and 37.3% had malnutrition with inflammation. The patients in severe group of malnutrition had low BMI, skinfold measurements, body fat percent, and dry weight. Age $>$ 50 years, serum albumin ($<$3.5gm/dl), low serum protein ($<$6.0 mg/dl), hyper-ferritinemia ($>$200 ng/ml), low TIBC ($<$270 μg/dl), $<$10% body fat, high risk group with comorbidities, and severe degree of malnutrition were associated with reduced overall survival.

On studying the inflammatory markers, it appears that inflammation has a major role in inducing malnutrition. Inflammation apart from uremia along with other

associated factors is also governed by the genotypic makeup of patients. Our study suggests that TNF-α is a key marker as it is related with both higher risk associated of ESRD (TNF-α-238 AA, TNF-α-308 A) and malnutrition (TNF-α-308 A) and higher death hazard (TNF-α-238 AA). IL-10-592 AA/IL10–819 TT genotype was associated with higher risk of inflammation in these patients on dialysis. Serum levels of IL-6 and TNF-α are elevated in severe malnutrition and in inflamed (elevated CRP group) patients. Thus inflammation both induces and accelerates malnutrition. IL-10 levels were increased in non-inflamed patients, suggesting its protective role against inflammation. The combined effect model of alleles associated with high IL-6, TNF-α, and low IL-10 revealed risk association with ESRD and poor nutritional and survival status (Sharma et al. 2013a).

In our study evaluation of the appetite and energy-related markers showed that ghrelin is a major marker and its genetic variants are associated with susceptibility to develop malnutrition (Ghrelin 72Met and 51Gln) and inflammation (Arg51Gln) in ESRD patients on hemodialysis. In silico analysis revealed that mutated allele of ghrelin 51Gln is associated with higher binding energy to the ghrelin receptor and hence lower stability binding as compared to the wild version of the ghrelin peptide. The combined effect model of alleles associated with low leptin, high ghrelin, and low RMR revealed association with ESRD and poor nutritional status. The levels of acyl ghrelin were lowest in the severe group of malnutrition. The appetite was lowest in severe SGA group followed by lower plasma levels of acyl ghrelin in the poor appetite group. Leptin levels are increased in the normal and mild category of SGA showing protective effect of higher leptin levels for malnutrition. Further studies on appetite and energy homeostasis regulating markers are required for establishing their exact role in malnutrition (Sharma et al. 2013b).

Policies and Protocols

There is no single objective method which can diagnose malnutrition accurately in dialysis patients. Height, weight, BMI, measures of body composition, anthropometric measurements, and biochemical tests like serum protein and albumin can give an estimate of degree of malnutrition. Assessment of malnutrition in dialysis patients also includes inflammatory status of patients. But inter-observer variation, lack of nutritional standards, and lack of objective criteria for malnutrition assessment in dialysis patients are important aspects of difficulties faced about nutritional evaluation.

Evaluation of nutritional status in HD patients:

- Dietary assessment: DPI, protein equivalent of total nitrogen appearance (PNA), dietary recall diary, and history
- Anthropometrics: body weight/height, BMI, skinfold thickness, and muscle strength
- Biochemical: serum albumin, serum transferrin, serum insulin-like growth factor (IGF)-1, serum prealbumin, total cholesterol, plasma and muscle amino acid

concentration, serum creatinine, C-reactive protein (CRP) which correlates negatively with serum albumin, and blood urea nitrogen (BUN) levels
- Body composition, BIA, DXA, SGA, and composite assessments; CNI, SGA + anthropometric indices and serum albumin; MIS, SGA + BMI, serum albumin, and total iron-binding capacity

Dietary recommendations for hemodialysis patients are (a) 1.2 g/kg body weight, protein intake per day of which at least 50% should be of high biologic value, and (b) 30 to 35 kcal/kg body weight of caloric intake per day. Patient's history of decreased protein intake and evidence of progressive decrease in BMI, serum albumin, and PNA need to be carefully evaluated for cause of malnutrition.

Indications for interventions and treatment with dietary supplementation contain an unintentional loss of 5% of dry (non-edematous) weight over 3 months or 10% of dry weight over 6 months or a serum albumin value of <3.8 g/dL. Patients should first be given treatment with oral dietary supplements, rather than intravenous hyperalimentation. An exception is patients who continue to lose weight despite oral supplementation and have very low serum albumin (<3.2 g/dL) despite oral supplementation given for 3 months or who are unable to take orally. For such patients, IDPN could be useful.

Dictionary of Terms

- **Malnutrition** – a term used to refer to any condition in which the body does not receive enough nutrients for proper function. Malnutrition may range from mild to severe and life-threatening.
- **Body mass index (BMI)** – is a measure of body fat based on weight in relation to height and applies to most adult men and women aged 20 and over. For children aged 2 and over, BMI percentile is the best assessment of body fat.
- **Chronic kidney disease (CKD)** – describes the gradual loss of kidney function. Kidneys act as filter wastes and excess fluids from blood, which are then excreted in urine. When chronic kidney disease reaches an advanced stage, dangerous levels of fluid, electrolytes, and wastes can build up in the body.
- **Dietary protein intake (DPI)** – protein and the CKD patient. When protein is ingested, protein waste products are formed. Unhealthy kidneys lose the ability to remove protein waste, and it starts to build up in the blood. Dietary protein intake for patients with CKD is based on the stage of kidney disease, nutritional status, and body size.
- **Glomerular filtration rate (GFR)** – is a test used to check how well the kidneys are working. Specifically, it estimates how much blood passes through the glomeruli each minute.
- **Maintenance hemodialysis (MHD)** – is renal replacement therapy for patients with end-stage renal disease. It prolongs survival, reduces morbidities, and improves quality of life.

- **Intradialytic Parenteral Nutrition (IDPN)** – is the infusion of an intravenous nutritional formula of hyperalimentation, such as amino acids, glucose, and lipids, during dialysis, to treat protein calorie malnutrition in an effort to decrease the associated morbidity and mortality experienced in patients with renal disease.
- **Protein nitrogen appearance (PNA)** – is also known as protein catabolic rate (PCR). It reflects the daily protein intake in maintenance hemodialysis (MHD) patients.
- **Protein-energy wasting (PEW)** – a term proposed by the International Society of Renal Nutrition and Metabolism (ISRNM). It refers to the multiple nutritional and catabolic alterations that occur in chronic kidney disease (CKD) which are associated with increased morbidity and mortality. Also defined as reduced somatic and/or circulating body protein mass and decreased fat mass, usually associated with reduced protein and energy intake. Its prevalence is variously estimated to be 18% to 75% in maintenance hemodialysis.
- **Proteinuria** – People with proteinuria have urine containing an abnormal amount of protein. This condition is often a sign of kidney disease. Glomerular filters damaged by kidney disease may let proteins such as albumin leak from the blood into the urine. Proteinuria can also be result of overproduction of proteins by the body.
- **Albuminuria** – indicates presence of albumin in the urine, typically as a symptom of kidney disease.
- **Hematuria** – denotes the presence of blood in urine.
- **End-stage renal disease (ESRD)** – is CKD when kidneys are functioning below 10% of their normal function.
- **Hyperparathyroidism** – is an excess of parathyroid hormone in the blood due to overactivity of one or more of the body's four parathyroid glands.
- **Inflammation** – is the immune system-regulated white blood cell response to infection and illness.
- **Dialysis adequacy** – The two methods generally used to assess dialysis adequacy are urea reduction ratio (URR) and Kt/V. A patient's average URR should exceed 65%. A patient's average Kt/V should be at least 1.2. A patient's URR or Kt/V can be increased either by increasing time on dialysis or increasing blood flow through the dialyzer.
- **Bioelectrical impedance analysis (BIA)** – is a method of assessing body composition, the measurement of body fat in relation to lean body mass. It is an integral part of health and nutrition assessment.
- **Dual-energy radiograph absorptiometry (DXA)** – is a means of measuring bone mineral density (BMD). Two X-ray beams, with different energy levels, are aimed at the patient's bones.
- **Subjective global assessment (SGA)** – This is a clinical technique called SGA, which assesses nutritional status based on features of the history and physical examination.
- **Composite nutritional index (CNI)** – It is worth identifying the indicators by different levels of poverty. As poverty is a multidimensional phenomenon, a composite index based on socioeconomic, demographic, health, and dietary

indicators is of interest for making comparison between various ethnic groups.

- **Malnutrition-inflammation score (MIS)** – is correlated with morbidity and mortality in maintenance hemodialysis patients. Malnutrition-inflammation complex syndrome (MICS) occurs commonly in maintenance hemodialysis (MHD) patients and is associated with increased morbidity and mortality.

Summary Points

- Protein malnutrition can be prevented and substantially reversed with ongoing dietary monitoring and nutritional therapy. It is common in patients with CKD, a growing patient population worldwide, and also is associated with increased morbidity and mortality.
- As per guidelines, daily energy intake in CKD patients should be 30–35 kcal/kg (ideal body weight), and protein intake should be of 0.6–0.8 g/kg/day for non-dialysis CKD patients and 1.0–1.2 g/kg/day for patients on peritoneal dialysis or hemodialysis, with >50% high biological value (HBV) proteins.
- Metabolic acidosis adversely affects both the kidney and patient outcome, and should be corrected, starting in patients with stage 3 CKD even without evidence of metabolic acidosis. Dietary modification with increased base content like vegetables and fruits may be initiated in stage 3 CKD to prevent metabolic acidosis which could be related to multiple metabolic derangements.
- Constipation and abnormal bowel habits can compromise gut epithelial cell integrity. This can also promote inflammation and uremic toxin accumulation. Based on patient's capacity, exercise should be incorporated as part of the CKD management.
- Current recommendation is to supplement 25(OH)-vitamin D for CKD patients with low vitamin D concentration.

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Malnutrition, Cachexia, and Quality of Life in Patients with Cancer

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Oliver Grundmann, Saunjoo L. Yoon, and Joseph J. Williams

Contents

Abstract

Cancer has become one of the most common and devastating health problems in the aging population worldwide. Patients often suffer from a range of complications associated with cancer and cancer treatments that impact their quality of life and may reduce overall survival. Among those secondary to cancer and treatment

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are malnutrition and cachexia, which may be a result of the malignancy itself or of the treatment approaches, especially chemotherapy. Depending on the specific cancer type and stage, the prevalence of cachexia ranges from 28% to 57% as evaluated by clinical trials. This chapter will address the following topics related to malnutrition and cancer cachexia:

- Contributing factors to the development of malnutrition and cachexia
- Underlying pathophysiology and biochemical derangement
- Potential biochemical screening markers for malnutrition and cachexia
- Current and future treatment approaches and preventative strategies

The chapter furthermore aims to provide a best practices approach to guide clinicians in the diagnosis, prevention, and treatment of cancer cachexia to optimize quality of life for the patient and improve survival.

Keywords

Malnutrition · Cancer cachexia · Inflammation · Chemotherapy · Hypothalamus · Orexigenic · Metabolism · Anorexigenic · Body composition · Ghrelin

List of Abbreviations

5-HT	5-hydroxytryptamine, serotonin
BIA	Bioelectrical Impedance Analysis
CaMKII	Calmodulin-dependent protein kinase II
CNS	Central nervous system
CRP	C-reactive protein
EMA	European Medicines Agency
EPA	Eicosapentaenoic acid
FDA	Food & Drug Administration
GHSR	Ghrelin/growth hormone secretagogue receptor
HPA	Hypothalamus pituitary adrenal
IGF	Insulin-like growth factor
IL	Interleukin
MNA-SF	Mini Nutritional-Assessment Short-Form
mRNA	Messenger ribonucleic acid
MST	Malnutrition Screening Tool
MUST	Malnutrition Universal Screening Tool
NRS-2002	Nutrition Risk Screening 2002
PG-SGA	Patient Generated Subjective Global Assessment
POMC	Proopiomelanocortin
RR	Relative risk
SARM	Selective androgen receptor modulator
TNF	Tumor necrosis factor

Introduction

Cancer is a leading cause of mortality and morbidity in the world, and access to medical treatment may be limited based on location and socioeconomic status of the patient (Ohlen et al. 2017). Depending on the cancer type, stage, and the treatment, a range of symptoms may present that can impact a patient's quality of life and require complementary treatment to adequately maintain health and well-being (Fig. 1). Cancer is defined as a group of disorders involving abnormal cell growth of an organ that may impact normal physiological function and spread to other body locations (Anand et al. 2008). Abnormal cell growth in general is referred to as a tumor and may not always be malignant in nature. One such example is benign prostate hyperplasia often encountered in older men (Ravery 1999). Depending on the location of the tumor, the rate of growth and signs and symptoms may have a profound impact on quality of life, morbidity, and mortality (Movsas et al. 2009; Guckenberger et al. 2013; Fernando et al. 2015). Common signs of malignant tumors of the liver, kidney, stomach, intestines, and pancreas are unexplained and sudden weight loss, loss of appetite, and change in bowel movements (diarrhea or constipation, especially for cancers of the small and large intestines) (Farhat et al. 2008).

Pharmacological treatment and the cancer itself can contribute to the loss of appetite in cancer patients, leading to malnutrition as the first stage of metabolic derangement. In addition, the abnormal cell growth diverts metabolic resources away from essential cellular maintenance of healthy tissues, thus leading to weight loss. Malnutrition is more prevalent in cancer patients who live in countries where

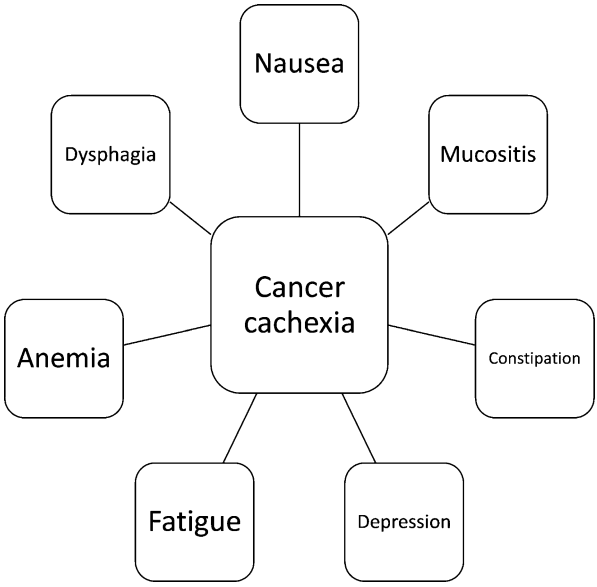

Fig. 1 Factors impacting quality of life in cancer cachexia. Symptomatic treatment is often best option to adequately treat patient and relief predominant symptoms. Consider physical and mental well-being

Table 1 Clinical differentiating factors between starvation, cachexia, and sarcopenia. +: present, −: absent, 0: unchanged

	Starvation	Cachexia	Sarcopenia
Weight loss	+	++	+/−
Fat loss	++	++	0
Muscle loss	+	+++	++
Proteolysis	−	++	+
Hypertriglyceridemia	−	++	+
Anemia	+	++	−
Insulin resistance	−	++	+
Elevated cytokines	+/−	++	+/−
Increased CRP	−	++	−

proper nutritional resources are limited (Isenring et al. 2010; Sala et al. 2012) although malnutrition in general can occur in all cancer patients initially if not recognized. Due to the metabolic demands of the tumor and accelerated protein turnover, malnourishment develops over an extended period of time during the initial stages of aggressive and invasive tumors such as pancreatic, stomach, and liver cancer (Arends et al. 2017). A gradual weight loss follows initial derangement of protein metabolism which is focused on muscle loss rather than fat loss due to the high demand of the tumor for amino acid sources (Horstman et al. 2016; Vanhoutte et al. 2016). Loss of muscle mass specifically is referred to as sarcopenia and is often a hallmark of the progression of malnutrition to cachexia (Santarpia et al. 2011; Peterson and Mozer 2017). Although sarcopenia can occur in the absence of cancer (Table 1), loss of skeletal muscle mass and malaise occurring together are strong indicators of a malignancy (Gonzalez and Heymsfield 2017; Peterson and Mozer 2017). Cachexia is the result of progressive malnutrition, loss of appetite, and development of sarcopenia including muscle mass related to a disorder with at least 5% body weight loss over the past 6 months (Arends et al. 2017). Cachexia is prevalent in 50% of cancer patients overall and rises to 80% in terminal cancer patients (Yoon et al. 2015; Arends et al. 2017) presenting a significant factor in morbidity, low quality of life, and mortality in this population (Fig. 1). In contrast to starvation and sarcopenia, cachexia presents with an inflammatory process as a differentiating factor (Table 1).

Contributing Factors to the Development of Cancer Malnutrition and Cachexia

Throughout life, abnormal cell growth may occur. Our body has various ways to detect and stop such cells from replicating and spreading to become a malignancy. However, once a tumor has formed, its metabolic demands will determine what nutritional resources are diverted from normal physiological processes. This initial step in tumor formation and metabolic deregulation may be small and insignificant

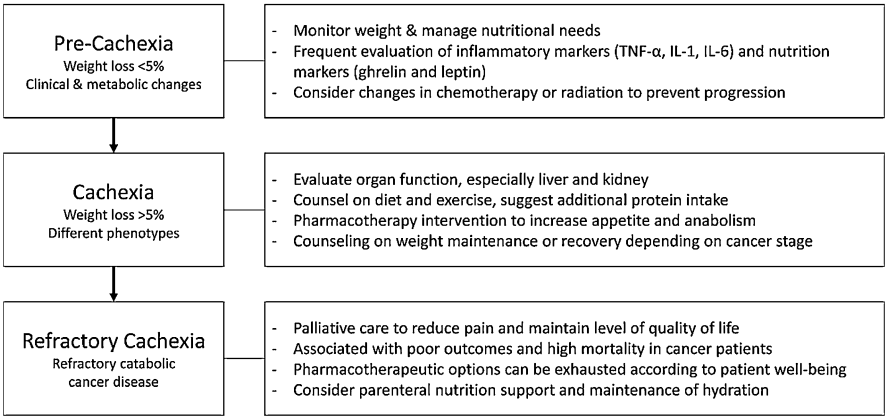

Fig. 2 Stages of cachexia in cancer patients. Clinical symptoms and management of precachexia, cachexia, and refractory cachexia. Depending on progression of cancer pharmacotherapy to prevent progression needs to be considered. *TNF-α* tumor necrosis factor α, *IL-1* interleukin-1, *IL-6* interleukin-6

but is an important predetermining factor in the development of malnutrition. Associated with metabolic deregulation are endogenous stress factors that contribute to the growth of a tumor and specialized cell types reverting to basic functions that disable proper functioning of the tissue (Hill et al. 2009). A number of independent factors that have been shown to contribute to the development of cancer malnutrition are age with adults older than 60 (adjusted RR 1.36), smokers (adjusted RR 1.21), lower socioeconomic status (adjusted RR 1.44), and impaired performance or inability to be physically active (adjusted RR 1.50) (Silva et al. 2015). It is imperative to detect early signs of malnutrition by frequently monitoring weight and food intake and prevent its progression to cachexia and metabolic deregulation which promotes stress and tumor growth (Santarpia et al. 2011; Arends et al. 2017). Early intervention is critical to improve quality of life and prolong survival time in cancer patients because it is extremely difficult to reverse once a patient reaches the refractory cachexia stage (Fig. 2).

Once malnutrition reaches a point of negative protein synthesis and positive proteolysis, the patient may be at risk of developing cachexia. The progression usually occurs over some time as the tumor advances and pro-inflammatory mediators reach concentrations that shift the balance from a positive protein balance that allows for maintenance of muscle mass to a negative protein wasting (Fearon et al. 2011; Peterson and Mozer 2017). It has not been determined what the critical point for this shift is, but it varies depending on the specific tumor location, its growth, and the general health of the patient, among other factors. Early detection and treatment access to prevent progression may impact the development of malnutrition and cachexia.

Weight change is the most commonly monitored indicator to differentiate between precachectic and cachectic patients. If weight remains stable or does not

decrease by more than 5% over the course of 6 weeks, the patient can usually continue their regular chemotherapy or radiation schedule. If weight loss reaches more than 5% or caloric intake is significantly impaired, delays in chemotherapy may become necessary to prevent progression of a pre-cachectic patient to cachexia. Once a patient has lost more than 5% of body weight within 6 weeks, more aggressive measures such as pharmacotherapy, nutritional counseling, and parenteral nutrition may have to be instituted (Fig. 2).

Underlying Pathophysiology and Biochemical Derangement

A key factor that has been identified in the development of cancer cachexia in the 1980s was the activation of the immune system with a resulting increased immune response and general inflammation (Moldawer et al. 1987). This increase in circulating pro-inflammatory mediators such as interleukins (IL), tumor necrosis factor (TNF), and cortisol has a global impact on the balance between anabolism and catabolism in the body (Suzuki et al. 2013). While our body will decrease metabolism and increase food intake during starvation, the opposite occurs during cachexia with increased energy expenditure and decreased food/caloric intake (Thomas 2007). This has been attributed to a profound dysregulation of the hypothalamic neuropeptide circuitry that is influenced by two key hormones released in the periphery, leptin and ghrelin (Ashitani et al. 2009).

Leptin is an adipocytokine that is released in response to food consumption and acts in the hypothalamus through the release of proopiomelanocortin which signals production of corticotropin-releasing hormone (Fig. 3) and suppresses further food intake (Kerem et al. 2008). It has been shown to be differentially expressed in cancer patients depending on the specific cancer type, being low in gastrointestinal cancers but high in breast and gynecological cancers (Wolf et al. 2006). Together with insulin, leptin is considered a major contributor to the development of obesity and anorexia. Both are intricately involved in the cellular uptake and metabolism of lipids and glucose, hence energy homeostasis, both in the periphery and in the CNS (Thon et al. 2016).

Ghrelin is a hormone excreted by enteroendocrine gastric cells to stimulate release of growth hormone and increase appetite (DeBoer 2011). It serves the function to balance energy homeostasis in conjunction with leptin and has been shown to fluctuate opposite to leptin in the bloodstream and stimulate appetite in cachectic patients following injection (Gonnissen et al. 2013). The mediated effects of growth hormone lead to the release of neuropeptide Y in the arcuate nucleus of the hypothalamus which then in turn signals the release of orexin (Fig. 3) in the left hypothalamic area with the resulting orexigenic (appetite-increasing) effect (Kerem et al. 2008).

During starvation, the physiological response would be to increase ghrelin release and thereby stimulate orexigenic processes to increase food intake and decrease energy expenditure to restore glucose and lipid stores until required levels have been reached. When this has been accomplished, leptin will be released and ghrelin will

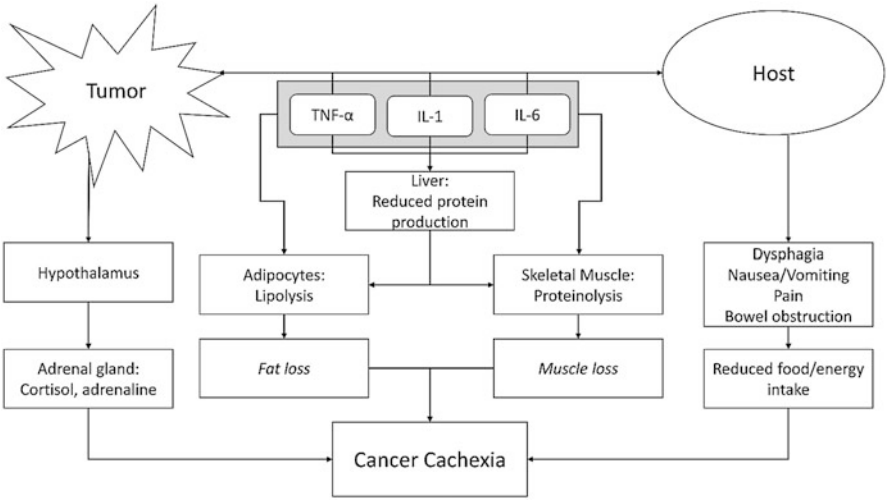

Fig. 3 Tumor-host interactions leading to lipolysis and proteinolysis. The major factors contributing to fat and protein loss as well as release of cortisol and increased activation of the sympathetic nervous system through release of epinephrine from the adrenal gland. Functional factors of the host may accelerate cancer cachexia through decreased food and caloric intake. *TNF-α* tumor necrosis factor α, *IL-1* interleukin-1, *IL-6* interleukin-6

be reduced to signal satiety (Williams et al. 2016). In cancer cachexia, the balance between ghrelin and leptin is not functioning due to systemic inflammation that profoundly impacts metabolic processes and impairs the ability of the body to properly metabolize macronutrients and maintain both adipose and muscle tissue.

Both TNF-α and IL-1 have been associated with cachexia and increased circulating levels of these pro-inflammatory mediators have been confirmed in cancer patients (Mantovani et al. 2000). While IL-6 levels are increased in patients with cancer cachexia, leptin levels are inversely correlated and appear to decrease with advanced cancer stages which shifts homeostasis towards ghrelin and therefore should stimulate appetite. Indeed, increased serum ghrelin levels have been reported in patients with cancer cachexia compared to cancer patients without cachexia or healthy individuals (Chopin et al. 2012). Since ghrelin stimulates the release of growth hormone, it may play a role in tumor growth although there is conflicting data to date. It also remains uncertain why cachectic patients do not gain appetite with increased circulating ghrelin levels as would be expected and it has been theorized that a resistance to ghrelin has evolved in these patients. Supporting evidence for this theory is a lack in increase of insulin-like growth factor-1 (IGF-1) levels, which would normally accompany ghrelin release (Garcia et al. 2013).

The pro-inflammatory environment that promotes tumor growth and often advances progression of malnutrition to cachexia in cancer patients is a crucial factor to consider in the diagnosis and treatment of the patient. The opposing mediators of food intake in the CNS are proopiomelanocortin for anorexic and neuropeptide Y for orexic effects (Fig. 3). Interleukin-6 is generated both in the periphery and centrally

to exert diverse effects on a global scale. It has been shown that IL-6 does promote tumor growth through increased proliferation, shaping of the microenvironment, and involvement in metastasis (Chang et al. 2013). In the CNS, IL-6-mediated intracellular signaling leads to less release of neuropeptide Y and hence decreased production of orexin which would stimulate food intake and appetite (Schele et al. 2013). Furthermore, IL-6 receptors are primarily located in the hypothalamic arcuate nucleus which is in close proximity to the anorexigenic pathway involving POMC and corticotropin-releasing hormone. Therefore, a decrease in neuropeptide Y may directly shift the balance towards decreased food intake.

Another pro-inflammatory mediator that is associated with the development of cancer cachexia and tumor growth is tumor necrosis factor α (TNF-α). Especially in the early development of tumors in promoting growth, invasion, and angiogenesis, circulating levels of TNF-α have been shown to play a significant role in various cancers. However, its levels are not directly correlated with cancer cachexia in the same manner as for IL-6 in a proportional manner. Rather, TNF-α is a global marker of inflammation (Fig. 4) and an indicator of potential progression of malnutrition to cachexia in conjunction with IL-6. While IL-6 decreases neuropeptide Y, TNF-α directly promotes proteolysis and muscle degradation of skeletal muscles (Fig. 4) by

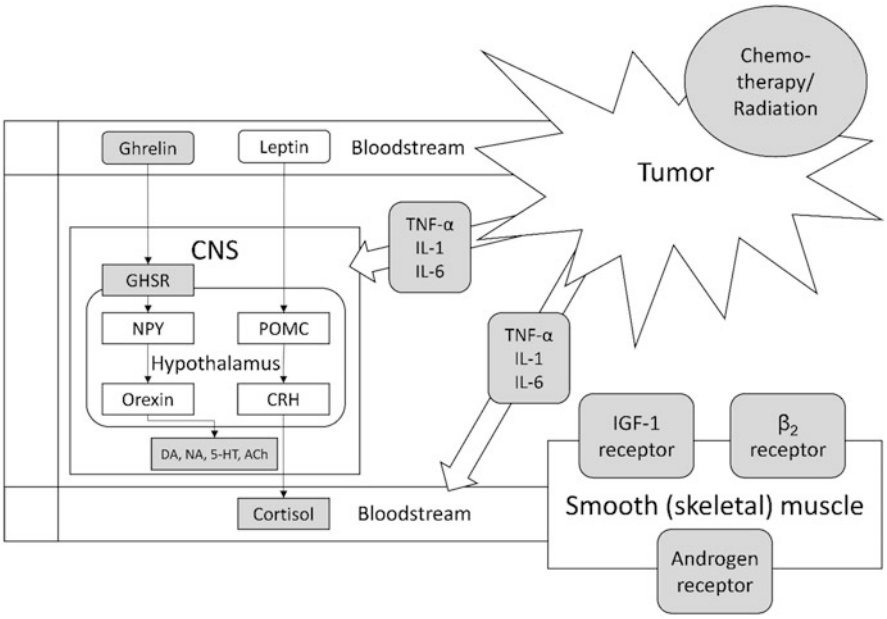

Fig. 4 Potential therapeutic targets for treatment of cancer cachexia. Grey shaded areas are current and potential targets for cancer cachexia including treatment of tumor. Other factors may not yet be explored or viable. *DA* dopamine, *NA* noradrenaline, *5-HT* serotonin, *ACh* acetylcholine, *CRH* corticotropin-releasing hormone, *POMC* proopiomelanocortin, *NPY* neuropeptide Y, *TNF-α* tumor necrosis factor α, *IL-1* interleukin-1, *IL-6* interleukin-6, *IGF-1* insulin-like growth factor-1, β_2: β_2 adrenergic, *GHSR* Ghrelin hormone secretagogue receptor

inducing apoptosis of fast muscle type II fibers through upregulation of the intracellular p38/MAP kinase pathway leading to reduced protein synthesis and muscle atrophy (Phillips and Leeuwenburgh 2005).

Given that the hypothalamus is part of the hypothalamic-pituitary-adrenal (HPA) axis, its activation will lead to the release of cortisol from the adrenal cortex into the bloodstream (Figs. 3 and 4). Cortisol is the endogenous glucocorticoid often associated with glucose, fat, and protein metabolism and suppression of the immune system (Nolten et al. 1993). In common terminology, it is often also referred to as the "stress hormone" since it stimulates the release of epinephrine from the adrenal medulla. Cortisol is released in response to production of proopiomelanocortin from the hypothalamus and release of corticotropin-releasing hormone and exerts anorexigenic effects by increasing lipolysis, proteinolysis, and both gluconeogenesis in the liver as well as glucose uptake by cells in the body (Cavagnini et al. 2000). Continued release of cortisol will cause loss of fat and muscle mass and eventually lead to a build-up resistance to sympathetic system activation (Fig. 4). This may be a positive modulator for tumor growth and progression of cancer cachexia although its exact role remains unclear.

Potential Biochemical Screening Markers for Malnutrition and Cachexia

The extensive search for biomarkers to predict a range of disorders and their severity has resulted in a wealth of information about the disorders themselves but not necessarily always yielded good markers for identifying or determining the severity of a disorder. An important determinant for a good biomarker of a disorder is its value in causation and correlation with the disorder. If the biomarker has a causative impact on the development of the disorder, then it can serve as a good predictor for its development. One drawback to this may be that the same biomarker can be indicative for the development of a range of disorders and hence lack specificity. Another limitation is the potential sensitivity of the biomarker and its careful interpretation in conjunction with other symptoms and observations. In many cases, biomarkers may be used after an initial diagnosis has been made or a suspicion for a disease state has sufficiently narrowed down potential choices.

With this in mind, the healthcare team needs to be aware to interpret any biomarker results within the context of the overall patient presentation and concomitant disorders. From prior discussions, it is clear that cancer patients are at risk of developing malnutrition with advancing cancer stages. Hence, weight, diet, and caloric intake should be frequently monitored. Biomarkers that can serve as indicators for malnutrition but are not specific to cancer malnutrition are low carnitine levels (Rabito et al. 2013), glutamine deprivation (Schlemmer et al. 2015), and to some degree total blood protein and serum albumin (Miyoshi et al. 2015). Malnutrition initially indicates a shift in caloric intake towards higher energy expenditure but may not yet involve proteolysis and lipolysis. Furthermore, malnutrition can develop both from the tumor growth and as a result of the chemotherapy

(Nicolini et al. 2013; Caillet et al. 2016). Serum albumin has often been used as a general indicator in conjunction with weight loss and complete blood counts to assess patients undergoing chemotherapy for potential malnutrition and chemotherapy-related toxicity (Arrieta et al. 2010; Hasenberg et al. 2010; Baicus et al. 2014). A range of nutritional assessment questionnaires have been utilized to evaluate patients, which can be used in conjunction with biomarkers such as the Nutrition Risk Screening 2002 (NRS-2002), Mini Nutritional-Assessment Short-Form (MNA-SF), Patient Generated Subjective Global Assessment (PG-SGA), Malnutrition Screening Tool (MST), and the Malnutrition Universal Screening Tool (MUST) (Isenring et al. 2010). Aside from the potential but unreliable use of carnitine, glutamine, and serum albumin as early indicators of malnutrition in cancer patients, a genomic marker study has revealed a correlation between the mRNA expression for the serine/threonine kinase enzyme CaMKII, which is directly related to exercise-induced inhibition of protein muscle synthesis and weight loss in cancer patients (Stephens et al. 2010). In this transcriptomic study, no correlation between the mRNA expression or protein levels of pro-inflammatory mediators or enzymes that generate cytokines and weight loss were observed in sampled skeletal muscle tissue, indicating that pro-inflammatory signaling mechanism alone may not explain the pathophysiological processes involved in cancer cachexia (Stephens et al. 2010).

Since pro-inflammatory cytokines are associated with cancer progression and appear to play a role in the development of cancer cachexia, a number of studies have focused on using interleukins, TNF-α, and C-reactive protein (CRP) as biomarkersand predictors of morbidity and mortality (McMillan 2009; Bilir et al. 2015). Morbidity and activities of daily living were significantly impacted with increasing CRP levels in patients with advanced cancer that also presented with weight loss and cachexia (Amano et al. 2017). There was no difference in regards to gender, but the researchers reported an increased odds ratio for patients with metastatic cancers and those with impaired performance status. Overall CRP levels showed a stronger correlation with activities of daily living than symptoms such as jaundice, anorexia, or pressure ulcers which is likely based on the specific cancer location. CRP can serve as a valuable predictor for the current health status and progression of a patient in regards to overall health and nutritional status (Dev et al. 2017).

In a small study with lung cancer patients, a correlation between the blood levels of the pro-inflammatory cytokines interleukin-1 and interleukin-6, TNF-α, and leptin and ghrelin was observed with the hypothalamic oxygen requirement in patients who were anorexic compared to controls and cancer patients who were not anorexic (Molfino et al. 2017). Lung cancer patients with anorexia had a lower hypothalamic oxygen level than their nonanorexic counterparts, which may explain their decreased appetite which also related to lower levels of leptin and ghrelin in anorexic patients compared to healthy controls. The authors therefore hypothesized that the hypothalamus may play a central role in appetite regulation and contributing to the development of both anorexia and cachexia in cancer patients.

TNF-α may not serve as the best biomarker for cancer cachexia since it is also released by many tumors and associated with local and systemic inflammation (Patel

and Patel 2017). Despite this dual role, TNF-α can often provide an indication on the progression of cancer cachexia in association with the weight loss and other parameters such as body fat mass and lean muscle mass. In early stages of tumor progression, TNF-α often initiates the loss of lean muscle mass and promotes lipolysis in fat tissue, whereas in later stages once catabolism has reached the stage of cachexia and TNF-α promotes the conversion of glucose into lactate leading to increased tumor lactate levels (Patel and Patel 2017).

Current and Future Treatment Approaches and Preventative Strategies

Malnutrition and cachexia in cancer patients are of obvious concern since it contributes to worse outcomes and lower quality of life, often leading to increased morbidity and mortality. Hence, early detection and intervention is essential and monitoring of weight changes and diet has been the primary measures for clinicians to initiate pharmacological and nutrition intervention. An additional factor contributing to weight loss and reduced quality of life are chemotherapy or radiotherapy itself that often impact immune function and weaken organ systems, especially liver and kidney function (Persson and Glimelius 2002). Pharmacological intervention most commonly employed includes the progesterone hormone, megestrol acetate, and other corticosteroids to stimulate appetite and increase weight (Table 2). However, a meta-analysis indicated that megestrol acetate compared to placebo only led to small weight gains and moderate appetite improvement and was associated with higher morbidities such as edema and thromboembolism as well as higher mortality (Ruiz Garcia et al. 2013). Other treatment options such as the cannabinoid dronabinol or the omega-3 fatty acid eicosapentaenoic acid (EPA) for appetite stimulation presented with conflicting result in clinical trials in regards to weight gain (Tuca et al. 2013).

Nonpharmacological approaches for the prevention or treatment of cancer cachexia and malnutrition often lack sufficient clinical data. However, a number of studies indicate that along with nutritional support and pharmacotherapy, certain complementary interventions may provide benefits to patients and improve quality of life and at least halt further progression of cancer cachexia. One such intervention is acupuncture which has been studied for a range of disorders since it has shown to improve immune function and hence may counteract pro-inflammatory mediators that contribute to the development and progression of cachexia (Yoon et al. 2015). In a feasibility study, improvement in body composition and appetite scores was observed, indicating a potential benefit for targeted acupuncture treatment in patients with gastrointestinal cancer (Yoon et al. 2015).

Based on the mechanism in this chapter that contributes to the development of cancer cachexia, there appear to be multiple targets for new drugs. However, it has been challenging to develop drugs that are not associated with significant adverse effects that also impact quality of life of patients that are often already weakened by chemotherapy and radiation treatment.

Table 2 Current treatment approaches for cancer cachexia

First line pharmacotherapy
Glucocorticoids
Dexamethasone
Methylprednisolone
Prednisolone
Progesterone derivatives
Medroxyprogesterone
Megestrol
Secondary therapy
Cannabinoids
Dronabinol
Cyproheptadine
Equivocal and investigational agents
Anticytokine agents
Melatonin
Omega-3 fatty acids
Pentoxiphylline
Thalidomide
Anabolic agents (testosterone derivatives)
Fluoxymesterone
Nandrolone
Oxandrolone
Metabolic inhibitors
Hydrazine
Beta-blockers
Albuterol
Espindolol
Appetite stimulants
Atypical antipsychotics
Olanzapine
Antidepressants
Mirtazapine

One target for the prevention of loss of skeletal muscle mass has been the androgen receptor with the administration of testosterone analogs (Dev et al. 2014). In case reports, clinical trials, and clinical practice, testosterone and its analogues (Table 2) are already used for this purpose but presented with unclear results in male patients (von Haehling and Anker 2014). Another selective androgen receptor modulator (SARM), enobosarm, has shown promising results in clinical trials by preventing muscle wasting and increasing lean skeletal muscle mass in patients already presenting with cachexia (Srinath and Dobs 2014). The drug is now awaiting FDA approval after two large phase III trials, POWER1 and POWER2, were completed in 2013.

Ghrelin has been a successful target for the treatment of cancer cachexia to stimulate appetite and increase food intake. The direct administration of the peptide ghrelin has to occur intravenously or subcutaneously since it is not orally available, making it an expensive treatment option to physicians and patients alike. In addition, ghrelin has a short half-life and has to be administered twice daily to achieve its desired effect (von Haehling and Anker 2014). A small-molecular peptidomimetic, the orally available anamorelin acts as an agonist at the central ghrelin/growth hormone secretagogue receptor (GHSR) stimulating the release of ghrelin and in turn increasing appetite and presenting with anabolic effects (Pietra et al. 2014). The increase in body weight observed in clinical trials was directly correlated with increased IGF-1 levels providing for the anabolic effect (Garcia and Polvino 2009). The drug was investigated in two phase III clinical trials and is now being considered for approval by the European Medicines Agency (EMA).

Another target for reversal of weight and muscle mass loss is the use of β_2-receptor agonists such as albuterol (Table 2). The use of β_2-receptor agonists for the treatment of asthma has a long tradition and one of its side effects has been a gain in muscle mass that has led to its occasional misuse as a doping agent. A novel partial β_2-receptor agonist, espindolol, which also acts as a β_1-receptor antagonist and a serotonin 5-HT_{1A} antagonist has been tested in patients with cancer cachexia and improved fatigue, gains in lean muscle and body weight, and increased grip strength (Stewart Coats et al. 2016). The drug requires further clinical studies to evaluate its effectiveness in cancer cachexia and potential adverse outcomes.

Policies and Protocols

Despite advances in the understanding of the pathophysiology and underlying contributors of malnutrition and cancer cachexia, the current best practices and protocols for the prevention and treatment of these conditions have not changed much over the past few decades (Molfino et al. 2014). The European Society for Clinical Nutrition and Metabolism (ESPEN) has issued guidelines for nutrition in cancer patients, which emphasizes early detection of malnutrition and weight loss to provide nutritional and exercise support and prevent the progression to cachexia with muscle loss (Arends et al. 2017). This general policy is repeatedly referred to in the literature as an essential hallmark in the prevention of cachexia and reduction in morbidity and mortality. One limitation of using changes in body weight as the sole predictor for malnutrition and cancer cachexia is its nonspecific nature in regards to body composition. Patients can present with relatively stable body weight due to water retention such as edema while losing lean body mass indicative of muscle wasting over many months. The metabolic derangement would potentially go unnoticed until diuresis coincides with weight loss. At that point, however, muscle wasting has already taken place and the patient presents with significant loss of lean body muscle mass. A better approach in this case is the use of body composition measurements using bioelectrical impedance analysis (BIA) which allows for measurement of body water, fat, and muscle distribution

(Kyle et al. 2004; Grundmann et al. 2015). It is worth noting that most oncologists will use a multimodal approach to preventing or treating malnutrition and cachexia in cancer patients: early recognition and intervention are essential which requires monitoring of the patient in consultation with the healthcare team. Referral and collaboration with other members and the support network of the patient can significantly improve quality of life and delay progression, especially if effective measures are implemented such as pharmacotherapy in conjunction with diet counseling, exercise, and complementary approaches that are well received by the patient, and continuous support to the patient and their environment in all activities of their daily living including cancer treatment and meeting their dietary and exercise goals (Bruggeman et al. 2016).

Dictionary of Terms

- **Cachexia** – A pathological condition involving involuntary weight loss involving decreases in lean muscle mass, fat tissue, and general increase catabolic activity. Loss of appetite, fatigue, and weakness are often symptoms of this wasting syndrome. Cachexia increases mortality.
- **Cancer** – A cell conglomerate that shows abnormal growth patterns absent the regular control of the surrounding tissue. Tumorous cancers may grow and expand to other tissues. Cancer cells show genetic abnormalities that allow them to resist apoptosis and regular growth patterns.
- **Inflammation** – A complex sequence of responses of the body to a harmful insult often involving activation of the immune system. Cardinal signs of inflammation include pain, swelling, functional loss, redness, and heat.
- **Malnutrition** – A condition involving a lack of proper supply of macro- and micronutrients as part of a balanced diet leading to an imbalance in nutritional needs for proper physiological functions. It can involve undernourishment or overnourishment in terms of caloric consumption. In association with diseases, it is most often undernourishment of macronutrients which leads to malnutrition.
- **Sarcopenia** – The loss of muscle strength due to atrophy. Sarcopenia can occur independent from cachexia as a progressive aging process due to decreased anabolic processes and protein metabolism as well as decreased quality and strength of muscle mass building.

Summary Points

- Cancer cachexia is prevalent in later stages of the disease and is often associated with poor outcomes leading to increased mortality and morbidity.
- Progression to cachexia has been linked to systemic inflammatory processes mediated by the tumor and perpetuated pro-inflammatory mediators tumor necrosis factor α (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6).

- Patients with cancer cachexia present with an underlying metabolic disorder with dysregulation in protein and fat metabolism as well as muscle wasting mediated by the neurohormones leptin and ghrelin.
- Early detection of malnutrition and adequate treatment can reduce morbidity and increase quality of life by preventing progression to cachexia.
- New drug targets are being investigated to treat cancer cachexia by stimulating appetite, reducing muscle wasting, and preventing protein and fat catabolism.

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Child Starvation and Neglect

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Contents

Abstract

Of the possible forms of child maltreatment, neglect is the most uncommon in industrialized countries. Neglect is associated with deprivation of necessities or caregiver's inability to provide for child's basic needs and/or failure to provide adequate supervision according to the child's developmental age. Starvation is an extreme form of nutrient deficiency which can be unintentional or deliberate,

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depending on the motivation of perpetrators. Lethal neglect, resulting from dehydration and failure to supply food and provide child medical care, remains a relatively uncommon and not well-identifiable cause of death in industrialized countries. When child neglect and starvation are suspected, forensic pathologists must perform a comprehensive assessment of all circumstantial data and medical records keeping in mind that many natural diseases can mimic abuse. In lethal cases, complete information about the child and their family life, crime scene investigation, and forensic autopsy coupled with laboratory findings orient the diagnosis and rule out other causes of death. Starvation can be acute or chronic. Assessing the duration of starvation is of paramount importance but it can rarely be done reliably. The reason for determining duration of fasting is to understand whether and how long prior to death any signs should have been recognizable by caregivers or health care workers, to enable the victim to receive medical care. In suspected child neglect and starvation a skeptical approach is fundamental. A neglected child can die in various ways, and neglected children may not show any signs of physical abuse.

Keywords

Neglect · Starvation · Child abuse · Failure to thrive · Physical growth · Caregivers · Body weight · Forensic · Autopsy finding · Forensic pathologist · Dehydration · Radiology

List of Abbreviations

AAPCN	American Academy of Pediatrics Committee on Nutrition
CT	Computed tomography
FTT	Failure to thrive
LCS	Cerebrospinal liquid
mEq	Milliequivalent
mg/dl	Milligrams per deciliter
NCANDS	National Child Abuse and Neglect Data System
NCHS	National Center for Health Statistics
NSPCC	National Society for the Prevention of Cruelty to Children
PAS	Periodic acid–Schiff
PEM	Protein energy malnutrition
UK	United Kingdom
US	United States
WCS	Waterlow Classification System
WHO	World Health Organization

Introduction

The literature contains a wide variety of definitions of child abuse, maltreatment, and neglect, including international and legal variations (Krug et al. 2002). The terms "child abuse" and "neglect" are included in the broad definition of child

maltreatment set out by the World Health Organization (WHO) Consultation on Child Abuse Prevention as follows:

Child abuse or maltreatment constitutes all forms of physical and/or emotional ill-treatment, sexual abuse, neglect or negligent treatment or commercial or other exploitation, resulting in actual or potential harm to the child's health, survival, development or dignity in the context of a relationship of responsibility, trust or power. (Krug et al. 2002, 59).

Welch and Bonner (2013) defined neglect as (1) deprivation of necessities or caregiver's inability to provide for child's basic needs (e.g., food, water, shelter, medical care, clothing, and education) and (2) neglect or failure to provide adequate supervision according to the child's developmental age.

According to the UK's National Society for the Prevention of Cruelty to Children (NSPCC), child abuse includes physical, psychological, or emotional abuse; sexual abuse; and neglect (NSPCC 2009).

A child can be neglected in many ways, including lack of attention to their primary needs, relative to their age, lack of education, shelter, protection, medical care, supervision, and lack of care to avoid harm.

Of the possible forms of abuse, neglect is probably the most uncommon in industrialized countries. Perhaps the worst form of neglect is failure to supply food and fluids, which can lead to death from starvation and dehydration. This form of neglect seems inexplicable in these countries, and although a cause of child death, it is not always identified as such.

It may manifest in some children as a failure to thrive (FTT), a form of neglect defined by American Academy of Pediatrics Committee on Nutrition (AAPCN) as "a significantly prolonged cessation of appropriate weight gain compared with recognized norms for age and gender after having achieved a stable pattern" (Block and Krebs 2005, 1234).

Starvation is an extreme form of nutrient deficiency which can be unintentional or deliberate, depending on the motivation of perpetrators (Block and Krebs 2005; Knight and Collins 2005; Smitherman 2011; U.S. Department of Health & Human Services et al. 2017).

Unintentional starvation can be related to organic diseases including cancer, chronic infection, hypoxemia from congenital heart defects, surgical interventions, gastrointestinal diseases (malabsorption, cystic fibrosis, celiac disease, liver diseases, and severe gastro-esophageal reflux), and malformations such as cleft palate, neurological impairment, and psychiatric illness. Starvation can also result from nonorganic causes including inadequate nutrition, for example, from prolonged breastfeeding without adequate supplementation, over-dilution of formula milk, diets low in protein, inappropriate composition of homemade formula milk, parental beliefs about food and feeding, and poor diet selection (Block and Krebs 2005; Knight and Collins 2005; Smitherman 2011; Friedman and Billick 2015; U.S. Department of Health & Human Services et al. 2017).

Deliberate starvation is an extremely life-threatening and uncommon form of child abuse (Catanesi et al. 2012). Knight and Collins in 2005 reported that victims of malnutrition/starvation and dehydration are usually aged between 6 and 18 months, and that the primary caregiver at the time of death is usually the mother

(Knight and Collins 2005; Ross and Juarez 2014; Barroso et al. 2016). Intentional starvation is more likely to be associated with particular circumstances, including a family structure complicated by divorce, religious beliefs, unconventional style of life, revenge against partner, and children from unplanned pregnancies. These children may come to personify parental frustration, and become the target of psychological and physical maltreatment, including the lethal complete deprivation of food and water (Ragan 2011). In the most extreme cases, this may be considered a form of torture including repeated physical injury, deprivation of the essential needs, severe psychological abuse, isolation, forceful restriction, and forced and long-lasting exposure to high or low temperature (Catanesi et al. 2012).

Generally speaking, there are two scenarios to consider for forensic pathologists facing cases of suspected child neglect with starvation:

1. *Unintentional neglect or harm*, where parents or caregivers can unintentionally put the child in danger, because they do not understand the child's needs and may be unaware of the potential danger of:
   - Not giving the child enough food, or food inadequate for the phase of growth; or.
   - Not asking for pediatric assistance if there are eating disorders or underlying organic diseases, because "*the situation is not so worrisome*" or the child is believed to be making a fuss.

   These situations occur regardless of the family's socio-economic status.
2. *Deliberate child neglect (and starvation).* Several factors may increase the risk of child neglect and starvation and can affect parents, family, and caregivers. The risk increases if the mother is very young and inexperienced, alone, with a stressful lifestyle, poorly educated, or affected by a mental or physical health issue or learning disability which may impair her ability to care for her children, or mean that she does not realize she is neglecting them. Fathers are more likely to physically abuse than neglect children (Ragan 2011; Catanesi et al. 2012; Miyamoto et al. 2017). A particular issue is obese caregivers who "compensate" for their own loss of control by restricting the diet of their children (Kudek et al. 2016).

The US National Child Abuse and Neglect Data System (NCANDS) defines "child fatality" as "as the death of a child caused by an injury resulting from abuse or neglect or where abuse or neglect was a contributing factor" (Catanesi et al. 2012, e14). Therefore, child deaths can result from a wide range of treatments, including those not directly related to child abuse and neglect (Ragan 2011). Data from the NCANDS for 2015 showed that 75.3% of children recorded had suffered from neglect and 17.2% from physical abuse; children under 3 years old accounted for 74.8% of fatalities because they are the most vulnerable (U.S. Department of Health & Human Services et al. 2017).

The physical and psychological effects of starvation were first studied in a group of conscientious objectors during World War II, known as "The Minnesota Starvation Experiment." In patients with a normal prestarvation weight, a weight loss of

5% impairs mental and physical function. After losing 10–15% of body weight, nutritional intervention may be necessary. A loss of 35% is life threatening (Madea et al. 2016).

When child neglect is suspected, forensic pathologists must perform a comprehensive assessment of all findings that can confirm abuse, bearing in mind that each family is unique. The investigation should broadly include:

1. The lifestyle and clinical history of the child.
2. Investigation of the scene.
3. A physical examination.
4. A radiographical assessment.
5. Autopsy and histological findings and.
6. Laboratory analyses.

Some investigations, such as an autopsy, cannot be performed on living people and certain clinical signs and symptoms are impossible to detect in a cadaver, but this sets out the broad steps.

Everything must be documented with detailed pictures, and if possible, including samples for laboratory testing (e.g., blood, residues of vomit, urine, feces, etc.).

Cause of Death in Child Neglect and Starvation

To confirm neglect and/or starvation, findings from the history and scene investigation must be coherent with the autopsy and laboratory results, and both point to this diagnosis. A neglected child can die in various ways, and neglected children may not show any signs of physical abuse. A diagnosis of fatal starvation is possible after ruling out all other causes of death, in the presence of circumstantial findings and with the victim's appearance indicative of some neglect (Fieguth et al. 2002).

Death from starvation is affected by several parameters, including age, preexisting disease, preexisting nutritional status and health, the amount of fat tissue, coexisting or intercurrent infectious diseases, physical exercise, intake of any fluid, and environmental factors (extreme temperature). Deprivation of food is often correlated with deprivation of water, which accelerates death. Hypertonic dehydration, resulting from liquid decrease in the body, causes hypovolemia and terminal shock. Survival with decreased or insufficient intake of water is possible only for a short period. Survival time has been reported as ranging from 17–76 days (median 40 days) to 60 days for food deprivation and 8–21 days for combined food and liquid deprivation (Dettmeyer et al. 2013). However, the loss of about 35–50% of body weight may cause death (Madea 2005).

In starvation, acute hypoglycemia or ventricular tachyarrhythmia can be the immediate cause of death, alongside secondary infections, especially of the lungs and the kidneys. Malnourished children are more susceptible to invasive infections, and aspiration of the stomach contents can also contribute to death (Madea 2005). Extremely high ketones body production and subsequent ketoacidosis may lead to

death, but only when all postmortem findings have excluded alternative causes of death (Palmiere et al. 2016).

Further Forensic Issues

All these investigations should allow the forensic pathologist to draw the following conclusions when facing a case of suspected neglect:

1. There is no coherent and comprehensible explanation for accidental injury and/or the history, as reported by the caregivers, does not match the established findings from the scene and physical and radiological examination.
2. A thorough examination of the victim reveals external or internal injuries highly suggestive of abuse (localization, patterned bruising, repeated and clustered injuries) and/or not congruent with the child's motor development and age or the situation described by the caregivers.
3. Injuries are not detected but findings from the scene and medical records are strongly indicative of neglect.
4. The manner of death is not natural or accidental, and all reasonable possibilities for the cause of death have been ruled out.

Manner of Death

In cases of starvation, specific issues have to be identified. The manner of death from inanition may be suicidal (hunger strike, protest, exhibitionism, psychiatric illness), homicidal (deliberate withholding of food and water), or accidental (shortage of food in famine, war, entrapment, and calamity, caregivers' ignorance and inability to provide care, and natural diseases). Homicidal and accidental inanition are both encountered in childhood, especially in infants under 3 years of age (Madea 2005).

Duration and Severity of Starvation

Assessing the duration of starvation is of paramount importance but it can rarely be done reliably, because data from literature are mainly limited to adults with acute food and water deprivation (e.g., hunger strikers), or to infants affected by malformations of the upper gastrointestinal tract (Madea et al. 2016). Starvation can be acute or chronic. Acute fasting is a sudden and complete interruption of food and water supply which leads to death. In chronic starvation, the lack of nutrient intake is prolonged and gradual. The duration of starvation also depends upon the severity of undernourishment, and whether it is associated with exacerbating factors such as concurrent diseases, which may often result, in turn, from starvation. The reason for determining duration of fasting is to understand whether and how long

prior to death any signs should have been recognizable by caregivers or health care workers, to enable the victim to receive medical care.

Classifications in the literature refer to Jelliffe's so-called protein-energy malnutrition (PEM), indicating the lack of protein associated with impaired intake of calories in developing countries (Batool et al. 2015).

The standard diagnosis of starvation was commonly made through comparison of anthropometrical data and organ weights of starved children with those of a reference population, using percentile charts (Madea et al. 2016). However, this method did not permit an estimation of degree of malnutrition, especially helpful in a forensic setting to classify infantile malnutrition in cases of deliberate neglect. PEM classifications can be helpful in distinguishing acute from chronic malnutrition (Ross and Juarez 2014).

Since the 1950s, different classifications have been proposed by several authors (Gomez, Wellcome, Kanawati and McLaren, Waterlow) based on parameters including weight, height, and age, the presence of edema, and midarm circumference/head circumference ratio (Gernaat and Voorhoeve 2000; Dettmeyer et al. 2013; Batool et al. 2015; Madea et al. 2016).

The Waterlow classification system (WCS) was used for forensic purposes in 1994 by Madea et al., investigating the death of monozygotic twins from starvation (Madea et al. 1994). In a more recent review published by Madea et al. in 2016, data from case reports of starved infants in the forensic literature were elaborated, and measurements and weights determined at autopsy were evaluated using the same system. The WCS was shown to be suitable for grading severity of starvation, even though a threshold of values for lethal starvation has not yet been described (Madea et al. 2016). The WCS, together with standard growth curves, is helpful in distinguishing between acute and chronic malnutrition and identifying duration (Fieguth et al. 2002).

Anthropometric parameters are not always available and precisely measurable. The examination of medical records has a key role in providing as much evidence as possible of the duration of starvation, considering that absolute withholding of nutrients results in a total body weight loss of about 0.7–1.0% per day (Madea 2005). It may therefore be possible to identify time interval prior to death. Alternative methods have been proposed where records are not available, including analysis of stable carbon and nitrogen isotopes of hair (Baković et al. 2017).

When a forensic pathologist is examining skeletonized bodies, assessing starvation or fatal neglect is even trickier. Medical and family history becomes fundamental together with skeletal indicators that could be diagnostic for malnutrition or undernutrition. These include discrepancies between different standards of hard tissue aging, abnormal dental development, and long bone lengths (Ross and Juarez 2014).

An early recognition of signs indicative of child neglect and abuse can be vital to prevent the situation from worsening, requiring prompt medical care and social support. Missed diagnoses of abuse and neglect because of carelessness towards children and/or records by health and social workers have been reported in literature (Koc et al. 2014). The reasons for this failure include inadequate training in

recognizing child neglect and a resistance to reporting suspected cases because of a wish to avoid legal involvement or not "betray" the child's family (Gunn et al. 2005). One survey demonstrated that more detailed medical records result in higher rates of conviction in cases of suspected abuse (Janßen et al. 2017).

Policies and Protocols

Policies: Forensic Approach

General Information About Child's Life The child's history is likely to be provided by parents or caregivers for very young people. General information about the child and their family life is acquired through a detailed narrative account of events around the death from social services or other agencies, particularly if the child was the subject of previous protection concerns, from neighbors, witnesses, school personal, and schoolmates, for example, if they noticed anything about the child's general appearance, clothing, or behavior that might be relevant (such as stealing or withholding food). Pediatricians may also provide information, if the child has ever seen one, so it is important to collect all medical records and details of any previous examinations. The mother is an important source of information about her pregnancy, and the child's birth, neonatal period, breastfeeding, growth, development, behavior, habits, immunizations, and dental care or treatments. It is important to understand whether the caregivers failed to bring the child to medical appointments and if they sought medical treatment when the child was ill. It is also vital to consider other children in the same family, especially if there are siblings to whom food and medical care were provided while the victim was starving (Kellogg and Lukefahr 2005; Platt et al. 2006; Ross and Juarez 2014).

Investigation of the Scene An extensive investigation of the house or facility where the child lives or has been living must be performed as soon as possible, to avoid alterations designed to misdirect investigation. The forensic pathologist must assess the appearance of the house, its suitability for infants or children (Fig. 1); the availability of food and liquids; how food is kept; whether food and water are adequate for the age of the children; whether clothing, shelter, blankets, and toys are in adequate places for sleeping and playing; the degree of cleanliness (if there are animals, insects infestation, excrement, residues of food, accumulated unwashed dishes, garbage, rubbish, etc.); any hazards such as weapons or drugs within the reach of children and safety measures taken to avoid harm; the presence of suspect objects as belt, or sticks, weapons, tools, cigarettes or cigars, drug paraphernalia; whether medication is available if the child is ill; the existence of school material; and the condition of the house's ventilation and heating (Platt et al. 2006; Ross 2011; Solarino et al. 2012; Lefebvre et al. 2017).

Physical Examination Physical examination in neglect and starvation cases aims to identify any inadequate/insufficient nutritional intake which could determine growth

Fig. 1 Scene of death. A 4-month-old infant, dead at hospital, lived in an apartment that was in a state of extreme neglect

defects, against the growth-curves provided by the National Center for Health Statistics (NCHS) (Smitherman 2011). All findings must be documented, including pictures and details of any injury, because these can all be signs of abuse (Fig. 2a–d).

It is important to consider whether external signs may be caused by physical violence (Smitherman 2011; Gilbert-Barness et al. 2014; Tsokos 2015) (Fig. 3). This will depend on the nature of the force involved and the extent of any injury. Indirect signs of neglect may include dermatitis, parasite infestations, insect bites, and scratching injuries. A careful and comprehensive intraoral and perioral examination is necessary in all cases of suspected abuse and neglect, because this is likely to include dental neglect (caries, gingivitis, etc) (AAPCN et al. 2008).

Any injury should be considered potentially abusive when (Tsokos 2015):

- There is no reasonable explanation for an accidental event.
- There are discrepancies between the injury and the caregiver's description of the incident.
- The child's motor development is not congruent with the history described.
- The child behaves abnormally during a medical examination.
- Cutaneous injuries show particular localization and patterned bruising, and they are repeated (juxtaposition of injuries of different ages) and clustered (three or more individual injuries in the same body region).

In *clinical starvation*, the skin may show atrophy, fissuring, and sores. Changes of hair and nails color and texture can result from decreased protein availability, and changes in cardiac rate and rhythm occur as a result of severe malnutrition/

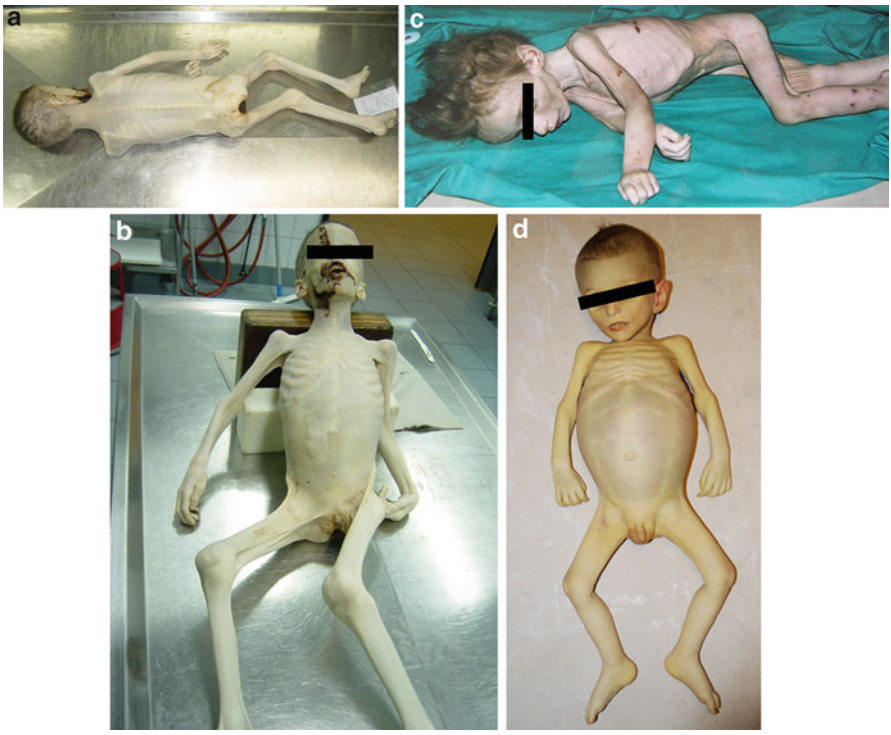

Fig. 2 Typical external appearance of the body in starvation. (**a**) The body of a 7-year-old girl (weight of 9.6 kg, height of 105 cm) with skeletal limbs, and signs of poor hygiene with fecal concretions; (**b**) extreme emaciation with "scaphoid" abdomen and protrusion of the iliac crests and the rib cage; (**c**) the body of a 16-month-old child (weight and height values less than the third percentile) with loss of Bichat's fat pad, sunken eyes, and bruises on the chest and on the limbs resulting from physical violence; (**d**) inanition in a 4-month-old infant resulting from malnutrition and concurrent acute lymphoblastic leukemia

undernutrition (Kellogg and Lukefahr 2005). Almost all organs are affected by nutrition deficiencies, including impairment of pulmonary and renal function (decreased ability to concentrate urine), bone marrow production, secretion of hormones, and brain growth and development. One of the major effects of severe malnutrition is atrophy of the thymus, which decreases immune defenses and increases the likelihood of opportunistic infections (Piercecchi-Marti et al. 2006). If a *fatal event* has occurred, algor, rigor mortis, livor mortis, bodily temperature, and possible putrefactive changes have to be registered to estimate the time of death. Anthropometric parameters including measurement of crown-heel, crown-rump, head diameter, foot length, exact weight, and height must be detected accurately; results are compared with standard pediatric percentile growth charts by age, birth weight, sex, and race. Diameter of limbs, thorax, and abdomen should be measured with a flexible tape (Saukko and Knight 2016). Photographs should be taken in a good light source, to highlight skin color and injuries, and show the general aspect of the body.

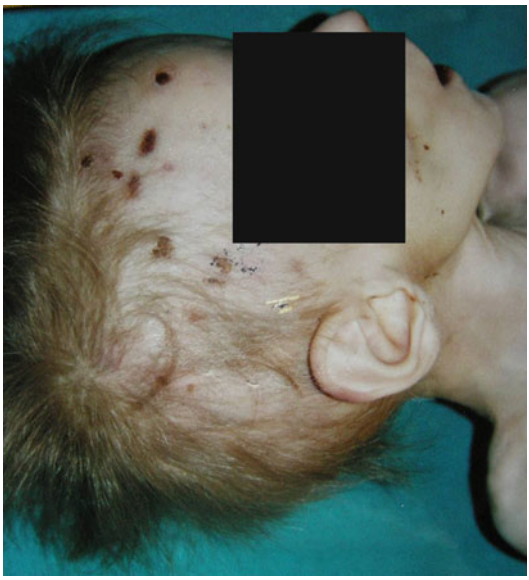

Fig. 3 Signs of physical abuse in a starvation death. Multiple abrasions and bruises at varying healing stages on the face and the scalp caused by objects thrown at her head and beating with a metal spoon

The classical external findings in cases of fatal starvation are extreme emaciation with loss of weight. Saukko and Knight (2016) identified two main patterns of starvation:

- *Wet-type starvation* (edema of face, trunk and limbs, pleural effusions, ascites) because of hypoproteinemia caused by breakdown of proteins to provide energy in the absence of sugar or inadequately intake of fat.
- *Dry-type starvation* resulting in loss of up to half the normal weight, with extreme emaciation, and leg edema.

Knight suggested that nutritional neglect and physical abuse are not always found together, because emaciated children are often uninjured, and battered children may be well-nourished. However, neglect and starvation are generally considered concurrently (Saukko and Knight 2016). Appearance in starvation is well defined and may enable a diagnosis of malnutrition/ undernutrition on sight (Madea 2005; Solarino et al. 2012; Madea et al. 2016). Concurrent dehydration is common, and this may be the main cause of death especially in newborns and infants. The skin's appearance may alter by duration and the type of nutritional deficiency. It can be dark brown or pale, but will be thin, wrinkled, and dry and remain ridged when pinched because of loss of subcutaneous fat and fluids (Fig. 4). Cheekbones are prominent because of the loss of Bichat's fat (the last subcutaneous adipose store to disappear), as are the occiput and ribs, with concavities in intercostal spaces and clavicles, and sunken supraclavicular fossae. In infants, fontanelles are depressed from loss of pressure of cerebrospinal liquid (LCS), eyes are sunken from loss of orbital fat and dehydration, tongue is dry and furred, lips are dry and cracked, hair are dry and

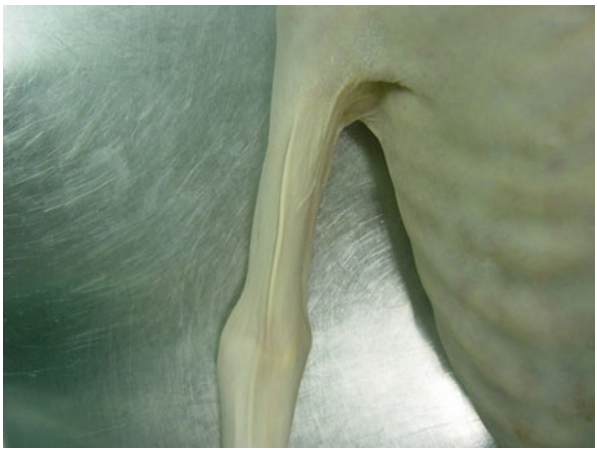

Fig. 4 Postmortem finding of extreme dehydratation. Skin of the upper arm remains ridged when pinched because of loss of subcutaneous fat and fluids

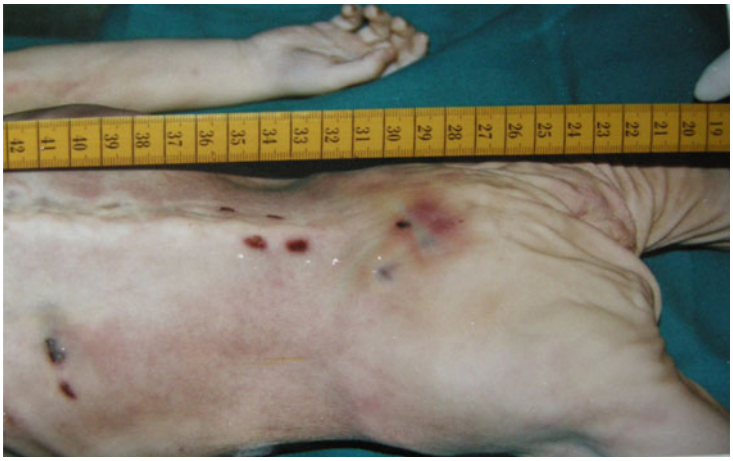

Fig. 5 Signs of neglect and physical abuse. Loss of subcutaneous tissue with protruded spine and pressure sores on the buttocks and spine in infant who have been forced to spend all time sitting in a stroller facing the wall (each injury measured 0.8 mm in diameter)

brittle, and alopecia may be present. The neck is thinned, with very little fat; the abdomen has a scaphoid shape, with concavity from the costal margin to the iliac crests, which will protrude. The limbs may be skeletal from loss of muscular and fatty tissue; edema may also be present, though this is rarely seen, as a result of hypoproteinemia. Signs of insect activity, ante- or postmortem, must be noted. Several signs may indirectly indicate neglect, including cutaneous infections and dermatitis as the result of poor hygiene, pressure sores on the occiput, buttocks, heels, and spine in infants who have been lying inert because they are either very weak or have been forced to do so (Fig. 5). Malnutrition induces a vicious cycle, because it predisposes to decubitus ulcers and infections, which lead to increased

calorific use. This in turn worsens the malnutrition and strengthens the predisposition to ulcers and infections (Madea 2005; Solarino et al. 2012; Madea et al. 2016).

Radiographical Assessment of Neglect Before autopsy, a complete body scan (CT scan or radiographic) is mandatory, to find any traumatic bone lesions or alteration of ossification and mineralization. Some fractures are strongly indicative of inflicted injury, especially multiple unexplained fractures in various stages of healing (Solarino et al. 2009; Ross and Juarez 2016). In starvation, osteopenia/osteoporosis and a deficiency of vitamin C can occur as scurvy, related to defective osteoid matrix formation and cartilage maturation. Radiological assessment can reveal Harris lines (or growth arrest lines) with delayed bone maturation as a nonspecific marker of stress. These appear as a radiopaque transverse trabeculation in metaphyses of long bones, instead of normal trabeculation, which is longitudinal. This finding results from missed mineralization of cartilage consequent on temporary arrest of biological processes of calcification (Piercecchi-Marti et al. 2006).

Autopsy and Histological Findings Typical findings are loss of body and organ weights. The decrease becomes slower after 3 months of starvation, after an initial rapid decrease. Decreasing body weight is the result of loss of subcutaneous adipose tissue and fat surrounding organs, and of organ and muscles atrophy (Fig. 6). Major viscera have to be weighted and compared with standard tables for sex and age. At section, internal fat stores such as the omentum, mesentery, and perirenal area will be lacking; organs except the brain will be very small; the gallbladder will be distended and filled with greenish bile because no food stimulates its emptying; the small bowel will be swollen, with reddish discolored mucosa, the walls of the stomach and bowel translucent and thin, with loss of mucosal layers. The stomach and small bowel will normally be empty, with dry feces in the colon and rectum, especially in fluid restriction and dehydration. In infants, stools may be fluid if dehydration was not severe. The bowel lining can appear ulcerated by fecaliths; foreign bodies can be found in the colon because starving people may try to eat anything accessible before death. Edema and pleural or peritoneal effusion may also occur; atrophy of

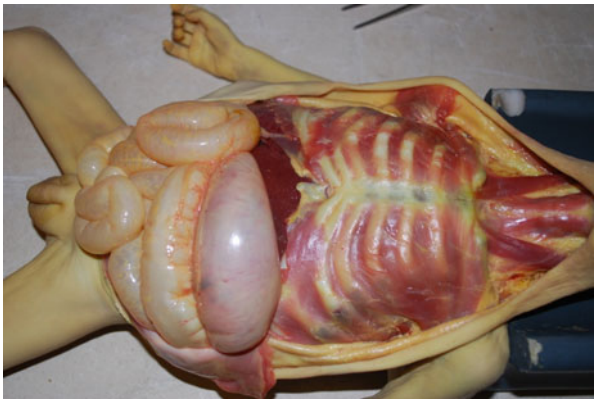

Fig. 6 Typical autopsy finding in starvation. Loss of subcutaneous adipose tissue and fat surrounding organs and severe atrophy of skeletal muscles

endocrine organs, lymphatic tissue, and reproductive glands (ovaries and testes) is a common finding. In infants, a complete atrophy of the thymus is symptomatic of starvation (Madea 2005; Tattoli et al. 2012; Solarino et al. 2012; Gilbert-Barness et al. 2014; Madea et al. 2016; Saukko and Knight 2016).

Histological findings are useful to support macroscopic observation; they are not specific for pediatric starvation but help to exclude underlying natural illnesses. The results reflect the alterations of the organ system, including the endocrine system (insulin, thyroid, and growth hormone are the main hormones affected), immune system (with atrophy of the thymus, lymph nodes, and tonsils), and gastrointestinal system (villous and pancreas atrophy, fatty infiltration of the liver). In particular, hepatocytes vacuolization and microvescicular steatosis from protein deficiency is often found in the liver; loss of glycogen in the liver can be observed on a periodic acid–Schiff (PAS) stain. A secondary siderosis as a result of protein deficiency in the spleen and the liver can occur. Food residues may be found in the lungs, as a sign of aspiration of the stomach contents, which could contribute to death. Bone histology may show vitamin deficiencies or growth disturbances, such as rickets with resorption lacunae and osteoclasts. Bone marrow can reveal gelatinous transformation, fat atrophy, and hypocellularity (Tattoli et al. 2012; Solarino et al. 2012; Osgood et al. 2014; Madea et al. 2016; Saukko and Knight 2016). Thymic involution is found with microscopic findings such as a "starry sky" appearance of the cortex, as a result of stress reactions of the thymus. Calcification of thymic Hassall's corpuscles and fibrofatty replacement have also been described (Kollins 2010).

Laboratory Analyses Supplementary analyses include biochemical investigations, toxicological dosages on available body fluids, virology and microbiology analyses, and metabolic screenings. These are mainly necessary to rule out other causes of death. Vitreous fluid is ideal for postmortem chemical analysis, because it is relatively isolated from other body fluids affected by postmortem changes of redistribution and concentration (Madea et al. 2016). An increase of electrolytes concentrations such as sodium, chloride, and urea nitrogen detected postmortem is a characteristic dehydration pattern. This measurement reflects electrolyte concentrations in serum at the time of death, considering the loss of selective membrane permeability occurring postmortem. A vitreous sodium level of more than 155 mEq and a chloride level of more than 13 mEq are likely to indicate dehydration (Dix and Calaluce 1999). Increased ketone (acetoacetate, beta-hydroxybutyrate, and acetone) body levels in blood, vitreous, pericardial fluid, and urine are linked to starvation-induced ketoacidosis and metabolic acidosis. Depletion of hepatic glycogen stores with mobilization of free fatty acids from adipose tissue increases ketone synthesis in starvation (Lee et al. 2016). Ketoacidosis is also, however, occasionally observed in other forensic settings, such as uncontrolled diabetes mellitus, exposure to low temperature, and alcoholic ketoacidosis (Palmiere et al. 2016). Starvation causes hypoglycemia, so glucose and lactate levels may be determined in LCS or vitreous fluid. Lower range sum values of 50–80 mg/dl and 10–160 mg/dl, respectively, are seen in fatal hypoglycemia. Hyperglycemia and diabetic coma may also occur, with

glucose level greater than 362 mg/dl in LCS (Dettmeyer et al. 2013). A combined analysis of ketones, glucose, and HbA1c in blood and body fluids has been proposed to support diagnosis of metabolic disorders in malnutrition as cause of death (Chen et al. 2015).

Protocols: Differential Diagnosis

An emaciated appearance of the body and signs of neglect may be not enough to identify starvation. A full investigation must include findings from the investigation of the scene and history, postmortem assessment including laboratory testing, and histopathological, neuropathological, ophthalmological, and radiological analysis. The forensic pathologist must be open-minded about all reasonable causes of death and aware that many potential mimickers of neglect exist as causes of starvation. Several natural illnesses, especially cutaneous diseases, may mimic child neglect or physical and sexual abuse (e.g., hemangiomas, allergic contact dermatitis, mongolian spots, purpura) (AlJasser and Al-Khenaizan 2008).

Anorexia-Cachexia Syndrome is found in up to 80% of patients with advanced cancer. It presents with anorexia, weight loss, wasting of muscle and adipose tissue, hyperlipidemia, and other metabolic abnormalities, so can mimic neglect (Tattoli et al. 2012; Madea et al. 2016). All natural diseases that can result in emaciation (e.g., malabsorbitive syndromes, underlying protein losing, endocrine and metabolic imbalances, cystic fibrosis, hematological diseases, and many others) must be excluded by autopsy, skeletal survey, toxicology, and chemical and metabolic testing (Block and Krebs 2005; Madea et al. 2016).

Several genetic problems, sometimes underestimated, including chromosomal number disorders, microdeletion/duplication syndromes, uniparental disomy/methylation disorder, disorders of DNA repair, teratogens, metabolic syndromes, and skeletal dysplasias may also cause starvation (Rabago et al. 2015).

The potential presence of eating disorders must be evaluated, as these can lead to starvation (Seeman 2014).

In the first few weeks or months of life, an infant can have difficulties in breastfeeding and feed poorly, potentially showing a significant FTT, or even starvation and inanition, which can be mistakenly considered to be a result of neglect (Lawrence and Lawrence 2016).

Palmiere et al. (2016) evaluated several postmortem biochemical markers in body fluids (including glycated hemoglobin, glucose, adrenaline and metanephrine, ethanol, urea nitrogen, creatinine and uric acid, proteins, thyroid hormones and thyroglobulin, and ethyl glucuronide) to enable exclusion of common causes of increased blood ketone levels.

The hypothesis of starvation may be formulated only after a comprehensive review of findings from the investigation at the scene, medical and social histories (even though antemortem biochemical data are often unavailable), autopsy, histology, toxicology, and biochemistry. If the pathologist suspects starvation-induced hyperketonemia, toxicology and postmortem biochemistry can orient the diagnosis.

Dictionary of Terms

- **Child** – every human being below the age of 18 years
- **Clinical forensic medicine** – accurate evaluation of the physical condition of living victims and alleged perpetrators
- **Forensic pathology** – a branch of medicine whose aim is the investigation of deaths with medico-legal implications (criminal such as murder, rape, and assault, or civil such as traffic or work-related fatality)
- **Histological analysis** – microscopic study of specimen from body tissues under a light or electron microscope, after samples have been sectioned, stained, and placed on microscope slides
- **Investigation of the scene** – careful examination of the death scene and medical evaluation of trace evidence found at the scene

Summary Points

- Child starvation is common in war scenarios and in undeveloped countries, and it is rare in industrialized country.
- Deliberate starvation of an infant or child is a rare but severe form of child abuse.
- In fatal starvation cases, forensic investigation should include thorough autopsy findings, full investigation of the crime scene, and information including past medical records, family history, and social background.
- Physical examination in neglect and starvation cases aims to identify any inadequate or insufficient nutritional intake.
- Symptoms of starvation need to be addressed in a broad spectrum of nutritional disease.
- Estimating protein-energy malnutrition (PEM) can be helpful in distinguishing acute from chronic malnutrition.
- Clinical starvation findings may include skin, hair, and nails abnormality and impairment of almost all organ systems.
- Performing radiological surveys helps to gather additional evidences when suspicion for nonaccidental neglect arises.
- Autopsy, histological, and laboratory findings contribute in diagnosing the cause and the manner of death in child starvation.
- Forensic pathologist must be open-minded about to all reasonable possibilities of death occurrence and aware that many potential mimickers of neglect exist as causes of starvation.

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Nutritional Consequences of Amyotrophic Lateral Sclerosis

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Rup Tandan, Waqar Waheed, and Connor Scagnelli

Contents

Abstract

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by degeneration of the motor neurons in the brain, brain stem, and spinal cord. Clinically, there are dysphagia and dysarthria from bulbar involvement and muscle weakness with atrophy that result in profound and progressive weakness, respiratory failure, and death within 3 to 4 years from onset. Malnutrition is present in a substantial proportion of patients, with energy or caloric deficiency predominating. The malnutrition is of multifactorial origin, resulting not only from decreased energy intake but also from increased caloric expenditure related to physical activity and hypermetabolism. Several techniques detect malnutrition, ranging from simple anthropometry to the estimation of body composition to

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define loss of muscle and body fat, indirect calorimetry to demonstrate increased resting energy expenditure, and doubly labeled water methodology to show increased total daily energy expenditure. Malnutrition has several adverse consequences, including accelerated disease progression and early death. This chapter describes an algorithmic approach to nutritional care in ALS, preferably undertaken through regular visits to a multidisciplinary clinic with staffed experienced healthcare providers. This chapter also provides recommendations for reliably estimating energy intake and expenditure needs of patients to ensure energy balance.

Keywords

Amyotrophic lateral sclerosis · Basal metabolic rate · Body composition · Body mass index · Dysphagia · Energy expenditure · Energy intake · Fat mass · Fat-free mass · Harris-Benedict equation · Hypermetabolism · Indirect calorimetry · Non-exercise activity thermogenesis · Percutaneous endoscopic gastrostomy · Resting energy expenditure · Weight loss

List of Abbreviations

AAN	American Academy of Neurology
AgRP	Agouti-related peptide
ALS	Amyotrophic lateral sclerosis
ALSFRS-R	Amyotrophic lateral sclerosis functional rating scale-revised
ALS-FTD	Amyotrophic lateral sclerosis-frontotemporal dementia
ALS-P	ALS-plus (cognitive and behavioral changes insufficient for diagnosis of FTD)
AMA	Arm muscle area
APOE	Apolipoprotein E
ATPase	Adenosine triphosphatase
BIA	Bioelectrical impedance analysis
BIS	Bioelectrical impedance spectroscopy
BMI	Body mass index
BMR	Basal metabolic rate
CI	Confidence interval
cREE	Calculated resting energy expenditure
DEXA	Dual-energy x-ray absorptiometry
DLW	Doubly labeled water
ECF	Extracellular fluid
EFNS	European Federation of Neurological Societies
ETF	Enteral tube feeding
FALS	Familial amyotrophic lateral sclerosis
FFM	Fat-free mass
FM	Fat mass
FUS	Fused in sarcoma
FVC	Forced vital capacity
GNRI	Geriatric nutritional risk index

H-B	Harris-Benedict
HR	Hazard ratio
ICF	Intracellular fluid
K^+	Potassium
MAC	Mid-arm circumference
MAMC	Mid-arm muscle circumference
mREE	Measured resting energy expenditure
MV	Mechanical ventilation
Na^+	Sodium
NEAT	Non-exercise activity thermogenesis
NGT	Nasogastric tube
NIV	Noninvasive ventilation
PA	Phase angle
PCMS	Protein-caloric malnutrition score
PEG	Percutaneous endoscopic gastrostomy
PN	Parenteral nutrition
POMC	Proopiomelanocortin
QOL	Quality of life
RDA	Recommended daily allowance
REE	Resting energy expenditure
RIG	Radiologically inserted gastrostomy
RR	Relative risk
SALS	Sporadic amyotrophic lateral sclerosis
SOD	Superoxide dismutase
T3	Triiodothyronine
T4	Thyroxine
TDEE	Total daily energy expenditure
TDP	Transactive response DNA-binding protein
TSF	Triceps skinfold
UK	United Kingdom

Introduction

Amyotrophic lateral sclerosis (ALS) (*Syn*: motor neuron disease, Charcot's disease) is a progressive neurodegenerative disorder of the aging population. The clinical manifestations, dysfunction, and disability in the disease result from degeneration of motor neurons in the spinal cord, brain, and brain stem with ensuing atrophy, fasciculation, and weakness of skeletal muscles from denervation. Concomitant spasticity in the oropharyngeal and limb muscles, due to corticospinal tract degeneration, adds to the morbidity in the disease (Van Damme and Robberecht 2013). The diagnostic criteria are well established and allow diagnostic classification of "definite," "probable," "laboratory-supported probable," and "suspected" cases. About 10% of cases with autosomal dominant inheritance harbor a mutation in the superoxide dismutase 1 (SOD1) gene (familial ALS, FALS), and in recent series, up

to 40% of patients in some regions have a mutation in the C9*orf*72 gene. Mutations involve several other genes in familial cases but are present in rare cases with apparent sporadic ALS (SALS). The incidence of the disorder is 1 – 2 cases/100,000 population, and the prevalence is 5 – 6 cases/100,000 population worldwide. The peak age of onset is in the sixth decade; it is almost twofold more common in men. Onset is insidious with limb muscle involvement in about 70 – 75% of cases and bulbar symptoms (dysarthria or dysphagia) in the remainder. Over time the weakness spreads to other body regions, usually in an orderly fashion, including to the oropharyngeal and respiratory muscles, while it continues to advance in muscles already involved. Death is from respiratory failure; the median survival is about 3–4.5 years from onset of weakness. Oropharyngeal and upper limb involvement limits the ability to handle food, in turn leading to progressive compromise of nutrition and hydration. Striking and often early and rapid loss of weight, usually accompanying dysphagia and malnutrition, is common but occurs in patients without dysphagia.

This chapter will discuss the frequency and causes of malnutrition, relevant studies in the literature, the consequences of energy imbalance, and an algorithmic approach to the evaluation and management of malnutrition in ALS patients.

Malnutrition

Malnutrition is common in ALS patients during the course of the disease. The nutritional deficiency resembles marasmus, a state of caloric deficiency, which commonly results from starvation in other parts of the world especially under conditions of war or famine. Protein-to-calorie intake, however, is unimpaired in ALS. Diminished caloric intake is mainly due to dysphagia resulting from bulbar muscle involvement with resultant impaired bolus formation, difficulty in chewing, and increased propensity for choking with aspiration.

Dysphagia is by far the single most important reason for nutritional impairment and is present relatively early in bulbar than limb-onset ALS due to swallowing dysfunction; with disease progression, it eventually affects almost 75% of patients. Advanced dysphagia is a result of difficulty in chewing and impaired bolus formation; it is associated with prolonged and effortful mealtimes and fear of coughing and choking which all result in decreased food and fluid intake. Anorexia affects many patients with ALS, often with dyspnea and contributes to reduced dietary intake with subsequent weight loss (Jesus et al. 2012). Compounding the effect of dysphagia on nutritional intake are feeding problems due to depression; weakness of the limbs with impaired dexterity and mobility that cause loss of independence; respiratory compromise; cognitive, behavioral, and psychological impairment; and lack of caregiver availability or willingness to help. The combination of these factors leads to difficulties with food preparation, consumption, and ultimately dependence on caregivers. Factors associated with malnutrition are also responsible for dehydration seen in the disease.

Prevalence of Malnutrition and Assessment Techniques

The prevalence and severity of malnutrition vary with the site of onset and stage of the disease, being more common in bulbar-onset patients and in those with advanced disease. The reported prevalence in different studies depends on the method used to study nutritional compromise (Table 1). Nutritional evaluation in ALS has generally adopted several approaches, sometimes in parallel. These include the following assessments:

1. Weight or body mass index (BMI) changes from the premorbid state at the initial visit and after diagnosis.
2. Body composition measured by anthropometry, bioelectrical impedance analysis (BIA)/bioelectrical impedance spectroscopy (BIS), or dual-energy x-ray absorptiometry (DEXA).
3. Daily energy (caloric) intake estimated by dietary analysis.
4. Daily energy expenditure from measured resting energy expenditure or basal metabolic rate.
5. Total daily energy expenditure (TDEE), a reliable surrogate for caloric requirement in humans, measured employing the doubly labeled water (DLW) method.

Based on the method employed, malnutrition is identified in 6% to 100% of patients at the time of first evaluation.

Studies of Nutritional Compromise

Loss of Weight or BMI

Weight

Several studies have examined weight loss and its prognostic value in ALS patients (Table 2). Weight loss is reported in 30 – 85% of patients, being more common in women, with bulbar-onset disease (although it is seen without bulbar involvement), and in advanced disease (Meininger et al. 1995; Korner et al. 2013). The mean loss ranges from 2% to 24% and may be a function of age, duration of disease and follow-up, rapidity of progression, and topography of involvement. The degree of weight loss and the proportion of patients with loss range from >5% in 12 – 50% to 10% in 36 – 48%, >15% in 55%, and >20% in 25% in different series. In general, weight loss of 5–10% from premorbid state is deemed significant for nutritional intervention; cachectic patients typically show loss of >20% of body mass. One study found weight loss to be similar in different BMI groups (Paganoni et al. 2011); in another, there was no difference in loss between normometabolic and hypermetabolic patients (Bouteloup et al. 2009). Weight loss correlates with ALSFRS-R scores and respiratory function, particularly in limb-onset patients. Generally, loss of FFM precedes depletion of FM that might even increase initially (Vaisman et al.

Table 1 Studies of nutritional state in amyotrophic lateral sclerosis

Reference	Country, # patients, M: F (%)	Age, mean ± SD (y)	Onset, bulbar: limb (%)	Disease duration, mean (range) [months]	BMI (kg/m^2)	FVC and ALSFRS or ALSFRS-R score	Nutritional measures examined	Frequency of malnutrition (outcome used)
Slowie et al. (1983)	USA, 20 (55:45) PEG not stated	57	NA	48 (6–132)	NA	NA	Weight TSF and MAMC Caloric intake	24% (weight loss >10%) 50% (anthropometry) 70% (caloric intake)
Shimizu et al. (1991)	Japan, 11 (45:55). All PEG+ and MV+	49 (range 23–70)	NA	85.2 (36–144)	NA	NA	Weight Calorie intake Indirect calorimetry BMR by H-B equation	0% (caloric balance) Energy intake > energy consumption in all Energy consumption < BMR in all
Kanda et al. (1994)	Japan 9 ALS (NA) 26 normal 43 muscle diseases	56 ± 8 24 ± 8 28 ± 17	NA	NA	16.6 ± 3.1 21.3 ± 3.1 17.9 ± 3.5	NA	Weight and BMI DEXA	78% (BMI*)
Mazzini et al. (1995)	Italy, 66 (52:48) PEG in 47%	59 ± 11(peg−) 61 ± 9 (peg+)	14:86	21 ± 12 (peg−) 26 ± 11 (peg+)	22.5 ± 3.9 (peg−) 19.7 ± 2.6 (peg+)	51.4 ± 20.3% Norris score 51–58	Weight and BMI	0–55% (PEG- and PEG+) (BMI*) 53% in PEG+ (weight loss >15%) ↑survival after PEG
Meininger et al. (1995)	France, 936 (57:43)	57.5 ± 12.3	31:69	23.2 ± 0.7	NA	NA	Weight	25% (weight loss >20%)
Nau et al. (1995)	USA, 12 ALS (100:0) 6 controls	51.3 ± 12.7 (ALS) 50.9 ± 12.3 (controls)	NA	17 ± 9	NA	NA	Weight DEXA	NA 1.45 kg weight loss over 6 months

Kasarskis et al. (1996)	USA, 16 (50:50) No PEG	58 ± 18, ♂ 58 ± 5, ♀	NA	24 ± 16, ♂ 31 ± 24, ♀	23	NA	Weight and BMI Caloric intake Prealbumin, urine nitrogen balance Anthropometry, BRI, CHI, CT scan Indirect calorimetry	31% (BMI*) 94% (↓ caloric intake)
Stambler et al. (1998)	USA, 245 (60:40) PEG not stated	53.1,@ 59.6@@	26:74	12.4,@ 8.3,@@	NA	80% @, 50% @@ 27 @, 23 @@	Weight	NA
Worwood and Leigh (1998)	UK, 47 (54:46) PEG in 6%	60	28:72	11	22.9 ± 9.5	NA	Weight and BMI TSF and MAMC	6% (BMI*) 21% (anthropometry)
Desport et al. (1999)	France, 55 (47:53) PEG in 5% (baseline), 35% overall	63.2 ± 11.2	44:56	29 ± 25	23.0 ± 5.1	76 ± 23% 28 ± 8	Weight and BMI	16% (BMI**)
Desport et al. (2000)	France, 30 (NA) PEG 100%	65.7 ± 10.3	47:53	26 ± 19	21.5 ± 3.5	67 ± 27% NA	Weight and BMI TSF and MAMC	NA
Desport et al. (2001)	France 62 ALS (52:48) 31 controls PEG NA	63 ± 11 66 ± 3	34:66	24 ± 26	ALS 24.6 ± 5.2 controls 25.1	78 ± 31% 29 ± 7	Weight, BMI. BIA Indirect calorimetry	NA

(*continued*)

Table 1 (continued)

Reference	Country, # patients, M: F (%)	Age, mean ± SD (y)	Onset, bulbar: limb (%)	Disease duration, mean (range) [months]	BMI (kg/m^2)	FVC and ALSFRS or ALSFRS-R score	Nutritional measures examined	Frequency of malnutrition (outcome used)
Desport et al. (2003)	France 32 (53:47), 15 (40:60)	62.9 ± 11.9, 64.1 ± 11.1	34:66 53:47	NA	24.7 ± 4.3 24.8 ± 3.9	FVC NA 28 ± 6, 32 ± 6	Weight and BMI BIA Indirect calorimetry	NA
Pessolano et al. (2003)	Argentina, 7 (57:43) PEG in 29%	53.2 ± 16.3	NA	NA	20.0 ± 4.8	NA (MV in 1) NA	Weight and BMI 24-h urine creatinine and weight for zero muscle mass	0%** Increased fat mass in 5 patients
Sherman et al. (2004)	USA, 34 (47:53) MV in 47% PEG NA	59.5 67 ± 3 (mv+) 56 ± 15 (mv−)	NA	56–67	24.6 25 ± 4 (mv+) 27 ± 9 (mv−)	64% 18	Weight. Indirect calorimetry	26% (BMI*) 21% obese (BMI)
Desport et al. (2005)	France, 168 (49:51) PEG in 35%	NA	37:63	19 ± 18	24.4 ± 4.4	77 ± 29% 28 ± 7	Weight and BMI BIA Indirect calorimetry	16% (BMI**)
Gil et al. (2007)	France, 222 (53:47) PEG not stated	64 [54, 70]***	30:70	8.2 [5.6, 13.3]***	NA	<80% in 25% patients NA	Weight and BMI	5% (BMI****) BMI NS in transition from milder to severer disease states

Desport et al. (2008)	France, 168 (49:51) PEG at onset NA	63.4 ± 12	37:63	18.6 ± 17.5	24.4 ± 4.4	77 ± 29 28 ± 7	Weight and BMI TSF BIA Indirect calorimetry	10% (BMI****) Phase angle ↓ more in malnourished patients Median survival 35.6 m Survival related to BMI, phase angle, and disease duration
Silva et al. (2008)	Brazil, 20 (80:20)	50 ± 10 (bulbar) 46 ± 13 (limb)	44:56	NA	22.0 ± 0.9 (bulbar) 23.3 ± 1.3 (limb)	54–84% ALSFRS-R 26.1 (bulbar), 34.3 (limb) Bulbar score 7 ± 4 (bulbar), 10 ± 2 (limb)	Weight and BMI Multiple skinfolds, MAMC PCMS score	0% (BMI) PCMS scores low (mean 77–80) Similar nutritional profiles in limb and bulbar patients
Bouteloup et al. (2009)	France, 61 (49:51) no PEG at baseline	64.3 ± 9.9	48:52	14.1 ± 9.9 (onset to first evaluation) 10.2 ± 8.7 (onset to diagnosis)	24.1 ± 3.8	83 ± 25% 31 ± 5	Weight and BMI Caloric intake DEXA Indirect calorimetry	9.8% (BMI****) 29.5% (weight loss >10%) F/u 10 m after diagnosis
Funalot et al. (2009)	France, 11 FALS (55:45) no PEG 33 SALS (55:45) PEG in 3%	60.7 ± 8.8 (FALS) 60.4 ± 8.7 (SALS)	18:72 (FALS) 25:75 (SALS)	15.5 ± 10.8 (FALS) 20.4 ± 21.2 (SALS)	27 ± 3.9 (FALS) 25.5 ± 5.7 (SALS)	FALS 82 ± 30%, SALS 84 ± 30% FALS 28 ± 7, SALS 29 ± 7	Weight and BMI Indirect calorimetry BIA	NA
Vaisman et al. (2009)	Israel, 33 (69:31) no PEG	59 ± 12	36:64	23 ± 14	23.3 ± 3.3	FVC NA. 25 ± 8	Weight and BMI Caloric intake Indirect calorimetry	NA

(*continued*)

Table 1 (continued)

Reference	Country, # patients, M: F (%)	Age, mean ± SD (y)	Onset, bulbar: limb (%)	Disease duration, mean (range) [months]	BMI (kg/m^2)	FVC and ALSFRS or ALSFRS-R score	Nutritional measures examined	Frequency of malnutrition (outcome used)
Jawaid et al. (2010)	USA, 274 (66:34) PEG NA	52.4 ± 13.5	24:76	NA	NA	FVC NA. AALSRS^ 55 (36–90)	Weight and BMI	1% (BMI*) 24% obese
Limousin et al. (2010)	France, 63 (51:49) PEG in 52%	66 ± 12	44:56	32 ± 25 Onset to diagnosis 11 m Diagnosis to final visit 21 m	25 ± 4	NA	Weight and BMI	0% at diagnosis, 14% at final visit-all (BMI*) 21% at diagnosis, 48% at final visit (>10% weight loss group)
Rio et al. (2010)	UK, 159 (NA) PEG 13% RIG 76% NGT 11%	62 ± 12	51:49	20.4 (NGT + ^^) 27.0 (rig+^^) 28.5 (peg+^^)	20.9 ± 3.6	NA	Weight and BMI Caloric intake	20% (BMI**)
Siirala et al. (2010)	Finland, 5 (80:20) PEG 100%	55 (50–76)	NA	78 (64–122)	25 (23–27)	MV in 100%. NA	Weight and BMI Indirect calorimetry	NA
Marin et al. (2011)	France, 74 (50:50) PEG 58% at 10 m follow-up	65.6 (56.5–73.3)+	48:52	11.0 ± 11.0	24.1 At diagnosis median ↓ from premorbid −0.55 (IQR-1.99 to 0.15) Median ↓ before death −1.70 (IQR −3.62 to −0.25)	NA	Weight and BMI TSF and MAMC BIA	9% (BMI****)

Paganoni et al. (2011)	USA, 427 (64:36) PEG NA	54.1 ± 13.10	20:80	NA	26.5 ± 4.3 (n = 188)	88 ± 18% (n = 188) 43 ± 6 (n = 188)	Weight and BMI	2% (BMI**) 19% obese Median f/u 13.1 m 14% ↑ adjusted survival for each higher BMI unit U-shaped survival; best in class I obese, worst in class III obese or malnourished
Ichihara et al. (2012)	Japan, 10 (70:30) PEG in 100%	66.0 ± 11.0	NA	76.8 ± 36.0	19.6	MV in 100%. NA	Weight and BMI Indirect calorimetry DLW	40% (BMI*) 20% (caloric balance)
Jesus et al. (2012)	France, 40 (66:34) no PEG	68.4 ± 10.8	45:55	7.4 (diagnosis to study)	24.9 ± 3.7	NA	Weight and BMI TSF and MAMC Caloric intake	7.5% (BMI****) 7.5% obese
Shimizu et al. (2012)	Japan, 77 (58:42) PEG not stated	66.4+ @	26:71 @@	25.2 (16.8–38.4) ++	22.9 (20.9–25.1) before onset ++ 19.9 (17.9–22.2) at first visit++	NA	Weight and BMI	NA
Clavelou et al. (2013)	France, 382 (55:45) PEG in 20% by 17 m follow-up	61.0 ± 12.4	23:77	9.6 ± 8.1 (0.5–54) (onset to diagnosis)	24.5 ± 4.1 (16.0–50.1)	94 ± 18% (53–165%) ALSFRS 33 ± 5	Weight and BMI	NA
Korner et al. (2013)	Germany, 121 (67:33) PEG 26% with dysphagia at follow-up	59.7	12:88	41.4	NA	FVC NA. ALSFRS-R 29, bulbar score 9	Weight. Survival	NA

(*continued*)

Table 1 (continued)

Reference	Country, # patients, M: F (%)	Age, mean ± SD (y)	Onset, bulbar: limb (%)	Disease duration, mean (range) [months]	BMI (kg/m^2)	FVC and ALSFRS or ALSFRS-R score	Nutritional measures examined	Frequency of malnutrition (outcome used)
Reich-Slotky et al. (2013)	USA, 150 (55:45) PEG NA	57.0 ± 10.9	16:84	<60	26.2 ± 4.6	FVC 88 ± 18% ALSFRS-r 35 ± 5	Weight and BMI	5% (BMI*)
Atassi et al. (2014)	USA, 8635 (60:40) PEG NA	56.2 ± 11.8	76:24	23.0 ± 17.0 11.6 ± 9.2 (onset to diagnosis)	25.4 ± 4.4	86 ± 24% ALSFRS 30 ± 6, ALSFRS-r 38 ± 5	Weight and BMI. Survival	NA
Georges et al. (2014)	France, 16 (75:25) no PEG at baseline	68 (57–73)***	31:69	26.5 (14–39)***	21.4 (19–27)	47% (35–54%) 31 (27–35)	Weight and BMI Indirect calorimetry	37% (BMI*)
Kasarskis et al. (2014)	USA, 80 (65:35) PEG 6%	58.8 ± 11.9	26:73@@@	24.5 ± 2.7	27.1 ± 4.6	76 ± 16% 36 ± 6	Weight and BMI Caloric intake Indirect calorimetry Physical activity BIS and DEXA DLW	3% (BMI*) 51% (caloric intake) 73% (caloric balance)
Wolf et al. (2014)	Germany, 176 (55:45) PEG NA	66.2 ± 10.3	35:65	12.5 ± 12.8 ≤12 m in 69%	<25 (53%)	<80% in 20% patients <32 in 42% patients	Weight and BMI	NA
Park et al. (2015)	South Korea, 193 (62:38) PEG NA	53.8 ♂ 57.4 ♀	19:81	22.1–39.3	21.2–23.4	FVC NA. ALSFRS-R 29–44, bulbar score 9–11	Weight and BMI GNRI Caloric intake TDEE	9% (BMI**) 20% (GNRI)

Ahmed et al. (2016)	Australia 29 ALS (69:31) 12 ALS -P (83:17) 21 ALS-FTD (57:43) 25 healthy controls PEG NA	61.5 ± 10.8 67 ± 19.2 65.7 ± 6.7 70.0 ± 5.3	28:72 33:67 33:67 33:67	24 ± 20 45.6 ± 36.0 64.8 ± 43.2	26.4 ± 3.5 27.1 ± 5.0 29.82.7	FVC NA. ALSFRS-r 41 ± 1 (ALS), 38 ± 2 (ALS-p), 34 ± 3 (ALS-FTD)	Weight and BMI Survival Caloric intake Eating behavior, hunger, and satiety	NA 12.5y f/u BMI not associated with survival Increased eating behavior changes with disease progression
Marin et al. (2016)	France, 261 (58:42) PEG NA	69.6 (61.5–76.3)++	31:69	8.6 (5–13)++	23.9 (21.1–26.8)++	NA. ALSFRS-R 38 (32–42)	Weight and BMI	13% (BMI****) 8% obese F/u 21.6 m, HR for death 1.16 for each unit BMI loss at diagnosis
Lee et al. (2017)	South Korea, 341 (55:45) PEG NA	57.2–61.6 ♂, 56.4–61.3 ♀	24:76	3.8–5.3 ♂, 3.8–4.8 ♀	19,5–23 ♂, 19.5–22.8 ♀	Men 63–88%, women 62–88% Men 21–41, women 20–39	Weight and BMI Caloric intake TDEE from several equations	Negative caloric balance related to stage of ALS
Shimizu et al. (2017)	Japan, 26 (50:50) PEG in 27%	64.5++	18:82	27.6++	19.8++	83%++ ALSFRS-R 37	Weight and BMI Caloric intake DLW	58% (caloric balance)

BIA Bioelectrical impedance analysis, *BIS* Bioelectrical impedance spectroscopy, *BMI* Body mass index, *BMR* Basal metabolic rate, *BRI* Bioelectrical resistive impedance, *CHI* Creatinine height index, *CT* Computed to mography, *DEXA* Dual-energy x-ray absorptiometry, *DLW* Doubly labeled water, *FALS* Familial ALS, *FTD* Frontotemporal degeneration, *FVC* forced vital capacity, *GNRI* geriatric nutritional risk index (risk of malnutrition), *H-B* Harris Benedict, *HR* hazard ratio, *IQR* Interquartile range, *MAMC* Mid-arm muscle circumference, *MV +* With mechanical ventilation, *MV −* Without mechanical ventilation, *NA* Not available, *NS* Not statistically significant, *PCMS* Protein calorie malnutrition score, *PEG+* With gastrostomy, *PEG−* Without gastrostomy, *SALS* Sporadic ALS, *TDEE* Total daily energy expenditure, *TSF* triceps skinfold.

* = BMI <20 kg/m2, ** = BMI < 18.5 kg/m2, *** = Median (quartile 1, quartile 3), **** = BMI <18.5 if age <70 year, <21 if age ≥ 70 year.

^= Appel ALS score, ^^= Onset to enteral feeding, + = Median, ++ = Median and interquartile range, @ = At disease onset, @@ = Additional 3% with respiratory onset, @@@ = Additional 1% with generalized onset, *AALSRS* Appel ALS Rating Scale, *ALSFRS* ALS functional rating scale, *ALSFRS-R* ALS functional rating scale-revised, *ALS-P* ALS-plus.

Table 2 Studies of weight loss in amyotrophic lateral sclerosis

Reference	Country, # of patients: with/ without dysphagia	Premorbid weight loss	Weight at initial visit (kg)	Duration of follow-up (months)	Weight loss (% patients, mean)	Weight loss with/ without dysphagia (mean, range %)	Survival with weight loss/no weight loss (months)	Comments
Slowie et al. (1983)	USA, 16/4	NA	NA	48 (6–132)*	In 85% overall ≥ 10% in 24%	5% (0–12%) (mild dysphagia) and 25% (10–44%) (severe dysphagia)	NA	No weight loss in 15% with dysphagia In some, weight loss without dysphagia
Mazzini et al. (1995)	Italy, 66	NA	NA	26 ± 11 (peg+) 21 ± 12 (peg−)	17 (peg+), 10 (peg−) >15% loss in 55%	NA	Mortality ↓ 6 months after PEG	Average 2.5 kg gain 12 months after PEG
Meininger et al. (1995)	France, 936	NA	NA	28 ± 23	>20% loss in 25% patients	Weight loss and bulbar score ↓ fastest with bulbar onset	NA	Risk factors for >20% weight loss: Females, older age, bulbar onset, fast bulbar decliners
Nau et al. (1995)	USA, 12	NA	NA	6	NA	NA	NA	Mean weight ↓ 1.45 kg, FFM ↓ 2.0 kg, FM ↑ 0.55 kg
Kasarskis et al. (1996)	USA, 16	NA	NA	50	24% weight loss (mean)	NA	NA	Greater FFM and FM loss in men
Stambler et al. (1998)	USA, 245	0.5%@, 2.7% @@ over 2–6 m screening	71.4 ± 1.0 @, 62.8 ± 2.0 @@ at 9 m	9	NA	NA	NA	Greater weight loss 2–6 m before 9-month study entry predictor of death

Desport et al. (1999)	France, 55	NA	60.3 ± 12.6	7 ± 4	5–10% weight loss in 30%	Unrelated to onset type	RR of death ↑ by 7.4 if malnourished	
Desport et al. (2000)	France, 30	NA	NA	26 ± 19 (from onset)	9.6% weight loss (in patients who had PEG)	NA	NA	
Desport et al. (2005)	France, 168	NA	64.5 ± 13.9	11 ± 9	3.5% weight loss (44 patients)	NA	Weight not correlated with survival (44 patients)	
Silva et al. (2008)	Brazil, 20	18% bulbar, 14% limb (NS)	64 ± 1.4 (bulbar) 68.5 ± 1.1 (limb)	NA	NA	NA	NA	Weight loss correlated with ALSFRS-R and respiratory scores in limb-onset patients
Bouteloup et al. (2009)	France, 61	6.4 ± 7.5% overall 5.6% bulbar, 7.2% limb >10% loss in 29.5% patients	NA	Up to 24 months (8 patients)	2.4% at 6 m and 4.1% at 12 m (28 patients)	NA	NA	Premorbid weight loss similar in normo- and hypermetabolic patients
Vaisman et al. (2009)	Israel, 33 (most with some dysphagia)	NA	65.4 ± 11.0	6	Weight ↓ 4.4%	NA	NA	FFM ↓ 8.5%, FM ↑ 1.3% Caloric intake unchanged
Limousin et al. (2010)	France, 63	3.9% loss over 24 m prior to diagnosis ≥10% loss in	66 ± 12	21	9% loss at final visit >10% loss in 48%, with mean loss of 18%	At diagnosis, premorbid weight loss >10% not correlated with bulbar-onset	Survival longer if no malnutrition at diagnosis and onset to diagnosis longer	Disease duration and premorbid weight loss correlated only if weight loss >10% Onset age and type,

(*continued*)

Table 2 (continued)

Reference	Country, # of patients: with/ without dysphagia	Premorbid weight loss	Weight at initial visit (kg)	Duration of follow-up (months)	Weight loss (% patients, mean)	Weight loss with/ without dysphagia (mean, range %)	Survival with weight loss/no weight loss (months)	Comments
		21%, with mean loss of 15%						gender, time to diagnosis, PEG, and NIV use not different if weight loss >10% vs <10%
Rio et al. (2010)	UK, 159	16.3 ± 10.6 (timing not given) >20% loss in 36% patients	58.2 ± 11.9 (at ETF) 47.5 (NGT), 59.4 (rig), 59.6 (peg)	0.9 (NGT) ** 7.2 (rig)** 6.7 (peg)**	NA	NA	Median survival 235d (weight loss ≤20%), 86d (weight loss >20%)	Gastrostomy prolonged survival vs NGT PEG vs NGT, RR for death 0.72 RIG vs NGT, RR for death 0.47
Marin et al. (2011)	France, 92	Median loss 2.3%. 5–10% loss in 15% >10% loss in 36%	65.6 (56.7–75) +	24 (or time of death)	7.1% (1.2–14.4) + before death	Median loss before death −7.05% (IQR −14.36 to −1.16)	Mortality 80.4% Median survival 27.8mos If loss ≥5% vs <5% at diagnosis, survival ↓ by 8 months and adjusted RR for death 1.92	At diagnosis, adjusted 30% ↑ risk of death for each 5% loss, 45% ↑ for ≥10% loss At follow-up, adjusted 34% ↑ risk of death for each 5% loss Survival ↑ 10% for each 2.5 kg higher FM Weight loss rate/ month not related to survival

Paganoni et al. (2011)	USA, 427	NA	NA	11.2 ± 8.4	NA	NA	U-shaped survival curve Best in obese class I BMI group vs other groups	Weight loss rate same by BMI groups (WHO criteria)
Ichihara et al. (2012)	Japan, 10	NA	48.9 ± 9.8	0.5/6	1.6% (0.4–2.5%) loss in 60% over 14 days	NA	NA	Weight gain in 40% and loss in 20% after 6 months of PEG feeding
Shimizu et al. (2012)	Japan, 77	NA	NA	Until death or on MV	NA	NA	HR for death 2.54 if BMI-RR <2.5 vs ≥2.5	Faster disease progression with greater BMI-RR
Clavelou et al. (2013)	France, 382	Mean 4% loss	69 ± 14	17.1 ± 9.8	Loss over 30 months: 3% (arm onset), 3.4% (leg onset), 8.7% (bulbar onset)	Loss greater in bulbar onset in first 15 months of follow-up	Median survival 26% shorter in bulbar onset	45% dead at follow-up
Korner et al. (2013)***	Germany, 121	NA	NA	24 (by survey)	Loss in 56% from onset Disease duration longer if no weight loss	Loss in 62% with dysphagia and 38% without dysphagia	Shorter survival if weight loss	Weight loss 2x greater in bulbar- vs limb-onset patients Lower total and bulbar ALSFRS scores; poorer vitality, physical and social functioning if weight loss

(*continued*)

Table 2 (continued)

Reference	Country, # of patients: with/ without dysphagia	Premorbid weight loss	Weight at initial visit (kg)	Duration of follow-up (months)	Weight loss (% patients, mean)	Weight loss with/ without dysphagia (mean, range %)	Survival with weight loss/no weight loss (months)	Comments
Marin et al. (2016)	France, 261	6 months prior to onset Median loss 3 kg (>5% weight) 2% loss age <65 year), 4.2% ages 65–75 year, 10.6% age > 75 year < 5% loss in 18%, 5–10% in 15%, ≥10% in 36% patients No loss in 32%	66 (57–74)+	21.6+	NA	NA	RR of death at 12 months for weight loss at diagnosis: ↑ 14% for each 5% loss, ↑ 45% for ≥10% loss vs no loss	Weight loss by gender and site of onset NS

* = Mean (range), ** = Median survival after enteral tube feeding procedure, *** >3.0 kg weight loss from disease onset.
@ Survivors, @@ Deceased, + Median (interquartile range).
ALSFRS ALS functional rating scale, *ALSFRS-R ALS* functional rating scale-revised, *BMI-RR BMI* reduction rate (BMI before onset minus BMI at first visit/time interval), *ETF* Enteral tube feeding, *FFM* Fat-free mass, *FM* Fat mass, *HR* Hazard ratio, *IQR* Interquartile range, *NA* Not available, *NGT* Nasogastric tube, *NIV* Noninvasive ventilation, *NS* Not statistically significant, *PEG +* With gastrostomy, *PEG −* Without gastrostomy, *RIG* Radiologically inserted gastrostomy, *RR* Relative risk, *WHO* World Health Organization.

2009) as muscle loss progresses; in one study, loss of FFM and FM was greater in men (Kasarskis et al. 1996). Malnutrition and denervation cause loss of weight, skeletal muscle, and body fat and account for cachexia of the disease and impair muscle function.

Low body weight and leanness in the premorbid state increase the risk of developing ALS by almost threefold (Gallo et al. 2013). Weight loss from the premorbid state, and after diagnosis, can predict survival. At diagnosis, greater weight loss is a predictor of death over the next year; the adjusted risk of death increases by 30% for 5% loss and 45% for ≥10% loss. After diagnosis, the adjusted risk of death increases by 34% for each 5% loss, and for each 2.5 kg higher FFM, the survival increases by 10% (Marin et al. 2011).

BMI

BMI (weight [kg] ÷ √ height^2 [m]) (kg/m^2) is easily measured and has been utilized in several studies of malnutrition in ALS (Table 2). The World Health Organization classifies subjects according to BMI as follows: < 20, malnourished; 20 – <25, normal; 25 – <30, overweight; 30 – <35, obese class I; 35 – <40, obese class II; and 40 or >, obese class III. In other classification systems, a BMI of <18.5 kg/m^2 if age is ≤65 year, or <20 if age is >65 year, denotes malnutrition (Desport et al. 2000). ALS patients showing malnutrition by other indicators, or those requiring gastrostomy, generally show lower BMI (Mazzini et al. 1995; Kasarskis et al. 1999).

Low premorbid BMI carries an increased risk of developing ALS (Gallo et al. 2013). Further, the premorbid BMI reduction rate correlates with total disease duration in some but not all studies and with survival as assessed by the hazard ratio (HR) or relative risk (RR) for death (Table 2). The risk of death increases by 16–24% for each unit of premorbid BMI lost.

In general, malnutrition by BMI criteria at diagnosis occurs in 6–53% of patients and correlates with a general nutritional risk index and respiratory parameters, but not in all studies (Table 3). Other studies show no correlation between BMI and site of onset (bulbar versus limb), neurological disability, measured resting energy expenditure, presence of hyper metabolism, and time to transition from possible/probable to definite ALS by El Escorial criteria. The risk of death is 2.2–7.4-fold higher in patients malnourished at presentation (Desport et al. 1999; Marin et al. 2011; Paganoni et al. 2011), but not in all studies (Shimizu et al. 2012). Several studies report a U-shaped relationship between BMI at first visit and progression of disease as assessed by a decline in the ALS functional rating scale-revised (ALSFRS-R) score or survival, with the best outcome in class I obese patients and the worst in those with malnutrition or class III obesity. In patients with BMI <30 kg/m^2, a higher BMI is associated with slower decline of ALSFRS-R scores (Reich-Slotky et al. 2013). The progression of ALS is about 1/3 slower, and survival is longer, if follow-up BMI is stable or increases by >1 unit versus declines by 1 unit. Conversely, a faster decline of BMI over 2 years portends faster disease progression and lower survival (Jawaid et al. 2010; Shimizu et al. 2012). Malnutrition at the time of insertion of a percutaneous endoscopic gastrostomy (PEG) tube also shortens survival.

Table 3 Studies of change from premorbid body mass index in amyotrophic lateral sclerosis

Reference	Country, # patients	Premorbid BMI	BMI at first visit (kg/m2)	Follow-up (months)	BMI at follow-up (kg/m2)	BMI change (kg/m2)	Comments
Limousin et al. (2010)*	France, 63	25 ± 4 (24 m prior to diagnosis)	24 ± 4	21	22 ± 6	↓ by 2 units	BMI change 2 years premorbid to diagnosis ↑ by 1.0 unit, to final visit ↓ by 3.3 units If premorbid weight loss >10%, BMI ↓ 3.8 at diagnosis and 5.2 at final visit No correlation between BMI or BMI change and disease duration
Marin et al. (2011)**	France, 92	24.7++ (6 m prior to diagnosis)	24.1 (21.2–27.0)+	28++	23.5 (20.9–26.6) + ↓ from premorbid to diagnosis by 0.55 units (IQR-1.99 to 0.15) ↓ from diagnosis to before death by 1.70 (IQR-3.62 to −0.25)		24% ↑ risk of death for each unit lost from premorbid BMI Malnourished BMI → RR of death 2.15 Overweight and obese BMI → RR of death 0.71 and 0.36, respectively

Shimizu et al. (2012)**	Japan, 77	22.9 (20.9–25.1)+	19.9 (17.9–22.2)+	25.2 (16.8–38.4) + (onset to MV or death)	NA	NA	BMI-RR correlated with total disease duration HR 2.5 if BMI-RR ≥2.5 versus <2.5 cox analysis HR for death 2.7 and 1.9 for BMI-RR and FVC at first visit survival similar if BMI ≥18.5 vs <18.5 at first visit
Wolf et al. (2014)	Germany, 176	NA (6 m preceding diagnosis)	<25 (53%) ≥25 (47%)	≥18	NA	Decline in units by <1 (16%), 1 to <2 (35%) or >2 (60%) versus premorbid	OR for death 2.8 if premorbid BMI loss ≥2 units versus <1 unit at diagnosis
Marin et al. (2016)	France, 261	NA recorded 6 m premorbid	23.9 (21.1–26.8)+	17.5 (13.5–18.2)@ (survival)	NA	1.26 (0.00–3.3)	HR for death 1.16 for each unit ↓ BMI at diagnosis from premorbid BMI

+ = Median and interquartile range ++ = median, +++ = Mean ± SEM, @ = Median and 95% confidence interval
BMI Body mass index, *BMI-RR* BMI reduction rate (premorbid before onset minus BMI at first visit/time interval), *FVC* Forced vital capacity, *HR* Hazard ratio, *IQR* Interquartile range, *NA* Not available, *OR* Odds ratio, *RR* Relative risk.
*BMI < 18.5, **BMI <18.5 if age <70 year, <21 if age ≥ 70 year, *** Median (Q1, Q3)

The inconsistency in the BMI criterion defining nutritional deficiency probably accounts for the wide ranges reported in the prevalence of malnutrition. Further, BMI cannot differentiate between lean and fat components of soft tissue, both of which are affected. For example, BMI did not suggest malnutrition in 70% of patients who were malnourished by other anthropometric criteria (Worwood and Leigh 1998). Caloric deficiency by the DLW method occurred in 10 ALS patients, none of whom had a BMI <20 kg/m^2 (Tandan 2000). Thus, reliance on BMI alone for diagnosing and following undernutrition in ALS is a questionable practice.

Alterations in Body Composition

Skin Folds and Circumferences

Many researchers have developed and validated equations from skinfolds, body circumferences and ratios from different anatomical regions in healthy subjects to estimate body density, and subsequently body fat and fat-free mass, to assess body composition and gleam the nutritional state (Kasarskis et al. 1996; Nau et al. 1997). Several investigators have measured triceps skinfold (TSF) thickness and mid-arm circumference (MAC) to derive mid-arm muscle circumference (MAMC), thus enabling estimation of arm muscle area (AMA), fat mass, and muscle mass to allow determination of age-gender percentiles as compared to normal populations (Table 4). AMA with other indices of muscle mass and disease progression also permit monitoring of muscle atrophy, in addition to establishing the nutritional state (Kasarskis et al. 1997). Generally, anthropometry overestimates FFM and underestimates FM in ALS as compared to DEXA-measured values (Nau et al. 1997).

Moderate malnutrition (TSF below 30th percentile) occurs in 15% and severe malnutrition (TSF below 24th percentile) in 10% of patients, although in 70% dietary inquiry reveals decreased caloric intake compared to RDA values (Slowie et al. 1983). There is a 41% reduction of AMA, denoting loss of muscle, over 50 months prior to death (Kasarskis et al. 1996), and no significant relationship to presence of dysphagia, as reported by others (Jesus et al. 2012). In the United Kingdom (UK), malnutrition (at least one anthropometric measure below the fifth percentile) occurred in 21% of patients irrespective of site of onset, but reduced daily caloric intake as compared to the UK standard was present in all (Worwood and Leigh 1998). Moderate malnourishment by protein-caloric malnutrition scores (PCMS) is evident in Argentinian patients, again unrelated to site of onset (Silva et al. 2008). Increased TSF thickness, an indicator of FM, is also associated with improved survival; in one study, each unit of higher TSF carried a survival advantage of 6% over 28 months of follow-up (Marin et al. 2011).

BIA and BIS

By measuring the resistance, conductance, and impedance of body tissues, single and multiple frequency BIA and BIS estimate body composition and phase angle to assess the nutritional state (Desport et al. 2003; Vaisman et al. 2009) (Table 5). BIS also evaluates the hydration status by measuring total body water and extracellular fluid (ECF) volume and subsequently extrapolates intracellular fluid (ICF) volume.

Table 4 Malnutrition assessed by first visit and follow-up body mass index in amyotrophic lateral sclerosis

Reference	Country, # patients	BMI at first visit (kg/m2)	Follow-up (months)	BMI at follow-up (kg/m2)	BMI change (kg/m2)	Comments
Mazzini et al. (1995)*	Italy, 31	22.5 (PEG−), 19.7 (PEG+) <20 in 53% (PEG+)	12	18.4 (PEG−), 20.5 (PEG+)	↓ by 4.1 units in PEG-, ↑ by 0.8 units in PEG+ at follow-up	BMI in normal range at follow-up in 64% PEG+
Kasarskis et al. (1996)	USA, 16	21.7 dysphagia+, 23.0 dysphagia-	6	Regression line showed 23% ↓ at time of death over 50 months	BMI ↓ correlated better with muscle loss in ♂ and fat loss in ♀	BMI <20 correlated with ↓ respiratory parameters
Worwood and Leigh (1998)*	UK, 47	22.9 (16.1–35.7)	None	NA	NA	BMI only identified 30% malnourished by anthropometry
Desport et al. (1999)**	France, 55	23 ± 5.1	7 ± 4	NA	NA	mortality 32% at follow-up malnutrition unrelated to onset site or neurologic disability RR of death 7.4x if malnourished
Desport et al. (2000)****	France, 30	21.5 ± 3.5 (at time of PEG)	NA	NA	NA	survival ↓ if BMI at time of PEG <18.5
Desport et al. (2001)	France, 62 ALS, 31 healthy controls	24.6 ± 5.2 (ALS), 25.1 ± 2.6 (healthy controls) (NS)	None	NA	NA	BMI not correlated with mREE
Pessolano et al. (2003)**	Argentina, 7	20.0 ± 4.8	None	NA	NA	BMI not correlated with lean or fat mass BMI underestimated fat, inaccurately represented body composition

(*continued*)

Table 4 (continued)

Reference	Country, # patients	BMI at first visit (kg/m2)	Follow-up (months)	BMI at follow-up (kg/m2)	BMI change (kg/m2)	Comments
Sherman et al. (2004)*	USA, 34	26.5 ± 8.9 (MV-), 24.5 ± 4.3 (MV+)	None	NA	NA	
Desport et al. (2005)*	France, 168	24.4 ± 4.4	10.9 (n = 44)	23.7	↓ by 0.9 units	BMI < 20 at first visit associated with ↓ survival, after adjusting for other prognostic variables
Gil et al. (2007)**	France, 222	NA	28.0 (bulbar), 36.9 (spinal)	NA	NA	BMI NS in transition from possible/probable to definite ALS or to death/permanent tracheostomy
Bouteloup et al. (2009) ****	France, 61	24.1 ± 3.8 (all cases) 23.6 ± 3.6 (12 m survivors)	24	22.3 ± 3.2 (at 12 months) NA at 24 m	↓ by 1.3 units at 12 m ($p < 0.001$)	BMI similar in normo-metabolic and hypermetabolic groups BMI not correlated with mREE, mREE/FFM, mREE-cREE/cREE, mREE/cREE
Vaisman et al. (2009)	Israel, 33 ALS, 33 healthy controls	23.3 ± 3.3 (ALS), 28.7 ± 4.8 (healthy controls) ($p < 0.001$)	6 (n = 10)	22.4 ± 3.4	↓ by 1.1 units ($p = 0.07$)	
Jawaid et al. (2010)	USA, 274	NA	24	NA	↓ by >1unit in 48% patients	BMI loss >1 unit correlated with bulbar-onset disease Faster adjusted progression rate and ↓ survival if BMI loss >1 unit vs stable or ↑ by >1 unit Progression 34% slower and survival 16 m longer if BMI stable or ↑ by >1 unit vs ↓ by >1 unit

Paganoni et al. (2011)	USA, 427	26.5 ± 4.3 (n = 188)	11	26.6 ± 4.9 (n = 188)	↑ by 0.1 unit (n = 188)	Median f/u 13.1 m 14% ↑ adjusted survival for each unit of BMI U-shaped survival; best with class I obesity, worst in malnourished and class III obese groups
Ichihara et al. (2012)	Japan, 10	19.6	NA	NA	NA	
Jesus et al. (2012)****	France, 40	24.9 ± 3.7	NA	NA	NA	Home assessment after ALS center visit ↑ calories and protein intake if weight and BMI low
Reich-Slotky et al. (2013)	USA, 150	26.2 ± 4.6	9 (study duration)	NA	NA	U-shaped relation between initial BMI and ALSFRS-R decline If BMI <30, ALSFRS-R decline slower in patients with higher BMI If BMI ≥30, ALSFRS-R decline faster in patients with higher BMI
Atassi et al. (2014)	USA, 8635	25.4 ± 4.4	NA	NA	NA	Kaplan Meier HR for death 0.65 (overweight) and 0.46 (obese) compared to those with entry BMI < 25
Kasarskis et al. (2014)	USA, 80	27.1 ± 4.0 ♂ 27.1 ± 5.7 ♀	12	NA	NA	No correlation between initial BMI or BMI change and survival

(*continued*)

Table 4 (continued)

Reference	Country, # patients	BMI at first visit (kg/m2)	Follow-up (months)	BMI at follow-up (kg/m2)	BMI change (kg/m2)	Comments
Park et al. (2015)**	South Korea, 193	21.2 ± 0.4 to 23.4 ± 0.3+++	NA	NA	NA	BMI lowest in lowest tertile group of ALSFRS-R score 22% patients in lowest tertile group of ALSFRS-R score were malnourished BMI correlated with general nutritional risk index, ALSFRS-R bulbar score, albumin, creatinine, BUN, total lymphocyte count, and TDEE
Ahmed et al. (2016)	Australia 29 ALS 12 ALS-P 21 ALS-FTD 56 BvFTD 25 Controls	26.4 ± 3.5 27.1 ± 5.0 29.8 ± 2.7 29.2 ± 4.2 25.3 ± 4.5	Up to 150	NA	NA	BMI lower in controls and ALS vs ALS-FTD and BvFTD neither initial BMI nor BMI groups (normal, overweight, obese) associated with survival increased eating behavior changes seen with disease progression
Shimizu et al. (2017)	Japan, 26	19.8 (17.6–22.3)+	NA	NA	NA	BMI correlated with TDEE

+Median and interquartile range ++Median, +++Mean ± SEM, +*Median and 95% confidence interval

ALSFRS-R ALS functional rating scale-revised, *BMI* Body mass index, *BUN* Blood urea nitrogen, *BvFTD* Behavioral variant frontotemporal dementia, *cREE* Calculated resting energy expenditure, *FFM* Fat-free mass, *FTD* Frontotemporal dementia, *HR* Hazard ratio, *mREE* Measured resting energy expenditure, *NA* Not available, *NS* Not statistically significant, *PEG* Percutaneous endoscopic gastrostomy, *PEG −* Without PEG, *PEG +* With PEG, *RR* Relative risk, *TDEE* Total daily energy expenditure.

* BMI <20.0, ** BMI < 18.5, *** Median (Q1, Q3), **** BMI < 18.5 if age < 70 year, <21 if age ≥ 70 year

Table 5 Studies of body composition in amyotrophic lateral sclerosis

Reference	Country, # of patients	FFM T1 (kg)	FM T1 (kg)	TSF T1	MUAC	MUAMC	FFM change T2 (kg or %)	FM change T2	TSF change T2	MUAC change T2	MUAMC change T2	Comments
Anthropometry												
Slowie et al. (1983)	USA, 20	NA	NA	Increased by 26% in ♂ Reduced in 5% ♂ and 100% ♀ vs controls	Reduced 9% ♂, 19% ♀ vs controls	Reduced in ♂ and ♀	NA	NA	NA	NA	NA	TSF below 30th and MUAMC below 35th percentile in 15% (moderate malnutrition) TSF and MUAMC below 24th percentile in 10% (severe malnutrition)
Kasarskis et al. (1996)	USA, 16	NA	NA	NA	NA	NA	↓ 23% over 50 months	% body fat ↓ 10% over 50 months	NA	NA	NA	BIA measured body composition AMA ↓ by 41% over 50 months Muscle and fat loss greater in ♂
Worwood and Leigh (1998)	UK, 47		♂ 29% ♀ 32% of body weight	♂: 11.5 mm ♀ 16.5 mm	NA	♂ 24 mm ♀ 21 mm	NA	NA	28.7 ± 4.33 ♂ 32.3 ± 4.58 ♀	NA	NA	By at least one measure, 21% malnourished (<fifth percentile values) Arm measures not different in bulbar- and limb-onset
Silva et al. (2008)	Brazil, 20	84%	26%	14.7 ± 0.5	25.7 ± 0.4	22.2 ± 1.8	NA	NA	NA	NA	NA	Arm measures not different with bulbar- and limb-onset Moderate malnutrition by PCMS scores in bulbar- and limb-onset

(*continued*)

Table 5 (continued)

Reference	Country, # of patients	FFM T1 (kg)	FM T1 (kg)	TSF T1	MUAC	MUAMC	FFM change T2 (kg or %)	FM change T2	TSF change T2	MUAC change T2	MUAMC change T2	Comments
Jesus et al. (2012)	France, 66	48 ± 13	20 ± 9	13.4 ± 7.8	NA	24.6 ± 4.6	NA	NA	NA	NA		Similar body composition if dysphagia present or absent
Gallo et al. (2013)	Western Europe, 220	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Reduced risk of dying if ↑ body fat HR for ALS risk 2.79 if underweight
Bioelectrical impedance analysis or spectroscopy												
Desport et al. (2005) And 2008	France, 168	44 ± 11	21	NA	NA	NA	3% ↓ (NS) over 11 m (n = 44)	5% ↓ over 11 m (n = 44)	NA	NA	NA	PA ↓ more in malnourished ALS patients Survival correlated with BMI, phase angle and disease duration
Marin et al. (2011)	France, 92	45 (36–53) +	20 (14–24) +	12.5 (9.3–16.3)+	NA	23.9 (22.2–26.5) +	6% ↓ over 28 m	5% ↑ over 28 m	7.8% ↑ over 28 m	Not given	7.8% ↓ over 28 m	Before death PA ↓ 32% and EC/IC fluid ratio ↑ 7% Survival 6% ↑ for 1 unit ↑ TSF, 10% ↑ for 2.5 unit ↑ FM, 67% ↑ for 0.2 unit ↑EC/IC fluid ratio
Ellis and Rosenfeld (2011)	USA, 56	43 ± 12	29	NA	NA	NA	NA	NA	NA	NA	NA	FFM 6%/11% lower if on NIV/ MV FM 15% higher if on NIV, 3% lower if on MV
Dual-energy x-ray absorptiometry												
Kanda et al. (1994)	Japan, 9 ALS 26 normal controls	35.2 ± 8.1 43.5 ± 4.5	7.4 ± 4.3 14.1 ± 6.3	NA	NA	NA	NA	NA	NA	NA	NA	Lower FM and FFM in ALS Lower fat to muscle ratio in ALS

Nau et al. (1995)	USA, 12 ALS 6 controls	NA	NA	NA	NA	NA	↓ by 2 kg over 6 m	↑ by 0.55 over 6 m	NA	NA	NA	Net gain of 3100 kcal of stored energy
Nau et al. (1997)	USA, 23	46 ± 2*	28 ± 2*	NA	NA	NA	NA	NA	NA	NA	NA	FFM overestimated and FM underestimated by anthropometry and BIA
Desport et al. (2003)	France, 47	44 ± 11	NA	14.2 ± 5.4	28.4 ± 3.9 (R) 28.8 ± 3.6 (L)	NA	11% ↓ (DEXA) 7% ↓ (BIA) over 10 m (n = 18)	NA	NA	NA	NA	FFM from DEXA and BIA ≤ 3%
Bouteloup et al. (2009)	France, 61	44 ± 12	NA	NA	NA	NA	5% ↓ (n = 22) over 12 m	↑ 3% and 10% over 6 and 12 m	NA	NA	NA	
Vaisman et al. (2009)	Israel, 33 ALS 33 controls	42 ± 7 (ALS) 55 ± 12 (controls)	22 (ALS) 28 (controls)	NA	NA	NA	9% ↓ over 6 m (n = 10)	1% ↑ over 6 m (n = 10)	NA	NA	NA	Daily caloric intake unchanged over 6 m FFM contributed 60% to mREE
Doubly labeled water												
Ichihara et al. (2012)	Japan, 10	27 ± 6	21.7 (10.8–29.5)	NA	NA	NA	NA	NA	NA	NA	NA	DLW measured body composition All patients on MV and PEG feeding
Shimizu et al. (2017)	Japan, 26	38.5 (35.9–40.9)	NA	NA	NA	NA	NA	NA	NA	NA	NA	DLW measured body composition FFM 23% lower in women FFM similar in PEG + and PEG− patients

Some studies utilized multiple techniques to estimate or measure body composition

MUAC Mid upper arm circumference, *MUAMC* Mid upper arm muscle circumference, *mREE* Measured resting energy expenditure, *MV* Mechanical ventilation, *NA* Not available, *NIV* Noninvasive ventilation, *PA* Phase angle, *PCMS* Protein-calorie malnutrition score, *PEG* Percutaneous endoscopic gastrostomy, *PEG −* Without PEG, *PEG +* With PEG, *TSF* Triceps skinfold thickness, *AMA* Arm muscle area, *BIA* Bioelectrical impedance analysis, *EXA* Dual-energy x-ray absorptiometry, *DLW* Doubly labeled water, *EC/IC* Extracellular/intracellular, *FFM* Fat-free mass, *FM* Fat mass, *HR* Hazard ratio, *mREE* Resting energy expenditure measured by indirect calorimetry

*=Mean ± SEM, + = Median (inter quartile range)

Generally, FFM and FM are lower in ALS patients than in healthy controls, particularly in those with relatively advanced disease on noninvasive ventilation (NIV) or mechanical ventilation (MV) (Ellis and Rosenfeld 2011), and decrease as the disease progresses. Phase angle (PA) is lower in ALS, especially in malnourished patients, and adversely affects survival (Desport et al. 2005, 2008; Marin et al. 2011). Body composition and hydration status affect survival; thus, each 2.5 unit higher FM has a survival advantage of 10%, and each 0.2 unit increase in ECF/ICF ratio has a favorable survival effect of 67% (Marin et al. 2011).

DEXA

The technique employs electron beams to produce x-rays containing photons, which demonstrate low- and high-energy peaks when passed through a cerium photon "K-edge" filter. Passing through the body causes differential attenuation of the photons dependent upon the mass attenuation coefficient of the tissue – the ratio of attenuation of the two energy peaks describes the tissue property and allows measurement of the mass of three principal body components: fat, bone mineral, and fat-free (lean) soft tissue. The DEXA system acquires and the software analyzes a pixel image of the body components and solves for two components in each pixel: fat and fat-free tissue and bone minerals.

Body composition studies show decreased FFM and FM in ALS patients compared to healthy controls in cross-sectional analyses (Table 5). With progressive disease, there is a 2.0 kg decline over 6 months (Nau et al. 1995); others have noted 5–23% decrease over 6–10 months. This FFM loss is largely due to denervation-induced muscle atrophy (Kasarskis et al. 1996; Desport et al. 2003; Bouteloup et al. 2009; Vaisman et al. 2009), with additional contribution from malnutrition.

Body fat percentage is higher in women with ALS, as in the healthy state. In early or mild disease, when nutrition is relatively unaffected, FM may initially increase by 1–10% during 6–28 months of follow-up as FFM declines (Nau et al. 1995; Bouteloup et al. 2009; Vaisman et al. 2009; Marin et al. 2011). As the disease progresses and nutrition compromises, FM declines by 5–10% over 11–50 months of observation (Kasarskis et al. 1996; Desport et al. 2005, 2008).

Deficiency of Energy Intake

Dietary assessment by the retrospective 24-hour recall or the prospective 3-day food diary estimates daily caloric intake (Kasarskis et al. 2014) using appropriate software; the intake is compared to the recommended daily allowance (RDA) or some other national standard (Kasarskis et al. 1996, 2014; Worwood and Leigh 1998) (Table 6). In patients with early disease without dysphagia, respiratory compromise, or nutritional supplementation, daily caloric intake by the 3-d food diary is similar to that in age- and gender-matched healthy controls (Tandan 2000). In a subsequent study from the United Kingdom, 24-h caloric intake in ALS patients and younger healthy controls was similar (Worwood and Leigh 1998). In contrast, a previous study that included patients with gastrostomy or advanced disease found caloric deficiency by 24-h recall in up to 70% of patients (Kasarskis et al. 1996). In general, some studies report 28–54% lower caloric intake in ALS patients compared to

Table 6 Studies of energy intake in ALS

Reference	Country, # Patients	Dietary enquiry	Mean intake in ALS (kcal/d) (mean, range)	Recommended intake by RDA or in controls (kcal/d)	% patients with intake < RDA or in controls	Comments
Slowie et al. (1983)	USA, 20	24-h recall	2100 (1150–3300) (none or mild dysphagia), 1480 (1275–1750) (advanced dysphagia) 2000 men, 1200 women	2400 men, 1800 women (RDA)	70% (< RDA)	Mild dysphagia in 80%: weight loss 5%, intake < RDA in 50% Advanced dysphagia 20%: weight loss 25%, intake < RDA in all
Shimizu et al. (1991)	Japan, 11	24-h	1050 (836–1311)	No comparison undertaken	Energy intake > expenditure in all	Energy expenditure < BMR in 82% All on PEG and MV
Kasarskis et al. (1996)	USA, 16	3-d food diary	NA	84 ± 8% of RDA	94% (< RDA) Caloric intake low 50 months prior to death	Decreased intake unrelated to dysphagia or proximity of death Protein intake > RDA in 84%
Worwood and Leigh (1998)	UK, 47	24-h recall	1604 men, 1511 women 1556 limb-onset, 1578 bulbar-onset	2380 ♂, 1900 ♀ (UK reference)	100% (< UK reference) Mean caloric intake 116% of BMR	Intake < BMR in 4% Intake unrelated to the presence of dysphagia Protein intake >RDA in 83%
Bouteloup et al. (2009)	France, 61	NA	1995 (all), 1847 (bulbar-onset), 2120 (limb-onset)	NA	NA	Intake 1582 kcal/d (normometabolic) and 1954 kcal/d (hypermetabolic)
Vaisman et al. (2009)	Israel, 33	7-d food diary	1384	1912 (controls)	NA	Intake 28% lower in ALS than controls Intake 14% > REE
Siirala et al. (2010)	Finland, 5	PEG feeding	1340	NA	NA	
Ichihara et al. (2012)	Japan, 10	PEG feeding	1000	2206 (Japanese reference)	Caloric intake < expenditure in 20% patients	Caloric intake 54% lower in patients than Japanese reference
Jesus et al. (2012)	France, 40	3-d recall	1932 (all), 1916 (with dysphagia), 1967 (no dysphagia)		73% of French recommendations	Lower protein intake in 33% Higher calorie and protein intake if lower weight, TSF, and FM

(*continued*)

Table 6 (continued)

Reference	Country, # Patients	Dietary enquiry	Mean intake in ALS (kcal/d) (mean, range)	Recommended intake by RDA or in controls (kcal/d)	% patients with intake < RDA or in controls	Comments
Kasarskis et al. (2014)	USA, 80	3-d food diary	2198 men, 1609 women	No comparison with reference intake	66%	Intake 27% lower in males Intake not significantly lower in bulbar- versus limb-onset; not different if BMI <25 versus 25–29 versus ≥30
Park et al. (2015)	South Korea, 193	24-h recall	Range 1229–1621	Korean dietary reference intake	Caloric intake 79%, 61%, and 54% of reference by tertile of ALSFRS-R score	Progressive ↓ of nutrient intake by tertile of ALSFRS-R score
Ahmed et al. (2016)	Australia, 87	Dietary questionnaire	1810 (ALS), 2053 (ALS-P), 2530 (ALS-FTD)	1573 (controls)	NA	Increasing severity of eating behavior change associated with longer survival in ALS-FTD variants
Lee et al. (2017)	South Korea, 341	24-h recall	1425–1649 ♂ 1461–1261 ♀	NA	Caloric intake lower than TDEE by 6.5–32.6% in ♂ and 8–35.4% in ♀	Negative caloric balance related to ALS stage Physical activity EE ↓ with more severe disease
Shimizu et al. (2017)	Japan, 26	3-d intake (oral and PEG)	1581 (all)* 1786 men, 1484 women* 1166 (PEG), 1712 (no PEG)*	No comparison undertaken**	NA	Higher TDEE per body weight compared to healthy Japanese population

Values of caloric intake expressed as mean except where stated

ALSFRS-R ALS functional rating scale-revised, *ALS-P* ALS-plus (ALS with cognitive and behavioral changes not meeting criteria for FTD), *BMI* Body mass index, *BMR* Basal metabolic rate, *EE* Energy expenditure, *FM* Fat mass, *FTD* Frontotemporal dementia, *MV* Mechanical ventilation, *NA* Not available, *PEG* Percutaneous endoscopic gastrostomy, *RDA* Recommended daily allowance, *REE* Resting energy expenditure, *RMR* Resting metabolic rate, *TDEE* Total daily energy expenditure, *TSF* Triceps skinfold thickness, *UK* United Kingdom

*=Median values, ** Men: 2665–2710 kcal/d, women: 2138–2254 kcal/d, Takayama Cohort Study. Sasaki KM et al., Br J Nutr, 117:822–28, 2017

healthy controls or national standards from several countries; protein intake is relatively normal (Table 6).

In some studies, reduced caloric intake correlates with weight loss and decreased TSF thickness, but not with the presence of dysphagia (Worwood and Leigh 1998; Kasarskis et al. 1996). Recently, no significant difference in caloric intake by 3-d diary was found between bulbar- and limb-onset patients or in patients without or with dysphagia (Kasarskis et al. 2014). Caloric intake is not different between BMI groups (Kasarskis et al. 2014), is higher if hypermetabolism is present (Bouteloup et al. 2009), and progressively decreases by tertiles of ALSFRS-R score (Park et al. 2015). Increased severity of change in eating behavior in ALS-FTD, leading to higher caloric intake, is associated with longer survival (Ahmed et al. 2016).

The use of 24-h intake provides a cheap, convenient, and easy method of assessing caloric intake. Further, intake from 24-h recall and 3-d food diary is not significantly different, thus validating the use of the 24-h recall method to assess intake (Tandan 2000). However, there are several caveats in using the 24-h method for assessing caloric intake: recording intake is prone to recall bias, depends on patient cooperation, can alter eating behavior, and can be imprecise, particularly when caregivers are doing the reporting, as is often the case with many ALS patients.

Enhanced Energy Expenditure

Energy Expenditure Estimated from Harris-Benedict Equation

The Harris-Benedict (H-B) equation is widely used by dieticians to calculate caloric needs in healthy subjects and in disease states (Harris and Benedict 1918). It takes into account gender, age, and height of the subject to calculate resting energy expenditure (REE) in kcal/d; using a factorial approach, the REE value is multiplied by a factor corresponding to the physical activity level of the subject to derive daily caloric requirement. This method does not account for differences in body composition and physical activity levels of subjects, which can be considerable in ALS patients and cause prediction errors. Daily caloric requirement estimated by the H-B equation is imprecise compared to TDEE measured by the gold standard DLW method, with a mean underestimation by 1475 kcal/day or overestimation by 990 kcal/day (Kasarskis et al. 2014) in patients from the USA; underestimation by 244 kcal/d is also noted in Japanese patients (Shimizu et al. 2017). Using a correction factor that accounts for disease severity improves the predictability of the modified H-B equation, resulting in a mean difference of <30 kcal/d (Kasarskis et al. 2014; Shimizu et al. 2017).

Measured and Calculated Resting Energy Expenditure

Several studies have compared the calculated REE (cREE) using the H-B equation with REE measured by indirect calorimetry (measured REE or mREE) using the ventilated hood technique after an overnight fast (Kasarskis et al. 2014) to examine the metabolic state in ALS patients (Table 7); an mREE/cREE ratio of >1.1 denotes hypermetabolism (Desport et al. 2001). Using this method, absolute mREE is higher in ALS patients with early disease as compared to age- and gender-matched healthy

Table 7 Metabolism assessed by measurement of resting energy expenditure from indirect calorimetry in amyotrophic lateral sclerosis

Reference	Country, # patients	mREE ALS (mean ± SD or range) (kcal/d)	FFM ALS (kg)	mREE controls (kcal/d)	FFM controls (kg)	mREE/ FFM ALS (kcal/kg)	mREE/ FFM controls (kcal/kg)	cREE ALS from H-B Equation	mREE/ cREE ALS (%)	mREE/ cREE controls (%)	T2 mREE in ALS	Interval between T1 and T2 (months)	Comment
Shimizu et al. (1991)	Japan, 11	886 (742–1037)	NA	NA	NA	NA	NA	NA	73–89%	NA	NA	NA	EI > EE in all by mean of 15% EE < BMR in 82%
Nau et al. (1995)	USA, 12	NA	NA		NA	NA	NA	NA	NA	NA	NA	6	EE-EI = <30 kcal/d at baseline EE-EI = −130 kcal/d at T2
Kasarskis et al. (1996)	USA, 16	NA	NA		NA	NA	NA	NA	94% at T1 to 110% before death	NA	Na+	About 50	↑ in mREE/cREE before death Negative correlation between mREE and FVC
Desport et al. (2001)	France, 62 ALS 31 controls	1561 ± 342	47 ± 12	1418 ± 191	49 ± 10	33	29	Not given	116%	105%	NA	NA	mREE/ cREE>110% in 67% ALS patients mREE and FVC not correlated
Sherman et al. (2004)	USA, 34 18 on MV and PEG	1655 ± 363 (MV+) 1341 ± 472 (MV-)	NA	NA	NA	NA	NA	NA	112% (MV+), 92% (MV-)	NA	NA	NA	ΔREE 43 ± 317 (range − 677 to +591) kcal/d
Desport et al. (2005)	France, 168	1522 ± 308	44 ± 11	NA	NA	35	NA	1334 ± 235	114%	NA	1466 (n = 44)	11	mREE/ cREE>110% in 57% at T1 and 48% at T2 ΔREE correlated with age and gender

Bouteloup et al. (2009)	France, 61	1449 ± 301	44 ± 12	NA	NA	33	NA	1316 ± 242	111%	NA	1485	12 (n = 28)	mREE/cREE 120% at T1 in 50% patients mREE/cREE >110% in 80% at T2
Funalot et al. (2009)	France, 33 SALS 11 FALS	1582 ± 300 (SALS) 1784 ± 340 (FALS)	46 ± 12 (SALS) 45 ± 7 (FALS)	NA	NA	34 (SALS) 40 (FALS)	NA	NA	112% (SALS) 127% (FALS)	NA	NA	NA	mREE/cREE >110% in 52% SALS and all FALS patients mREE correlated with FFM, not with disease duration or severity or FVC
Vaisman et al. (2009)	Israel, 33 ALS 33 controls	1467 ± 218	42 ± 7	1744 ± 367	55 ± 12	35	32	NA	104%	104%	1387	6 (n = 10)	mREE lower in ALS mREE/FFM higher in ALS at T1, increased at T2 mREE/cREE similar in ALS and controls at T1 and T2 mREE/cREE ↑ in 39%, ↓ in 9%, ↔ in 52%
Siirala et al. (2010)	Finland, 5 (all on MV and PEG)	1130 ± 170	NA	NA	NA	NA	NA	1580	66%	NA	NA	NA	mREE/cREE from several equations lower in MV patients

(*continued*)

Table 7 (continued)

Reference	Country, # patients	mREE ALS (mean ± SD or range) (kcal/d)	FFM ALS (kg)	mREE controls (kcal/d)	FFM controls (kg)	mREE/ FFM ALS (kcal/kg)	mREE/ FFM controls (kcal/kg)	cREE ALS from H-B Equation	mREE/ cREE ALS (%)	mREE/ cREE controls (%)	T2 mREE in ALS	Interval between T1 and T2 (months)	Comment
Ellis and Rosenfeld (2011)	USA, 56	1489 ± 326	43 ± 12	NA	NA	35 ± 6	NA	1522 ± 291	98%	NA	NA	NA	mREE best predicted by FFM, age and gender mREE/cREE error − 2.7 to +13.9% from several equations Bias ↑ with declining respiratory function
Ichihara et al. (2012)	Japan, 10 (all on MV and PEG)	807 ± 116 (n = 3)	27 ± 6	NA	NA	28.2	NA	1109 ± 211	75%	NA	NA	NA	mREE/cREE from several equations <1 in all 3 MV patients
Georges et al. (2014)	France, 16 (all on NIV)	1197 (no NIV) 1149 (on NIV)	NA	NA	NA	NA	NA	1390	90% (no NIV)	NA	NA	NA	mREE on NIV ↓ by 7% mREE correlated with FVC and MIP
Kasarskis et al. (2014)	USA, 80	1539 ± 366	51 ± 11	NA	NA	30	NA	1596 ± 283	96%	NA	NA	11	Compared to mREE, equations estimate REE well in ♂ but overestimate in ♀ EI and TDEE vary widely in ALS

MIP Maximum inspiratory pressure, *mREE* Measured resting energy expenditure, *MIP* Maximum inspiratory pressure, *MV+* With mechanical ventilation, *MV-* Without mechanical ventilation, *NA* Not available, *NIV* Noninvasive ventilation, *REE* Resting energy expenditure, *SALS* Sporadic ALS, *TDEE* Total daily energy expenditure, *T1* Initial evaluation, *T2* Subsequent evaluation, +Average of several estimations over 50 months before death, *ΔREE* mREE minus cREE, *cREE* Calculated resting energy expenditure, *BMR* Basal metabolic rate, *EE* Energy expenditure, *EI* Energy intake, *FALS* Autosomal dominant and autosomal recessive, all SOD1 mutation negative, *FFM* Fat-free mass, *FVC* Forced vital capacity

controls (Tandan 2000). In the progressive phase of the disease, mean mREE/cREE ratios of >1.1 are reported in 57–67% of patients with SALS and all patients with FALS. Some researchers (Kasarskis et al. 1996), but not all (Desport et al. 2001), noted a negative correlation between mREE and FVC in proximity to death, implying that effortful breathing from falling FVC could also account for the high mREE/cREE ratio. The fact that the use of NIV in the resting state decreases mREE by an average of 7% (Georges et al. 2014) would confirm at least a partial role for increased respiratory effort in producing hypermetabolism. Other investigators, however, have not found hypermetabolism in ALS patients as compared to matched controls from estimates of the mREE/cREE ratio (Vaisman et al. 2009). In patients with advanced or terminal disease who are immobile, with a PEG tube and on MV, the mREE/cREE ratio becomes <1.0, indicating that these patients are no longer hypermetabolic. In some such patients, the TDEE approximates mREE values (Ichihara et al. 2012; Kasarskis et al. 2014).

Energy Expenditure Measured by Doubly Labeled Water

The DLW technique directly measures TDEE in free-living humans over a 10–14-day period (Kasarskis et al. 2014). Few studies have measured TDEE along with simultaneous estimates of caloric intake and body composition (Tandan 2000; Ichihara et al. 2012; Kasarskis et al. 2014; Shimizu et al. 2017). An earlier study found TDEE up to 30% higher in ambulatory ALS patients with early disease as compared to age- and gender-matched healthy controls (Tandan 2000). The mean TDEE was 339 kcal/d higher in patients than in controls; about 2/3rds of the difference was due to increased caloric use during physical activity and the rest due to hypermetabolism (Tandan 2000). TDEE exceeded daily caloric intake by 24-h recall and 3-d food diary in 90% of patients by a mean of 371 kcal/d indicating negative caloric balance.

In Japanese patients with advanced disease, very low FFM, and nutritional and ventilator support, negative caloric balance occurred in 20%, despite PEG feeding (Ichihara et al. 2012). In the largest DLW study to date in ALS, 73% of patients exhibited negative caloric balance; the mean caloric deficit was 430 kcal/d in men and 466 kcal/d in women. Risk factors for negative caloric balance were lower caloric intake, and higher FVC and TDEE, including an almost fourfold higher physical activity-associated caloric expenditure (Kasarskis et al. 2014). In Japanese patients not using assisted ventilation, 58% showed negative caloric balance (Shimizu et al. 2017). The median daily caloric intake was lower in patients with PEG; TDEE per body weight was higher in patients compared to the healthy Japanese population (Shimizu et al. 2017).

Thus, up to 75% of ALS patients can be in negative caloric balance, particularly in early or progressive phases of the disease, while ambulation is possible. Later in the disease, as nutrition through a PEG and ventilator assistance are required, caloric requirements decline. Greater caloric needs in early ALS are largely due to increased caloric use during physical activity, with a hypermetabolic state contributing particularly as the disease advances. Appropriate nutritional evaluation and management

are therefore paramount and need implementation with other therapies throughout the disease course.

Energy Balance in ALS

Energy homeostasis results from balance of energy intake and energy expenditure. In healthy subjects, the glucose-insulin axis that is intrinsically linked to glucose and lipid metabolism controls energy intake. Energy intake is in balance with basal and activity-dependent energy expenditure, thus leading to stable energy stores, mostly in the form of triglycerides in adipocytes, and a stable BMI (Fig. 1).

Energy Intake

In ALS, energy homeostasis is impaired from a combination of decreased energy intake and increased energy expenditure (Fig. 2). The decreased energy intake as compared to requirements is multifactorial, as discussed previously, and varies between patients due to topographical and disease severity factors (Fig. 3). Another factor with potential effects on the nutritional state in ALS is insulin resistance (Lekoubou et al. 2014). Energy homeostasis depends on the uptake of nutrients,

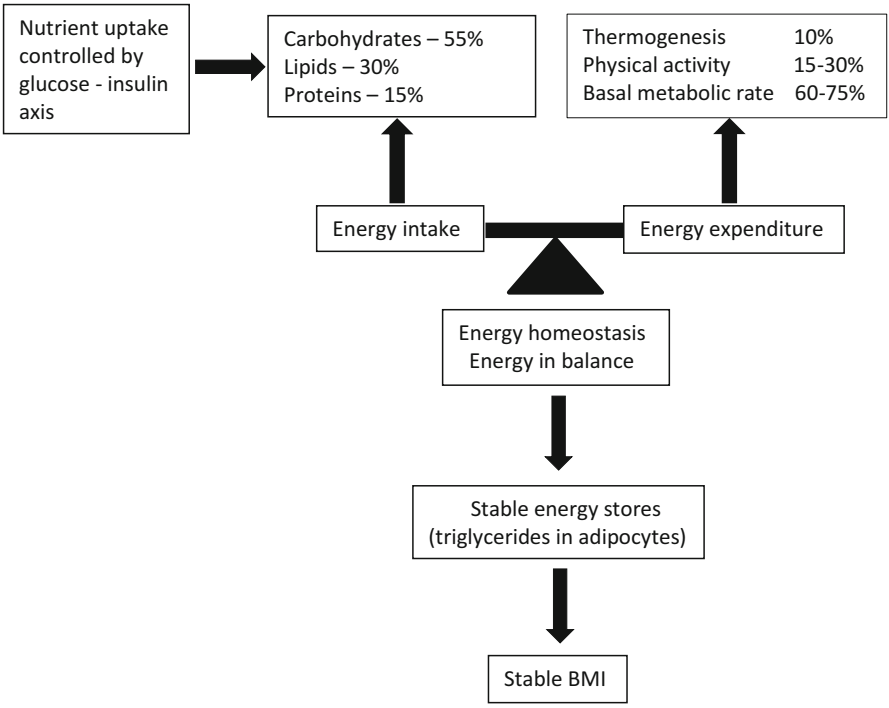

Fig. 1 Energy homeostasis in the healthy state

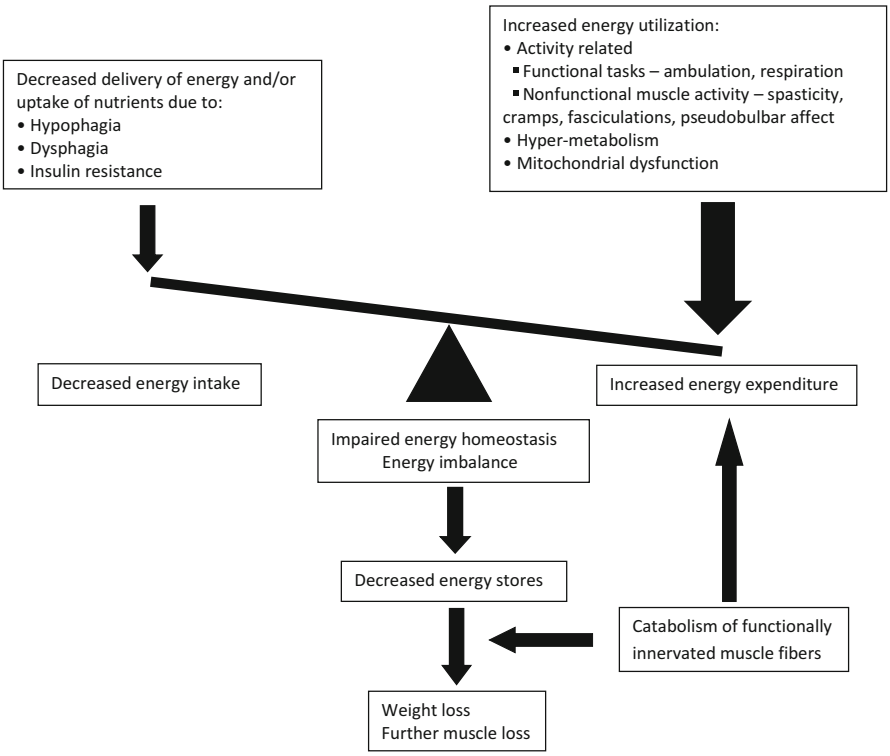

Fig. 2 Energy homeostasis in ALS

including glucose and lipids, controlled by the glucose-insulin axis. Whether the increased prevalence of diabetes and insulin resistance drives the neurodegeneration, or has a neuroprotective effect, remain to be determined.

Energy Expenditure

Compounding the reduced energy intake is the increased energy use due to hypermetabolism (Desport et al. 2005; Muscaritoli et al. 2012), during labored physical activity (Tandan 2000; Kasarskis et al. 2014) and presumably during non-ambulatory muscle use such as with emotional lability, cramps, and fasciculations to overcome spasticity (labeled non-exercise activity thermogenesis or NEATS). Factors potentially causing hypermetabolism include sympathetic over activity, mitochondrial dysfunction, and infections such as aspiration pneumonia; these can potentially exacerbate malnutrition and sarcopenia (Figs. 2 and 3).

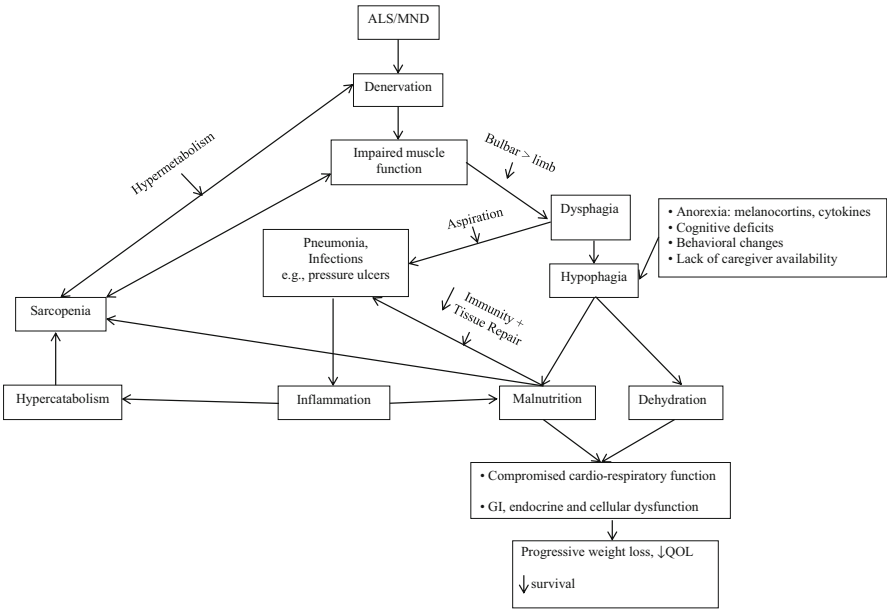

Fig. 3 A schematic representation of the vicious circle of clinical deterioration seen in ALS. See text for details

Consequences of Energy Imbalance and Malnutrition in ALS

Impaired energy homeostasis due to decreased energy intake, increased energy expenditure, or both leads to chronic energy deficiency. This poses excessive metabolic demands and has a catabolic effect on remaining functionally innervated muscles to maintain tasks such as mobility and ventilation, thus further aggravating energy expenditure (Fig. 2). The neuronal degeneration and its consequences result in loss of weight, FFM, and eventually FM, with muscle weakness and atrophy (sarcopenia) and negative consequences on muscle function, including of the diaphragm. Additionally, there is fat redistribution causing loss of subcutaneous and increased abdominal fat that initially correlate with functional status and survival and later explain the proclivity to insulin resistance and hypercholesterolemia (Lindauer et al. 2013).

Malnutrition also links to a series of detrimental consequences, which aggravate the underlying condition in ALS by setting off a vicious cycle of further clinical deterioration (Fig. 3). Dysphagia and multifactorial hypophagia lead to malnutrition, which depresses immunity and impairs tissue repair that make patients susceptible to infections. This sets off an inflammatory response, contributing to excessive energy expenditure that further impairs energy homeostasis, one of the many reasons for the hypermetabolic state. This completes the vicious cycle by worsening sarcopenia. There are multiple interrelated aspects in this cycle: *malnutrition*, largely contributed

by dysphagia; *infections*, most commonly aspiration pneumonia; *inflammation*, which produces a hypermetabolic state and impairs substrate utilization which worsens malnutrition; *denervation-induced sarcopenia*, which is worsened by hypermetabolism and malnutrition; and lastly the two-way relationship between sarcopenia and impaired muscle function.

There are several other potential adverse consequences of malnutrition, in general (Sheard 2014). These include compromised respiratory and cardiovascular functions and endocrine dysregulation that decreases T4 and T3 levels, increases reversed T3 levels, and suppresses gonadotrophins leading to reduced testosterone, estrogen, and progesterone levels. Gastrointestinal dysfunction causes changes in pancreatic exocrine function, intestinal blood flow, villous morphology, and intestinal permeability. General cellular dysfunction results from downregulation of Na^+/K^+ ATPase activity. The clinical consequences of these alterations are fatigue, increased risk of pressure ulcers, poor wound healing, weight loss, and indeed faster disease progression with reduced survival (Desport et al. 1999; Marin et al. 2011).

Metabolic Impairment in ALS

Neural mechanisms and pathways underlying the metabolic impairment in ALS remain elusive. Recent data suggest that perturbations in the hypothalamic melanocortin pathway controlling food intake, energy expenditure, and weight control may be relevant (Fig. 4). The anorexigenic pathway involves release of leptin from adipose tissue, which acts on pro-opiomelanocortin (POMC) and cocaine-related and amphetamine-related transcript (CART) neurons in the hypothalamus. POMC undergoes posttranslational modification to produce peptides such α- and β-melanocyte-stimulating hormone (α- and β-MSH) which act on the MCR3 and MCR4 melanocortin receptors in the paraventricular nucleus (PVN), leading to a decrease in food intake. The orexigenic appetite-stimulating pathway is controlled by the release of ghrelin peripherally, which targets neurons of the arcuate nucleus (ARC) in the hypothalamus that contain neuropeptide Y (NPY) and agouti-related peptide (AgRP); the latter promotes food intake by antagonism of MCR3 and MCR4 receptors.

The failed clinical trial with pioglitazone, an oral antidiabetic agent, when added to riluzole therapy (Dupuis et al. 2012) showed decreased hyperglycemia and liver enzyme levels and increased circulating adiponectin peripherally in ALS patients (Vercruysse et al. 2016). However, a central effect on weight gain mediated through inhibition of the hypothalamic melanocortin system, as reported in prior clinical trials, was lacking. Reduced POMC-positive neuronal counts, and increase in density of AGRP fibers in the hypothalamic arcuate nucleus of multiple transgenic rodents (including SOD1, G86R, TDP-43, and FUS models) carrying human gene mutations, support selective involvement of the melanocortin system. The melanocortin defect is primarily caused by loss of serotonin, a major activator of POMC neurons through the 5HT2C receptor (Wang and Chehab 2006); markers of other stressors which could affect POMC neurons, including oxidative stress,

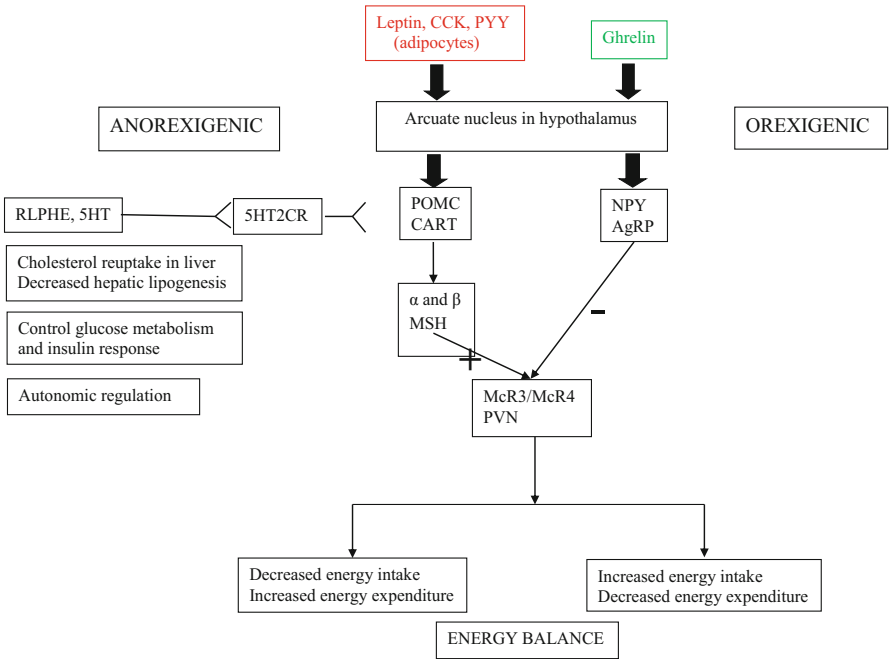

Fig. 4 Central pathways modulating energy intake and expenditure

endoplasmic reticulum stress, defective peroxisome biogenesis, or mitochondrial abnormalities, did not account for the decreased POMC neuronal counts. Nevertheless, other central or peripheral mechanisms of weight loss in human ALS need definition, as the POMC deficiency resulting from the melanocortin defect triggers an increased orexigenic drive to produce a compensatory increase in food intake.

The observed melanocortin defect could potentially explain alterations in other peripheral metabolic pathways in ALS patients. The melanocortin system is important in controlling glucose metabolism and insulin response (Obici et al. 2001), and in regulating peripheral lipid metabolism, by activating cholesterol reuptake in the liver (Perez-Tilve et al. 2010) and reducing hepatic lipogenesis (Leckstrom et al. 2011). Therefore, the observed increased circulating cholesterol (Dupuis et al. 2011) and glucose intolerance in ALS are explained by the melanocortin defect. Moreover, impaired regulation of the autonomic nervous system (Sohn et al. 2013) by the melanocortin defect also explains the dysautonomia sometimes seen in ALS patients (Baltadzhieva et al. 2005).

Taken together, the combined clinical and experimental evidence provides a mechanistic explanation for abnormalities of food intake and weight control in ALS patients. Importantly, these results also show that ALS progression impairs responsiveness to classical drugs, thus leading to weight gain. This has important implications for the pharmacological management of weight loss in ALS, in addition to nutritional supplementation in the disease.

Policies and Protocols

Protocols for Nutritional Management of ALS

In view of the grave consequences of malnutrition and its association with increased morbidity and mortality, nutritional care of patients with ALS is undoubtedly an important element of disease management. The aims of nutritional management are to maintain energy (calorie) balance by optimizing intake and to minimize the effects of weight loss, by both preventative intervention and rehabilitation of weight loss. These goals are best achieved by caring for patients in multidisciplinary clinics with an experienced team of healthcare personnel. Specifically, for nutritional management, patients should undergo regular neurological, nutritional/dietary, and speech and swallowing evaluations. This testing should occur at the time of diagnosis, and then at least every 3 months and occasionally more often, depending on the nutritional status and/or rate of disease progression.

Each clinic visit should include recording of weight, height, and body mass index (BMI) and collection of data from 24-h dietary intake. In addition, assessment of the nutritional state by using other methods should be undertaken, if appropriate equipment is available. Collecting anthropometric data using a Harpenden caliper or measuring tape is simple and cheap to undertake and widely used. TSF is the skinfold thickness most commonly used to assess percent body fat and then derive FM and FFM. When other skinfolds or circumferences are measured, equations published for use in healthy subjects are used. Despite its simplicity, some limitations of anthropometric data include the lack of precision due to asymmetric neurogenic atrophy or uneven fat distribution, dependent edema caused by immobility, inability to accurately distinguish components of body composition (FM and FFM), and the fact that these equations are not validated in ALS.

Other techniques used to measure body composition include BIA, BIS, and DEXA (Desport et al. 2003); BIS also assesses hydration status. However, these methods have some limitations; while BIA and BIS are simple, portable, noninvasive, and inexpensive and are validated in ALS, asymmetrical muscle involvement from denervation influences data. DEXA, while ideal for body composition assessment to differentiate FM from FFM and bone mass (Kanda et al. 1994; Nau et al. 1995, 1997), has limited clinical utility because of high cost, technical difficulties in patients who cannot lie flat, and cannot be used in homebound patients.

Laboratory tests assess the nutritional status, as some correlate with disease progression and survival in ALS. These include serum albumin, prealbumin, total leukocyte count, hemoglobin, magnesium, calcium, phosphorous, serum zinc and copper, retinol-binding protein, creatinine height index, uric acid, lipid profile, and APOE (Lacomblez et al. 2002; Chiò et al. 2014). The relevance of these biochemical indices is not certain in the clinical care of ALS patients. When performed, correlation with other traditional markers of malnutrition should be undertaken; longitudinal studies are required to understand their significance as nutritional markers.

Policies for Nutritional Management of ALS

Energy balance is assessed by utilizing a validated and practical equation, which is a modification of the H-B equation with additional factors relating to disease severity (ALSFRS-6) (Kasarskis et al. 2014). This requires the following actions to be undertaken sequentially:

(1) At home: patient or caregiver will record 24-hour food intake in diary prior to clinic visit.
(2) In the clinic: dietitian will review the 24-h food diary to calculate energy intake using appropriate software.
(3) In the clinic: personnel will obtain ALSFRS-6 score on patient.
(4) In the clinic: dietitian will estimate TDEE using the ALSFRS-6 modification of the H-B equation (Kasarskis et al. 2014).

A web-based calculator, based on the ALSFRS-6 modification of the H-B equation, is available at https://mednet.mc.uky.edu/alscalculator.

The ALSFRS-6 score is the total score of six questions from the ALSFRS-R questionnaire (each rated from 4 = normal to 0 = cannot do; normal score = 24):

Questions 1 (speech), 4 (handwriting with dominant hand as compared to prior to ALS), 6 (dressing and hygiene), 7 (turning in bed and adjusting bed clothes), 8 (walking), and 10a (dyspnea).

The H-B equation and the ALSFRS-6 modification of the H-B equation are below:

H-B equation for men:

Basal metabolic rate (kcal/d) = 66 + (6.23 × weight in pounds) + (12.7 × height in inches)−(6.76 × age in years)

H-B equation for women:

Basal metabolic rate (kcal/d) = 655 + (4.35 × weight in pounds) + (4.7 × height in inches) – (4.7 × age in years)

ALSFRS-6 modification of H-B equation:

TDEE = BMR from H-B equation (kcal/d) + (55.96 × ALSFRS-6 score)− 168 (kcal/d)

A practical example of calculation of TDEE using the ALSFRS-6 modification of the H-B equation is as follows:

Age: 60-year-old ALS patient
Sex: Male
Weight: 180 lbs

Height: 5′ 10″ (70″)
Moderate weakness in upper extremities
ALSFRS-6 score: 19 of 24
Speech: 4
Handwriting: 2
Dressing: 2
Turning in bed: 3
Walking: 4
Calculate Harris-Benedict BMR

$$\text{BMR (kcal/d)} = 66 + (6.23 \times 180 \text{ lbs.}) + (12.7 \times 70'') - (6.76 \times 60 \text{ years}) = 66 + 1121 + 889 - 406 = 1670$$

Adjust for ALS functional status

$$(55.96 \times 19) - 168 = 1063 - 168 = 895 \text{ kcal/d}$$

Calculated TDEE = 1670 kcal/d + 895 kcal/d = 2565 kcal/d

If the combination of these assessments suggests a negative energy balance (daily caloric intake < TDEE), especially during the early stages of the disease, several strategies exist to maintain nutrition in patients who can safely swallow. These involve dietary advice, food fortification with oral nutritional support and prescription of high-caloric supplements, support with safe-swallowing techniques and adaptive eating utensils, and modification of diet texture (Fig. 5).

If these dietary modifications cannot ensure adequate nutrition, then positive energy balance cannot be restored, and significant weight loss progresses making artificial nutrition essential (Shimizu et al. 1991; Kasarskis et al. 2014) (Fig. 6). Artificial nutrition is implemented if weight loss ≥5–10% from premorbid weight, or BMI is <18.5 kg/m^2 if age is 18–65 years and <20 kg/m^2 if age is >65 years

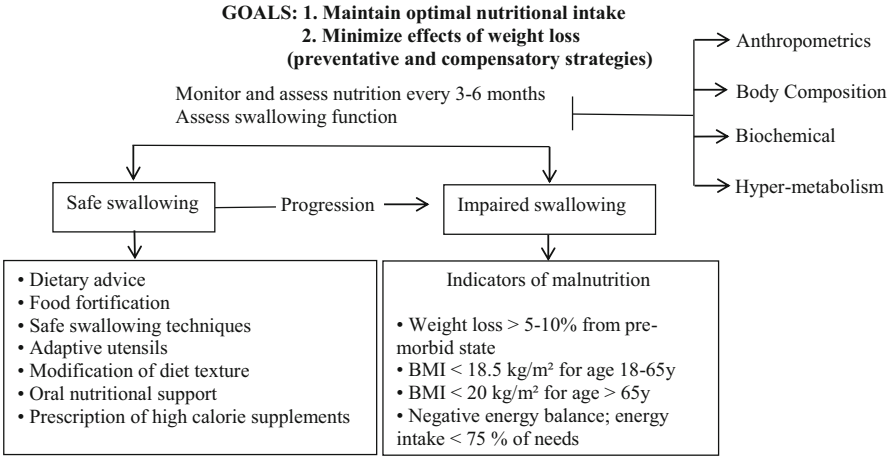

Fig. 5 Suggested algorithm for nutritional care in ALS

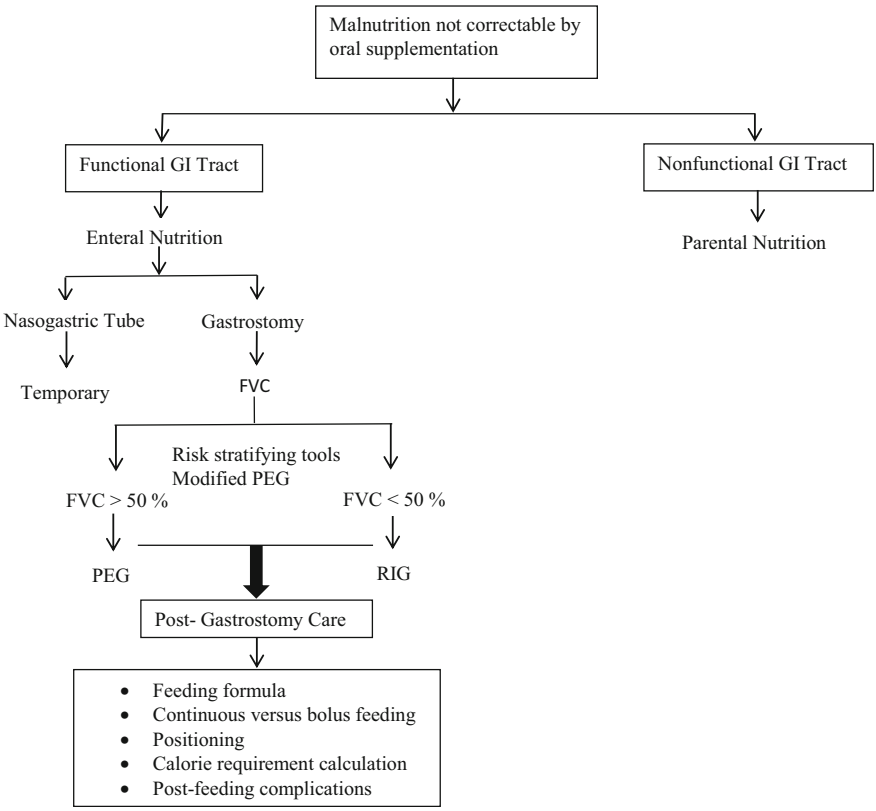

Fig. 6 Suggested algorithm for enteral feeding in ALS

(Desport et al. 2000), or there is persistent negative energy balance, defined as energy intake of <75% of energy needs. For patients who have a functional gastrointestinal tract, enteral tube feeding (ETF) can be delivered either via nasogastric tube (NGT) or gastrostomy insertion, the latter being the recommendation by the American Academy of Neurology (AAN) (Miller et al. 2009) and the European Federation of Neurological Societies (EFNS) (EFNS Task Force on Diagnosis and Management of Amyotrophic lateral Sclerosis 2012).

NGT feedings are indicated only for short-term nutritional support due to the high risk of aspiration, propensity for increasing oropharyngeal secretions, nasopharyngeal discomfort or even ulceration, and social embarrassment. Parenteral nutrition (PN) is rarely indicated in ALS patients and can be considered when consent to ETF is denied or ETF is contraindicated; or there is an inability to access or use the gastrointestinal tract due to malabsorption, dysmotility, or enterocutaneous fistulas. Nasogastric or nasojejunal feeding is not appropriate for long-term ETF due to

Table 8 Comparison of PEG and RIG procedures

	PEG	RIG
FVC requirement	>50%	<50%
Access	Endoscopic	Percutaneous, under fluoroscopic guidance
Sedation	Generalized	Local
Positioning	Recumbent	Upright
Respiratory complications	Higher	Lower
Occlusion risk due to small diameter of tube	Lower	Higher

PEG Percutaneous endoscopic gastrostomy; *RIG* radiologically inserted gastrostomy

patient discomfort, increased likelihood of mechanical complications, and risk of worsening respiratory insufficiency (Verschueren et al. 2009).

Gastrostomy tube insertion uses one of two methods, percutaneous endoscopic gastrostomy (PEG) or radiologically inserted gastrostomy (RIG); differences between the two procedures are highlighted (Table 8). Recent data from a large prospective cohort of ALS patients undergoing gastrostomy found no difference in mortality in patients who underwent PEG versus RIG (overall mortality 4%, 95% CI 2–6%; PEG mortality 3%, 95% CI 1–8%; RIG mortality 3%, 95% CI 1–9%) (ProGas Study Group 2015). In general, both the AAN and EFNS recommend PEG insertion to be performed when FVC is >50% of predicted. However, gastrostomy insertion is successfully undertaken in patients with FVC <50% of predicted, usually with noninvasive respiratory support (Czell et al. 2013). In a recent observational study from a single center, which utilized a "traffic light" risk stratification tool, the authors successfully performed a modified PEG procedure despite respiratory compromise (Thompson et al. 2017).

There is currently scarcity of evidence to guide postgastrostomy feeding in patients with ALS. Even though a handful of observational studies suggest that gastrostomy feeding improves quality of life (QOL) in ALS patients (Mitsumoto et al. 2003), results from a large prospective study indicated that the effect of gastrostomy on QOL was neutral (ProGas Study Group 2015). These results are due to the advancing disease with loss of FFM and its effect on progressively worsening function and to ineffective and non-standardized postgastrostomy care in patients. The lack of standardization may be due to a choice of enteral formula that is not calorically dense to reduce volume, high in fat and low in carbohydrates to minimize carbon dioxide production and possibly slow disease progression, high in omega-3 fatty acids and antioxidants to modify the inflammatory state, and high in fiber to reduce constipation (Muscaritoli et al. 2012). Currently no specific enteral formula confers a benefit to ALS patients; rather, guidance derives from the use in other disease states, such as formulas devised for acute lung injury and acute respiratory failure (Pontes-Arruda et al. 2008). Assessing the effectiveness of specific formulas on disease progression in ALS is an area of needed research.

Several other postgastrostomy feeding logistics need consideration. These include:

1. Bolus feeding versus continuous feeding using a pump, the choice being dependent on resources and patient comfort
2. Patient positioning, to include raising the head end to at least 30 degrees during and for 1 hour after feeding
3. Post-feeding flushing with water to clean the tube, prevent tubal obstruction, and provide adequate hydration
4. Calculation of postgastrostomy calorie requirement
5. Prevention and treatment of postgastrostomy complications, including diarrhea and refeeding syndrome (Hisham et al. 2008)

There is an urgent need to understand the poor nutritional outcomes following gastrostomy in the majority of ALS patients and to further study and develop evidence-based guidance on nutritional management following gastrostomy.

Dictionary of Terms

- **Amyotrophic Lateral Sclerosis** – Also called motor neuron disease (in the United Kingdom), Charcot's disease (in France), or Lou Gehrig's disease (in the USA), this is a neurological condition due to loss of nerve cells in the brain and spinal cord that control muscle function.
- **Body Mass Index** – An index of body size, derived from the ratio between weight and height.
- **Daily Energy Intake** – The number of calories eaten daily through food.
- **Daily Energy Expenditure** – The number of calories spent daily in supporting functions of essential organs (e.g., the brain, heart, lungs, etc.) and physical activity.
- **Fat Mass** – The amount of fat in the body, which can reflect the nutritional state.
- **Fat-Free Mass** – The amount of lean tissue, usually muscle, in the body.
- **Gastrostomy** – An artificial "hole" in the stomach to facilitate delivery of nutrition in individuals who are unable to swallow.
- **Harris-Benedict Equation** – An equation that utilizes a person's weight, height, age, and gender to estimate the number of calories needed daily for bodily functions, found to be inaccurate to predict calories needed daily in ALS subjects.
- **Hypermetabolism** – Excessive use of calories to support the functions of essential organs.
- **Modified Harris-Benedict Equation** – A valid equation using some ALS severity measures to modify the Harris-Benedict equation and correctly estimate the number of calories needed daily for bodily functions.

- **Malnutrition** – A deviation from the normal nutritional state (could be undernutrition that results in excessive thinness or overnutrition that produces obesity).
- **Marasmus** – A state of undernutrition resulting from decreased intake of calories.

Summary Points

- This chapter reviews the frequency, causes, and approach to management of malnutrition in amyotrophic lateral sclerosis (ALS).
- Depending on the method uses, malnutrition is identified in from 6 to 100% of patients.
- Malnutrition is primarily due to energy or caloric deficiency; protein deficiency is uncommon.
- Dysphagia reduces energy intake; labored physical activity and hypermetabolism produce excessive energy expenditure, which alone or in combination result in negative energy balance.
- Regular nutritional care is best provided in multidisciplinary clinics where patients are evaluated by a team of experienced providers.
- At clinic visits, the team members identify the causes of malnutrition, chart energy intake from food diaries, and estimate energy needs based upon disease severity and topography of involvement.
- A validated published tool, which is a modification of the commonly used Harris-Benedict equation, is used to assess energy needs and incorporates disease severity measures.
- At clinic visits, energy balance is critically examined, and appropriate oral dietary modifications with regard to food type, food consistency, and energy supplementation are recommended.
- At subsequent clinic visits, if energy balance is not met, then a recommendation is made for an alternative route for feeding, which is typically a percutaneous or radiologically inserted gastrostomy.

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Hydration in Amyotrophic Lateral Sclerosis 53

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Abstract

Water is essential for health and survival. Water needed for health exceeds the amount the body produces through metabolic activity. Although water intake is predominantly as free water or in liquid drinks, about a quarter comes from solid foods such as meats and vegetables. Water exists as total body water (TBW) that constantly replenishes and occurs in intracellular and extracellular compartments. Recommended daily water intake is 3.0 L in men and 2.5 L in women and decreases with normal aging; inadequate intake in healthy elderly results in

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increased morbidity and mortality. Amyotrophic lateral sclerosis (ALS), a neurodegenerative disease of middle age, causes diffuse weakness with dysfunction in the limbs and bulbar muscles accompanied by erosion of quality of life and early death from respiratory failure. Although often discussed in ALS circles, the assessment and management of dehydration are frequently overlooked. Dehydration assessed by the gold standard-labeled water method to examine daily water turnover, a surrogate for water intake, occurs in 20% of ALS patients at first evaluation; its degree increases as the disease progresses. TBW and water turnover are 1,200 ml (3.4%) and 260 ml/day (8.6%) lower, respectively, in ALS patients compared to age- and gender-matched healthy controls. Risk factors for poor hydration are female gender, bulbar-onset disease, malnutrition estimated by body mass index, and low lung function measured by incentive spirometry. As in the healthy elderly population, decreased water intake adversely affects survival in ALS patients. Equations used to estimate daily water requirement, recommended by the US Centers for Medicare and Medicaid Services in healthy elderly, are inaccurate for use in ALS patients. Validated equations are now available to accurately estimate hydration using endpoints routinely assessed in ALS clinics. As with nutrition, hydration should be regularly examined at ALS clinic visits using these equations to assess water requirements and improve clinical care.

Keywords

Amyotrophic lateral sclerosis · Dehydration · Doubly labeled water · Harris-Benedict equation · Hydration status · Total body water · Total daily energy expenditure · Water intake · Water turnover · Survival

List of Abbreviations

ALS	Amyotrophic lateral sclerosis
ALSFRS-6	Summed scores for speech, handwriting, dressing & hygiene, turning in bed and adjusting bedclothes, walking and dyspnea components of ALSFRS-R (normal = 24)
ALSFRS-R	Amyotrophic lateral sclerosis functional rating scale-revised
BMI	Body mass index
DLW	Doubly labeled water
FVC	Forced vital capacity
H-B	Harris-Benedict
OR	Odds ratio
TBW	Total body water
TDEE	Total daily energy expenditure

Introduction

Water is an essential component of the diet and vital for health and sustenance of life. Total body water (TBW) is present in intracellular and extracellular compartments and accounts for 75% of body weight in infants and 55% in the elderly

(Nicolaidis 1998). TBW turns over continuously; the amount required for maintaining normal hydration and health needs supplementation from external sources (Kleiner 1999). The average daily water consumption to maintain normal hydration is determined to be 3.0 L in males and 2.5 L in females (Raman et al. 2004). Dehydration results from depleted TBW due to inadequate intake or excessive loss in the healthy state or due to illness (Begum and Johnson 2010). Dehydration and associated morbidities are reversible; however, if left untreated the risk of mortality is significantly increased.

Amyotrophic lateral sclerosis (ALS) affects middle- to late-age subjects (Li et al. 1990) who already have an increased risk of dehydration (Phillips et al. 1984; Silver and Morley 1992; Phillips et al. 1993). Malnutrition is frequent in the disease; the multiple causes of malnutrition in ALS are also relevant in producing dehydration. Contributory factors include poor oral intake due to bulbar dysfunction, weakness of arms that interferes with feeding, anorexia, depression, cognitive impairment, impaired mobility, and lack of caregiver availability (Scagnelli et al. 2017). Additionally, impaired thirst and fear of incontinence also contribute to poor hydration. Dehydration is a chronic concern in ALS but is often overlooked in the management of patients (Scagnelli et al. 2017).

Prevalence of Dehydration in the Elderly and in ALS

Preformed water intake decreases by 15 mL/decade in healthy men and women between the ages of 40 and 79 years (Raman et al. 2004). This decrease with normal aging also occurs in TBW (Lesser and Markofsky 1979; Weitzman and Kleeman 1999). Dehydration is the most common cause of electrolyte abnormality in the elderly (Lavizzo-Mourey et al. 1988), and estimates are that 1.5% of elderly subjects living in institutions undergo hospitalization annually for dehydration (Warren et al. 1996). In the early 1990s, dehydration was the principal diagnosis in 6.7% of all hospitalized Medicare patients (Warren et al. 1994). Dehydration occurs in 31% of nursing home subjects by using urine-specific gravity, urine color, bioelectrical impedance analysis, and dietary records when hydration is tracked for 6 months (Mentes 2006).

There is no prior epidemiological data on the prevalence of dehydration in ALS. Data on prevalence is now available from a recent multicenter study in the USA (Scagnelli et al. 2017). In this study, 80 patients with “definite” or “probable” ALS from 5 academic centers (Kasarskis et al. 2011, 2014; Scagnelli et al. 2017) underwent 250 observations for measurement of hydration status using the gold standard doubly labeled water (DLW) method over a 10-day period (Scagnelli et al. 2017). Data were compared to that in age- and gender-matched healthy subjects from a Clinical Research Center Database from the Robert Larner, MD College of Medicine at the University of Vermont, Burlington, VT, USA. Daily water turnover, a surrogate for water intake in weight stable subjects, was less than two standard deviations below the healthy control mean in 20% of patients. Among hospitalized patients in a Center for Medicare and Medicaid Services (CMS) national inpatient

database, dehydration and malnutrition were the most frequent concurrent diagnoses, affecting 36% of ALS patients (Lechtzin et al. 2001).

Hydration in ALS

Water Turnover

The average daily water turnover by DLW is 9% or 260 mL lower in ALS patients than in age- and gender-matched healthy controls (Scagnelli et al. 2017) (Table 1). Water intake in ALS patients from this study is also lower than that reported in healthy controls from intake questionnaires (Zizza et al. 2009) and by DLW in another study of a larger number of healthy subjects (Raman et al. 2004). Risk factors for lower water intake are bulbar-onset disease and values below the median in the groups for amyotrophic lateral sclerosis functional rating scale-revised (ALSFRS-R) (Table 2), body mass index (BMI), and percent predicted forced vital capacity (FVC) (Fig. 1). The odds ratio (OR) for low water turnover in the below median groups ranged between 2.03 and 3.75 and was the highest for BMI.

Total Body Water

In the multicenter study from the USA (Scagnelli et al. 2017), TBW was 1,200 ml (3.5%) lower in ALS patients as compared to that in matched healthy controls (Table 2). Although there are no data from other studies in ALS, in an isotopic dilution study using tritiated water, TBW was not significantly different in groups of patients with muscular dystrophy, myasthenia gravis, and neurogenic atrophy (Delwaide et al. 1972). This study only had one ALS patient in the neurogenic atrophy group.

Table 1 Total body water and water turnover by the doubly labeled water method in ALS patients and matched healthy controls ($n = 57$ in each group)

	ALS (mean ± SD)	Healthy controls (mean ± SD)	p-value (paired t-test)
Age (y)	64.1 ± 9.4	64.8 ± 10.0	0.07
Height (cm)	168.0 ± 10.2	167.8 ± 8.9	0.99
Weight (kg)	76.2 ± 15.9	73.1 ± 14.4	0.53
BMI (kg/m2)	26.9 ± 4.5	25.9 ± 4.2	0.20
Fat-free mass (kg)	45.7 ± 10.4	49.3 ± 10.1	0.003
Fat mass (kg)	31.3 ± 11.2	24.0 ± 11.0	0.004
TBW-DLW (L)	34.6 ± 7.7	35.8 ± 7.5	0.15
Water turnover-DLW (L/d)	2.75 ± 0.83	3.01 ± 0.66	0.05

Table 2 ALS functional rating scale-revised

Questions
1. Speech
4 = Normal speech processes
3 = Detectable speech disturbances
2 = Intelligible with repeating
1 = Speech combined with nonvocal communication
0 = Loss of useful speech
2. Salivation
4 = Normal
3 = Slight but definite excess of saliva in mouth, may have nighttime drooling
2 = Moderately excessive saliva, may have minimal drooling
1 = Marked excess of saliva with drooling
0 = Marked drooling, requires constant tissue or handkerchief
3. Swallowing
4 = Normal eating habits
3 = Early eating problems – occasionally
2 = Dietary consistency changes
1 = Needs supplemental tube feeding
0 = NPO (exclusively parenteral or enteral feeding)
4. Handwriting
4 = Normal
3 = Slow and sloppy, all words are legible
2 = Not all words are legible
1 = Able to grip pen but unable to write
0 = Unable to grip the pen
5a. Cutting food and handling utensil (patients without gastrostomy)
4 = Normal
3 = Somewhat slow and clumsy but no help needed
2 = Can cut most foods, although clumsy and slow; some help needed
1 = Food must be cut by someone but can still feed slowly
0 = Needs to be fed
or
5b. Cutting food and handling utensils (alternate scale for patients with gastrostomy)
4 = Normal
3 = Clumsy but able to perform all manipulations independently
2 = Some help needed with closures and fasteners
1 = Provides minimal assistance to caregivers
0 = Unable to perform any aspect of task
6. Dressing and hygiene
4 = Normal function
3 = Independent and complete self-care with effort or decreased efficiency
2 = Intermittent assistance or substitute
1 = Needs attendant for self-care
0 = Total dependence

(*continued*)

Table 2 (continued)

7. Turning in bed and adjusting bedclothes
4 = Normal
3 = Somewhat slow and clumsy but no help needed
2 = Can turn alone or adjust sheets but with great difficulty
1 = Can initiate, but not turn or adjust sheets alone
0 = Helpless
8. Walking
4 = Normal
3 = Early ambulation difficulties
2 = Walks with assistance
1 = Non-ambulatory functional movement
0 = No purposeful leg movement
9. Climbing stairs
4 = Normal
3 = Slow
2 = Mild unsteadiness or fatigue
1 = Needs assistance
0 = Cannot do
10. Breathing
4 = Normal
3 = Shortness of breath with minimal exertion (e.g., walking, talking)
2 = Shortness of breath at rest
1 = Intermittent (e.g., nocturnal) ventilatory assistance
0 = Ventilator dependent
R-1. **Dyspnea**
4 = None
3 = Occurs when walking
2 = Occurs with one or more of the following: eating, bathing, dressing
1 = Occurs at rest, difficulty breathing when either sitting or lying
0 = Significant difficulty, considering using mechanical ventilatory support
R-2. **Orthopnea**
4 = None
3 = Some difficulty sleeping at night due to shortness of breath, does not routinely use more than two pillows
2 = Needs extra pillows in order to sleep (more than two)
1 = Can only sleep sitting up
0 = Unable to sleep
R-3. **Respiratory insufficiency**
4 = None
3 = Intermittent use of NIPPV
2 = Continuous use of NIPPV during the night
1 = Continuous use of NIPPV during the night and day
0 = Invasive mechanical ventilation by intubation or tracheostomy

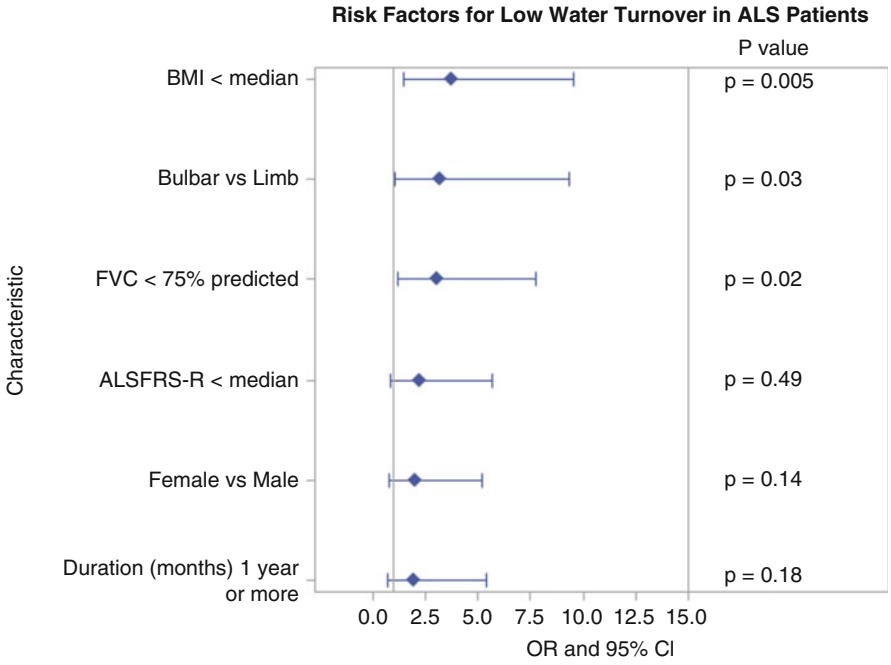

Fig. 1 Risk factors for low water turnover in ALS patients using the doubly labeled water method ($n = 79$)

Hydration Parameters with ALS Progression

With progressive worsening of clinical measures over a year, decreases in average daily water turnover by 14% or 40 mL, and in TBW by 6% or 210 mL, are seen (Fig. 2). This rapid worsening of hydration is largely a consequence of declining intake and can account for several adverse biological effects.

Consequences of Dehydration in Elderly

Poor hydration has several health-related adverse consequences in normal subjects. In the elderly, reduced water intake, resulting in dehydration, is the most frequent cause of electrolyte disturbances (Lavizzo-Mourey et al. 1988), particularly hypernatremia, and produces increased mortality and morbidity (Borra et al. 1995). In the elderly, dehydration alone has serious consequences, including an increased likelihood of pneumonia, urinary tract infections, bowel obstruction, vomiting, fever, delirium, and cardiovascular complications. Dehydration in the elderly increases the risk of morbidity and mortality not only in of itself but also when it

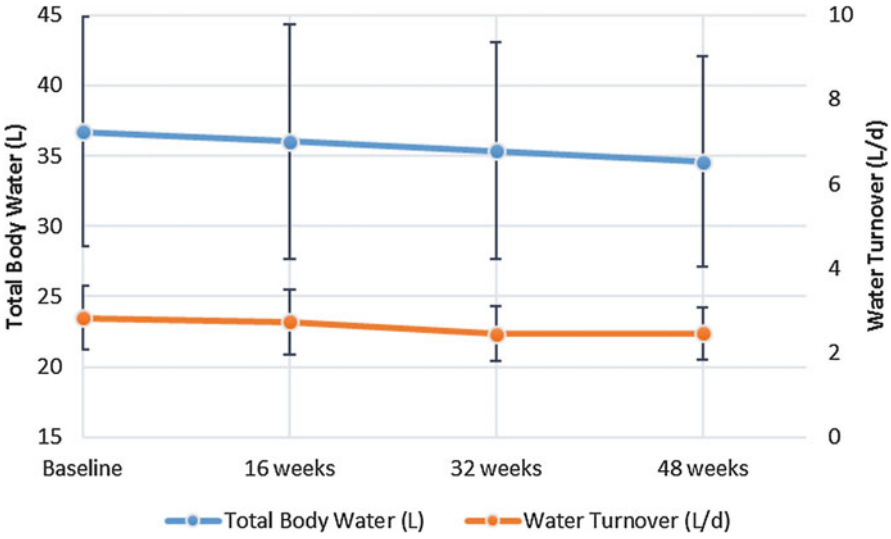

Fig. 2 Change in total body water and water turnover measured by doubly labeled water over 48 weeks in ALS patients ($n = 49$)

co-occurs with several diseases, including cancer, diabetes, urinary infections, and respiratory illnesses (Warren et al. 1994).

Consequences of Dehydration in ALS

The effect of dehydration on mortality in ALS has been studied recently. Poor hydration is associated with increased mortality in ALS patients, as shown by the multicenter study from the USA (Scagnelli et al. 2017). The median Kaplan-Meier survival in a clinic cohort is reduced by 16 months in patients with estimated daily water intake values below the median as compared to above the median (Fig. 4). By quartiles of water intake, patients in the lowest quartile show reduced median survival by 21 months as compared to that in the highest quartile (Fig. 5). Mortality 12 months after the first clinic visit is 45% in the lowest quartile compared to 9% in the highest quartile (Fig. 6); after adjusting for the known prognostic factors in ALS, the hazard ratio for increased likelihood of death from reduced water intake is still significant at 12 months. These data suggest that lower water intake is an independent risk factor for survival.

In dichotomous groups separated at median values of estimated daily water intake and BMI, median Kaplan-Meier survival was 14, 18, 31, and 29 months, respectively, in groups with low BMI/low water intake, high BMI/low water intake, low BMI/high water intake, and high BMI/high water intake (Fig. 7) (Scagnelli et al. 2017). The proportion of patients in the above median and below median groups

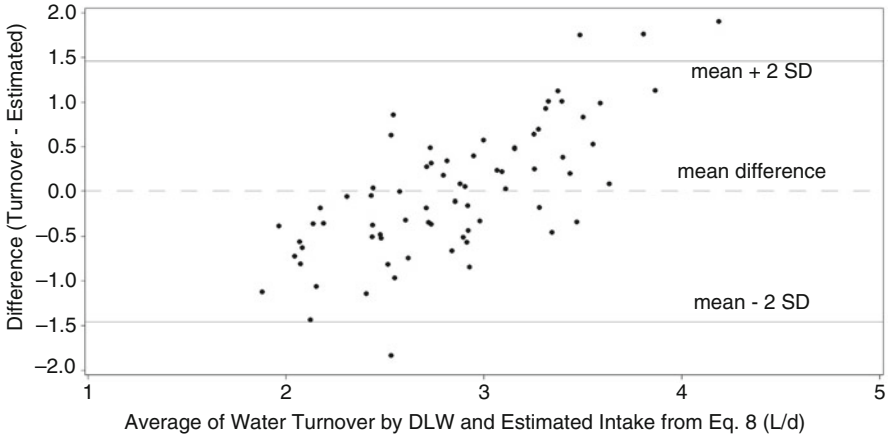

Fig. 3 Bland-Altman plot of water turnover from doubly labeled water and water intake estimated from *Equation 8* in ALS patients ($n = 79$)

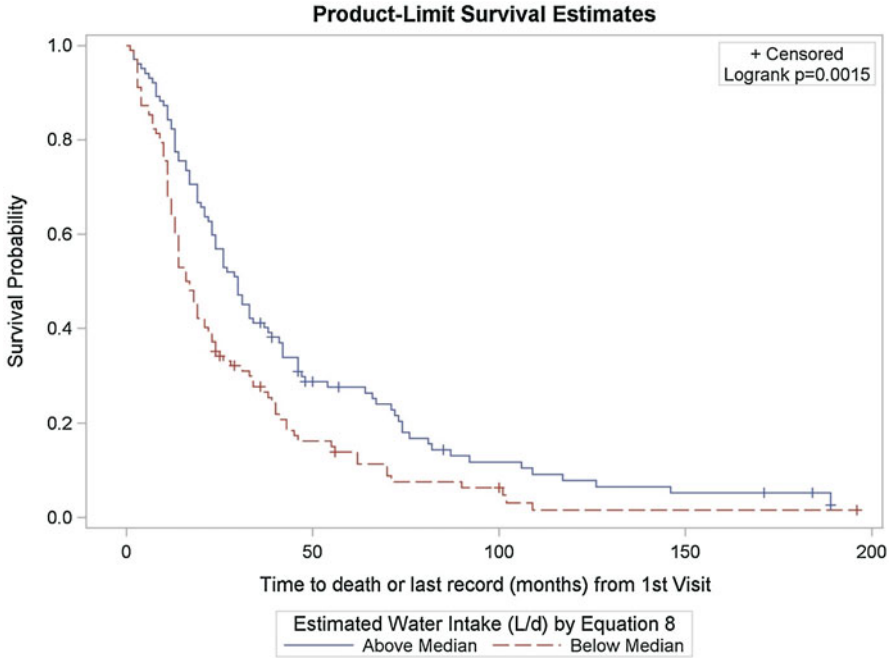

Fig. 4 Kaplan-Meier survival curves for baseline daily water intake estimated from *Equation 8* in patients seen at University of Vermont ALS Center clinic ($n = 208$) with intakes above and below median value of 2.88 L/d. Proportional hazards regression, hazard ratio (HR) = 1.59 ($p = 0.002$)

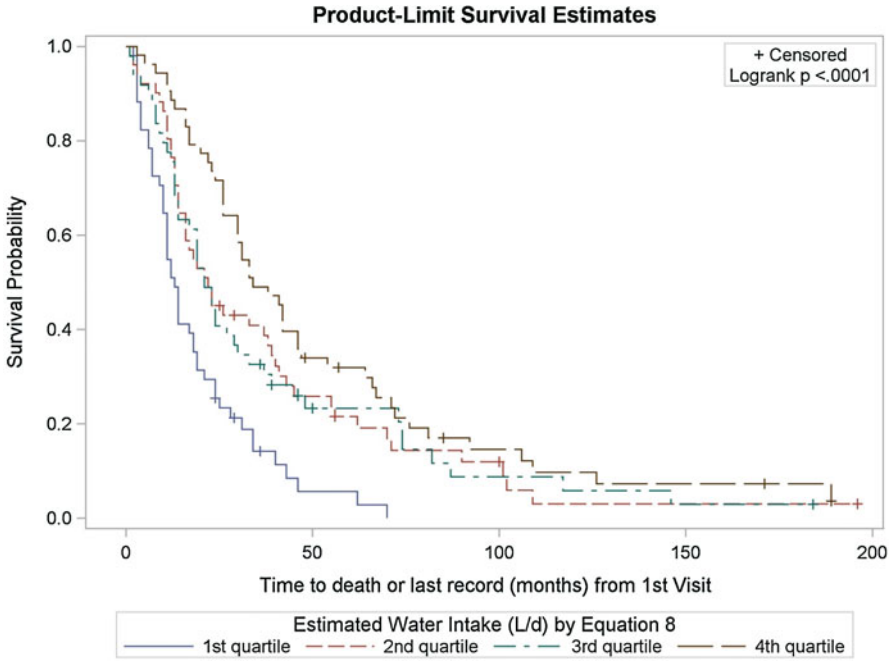

Fig. 5 Kaplan-Meier survival curves for baseline daily water intake estimated from *Equation 8* in patients seen at University of Vermont ALS Center clinic ($n = 208$), by quartiles

for BMI and water intake is comparable (Fig. 8), showing concordance between the nutritional state and dehydration based on water intake employing *Equation 3*. These data demonstrate that low water intake can have a more significant negative impact on survival than low BMI. Daily water intake, water intake relative to TBW, and TBW corrected for body weight and lean body mass are for the first time shown to be significant predictors of survival in ALS (Fig. 9). Taken together, these data indicate that both water intake and TBW are probably chronically low in ALS patients and add to the adverse risk conferred by the other known prognostic factors toward shortened survival.

Equations for Estimating Water Intake and TBW

Scagnelli et al. (2017) have shown that the two equations published in the USA by CMS (Recommended Daily Allowances 1989; Chidester and Spangler 1997) for estimating daily water intake in healthy adults are inaccurate in predicting water requirement in ALS (*Equations 1 and 2*, Table 2). Using DLW data for validation, they created equations to estimate daily water intake and TBW using variables easily obtained at routine ALS clinic visits (*Equations 3–5*, Table 2).

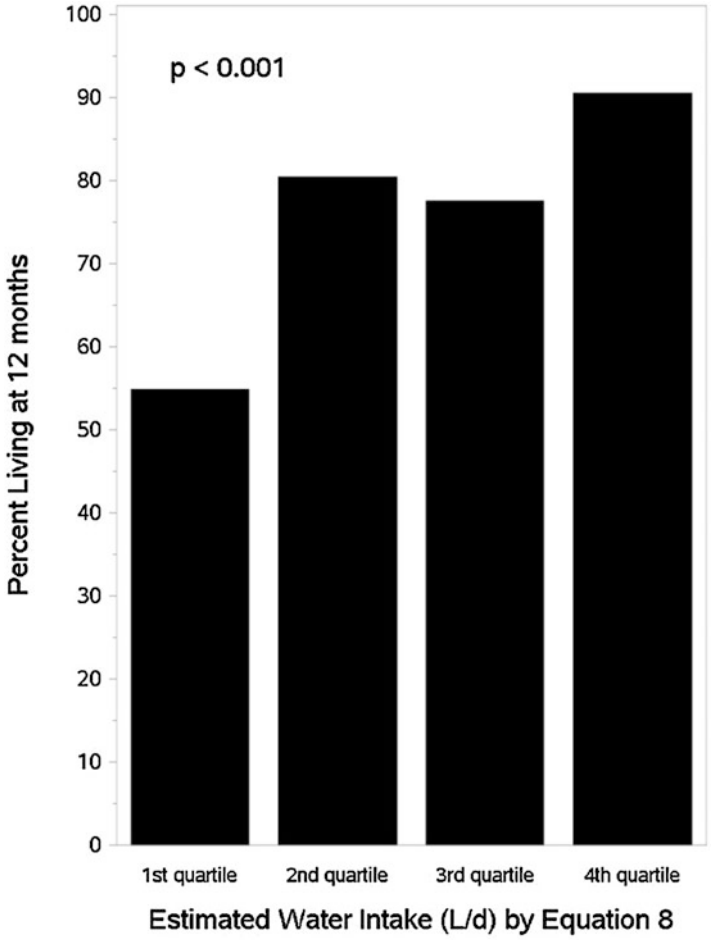

Fig. 6 Bar graph of survival at 12 months in quartiles of clinic patients ($n = 204$) based on daily water intake estimated by *Equation 8* at first visit in patients seen at University of Vermont ALS Center clinic (p-value is for Kendall's Tau correlation)

The validated *Equations 3* and *4* accurately estimate TBW in men and women with ALS.

An accurate estimate of total daily energy expenditure (TDEE) is a prerequisite for using *Equation 1* to predict water intake. The Harris-Benedict (H-B) equation, when used to predict daily energy requirements, significantly underestimates TDEE in ALS patients. However, the predictability is much more accurate if an ALS disease-specific modification (the ALSFRS-6 modification) of the H-B equation (*Equation 5*, Table 2) is utilized (Kasarskis et al. 2014) to estimate TDEE. Thus, TDEE obtained from *Equation 5* provides an accurate estimate of daily water intake in ALS patients (Scagnelli et al. 2017).

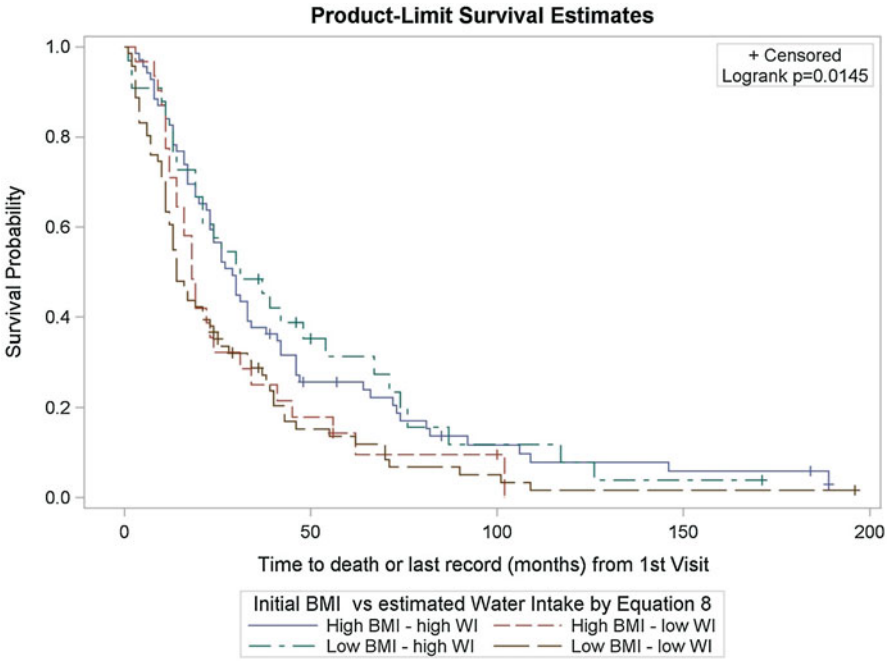

Fig. 7 Kaplan-Meier survival curves for combined effect of body mass index and estimated water intake from *Equation 8* at first visit using dichotomized groups above and below the median in patients seen at University of Vermont ALS Center clinic

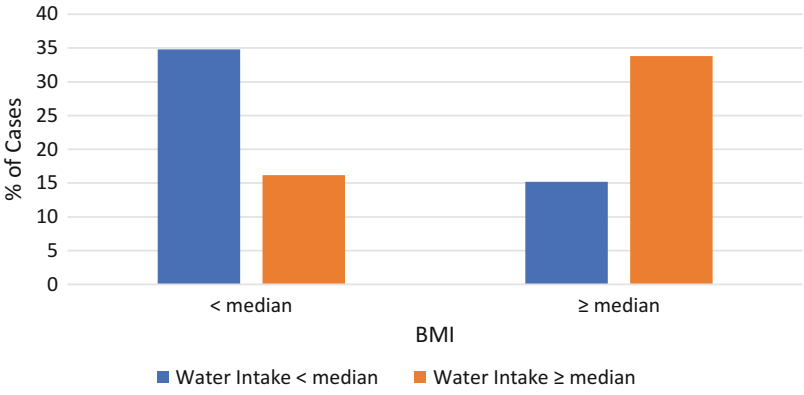

Fig. 8 Close relationship between body mass index and water intake estimated from *Equation 8* at first visit in patients seen at University of Vermont ALS Center clinic ($n = 208$)

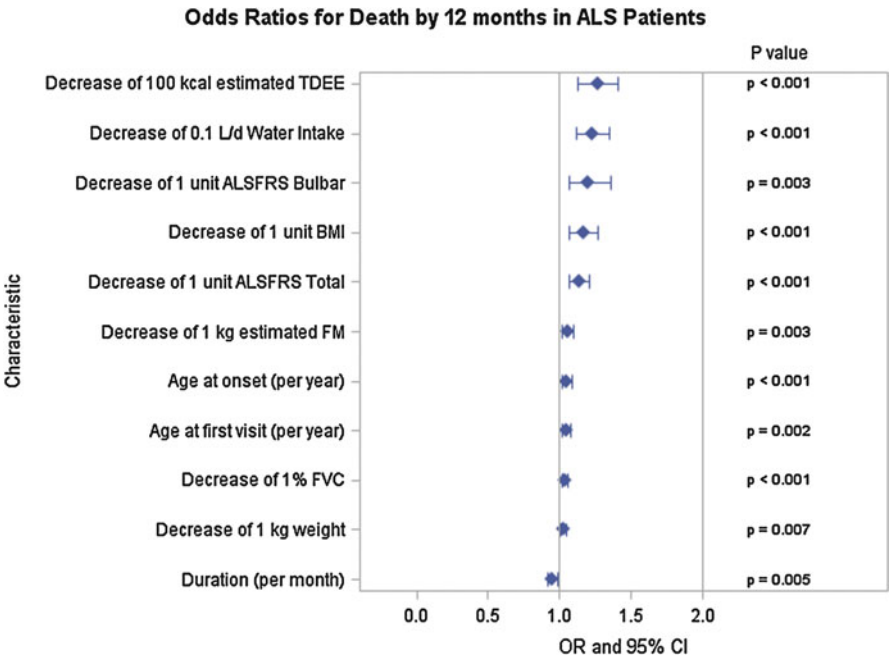

Fig. 9 Odds ratio for mortality at 12 months from first visit based on baseline demographic and hydration measures in patients seen at University of Vermont ALS Center clinic ($n = 208$)

Policies and Protocols

Protocols

At each ALS clinic visit, the following tools are essential to provide accurate estimates of hydration status:

1. *Equations 3* and *4* to estimate TBW
2. Basal metabolic rate using the H-B equation
3. Total ALSFRS-6 score
4. *Equation 5* to estimate TDEE
5. *Equation 8*, using the TDEE value from *Equation 5*, to estimate daily water intake

Policies and Recommendations

Figure 10 is a flow diagram of the recommended course of action to estimate TBW and water intake in ALS patients. The individual steps to predict nutrition and hydration requirements in ALS patients at each clinic visit, therefore, are:

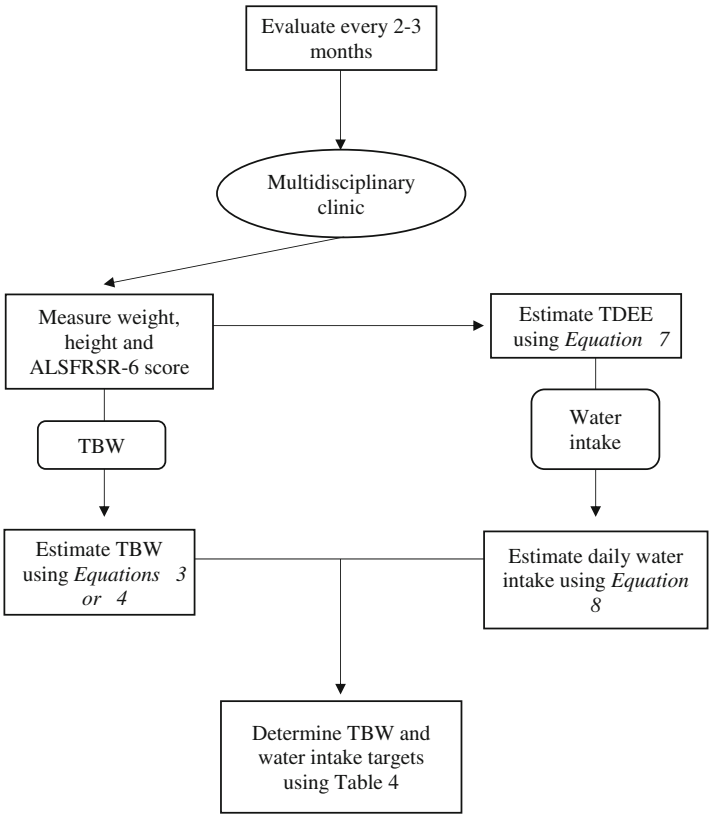

Fig. 10 Suggested algorithm for assessment of hydration in ALS patients

1. Measure, not estimate, body weight.
   (i) Record height.
2. Record the consensus responses (from the ALS patient, family members, ALS clinician) to the ALSFRS-6 (Questions 1, 4, 6, 7, 8, and 10 from the ALSFRS-R questionnaire) (Table 2).
3. Calculate the total daily energy (calorie) needs according to the ALSFRS-6 modification of the H-B equation to provide energy intake targets for the ALS patient (Kasarskis et al. 2014).
4. Calculate the daily water needs according to *Equations 3–5* to provide TBW and water intake targets (Table 3).
   (i) Recommendations from the dietician and speech pathologist can help the patient achieve the goals set forth in #3 and #4.
5. Consult Table 4 to determine recommended values of TBW and water turnover in men and women by age.

Table 3 Equations for estimating total body water, daily water intake, basal metabolic rate, and total daily energy expenditure (modified from Table 1, Scagnelli et al. (2017))

	Equation	Comment
Equation 1 (CMS)	**Water requirement** (L/d) = 0.001* total daily energy expenditure	Total daily energy expenditure estimated from H-B equation and factorial approach
Equation 2 (CMS)	**Water requirement** (L/d) = 100 mL/kg body weight for first 50 kg + 50 mL/kg for remainder of body weight	From body weight
Equation 3 (TBW)	**Total body water** (L) (men) = [0.209* weight (kg)] + [0.140* height (cm)] − 1.809	From body weight and height
Equation 4 (TBW)	**Total body water** (L) (women) = [0.209* weight (kg)] + [0.140* height (cm)] – 8.064	From body weight and height
Equation 5 (H-B equation)	**Basal metabolic rate** (kcal/d) = 66 + (6.23 × weight in pounds) + (12.7 × height in inches) − (6.76 × age in years)	For men
Equation 6 (H-B equation)	**Basal metabolic rate** (kcal/d) = 655 + (4.35 × weight in pounds) + (4.7 × height in inches) − (4.7 × age in years)	For women
Equation 7	**Total daily energy expenditure** (kcal/d) = BMR + (55.96 × ALSFRS-6 score) − 168	From ALSFRS-6 modification of Harris-Benedict equation
Equation 8	**Water requirement** (L/d) = 0.087 + 0.001151* total daily energy expenditure (Fig. 3)	Total daily energy expenditure estimated from ALSFRS-6 modification of Harris-Benedict equation

6. Reassess at subsequent clinic visits.
7. If the patient fails to achieve the goals for energy and water intake, then recommend a gastrostomy for an alternative route for nutrition and hydration.

Dictionary of Terms

- **Amyotrophic lateral sclerosis** – Also called motor neuron disease (in the UK), Charcot's disease (in France), or Lou Gehrig's disease (in the USA), this is a neurological condition due to loss of nerve cells in the brain and spinal cord that control muscle function.
- **Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised** – A 13-item questionnaire completed by patients, caregivers, or clinic staff that evaluates activities of daily living and the functional state of patients.

Table 4 Water turnover and TBW in healthy men and women by decade

		Age	40–49	50–59	60–69	70–79
		Water turnover*	3.8 ± 1.2	3.6 ± 0.9	3.6 ± 0.9	3.4 ± 0.8
	Raman et al. 2004					
		TBW**	42.0 ± 5.0	43.5 ± 6.0	42.0 ± 6.0	39.0 ± 5.0
Men						
		Water turnover	NA	3.7 ± 0.6	3.3 ± 0.7	2.6 ± 0.5
	Scagnelli et al. 2017					
		TBW	NA	43.0 ± 7.0	42.0 ± 6.0	39.0 ± 5.0
		Water turnover	3.3 ± 0.8	3.0 ± 0.8	2.9 ± 0.7	2.8 ± 0.7
	Raman et al. 2004					
		TBW	33.0 ± 5.0	30.0 ± 4.0	30.0 ± 3.0	28.0 ± 4.0
Women						
		Water turnover	2.7 ± 0.4	3.3 ± 0.5	2.8 ± 0.6	3.1 ± 0.7
	Scagnelli et al. 2017					
		TBW	28.0 ± 5.0	27.0 ± 1.0	30.0 ± 4.0	32.0 ± 2.0

* = L/d; ** = L

- **Amyotrophic Lateral Sclerosis Functional Rating Scale-6** – A six-item subcomponent of the ALS Functional Rating Scale-Revised that evaluates the summed scores for speech, handwriting, dressing and hygiene, turning in bed and adjusting bedclothes, walking, and dyspnea and helps to accurately estimate daily water intake needs.
- **Daily Energy Requirement** – The number of calories needed in the daily diet through food to maintain a healthy state.
- **Daily energy Expenditure** – The number of calories spent daily in supporting functions of essential organs (e.g., the brain, heart, lungs, etc.) and in carrying out physical activities.
- **Dehydration** – A state of water deficiency in the body.
- **Gastrostomy** – An artificial "hole" in the stomach to facilitate delivery of nutrition and hydration in individuals who are unable to swallow.
- **Harris-Benedict Equation** – An equation that utilizes a person's weight, height, age, and gender to estimate the number of calories needed daily for bodily functions.
- **Hydration** – A measure of the body's water status.
- **Modified Harris-Benedict Equation** – A valid equation using some ALS severity measures to modify the Harris-Benedict equation and correctly estimate the number of calories and amount of water needed daily to maintain nutrition and hydration and also bodily functions.

- **Total Body Water** – The volume of water, in liters, contained in the body inside the tissues and in the circulation.
- **Water Intake** – The volume of water consumed largely as liquids; some are also available from solid foods.
- **Water turnover** – The volume of water that is added daily to the body's water compartments to maintain normal hydration.

Summary Points

- This chapter reviews the prevalence, consequences, and approach to management of dehydration in amyotrophic lateral sclerosis (ALS).
- Dehydration is identified in 20% of patients using the gold standard doubly labeled water method.
- Dehydration is an independent risk factor for early death in patients and is probably responsible for increased morbidity.
- Reduced oral intake due to dysphagia is the principle cause of dehydration; however, also contributory are anorexia, depression, cognitive dysfunction, weakness of arms, lack of caregiver availability or support, and decreased thirst associated with normal aging.
- As with nutritional care, attention to proper hydration is best provided by education of patients and caregivers in multidisciplinary clinics by a team of experienced providers.
- At clinic visits, team members can identify malnutrition and dehydration using validated published tools.
- It is essential to assess daily energy needs correctly so as to appropriately determine hydration requirements.
- If appropriate oral dietary modifications with regard to food type, food consistency, and energy supplementation cannot correct malnutrition and dehydration, then a recommendation is made for an alternative route for feeding, which is typically a percutaneous or radiologically inserted gastrostomy.

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Surgical Treatment for Severe Protein-Calorie Malnutrition After Bariatric Surgery

54

Reginaldo Ceneviva and Wilson Salgado Junior

Contents

Abstract

Obesity is a chronic and universal disease that has reached global epidemic proportions and is one of the ten greatest health risks that can be prevented. Medical treatment results in a limited and usually transitory weight loss, especially among the more obese patients. The frequent failure of conservative therapy, coupled with worsening of quality of life and possible reduction of survival, favors the indication of surgical treatment.

Bariatric surgeries are an efficacious treatment for morbid obesity, but they are not free of early or late complications, including severe protein-calorie malnutrition. Although few evidence-based studies are available to instruct decisions about strategies and managements, emphasis was placed on the description of

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the more accepted surgical revision procedures as based on relevant publications and on some anatomical and physiological features.

Keywords

Obesity · Bariatric surgery · Sleeve gastrectomy · Roux-en-Y gastric bypass · Malabsorptive bariatric procedures · Bariatric surgery complications · Malnutrition · Protein-calorie malnutrition · Protein-energy malnutrition · Surgical revision treatment

List of Abbreviations

AIDS	Acquired immunodeficiency syndrome
AL	Alimentary limb
BMI	Body mass index
BPD	Biliopancreatic diversion
BPD/DS	Biliopancreatic diversion/duodenal switch
BPL	Biliopancreatic limb
CC	Common channel
DRYGB	Distal Roux-en-Y gastric bypass
IFSO	International Federation for the Surgery of Obesity and Metabolic Disorders
PCM	Protein-calorie malnutrition
RYGB	Roux-en-Y gastric bypass
SG	Sleeve gastrectomy (or vertical gastrectomy)
SPCM	Severe protein-calorie malnutrition
SS	Stomach size

Introduction

Nutrient deficiencies constitute the most important long-term complications of bariatric procedures (Tack and Deloose 2014, 741; Nett et al. 2016). Protein-calorie malnutrition (PCM) may develop after bariatric surgery, and diagnosis should be made early before complications establish and physical conditions deteriorate, with medical treatment and dietary adjustment being promptly initiated. The demand for revision bariatric procedures increases with the increasing number of primary bariatric surgeries (Ma et al. 2016).

For a successful revision, it is essential to evaluate data about the previous surgical procedure and perform a careful preoperative clinical-laboratory study, including the assessment of nutritional status and other parameters such as imaging examinations and the behavioral and dietary habits of the patients (Salgado et al. 2014; Santarpia et al. 2014; Ma et al. 2016).

The proposition of revision surgery should be made preferably by a health multi-professional team, when there is an obstructive problem not solved by endoscopy or when there is a recognized failure of the appropriate conservative treatment.

According to Topart and Becouarn (2015), revision surgery should be indicated when there is failure of medical treatment for excessive and continuous weight loss (>100% excess weight loss) or biological and laboratory signs of SPCM (severe protein-calorie malnutrition), risk of liver failure, persistent hypoalbuminemia (<3.5 g/dL), and persistent hypoprealbuminemia (<0.02 g/dL).

The selection of a particular surgical revision procedure for the treatment of SPCM after each type of primary surgery has not been standardized in the literature. There is limited evidence due to the small number of studies published which are usually retrospective and involve small patient series. Furthermore, most studies on the treatment of malnutrition after bariatric surgery do not specify the type and severity of malnutrition, making it difficult to identify the PCM, particularly in its severe form. Revision surgeries are usually more complex and pose higher risk than the previous surgical procedures (Brethauer et al. 2014). Thus, it is desirable that they should be performed by experienced surgeons (Buchwald 2015; Ma et al. 2016).

Kellogg (2011) showed that the overall incidence of surgical revision after a primary bariatric operation is of 5–50%, including other indications besides PCM. According to Nesset et al. (2007), out of 1584 patients submitted to bariatric surgery, 218 (14%) underwent revision procedures, of which 97 (44%) for unsatisfactory weight loss and 26 (12%) for severe nutritional/metabolic problems.

Reoperations for treatment of SPCM involve three kinds of procedures:

Reversals – restoration to original anatomy

Conversions – which consist of another kind of procedure, considered to be better than the previous one, regarding the potential improvement of the nutritional status

Corrections – which solve complications or complement the treatment effect of the primary bariatric surgery

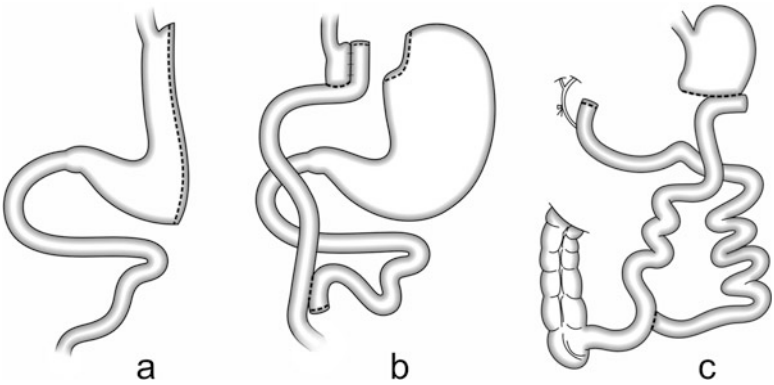

Fig. 1 Bariatric surgeries. (**a**) Sleeve gastrectomy. (**b**) Roux-en-Y gastric bypass. (**c**) Biliopancreatic diversion

Knowledge about the primary bariatric surgery and the anatomical features is essential for the surgeon to perform the therapeutic revision procedure efficaciously (Ma et al. 2016). Figure 1 shows schemes of three bariatric operations which represent three different groups: purely restrictive, mixed with predominant restrictive component, and mixed with predominant malabsorptive component.

Surgical Revision Techniques

For Purely Restrictive Surgeries

In general, reversal and conversion after restrictive surgeries are only indicated in the rare cases of occurrence of SPCM. Stenosis or obstructions are usually resolved by therapeutic methods involving an endoscopic or surgical approach to revise the complication itself, such as endoscopic dilatation of the stenosis and removal of the band (Brethauer et al. 2014). Milone et al. (2014) found, in a systematic review, that persistent vomiting was the major determinant of Wernicke encephalopathy in patients undergoing restrictive weight loss surgery.

For Purely Malabsorptive Surgeries

Revision of the jejunoileal bypass should be considered to prevent cirrhosis, oxalate nephropathy, renal failure, and chronic malnutrition (Singh et al. 2009; Chousleb et al. 2012).

For Mixed Surgeries with a Malabsorptive Component

Roux-en-Y Gastric Bypass

Hypoalbuminemia is uncommon after RYGB (Pajecki et al. 2007). Anatomical causes of malnutrition are usually indications for reoperation. PCM may result from complications of RYGB due to stenosis of the gastrojejunostomy accompanied by vomiting and, not infrequently, by dysphagia and psychological disorders such as fear of eating. Stenosis of the gastrojejunal anastomosis should be preferentially treated by endoscopic dilatation, and when this treatment fails, the therapeutic alternative is reoperation with a direct approach to the cause of obstruction (Kushner 2000), as is the case with other causes of mechanical obstruction of the alimentary tract. Chronic or intermittent pain during late postoperative period may result from intestinal obstructions of several types: internal hernia, stenosis of the jejunojejunal anastomosis and adhesions, or, more rarely, intussusception of the enteral loop (Kassir et al. 2016). Image exams may fail to confirm the hypothesis of obstruction due to adhesions or to an internal hernia, mainly if those exams are performed outside the episodes of pain. An early surgical intervention may be crucial because of the possibility of ischemia of the bowel loops involved.

Diarrhea and excessive weight loss are causes of PCM (Malinowski 2006) but are infrequent after RYGB. Crohn's disease, celiac disease, and lactose intolerance manifested before or after RYGB involve a particularly conservative treatment (Janczewska et al. 2011; Cuenca-Abente et al. 2013) but may indicate a revision procedure if marked malabsorption attributed to bariatric surgery occurs.

No established directives are available to guide the choice of the type of revision procedure for the treatment of PCM after RYGB (Fig. 2a). The choice between reversal and revision is frequently difficult.

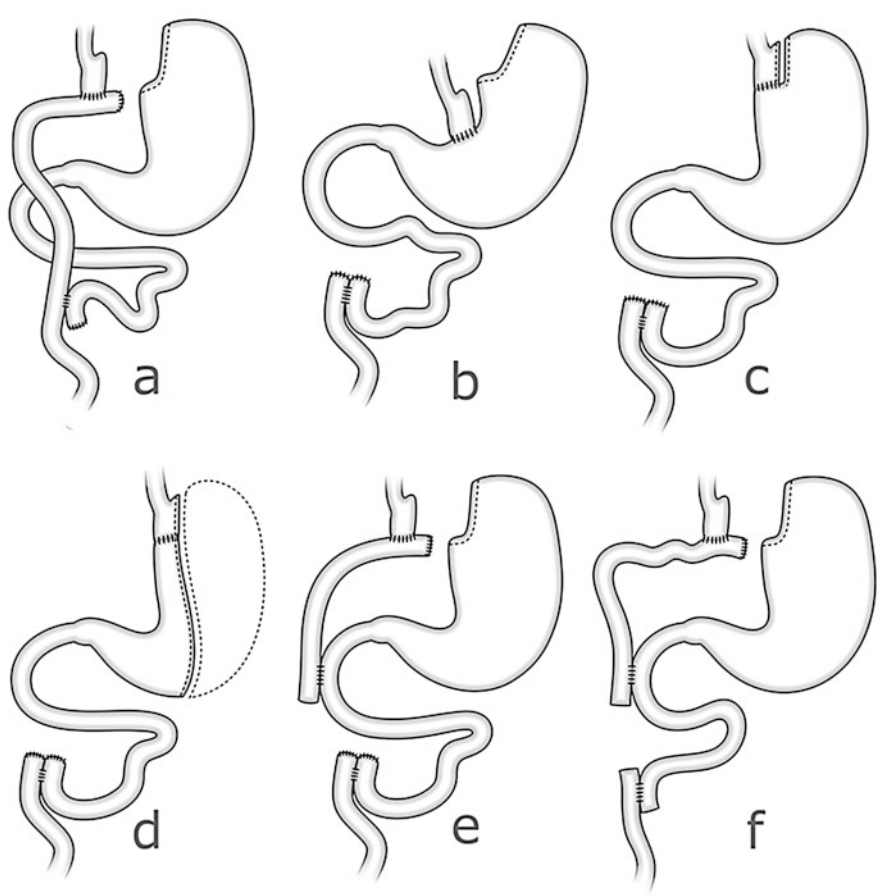

Fig. 2 Revision surgeries for Roux-en-Y gastric bypass. (**a**) Standard RYGB. (**b**) Reversal with an end-to-side anastomosis between the pouch and the body of the stomach. (**c**) Reversal with an end-to-side anastomosis between the pouch and the fundus of the stomach. (**d**) Conversion to sleeve gastrectomy. (**e**) Short jejunal interposition. (**g**) Jejunal interposition of the entire AL

Reversal of RYGB

In a systematic review of 175 articles selected, Brethauer et al. (2014) observed that the indications to reverse RYGB were rare and based, among other disorders, on severe intractable nausea, vomiting, excessive weight loss, psychological issues, recurrent anastomotic ulceration, malnutrition, and extensive bowel resections. Shoar et al. (2016) selected 100 patients in a systematic review of 35 papers. Although weight regain was the main reason for revision surgery after RYGB, malnutrition was the most common indication for reversal (12.3%), followed by severe dumping syndrome (9.4%), postprandial hypoglycemia (8.5%), and excessive weight loss (8.5%).

Reversal of RYGB involves the reconstruction of the stomach by means of anastomosis of the gastric pouch with the body (Fig. 2b) or with the fundus (Fig. 2c) of the remnant stomach, in addition to reconstruction of the small bowel continuity (Himpens et al. 2006). Measurement of the total length of the small bowel is obligatory, and the AL must be preserved, especially if longer than 100 cm (Vilallonga et al. 2016).

Reports of RYGB reversal include resection of the primary gastrojejunal anastomosis, what may eliminate or reduce the restrictive component of the operation, and reconstruction of the small bowel continuity, what abolishes the malabsorptive component. However, some related articles do not mention the extension of the AL and the CC in the primary operation nor whether intestinal restoration was associated with reconstruction of the stomach.

Results of reviewed studies evaluating procedures for PCM after RYGB are shown in Table 1.

The indications of RYGB revision procedures to treat PCM had an ample variation rate, from 4% (Zaveri et al. 2016) up to 58% (Chen et al. 2016). This difference was probably due to the variations in the series of patients studied or in the criteria adopted to establish the need for reoperation. Most of the articles do not make any reference to the severity of malnutrition. Several authors stressed the need for gastrostomy feeding in the preoperative and occasionally postoperative nutritional support. The reversal was the most common revision operation performed for the treatment of malnutrition reported in those publications, except for two cases in which the reoperation was an SG and one case which was a jejunal interposition. On some occasions, when weight regains were anticipated, reversal was complemented by an SG during the procedure, although this may increase the possibility of complications (Vilallonga et al. 2013). No mortality was referred after the revision procedures as treatment for PCM, and they generally involve short follow-up. These results favor the surgical revision procedures to treat selected cases of PCM. General results showed malnutrition and short gut syndrome resolution after the RYGB revision surgery. Jejunal interposition and intestinal restoration can also be efficacious to treat PCM after RYGB (Ceneviva et al. 2016). The significant variation of follow-up duration seems to explain different outcomes, mainly related to complications. Early postoperative assessment may fail to show complications, such as weight regain. In a recent review evaluating 100 reversals of RYGB to normal anatomy, followed up to 48 months, Shoar et al. (2016) reported a significant rate

Table 1 Surgical revision after Roux-en-Y gastric bypass

Authors	Primary surgery: n	Pouch (mL) AL/CC (cm)	PCM	Revision surgery	Follow-up	Results
Shoar et al. (2016) Review	RYGB reversal: 100	NR	Malnutrition: 12.3%	Reversal: 12.3%	3 weeks–48 months	No mortality reported Weight regain: 28.8% Severe GERD: 10.2% Abdominal pain: 6.8%
Zaveri et al.(2016) Multicenter study	RYGB reversal: 50	NR	Malnutrition: 2 (4%)	Reversal: 2	1 year	Mortality: 0
Chen et al.(2016)	RYGB conversion: 49	NR	Malnutrition: 28 (58)	Conversion to SG: 49	NR	Early mortality: 0
Vilallonga et al. (2013)	RYGB reversal: 10 RYGB reversal +SG:10	NR	PCM: 1 (10%) PCM: 2 (20%)	Reversal: 1 Reversal + SG: 2	11.5 months 11.5 months	Mortality: 0 Mortality: 0
Chousleb et al. (2012) Review	RYGB reversals: 8	NR	Malnutrition: 3	Reversal to NA	2–13 years	Satisfactory results
Faintuch et al. (2004)	RYGB: 205	AL: 100 cm BPL: 70 cm	11 (4.7%)	Revision of gastric stenosis: 3 Endoscopic dilatation: 1	NR	Mortality: 2/11 (18.2%) Mortality: 2/205 (1%)
Akusoba et al. (2016) Case report	RYGB: 1 FU: 2 years	AL: 150 cm	1	Reversal to NA	12 months	BMI pre-reversal: 18.9 BMI post-reversal: 25 Good results
Ceneviva et al. (2016) Case report	RYGB: 1 FU: 36 months	AL: 50 cm BPL: 70 cm CC: 180 cm	PCM/refractory diarrhea: 1	Jejunal interposition between the proximal gastric pouch and duodenum plus intestinal restoration	3 years	Complete remission of PCM and diarrhea without weight regain
Park and Kim (2014) Case report	RYGB: 1 FU: 32 months	AL: 120 cm BPL: 70 cm	1	Reversal to NA	21 days	Good early results

AL alimentary limb, *BPL* biliopancreatic limb, *CC* common channel, *PCM* protein-calorie malnutrition, *RYGB* Roux-en-Y gastric bypass, *SG* sleeve gastrectomy, *NA* normal anatomy, *GERD* gastroesophageal reflux disease, *BMI* body mass index, *FU* follow-up

of unfavorable results: weight regain (28.8%), severe gastroesophageal disease (10.2%), and abdominal pain (6.8%). RYGB reversal to normal anatomy, as with the reversal of other bariatric procedures, may result in weight regain and recurrence of obesity-related comorbid conditions (Pernar et al. 2016). However, there are particular circumstances in which the reversal of RYGB cannot be avoided (Chousleb et al. 2012). Judicious patient selection must be considered, due to the high incidence of weight regain after RYGB reversal (Shoar et al. 2016). RYGB reversal should be performed especially by experienced bariatric surgeons (Park and Kim 2014).

Conversion of RYGB to Sleeve Gastrectomy

The conversion to SG (Fig. 2d) associated with the restoration of intestinal continuity has the objective of reversing the malabsorptive effects of the partial exclusion of the intestine while maintaining the restrictive effect of the SG. As shown by Chen et al. (2016), the main reasons for conversion to SG in 49 of 2382 patients submitted to RYGB and miniRYGB were malnutrition (58%), weight regain (10%), food intolerance (18%), and others (14%). The conversion resulted in the improvement of malnutrition with no mortality, however with a high rate of early complications such as leakage (6.8%) and internal bleeding (5.1%) (Shoar et al. 2016).

Interposition of a Jejune Loop Between the Gastric Pouch and the Duodenum

Sometimes it is not easy to clarify the cause of PCM after RYGB, what explains the difficulty surgeons often have in choosing which revision process should be used in cases of SPCM refractory to medical treatment.

Many surgeons perform gastric bypass surgery in a conventional manner with a BPL of 50–70 cm and an AL of 100–150 cm, usually without worrying about how much of the CC should remain. This happens because with the small bowel with a length of more than 500 cm or 600 cm, as is usually the case, the expectation is that at least 280 cm of the CC will remain to prevent excessive malabsorption. The small bowel, however, may be shorter than 500 cm, and, depending on its length, standard RYGB can result in a CC length close to 100 cm or less, thus exacerbating malabsorption and favoring the development of PCM. Furthermore, if the small bowel is very short, for example, with a jejunoileal length of less than 300 cm, RYGB can result in short bowel syndrome and consequent PCM (Malinowski 2006), even when a CC of more than 150 cm is constructed (Ceneviva et al. 2016). The interposition of a jejune loop between the proximal gastric pouch and the duodenum was proposed for a patient with SPCM after RYGB, with a CC length of 180 cm and a total jejunoileal length of only 300 cm. The aim was to maintain the restrictive component and to increase the absorptive area by including the duodenum and proximal jejunum, as they are significant intestinal segments in the alimentary tract for nutrient absorption (Ceneviva et al. 2016). The surgical technique with interposition of a shorter jejune loop (Fig. 2e) involves the section of the AL of RYGB at 20–30 cm from the gastrojejunal anastomosis, with the jejunojejunal anastomosis being reversed by a section of the BPL and food transit being reinstated with a jejunoduodenal anastomosis and a jejunojejunal anastomosis. When the AL is

relatively short, jejunal interposition may be performed by sectioning that loop close to the jejunojejunal anastomosis and then anastomosing it to the second portion of the duodenum (Fig. 2f). Jejunal interposition and intestinal restoration eliminate almost the entire malabsorptive component of RYGB and have the advantage of maintaining the restriction conferred by the gastric capacity reduction and the gastrojejunal anastomosis.

Distal Roux-en-Y Gastric Bypass

Malnutrition, more frequently associated with malabsorptive procedures (DRYG, BPD, and BPD/DS), is the late complication of bariatric surgery that most requires revision. The appropriate revision procedure is performed either to increase the length of the common limb or reverse the intestine to its original anatomy. Reversal is rarely indicated because of the consequent regain of lost weight and resumption of the previous comorbidities. Distal RYGB, performed with different lengths of the AL (150 cm or more) and of the CC (120 cm or less from the ileocecal valve), with the objective of adding malabsorption to standard RYGB, has been demonstrated to result in long-term weight loss in the super obese and morbidly obese patients. However, there has been a high incidence of protein malnutrition and deficiency of various micronutrients requiring revision in a significant number of cases (Fox et al.1996; Fobi et al. 2001; Ciovica et al. 2008; Pitt et al. 2016). Results of reviewed studies evaluating revision procedures for PCM after malabsorptive bariatric surgeries are shown in Table 2.

Several articles have shown the relationship between common channel length, weight loss, and malnutrition rate. Sugerman et al. (1997) found that all 5 patients submitted to DRYGB with 50 cm long CC developed PCM requiring revision procedure, whereas out of 22 patients with 150 cm long CC, only 3 (13.6%) developed PCM needing reoperation. Kellum et al. (2011) cited that out of 23 patients with a 50 cm long CC, 13 (56.5%) required revision as compared with 8 out of 25 (32%) with CC $\geq$ 100 cm. Stefanidis et al. (2011), in an evidence-based review, demonstrated a significant positive impact to exist on weight loss when the length of the CC approaches 100 cm. Based on their findings, these authors recommended that to obtain the benefit of malabsorption for weight loss after RYGB, surgeons should focus rather on CC length than on AL or BPL length. BPD/SS $\geq$ 200 mL and BPD/DS/CC = 100 cm decrease the risk of revision surgery. No consensus has been observed to exist about the optimal length of the limbs of RYGB (Tacchino 2015), probably because other factors are participating. One example is the association between patient height and total small bowel length demonstrated by Tacchino (2015), what suggests that those measurements can also be of help in deciding on the appropriate length of the loops.

In the treatment of PCM after DRYGB (Fig. 3a), the aim with the revision procedures is to increase the absorptive area of the intestine, by means of lengthening the common channel (Fig. 3b, c) or by performing a proximal enteroenterostomy (Scopinaro 2008; Appresai and Murr 2012) (Fig. 3d).

Some conclusions concerning DRYGB are common in the studies shown in Table 2: a short CC (= or < 100cm) DRYGB results in an increase in weight loss but at the

Table 2 Surgical revision after malabsorptive procedures

Author	Primary surgery: n	SS(mL) AL/CC (cm)	PCM	Revision surgery (%)	Results
Topart and Becouam (2015) Review	BPD BPD/DS	300 mL 200/50 150 mL 200/100	Reason for revision and reversal up to 40–60%	Revision: 3–18.5 Reversal: 2.1–7 Revision: 0.5–4.9 Reversal: 0.2-0.6	PCM: 10%/years after BPD and BPD/DS Revision: remission of PCM
Scopinaro et al. (2005)	BPD: 429 BPD: 144	400 mL 200/50 200–500 mL 200/50	7% 2.1%	CCE 150 cm along BPL: 1.4 Intestinal restoration: 0.3	Mortality: 2.1% Remission of PCM
Scopinaro (2006)	BPD: 1217	200–250 mL 200/50	11%	CCE 150 cm	Learning curve dropped PCM from 15% to < 3%
Hess et al. (2005)	BPD/DS: 1000	40 Fr AL: 250–325	Reason for revision: 3.7% Reason for reversal: 0.61%	CCE: 3 proximal enteroenterostomy: 0.6	Follow-up ≥ 10 years: 95% good satisfaction
Marceau et al. (2007)	BPD/DS: 1423	250/100	Reason for revision: 0.7% Reason for reversal: 0.2%	CCE: 0.7 Total reversal: 0.5	Operative mortality: 1% Follow-up: 15 years
Marceau et al. (2009)	BPD: 248 BPD/DS: 438	250/50 250/100	Reason for revision: 21.7% Reason for revision: 1.4%	CCE 50–100 cm: 18.5 reversal: 2.7 CCE: 2 reversal: 0.5	Mortality 10 years: 0.8% Mortality 10 years: 8.4%
Fox et al. (1996)	DRYGB: 60	AL: 150 CC: 100	Hypoproteinemia: 40% PCM:33% Anemia: 37% Diarrhea: 20%	CCE: 3	Malnutrition requires close follow-up
Sugerman et al. (1997)	DRYGB: 27	CC 50 cm: 5 CC 150 cm: 22	100% 13.6%	Revision: 5/5 (100%) Revision: 3/22 (13.6%)	A 50cm CC: unacceptable mortality (40%) and morbidity NR
Fobi et al. (2001)	DRYGB: 65	CCS: 50%	15/65 (23%)	CCE: 6/65 (9.2%)	Mortality: 0 A DRYGB 100-150 cm CC increases PCM and WL
Kellum et al. (2011)	DRYGB: 48	CC 50 cm: 23 CC ≥100 cm: 25	PCM: 13 (56.5%) PCM: 8 (32%)	CCE: 56.5 CCE: 32	7–19 years after DRYGB Mortality: 8 (16.6%)

SS stomach size, *AL* alimentary limb, *BPL* biliopancreatic limb, *CC* common channel, *CCE* common channel elongation, *CCS* common channel shortening, *PCM* protein-calorie malnutrition, *BPD* biliopancreatic diversion, *BPD/DS* biliopancreatic diversion/duodenal switch, *DRYGB* distal Roux-en-Y gastric bypass, *NR* not reported, *FU* follow-up, *TPN* total parenteral nutrition *WL* weight loss

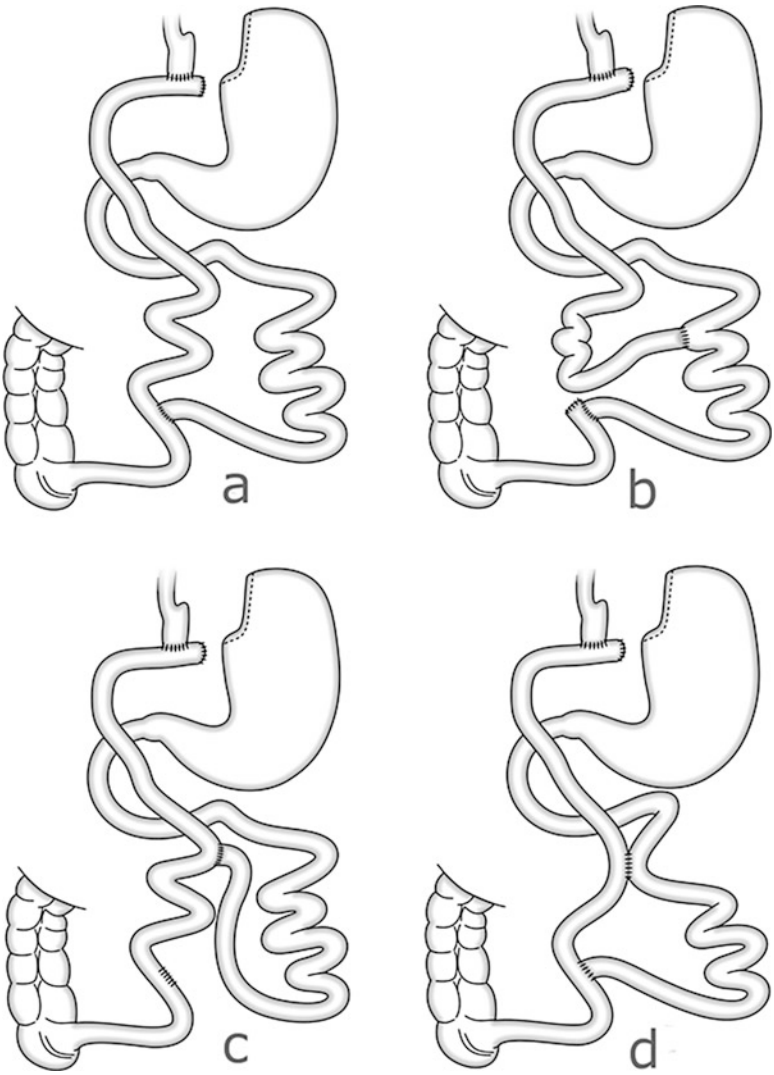

Fig. 3 Revision surgeries for distal Roux-en-Y gastric bypass. (**a**) DRYGB. (**b**) Elongation of 100–150 cm of the common channel along the biliopancreatic limb. (**c**) Elongation of the common channel along the alimentary limb. (**d**) Proximal enteroenterostomy

expense of an increased incidence of PCM and revision procedures requiring close monitoring and follow-up (Fox et al. 1996; Sugerman et al 1997; Kellum et al. 2011); results of CC lengthening revision to treat PCM are good. According to Sugerman et al. (1997), a 50 cm long CC results in unacceptable morbidity and mortality, and according to Kellum et al. (2011), DRYGB should not be the choice of primary operation for morbid or super obese patients.

Biliopancreatic Diversions

The prevalence of PCM after BPD or BPD/DS depends on the length of the common loop and ranges from 1% to 6% of the cases (Sudan and Jacobs 2011; Scopinaro 2006; Gagner 2010; Currò et al. 2015). Even though biliopancreatic diversion is one of the most efficacious surgical procedures for obesity, it is not very widespread because of the fear of long-term nutritional complications, besides complexity of the operation and lack of knowledge (Anderson et al. 2013; Ballesteros-Pomar et al. 2016). Despite the relatively small number of biliopancreatic diversions performed, the rates of revision surgery due to excessive protein malabsorption after those operations are relatively high. Topart and Becouarn (2015) found, in a review of 18 articles selected, a PCM rate of 10%; PCM was the cause for revision procedures up to 40–60% of the patients in 2 years after BPD and BPD/DS (Table 2). These authors also observed an incidence of those revision procedures to occur due to protein malabsorption of 3.0–18.5% after BPD and of 0.5–4.9% after BPD/DS. The prevalence of reversal surgeries is frequently reported to be in the 0.2–7.0% range after BPD. These rates are similar to those reported by other authors cited in Table 2. The prevalence of PCM after BPD or BPD/DS depends on the size of the stomach left and mainly on the length of the common loop and ranges from 1,4% (BPD/DS) to 21,7% (BPD) (Scopinaro et al. 2005; Scopinaro 2006; Marceau et al. 2009).

The revision procedures usually are common channel lengthening or enteroenterostomy and technically similar for BPD and BPD/DS. The length of the AL and of the CC has been, respectively, 200 cm and 50 cm for BPD and 200 cm and 100 cm for BPD/DS, although varying with the characteristics of the patient (Scopinaro 2008, 2012). Common channel lengthening is the revision procedure of choice for these diversions, usually with a 150 cm length for BPD and a 100 cm choice for BPD/DS, so that the length of the bowel in contact with food becomes 400 cm or longer with both techniques. The extension of CC lengthening reported in the literature varies among authors (Table 2), usually according to the severity of diarrhea and the occurrence or absence of associated PCM. Lengthening of the CC of the BPD (Fig. 4a) should be performed by proximally advancing the junction between AL and CC along the BPL as this will promote a greater increase of the absorptive area (Fig. 4b). The lengthening along the AL may be restricted to the rare cases of diarrhea due to excessive reduction of ileal absorption of bile salt (Fig. 4c) (Scopinaro 2008). The proximal enteroenterostomy is a simple and efficacious technique (Hamoui et al. 2007) (Fig. 4d). The longer the common channel, the greater the absorption capacity and the potential for PCM correction, but also the greater the possibility of weight regain.

The most frequently performed revision procedure for the treatment of PCM after standard RYGB is the reversal (Table 1) and for biliopancreatic diversions is the correction (Table 2). The term reversal of biliopancreatic diversion has been applied at times indistinctively to surgical revisions removing the malabsorptive component partially (proximal enteroenterostomy) or totally (anatomical continuity). It seems logical to restrict reversal or intestinal restoration after BPD to recover the entire absorptive area of the intestine, also including the duodenum in the food transit

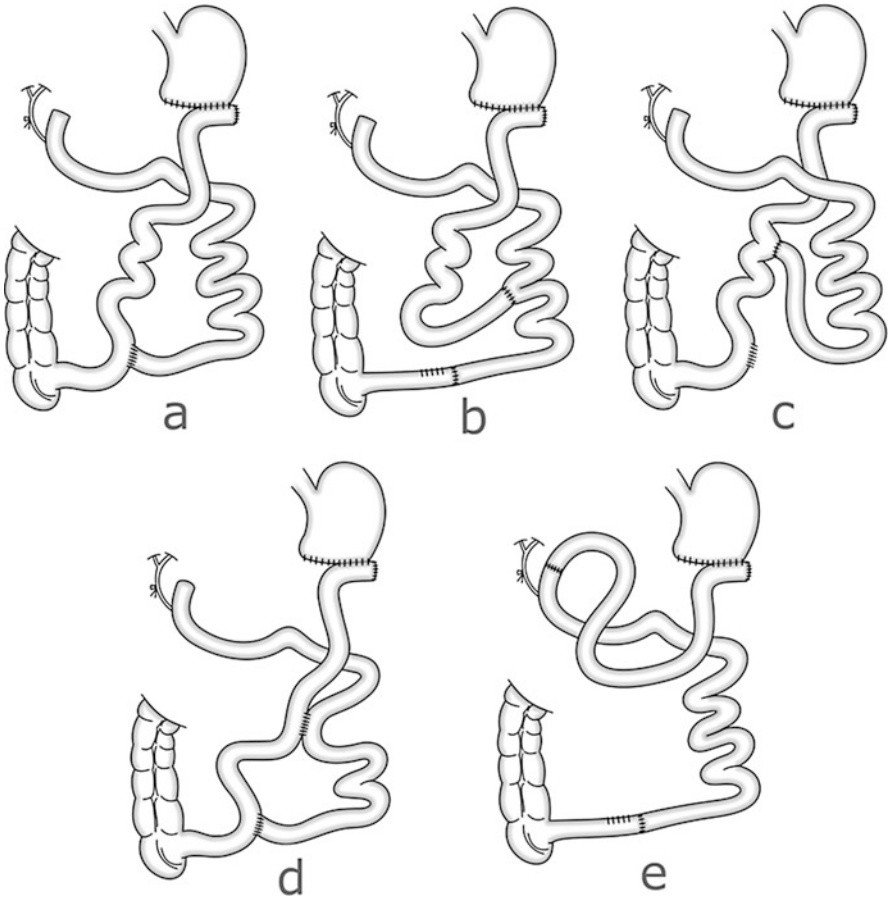

Fig. 4 Revision surgeries for biliopancreatic diversion. (**a**) BPD. (**b**) Elongation of about 150 cm of the common channel along the biliopancreatic limb. (**c**) Elongation of about 100 cm of the common channel along the alimentary limb. (**d**) Proximal enteroenterostomy close to angle of Treitz. (**e**) Restoration of the intestinal continuity

(Fig. 4e). The indications of reversal of biliopancreatic diversion are rare and usually performed after failure of revision (Scopinaro 2008). The procedure of choice for patients in hazardous physical condition is enteroenterostomy, which is faster to perform and less liable to postoperative complications. When malabsorption is serious, the enteroenterostomy should be performed close to the angle of Treitz. Some conclusions concerning BPD and BPD/DS have been drawn: BPD is a very efficacious surgery (Ballesteros-Pomar et al. 2016), and according to Scopinaro (2006), it is necessary to tailor the operation to the patient's characteristics, in order to achieve the best results with the minimum of complications; the BPD/DS is efficacious for all categories of morbidly obese patients, including the super obese (Hess et al. 2005); the DS improves the BPD, and according to Marceau et al. (2009)

and Sethi et al. (2016), it yields better results related to weight loss, revision, side effects, and absorption of nutrients.

Metabolic complications, including protein-calorie malnutrition, micronutrient deficiencies, and anemia, after bariatric procedures with a predominant malabsorptive component, can be fully reversed after appropriate lengthening of CC (Scopinaro 2008) and also after proximal enteroenteroanastomosis (Clare 1993; Appresai and Murr 2012) and reversal (Clare 1993).

Policies and Protocols

Policy

Prevalence of obesity is high and increasing worldwide, and in many countries, the supply of bariatric surgeries has not been enough to meet the existing demand. Patients who underwent bariatric surgery generally require medical follow-up throughout life, with more rigorous control in the first postoperative years. It should be advisable that the public health hospitals where bariatric surgeries are performed should be in charge of the postoperative control in the 1st years. However, it is not possible, due to the ever-increasing number of patients operated, to offer all necessary periodic follow-up. Thus, a scheme that has been followed at our Bariatric Centre provides clinical and hospital laboratory control at 1, 2, 3, 6, 12, 18, 24, and 36 months after surgery or at shorter intervals if needed. Then, if evolution is favorable, patients with good progress are counter-referred to the doctors who first referred them, instructing them through standard protocols containing guidelines on necessary follow-up, medication to be maintained, and exams to be requested in periodic checks. Those patients return to the hospital where they had been operated on at every 5 years after surgery or any time if the general practitioner deems it necessary. Schemes like this favor a better and sensible postoperative assistance to the patients and a standard survey of results.

Protocols

1. The preoperative preparation for bariatric surgery aims at improving the physical and psychic conditions of the patient. To reduce postoperative morbidity and mortality rates, the preoperative care should involve the treatment of frequent functional and metabolic disorders, such as severe sleep apnea and diabetes, as well as the improvement of the cardiorespiratory functional capacity through specific medical therapy, physiotherapy, and weight loss. By means of controlled diet and physical exercises, we aim, in the preparation for surgery, to reach, according to Tarnoff et al. (2008), a weight loss of about 10% and for the super obese (BMI > 50 kg/m^2) of 15%. This preparation is expected to result in several advantages: facilitation of surgical access, reduction in operative time, number and degree of complications, and length of hospitalization, besides contributing

to more rapid recovery and greater weight loss. Preoperative preparation may include the use of intragastric balloon to promote weight loss, particularly in patients with BMI $>$ 60 kg/m^2 and having uncontrolled comorbidities and/or difficulty to lose weight. The obese usually have inadequate eating habits, almost always eating hyper caloric diets and leading a sedentary lifestyle. It is important that they introduce the lifestyle changes already in the preoperative period, ingesting qualitatively and quantitatively correct food and doing regular physical activities, which should persist after surgery. The preparation time varies according to the patient's physical and psychic conditions and degree of engagement and may last a few to many months. Few patients, due to their bad health conditions, need a closer control with the use of the physiotherapeutic services of the Bariatric Centre itself.

2. The importance of adopting a healthy lifestyle before and after bariatric surgery has already been stated. Several schemes devised for losing and maintaining weight have been proposed. The WHO recommends doing moderate physical activity for 30–60 min a day, three times a week to maintain, and five or more times a week to lose weight. We follow a protocol for weight loss that includes physical exercises under the guidance of a physiotherapist and that has the following characteristics:
   - Frequency: 3 days a week up to 5–7 days a week.
   - Type of exercise: aerobic at least for 30 min and at most for 60 min, continuous or accumulated (walking, cycling, swimming, and/or water aerobics and treadmill, among others). Endurance exercises can be added to aerobic exercises.
   - Intensity: mild to moderate.
   - The exercise session should be divided into three stages: 5 min of warm-up, 50 min of constant intensity exercise, and 5 min of cooldown, plus stretches.
   - Wearing of loose clothes and comfortable shoes, preferably trainers, with soft soles and good impact absorption. Walking on stable surfaces.
   - Hydration before and during exercise.
   - The program should begin by doing a minimum of 30 min a day and increasing the session gradually up to the recommended 60 min.

The recommendation of rigid physical activity regimens for maintenance of weight loss is frequently difficult to follow by patients. It is important to know the limitations patients have to adapt their physical activity scheme, including by changing incidental and/or leisure activities.

Dictionary of Terms

- **Stenosis of the gastrojejunal anastomosis after gastric bypass** – a narrowing of the passage from the stomach pouch to the small intestine (gastrojejunal anastomosis). It is called anastomotic stenosis, also known as anastomotic stricture.

- **Laparotomy** – a surgical incision in the abdominal wall aiming at opening the peritoneal cavity, which offers surgeons a view inside the cavity, and being usually performed under general or regional anesthesia, with a therapeutic or exploratory purpose.
- **Gastroplasty** – any plastic surgery performed to reshape the stomach or repair any stomach defect or deformity. With respect to obesity, it is a surgical procedure to change the shape of the stomach aiming at limiting the gastric capacity and, therefore, reducing food ingestion. It is used in cases of severe obesity.
- **Gastrectomy** – a surgical procedure performed to remove all or part of the stomach.
- **Sleeve gastrectomy** – removal of the left portion of the stomach, performed typically in weight loss surgery, alone or combined with duodenal switch.
- **Distal gastrectomy** – removal of the distal portion of the stomach (the lower portion) in the biliopancreatic diversion (Scopinaro technique), used in cases of severe obesity.

Summary Points

- The demand for revision bariatric procedures increases with the increasing number of primary bariatric surgeries performed.
- Bariatric surgeries are an efficacious treatment for obesity, however are not free of early or late complications, including severe protein-calorie malnutrition.
- This chapter focuses on the surgical treatment of severe protein-calorie malnutrition (SPCM) after bariatric surgery, mainly on indications and surgical techniques used for revision.
- PCM after bariatric surgery may occur as a consequence of the restricted food ingestion and/or the malabsorptive effects of the bariatric surgery itself and also of other less frequent causes.
- The proposition of revision surgery should be made preferably by a health multidisciplinary team when there is an obstructive problem not solved by endoscopy or when there is a recognized failure of appropriate conservative treatment.
- An overview was given on the main surgical revision techniques.
- An attempt was made to describe the most accepted procedures by analyzing relevant published studies and the types of surgical treatment for SPCM after bariatric surgery.
- Surgical treatment has some particularities related to the type of primary surgery performed and mainly to the causes of the PCM.
- There are few clinical indicators or studies that guide strategies for the treatment for SPCM after standard Roux-en-Y gastric bypass.
- For malabsorptive surgeries, however, it is well established that the revision procedure of choice is the elongation of the common channel.

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Part VIII

Effects of Undernutrition, Endocrinology, Metabolism, and Tissue Systems

Endocrine Changes in Undernutrition, Metabolic Programming, and Nutritional Recovery

55

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Abstract

Undernutrition is a consequence of an unbalance between supply of nutrients/energy and the demand of the body to ensure its functions and growth. It has deleterious effects on the development of organs and growth, generating stunting and underweight in childhood. Globally, about 159 million children (<5 years of age) have stunting, and more than 50 million are underweight.

Undernutrition undermines economic growth, perpetuates poverty, and is associated with the development of noncommunicable diseases in the long term. Several studies have demonstrated that fetuses and infants under a limited supply of nutrients program their metabolism to ensure their survival by sparing energy and selectively preserving tissues and organs. This strategical programming results in specific metabolic and endocrine changes that remain throughout the life span and in the next generations. The aim of this chapter is to describe the major endocrine changes in undernutrition and, in addition, to present some results of an adequate recovery in height and weight in the first 5 years of life.

Keywords

Cortisol · IGF-1 · Thyroid hormones · Insulin · Leptin · Reproductive hormones · Undernutrition · Stunting · Low birth weight · Metabolic programming · Nutritional recovery

List of Abbreviations

ACTH	Adrenocorticotropic hormone
α-MSH	Alpha-melanocyte stimulating hormone
CART	Cocaine amphetamine-regulated transcript
CREN	Center for nutrition education and recovery
CRH	Corticotrophin-releasing hormone
FSH	Follicle stimulating hormone
GH	Growth hormone
GnRH	Gonadotropin-Releasing Hormone
GR	Glucocorticoid receptor
HOMA	Homeostasis model assessment
HPA	Hypothalamus-pituitary-adrenal
HPT	Hypothalamus-pituitary-thyroid
IGF	Insulin-like growth factor
LH	Luteinizing hormone
POMC	Proopiomelanocortin
T3	3,5,3′-Triiodothyronine
T4	3,5,3′,5′-Tetraiodothyronine (or thyroxine)

TNF	Tumor necrosis factor
TRH	Thyrotropin-releasing hormone
TSH	Thyroid stimulating hormone

Introduction

Undernutrition in the critical windows of body development promotes endocrine and metabolic changes to guarantee immediate survival that seems to have long-term deleterious effects (Martins et al. 2011). Some of these adaptations have programming effects that are being progressively clarified. Undernourished children and adolescents, for example, show metabolic and endocrine alterations that increase the risk of noncommunicable diseases (Reynolds 2013). Moreover, studies in short stature adults have demonstrated higher prevalence of diabetes, cardiovascular diseases, and obesity; and significantly lower labor capacity (Florêncio et al. 2008).

The aim of this chapter is to describe the major endocrine changes in undernutrition and, in addition, to present some results of an adequate recovery in height and weight in the first 5 years of life.

Hypothalamus-Pituitary-Thyroid Axis

3,5,3′-triiodothyronine (T3) and 3,5,3′,5′-tetraiodothyronine (T4) are the major hormones produced by the thyroid gland and have a significant role in several physiological processes such as linear growth, neural development, metabolic rate, and body temperature (McAninch and Bianco 2014). The thyroid produces T4 in higher concentrations than T3, but in peripheral tissues, T4 can be converted to T3, by deiodinases. T3 is the major active thyroid hormone. The production of these hormones is controlled by the hypothalamus-pituitary-thyroid (HPT) axis.

The hypothalamic arcuate nucleus integrates signals that originate in peripheral tissues, such as the hormones ghrelin, leptin, insulin, and the metabolites glucose and fatty acids. It responds to different conditions such as hunger or satiety by activating orexigenic neurohormones such as neuropeptide Y and agouti-related peptide, or anorexigenic hormones such as proopiomelanocortin (POMC), alpha-melanocyte stimulating hormone (α-MSH), and cocaine amphetamine-regulated transcript (CART), respectively (Joseph-Bravo et al. 2015). When orexigenic neurons are active, there is a decrease in TRH expression, while anorexigenic neurons activate TRH expression. In animals submitted to fasting, the central administration of α-MSH or CART avoids inhibition of TRH gene expression and can maintain or increase TRH release (Fekete and Lechan 2014). Fasting can also accelerate the inactivation of T4 and T3 by conjugation with glucuronic acid (McAninch and Bianco 2014). Lower concentrations of T4 and T3, such as observed in severe hypothyroidism, can decrease the total energy expenditure about 50% (McAninch

and Bianco 2014). These data together show the importance of the HPT axis in response to energy deficits.

Children with kwashiorkor, a severe form of undernutrition, show a decrease in circulating concentrations of T4 and T3 due to the decrease in the carrier proteins, thyroid hormone binding globulin and thyroid hormone binding prealbumin and albumin (Kumar et al. 2009).

The duration of undernutrition appears to be important in determining alterations in the HPT axis (Brown and Brasel 1990). In acute undernutrition, there is a reduction in total T3 and T4 concentrations secondary to the reduction on plasma proteins, with maintained euthyroid status, whereas prolonged and severe undernutrition damage the adaptive mechanisms resulting in hypothyroidism with low concentrations of free T3 and an increase of reverse T3 (inactive form) (Waterlow et al. 1992). The lower T3 reduces thermogenesis and oxygen consumption, which allows a better conservation of energy across the insufficiency of energy. In addition, the decrease in peripheral level (liver and kidney) of the enzyme activity that converts T4 to T3, 5′- deiodinase (which promotes the hormone action at the target cell) can contribute to the lower concentrations of thyroid hormones in undernutrition (Waterlow et al. 1992). Another physiological adaptation in undernourished children can be the inhibition of the thyroid function caused by higher activity of hypothalamus pituitary adrenal axis (HPA) (Joseph-Bravo et al. 2015).

It has been described that undernutrition during gestation programs thyroid status in adulthood. Women with low birth weight and low stature show increased risk of spontaneous hypothyroidism (Kajantie et al. 2006). One study with offspring rats undernourished during gestation and lactation found normal T4 and TSH but lower T3 at weaning, indicating normal thyroid status but decreased function in target tissues (Ayala-Moreno et al. 2013). The weaning animals then received ad libitum access to food and on the 90th day normal concentrations of T3, but lower free T4 and higher concentration of TSH were found compared to controls, indicating persistent thyroid impairment.

Stunting in less severely undernourished children promotes changes in thyroid status as well. Normal concentrations of TSH and free T3, but lower free T4, were found in stunted children when compared to nonstunted controls (Martins et al. 2016). Similar findings were observed in short stature Brazilian women (but not men) with overweight/obesity, since it was found lower T3 concentrations in these short stature women in comparison to overweight/obese women with normal stature (Sawaya et al. 2009).

Hypothalamus-Pituitary-Adrenal Axis

The adrenal gland produces the stress hormone cortisol through stimulation of HPA axis. Undernourished individuals have higher cortisol concentration (Romero et al. 2009). The production of cortisol depends on the release of corticotrophin-releasing hormone (CRH) by paraventricular nucleus in hypophyseal portal system that stimulates synthesis and secretion of adrenocorticotropic hormone (ACTH) by

adenohypophysis. ACTH then acts in the zona fasciculata of adrenal gland stimulating cortisol production (Gunnar and Quevedo 2007).

Cortisol has catabolic effects that are important during undernutrition, as it promotes the increase of gluconeogenic activity, proteolysis, and lipolysis. These effects are responsible to maintain normal glycemia and to ensure energy supply to the brain. Glucose is the major energetic substrate of the nervous system. Cortisol interacts with glucocorticoid receptors (GR) located in the cytosol and nucleus of the target cells to promote its effects. Marasmic children without infection show higher nuclear GR in leukocytes in comparison to well-nourished children with infection (Manary et al. 2006). However, cortisol concentrations in these children were lower than in those with marasmus and infection. This is important because it demonstrates that infection is a strong stimulus to increase cortisol concentrations. Moreover, the higher number of GR demonstrates that nutritional status modulates glucocorticoid receptor action, in addition to the increase in circulating glucocorticoid concentrations (Manary et al. 2006).

Changes in the epigenetic status of the GR were found in the liver of offspring rats that were fed a protein-restricted diet during the intrauterine period (Stevens et al. 2011). These animals showed a decreased GR methylation, with a 200% increase in GR expression in comparison to controls. Furthermore, these changes persisted in the offspring even though the dietary restriction had stopped, suggesting that the methylation status of genes is potentially permanent.

Epigenetic effects in cortisol response have also been described in human fetus that suffered intrauterine undernutrition (Weaver 2009). Children born at term but with low birth weight, for example, show higher cortisol concentration at 10 years of age (Cianfarani et al. 2002). Another example of the reprogramming of HPA axis is the change in the activity of the 11 Beta-hydroxysteroid dehydrogenase type 2 enzyme. This enzyme converts cortisol to cortisone and constitutes a placental barrier that protects the baby from the higher maternal cortisol concentration during stress conditions. Undernutrition promotes a down regulation of this enzyme and a fetal overexposure to maternal cortisol (Draper and Stewart 2005).

Higher maternal cortisol concentrations present in undernourished pregnant animals in the latter half of pregnancy contribute to change the set point of HPA axis in the offspring, making this axis more reactive to stress (Reynolds 2013). Thereafter, many studies have pointed out cortisol as one of those hormones responsible for the higher risk of noncommunicable diseases in adult life among undernourished individuals (Reynolds 2013).

Growth Hormone and Insulin-like Growth Factor-1 Axis

One of the most evident consequences of undernutrition is the restriction on linear growth. This is mainly a result of a disruption on the growth hormone (GH) –insulin-like growth factor-1 (IGF-1 or somatomedin C) axis. Children with marasmus or kwashiorkor have higher GH concentration, whereas the hepatic IGF-1 production is reduced when compared to normal children (Kilic et al. 2004). This can be explained

by a decreased negative feedback at the pituitary level due the lower concentration of IGF-1. IGF-1 acts in hypothalamus and pituitary decreasing secretion of growth hormone releasing hormone and GH, respectively. In addition, the lower IGF-1 concentrations, despite of higher GH, seem to be due to three factors: (1) lower expression of the hepatic GH receptor or defect in intracellular mechanisms postreceptor such as found in animal models of starvation and protein deficiency, respectively; (2) lower blood insulin and T3 concentrations (Fazeli and Klibanski 2014); and (3) lower essential amino acid concentrations (Thissen et al. 1999). This GH resistance allows the increase of lipolysis and gluconeogenesis and, consequently, brain energy availability (Fazeli and Klibanski 2014). The lower IGF-1 concentration, on the other hand, is an important determinant for the decrease in linear growth. For this reason, IGF-1 is considered a biomarker of nutritional status in children (Hawkes and Grimberg 2015).

Insulin and Glucose Metabolism

Changes in insulin concentration are common in undernourished individuals. Severe hypoglycemia is a signal commonly found in terminal cases, but in general, undernourished individuals show low or normal fasting glycemia concentrations accompanied by low insulin. The production of insulin by the pancreas appears to be particularly affected. Lower insulin release following oral glucose tolerance test was observed among undernourished children (Das et al. 1998). In addition, a decreased activity of beta-cell function was found in stunted adolescents (Martins and Sawaya 2006) (Fig. 1).

The alterations in glucose and insulin metabolism seem to have a programming effect as well. Higher insulin sensitivity was observed in small for gestational age newborns (Soto et al. 2003). A study in Brazilian stunted adolescents found higher insulin sensitivity (HOMA-S) as well as lower insulin production (HOMA-B) (Fig. 1). Other studies found that undernutrition in the first year of life independent of the birth weight was associated with higher insulin concentration and lower insulin sensitivity, which worsened as BMI increased in adult life (González-Barranco et al. 2003). Brazilian adult women with short stature and obesity showed higher insulin resistance, together with altered glycemic and lipid profile, in relation to obese normal height women (Florêncio et al. 2007). Although an increase in total body mass was associated with a moderate decline in peripheral insulin sensitivity, abdominal obesity showed a much steeper decline in insulin sensitivity and was accompanied by reduced peripheral glucose stimulation and insulin production. In addition, compared to women of medium height, women with short stature had higher concentrations of glycated hemoglobin, total cholesterol, and LDL, whereas HDL cholesterol concentrations were significantly lower. Stature was identified as the main factor associated with insulin resistance. These findings demonstrate that undernutrition when associated with overweight generates worst metabolic consequences in comparison to normal height individuals with overweight.

Changes in the metabolism of glucose and insulin may also be observed in subjects with mild stunting (height for age between −2 and −1 Z score). Overweight

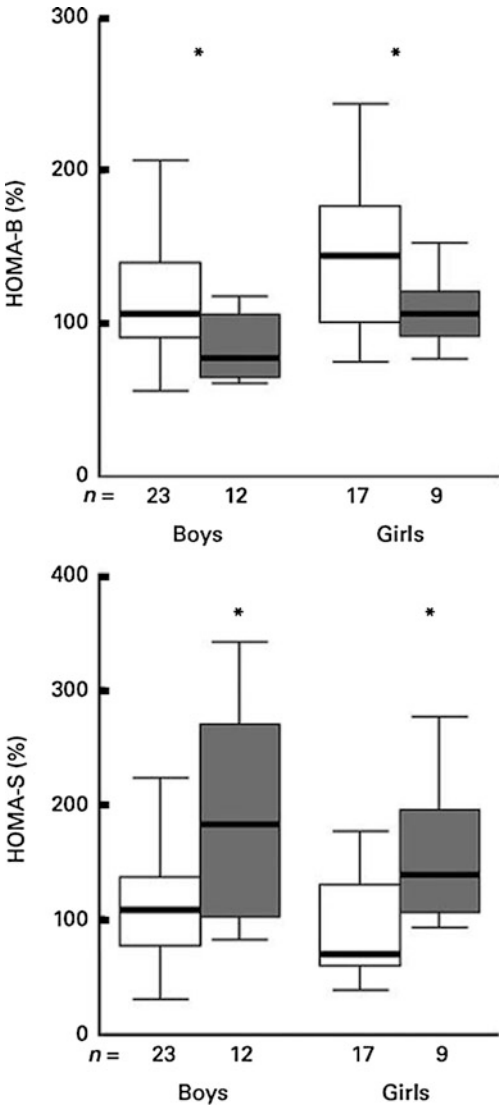

Fig. 1 Insulin production and sensitivity in stunted and well-nourished children. Homeostasis model assessment for pancreatic beta-cell function (*HOMA-B*) and for insulin sensitivity (*HOMA-S*) values of stunted (*black box*) and nonstunted (*white box*) boys and girls. The box represents the interquartile range which contains 50% of the values, the vertical bars indicate the highest and lowest values (excluding outliers) and the line across the box indicates the median. Significant difference between groups: $*P < 0.05$. Lower HOMA-B means lower insulin production and higher HOMA-S show higher insulin sensitivity, both in stunted children (Reprinted with permission from British Journal of Nutrition (2006), 95, 996–1001)

Brazilian adolescents with mild stunting showed higher blood glucose, insulin resistance, and lower insulin production (da Luz Santos et al. 2010). Moreover, adolescents with mild stunting presented elevated insulin concentration at a lower waist circumference deciles compared with nonstunted subjects (Fig. 2) (Clemente et al. 2014). The authors suggested that the increase in plasma insulin is one of the primary metabolic deviations that occur in stunted individuals and may be associated with the elevated risk of insulin resistance and diabetes found in short stature adults.

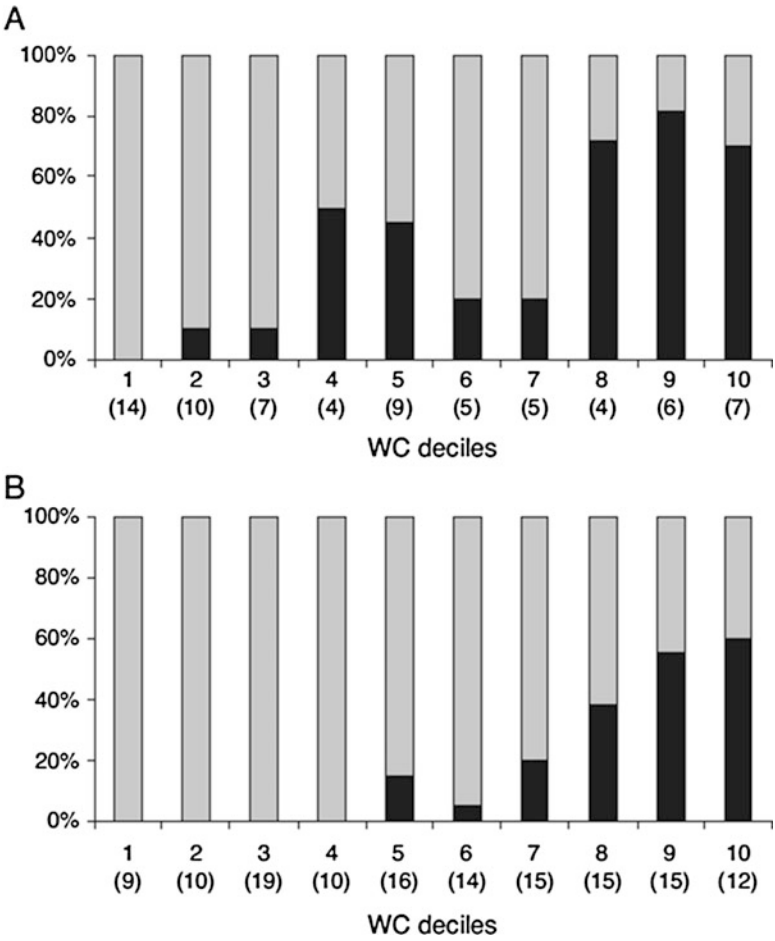

Fig. 2 Relationship between waist circumference and elevated insulin in stunted and well-nourished children. Distribution of stunted (**a**) and nonstunted (**b**) individuals according to waist circumference (*WC*) deciles and their respective prevalences of elevated insulin concentrations: (*black box*) > 75th percentile; (*gray box*) ≤75th percentile. The WC deciles correspond to the following absolute values of stature of studied population: (*1*) 53 cm, (*2*) 55.90 cm, (*3*) 57.50 cm, (*4*) 59.50 cm, (*5*) 62 cm, (*6*) 65 cm, (*7*) 68 cm, (*8*) 71 cm, (*9*) 76.74 cm. The numbers between parentheses represent the number of individuals of the sample in each decile of WC (Reprinted with permission from J Pediatr (Rio J). 2014;90(5):479–485)

Leptin

The use of fat stores is essential in situations of food restriction and undernutrition. Adipose tissue is the local synthesis of many hormones such as leptin, adiponectin, plasminogen activator inhibitor-1 (PAI-1), and others, which are collectively referred

to as adipokines. Leptin is considered an adipostat signal because it provides a good measure of the volume of the adipose tissue (Park and Ahima 2015).

Leptin is regulated by peripheral factors such as insulin, cortisol, estrogens and tumor necrosis factor alpha (TNF-alpha) (Park and Ahima 2015). It acts particularly at the hypothalamic level through binding to the ObRb receptor (Park and Ahima 2015); and its main action is to regulate (stimulatory effect) the expression of the anorexigenic peptides and inhibit orexigenic hormones in the nucleus arcuate. Leptin acts synergistically with the peripheral hormonal signals to influence the release or inhibition of these peptides and, consequently, the regulation of energy expenditure and eating behavior.

Leptin is considered a "starvation hormone" because of its strong signaling action in the central nervous system in energy deficit and the activation of counterregulatory mechanisms to conserve energy as the reduction of thyroid hormones, basal metabolic rate, and protein turnover (Prentice et al. 2002). Leptin also plays an important role in the control of linear growth, pubertal development, cardiovascular and immune function (Soliman et al. 2012).

Studies in children with kwashiorkor or marasmus have demonstrated lower leptin concentrations compared to healthy children (Soliman et al. 2000). Similar findings have also been observed in Brazilian children with mild to moderate undernutrition (Martins et al. 2014).

Reproductive Hormones

Undernourished children have delayed puberty and lower concentrations of FSH (Follicle Stimulating Hormone) and LH (Luteinizing Hormone) (Iwasa et al. 2015). The decrease in these hormones contributes to a delay in the menarche. It is well established that the organism has to reach a critical weight and body size for the initiation of puberty, regardless of the age at which started the spurt of adolescence growth, and leptin plays a key role in this mechanism (Iwasa et al. 2015). As the leptin concentrations are lower in undernourished individuals, the excitatory effect of leptin in the GnRH expression is impaired. In this condition, the activity of hypothalamus pituitary gonadal axis is decreased, explaining at least in part the delay in pubertal developmental in undernourished adolescents (Iwasa et al. 2015).

The major endocrine changes in undernutrition are summarized in Figs. 3 and 4.

Metabolic Programming

Changes in Body Composition

Undernutrition promotes long-term changes in body composition, by increasing central fat mass, and therefore, ensuring fast availability of energy (Martins et al. 2004; Hoffman et al. 2007). Undernourished children have also lower resting metabolic rate associated with lower lean mass and this decrease in energy expenditure helps the

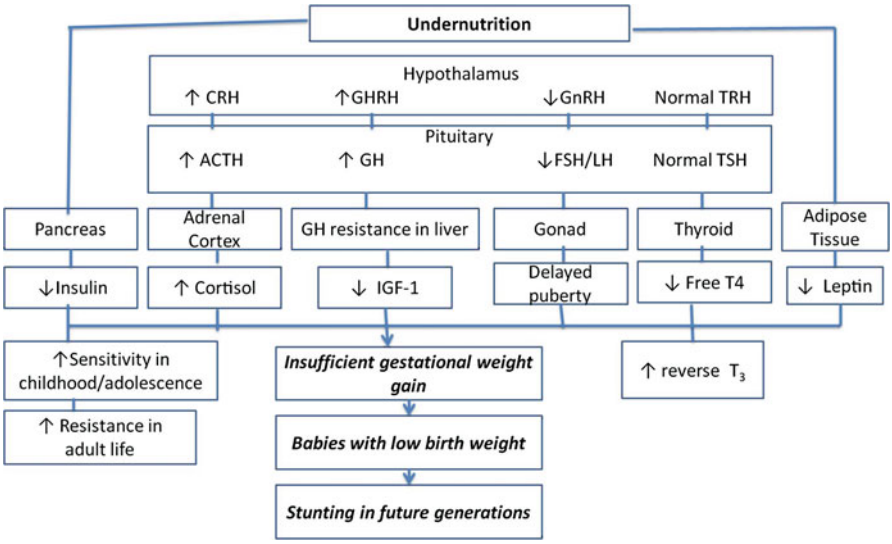

Fig. 3 Major endocrine changes found in undernutrition. These changes are associated with increased risk of development of noncommunicable diseases in adulthood and may impact next generations

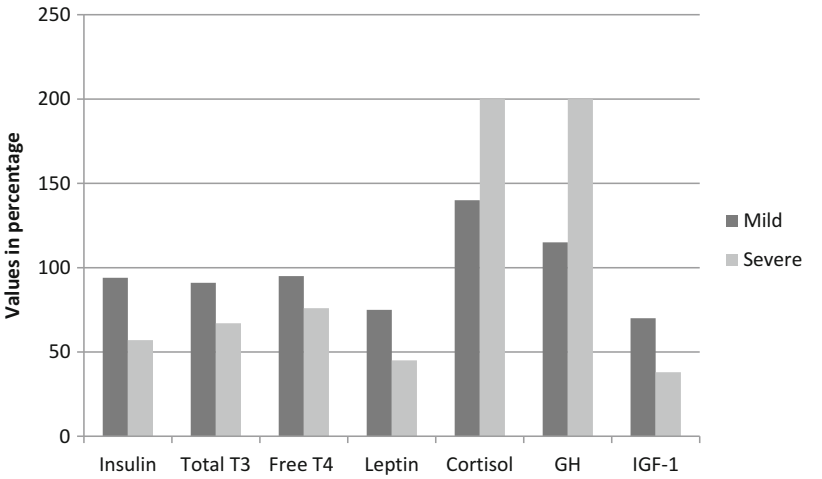

Fig. 4 Changes in hormonal concentrations in children that suffer mild or severe undernutrition. Hormonal concentrations are presented in percentage of normal hormone concentration (100%). Leptin, GH, and IGF-1 concentrations are influenced by age and gender. Cortisol concentration is positively associated to the degree infection. The increase or decrease in hormone concentrations depends of the degree of undernutrition, energy balance and protein intake. For example, acute severe undernutrition (72 h) can promote wide hormonal changes such as 75% decrease in leptin (accompanied by a weight loss). Children with kwashiorkor have high GH concentration and when treated with protein during 3 days show 50% decrease on GH concentration. This decrease does not occur when the children are treated with carbohydrate only

increase in fat mass (Soares-Wynter and Walker 1996; Sawaya et al. 2003). In addition, smaller increments in bone mineral density were described in undernourished adolescents of both sexes during prospective studies (Martins et al. 2011). A decrease in fat oxidation was also identified (Hoffman et al. 2000). These findings demonstrate that in environmental conditions where the consumption of energy and nutrients is insufficient or inadequate, the organism prefers to reduce growth and energy expenditure, while at the same time activating mechanisms of energy conservation.

Hypertension

High prevalence of hypertension has been found in children, adolescents (Fernandes et al. 2003), and adults (Florêncio et al. 2004) that suffer undernutrition. Intrauterine development of the kidney is particularly affected by maternal undernutrition due to the lower number of nephrons formed (Hinchliffe et al. 1992). The renal structure and specifically the number of nephrons are some of the main determinants of blood pressure and renal function, so that individuals with low numbers of nephrons have a predisposition to hypertension.

Maternal short stature is independently associated with obesity, abdominal obesity, and increased blood pressure and is an important determinant of children's health, as it is associated with low birth weight and stunting (Ferreira et al. 2009).

Some mechanisms have been proposed to explain the development of hypertension in this population. A deficit in elastin synthesis of the aortic wall and large vessels was described, and this deficiency may cause changes in the mechanical properties of the vessel (Martyn and Greenwald 2001). Changes in the renin-angiotensin-aldosterone and sympathoadrenal system also have been found. Girls born small for gestational age showed increased noradrenaline concentration when compared to those born with adequate weight for gestational age (Franco et al. 2008). The boys, on the other hand, showed increased activity of the angiotensin-converting enzyme and higher angiotensin II activity.

Diabetes

It is known that poor countries with accelerated urbanization have shown an increase in the prevalence of type 2 diabetes (Yajnik 2004). Diabetes among Ethiopian adults, for example, was shown to be associated with a history of undernutrition and lack of basic sanitation in childhood, reinforcing the importance of adequate postnatal development for long-term health maintenance (Fekadu et al. 2010). Adults who suffered intrauterine growth restriction have also higher risk of development of diabetes (Forsén et al. 2000).

Nutritional Recovery

One of the biological variables that have the greatest impact on the long-term health is stature. Special attention to the quality of the diet is then essential during nutritional recovery, especially in the quality of the protein intake, to allow the

gain in stature without an exaggerated increase in the energy supply. As an example, undernourished school-aged children treated with high-protein diet showed an increase in height directly related to the amount of protein supplementation compared to a group fed an oil-added diet (Kabir et al. 1998). Refeeding these children with normocaloric and normoprotein diet increased IGF-1 concentrations after 5 days by up to 70% of the basal levels before feed restriction, whereas refeeding with isocaloric but hypoprotein diet delayed recovery in IGF-1 for 2 days, and the concentrations of these hormones did not reach 50% of the prerestriction values (Thissen et al. 1999).

One strategy to adequate recovery in height and weight of undernourished children is the investment in the creation of rehabilitation centers with outpatient and day-hospital services. Few decades ago, some rehabilitation centers were established in Brazil in Southeast area in the city of São Paulo, and later in Northeast area, in the city of Maceió, one of the poorest areas of the country. These centers are called Centers for Nutrition Education and Recovery (CREN). They offer treatment to thousands of undernourished children living in urban slums every year. In the box, there is a detailed description of the methodology of the treatment developed at CREN in Brazil, aiming the recovery of height as well as weight.

Policies and Protocols

CREN Treatment Protocol at day Hospital

Active search is an important aspect of the CREN methodology to find undernourished children directly at the community level (Fig. 5). After identifying children with underweight and/or stunting by anthropometric census, families are visited at home and invited to CREN for treatment.

Any child under 5 years of age with weight-for-height and/or height-for-age Z score < -2.00 is eligible for day-hospital treatment. Children that present diseases which potentially could affect linear growth (e.g., hypothyroidism, deficiency of growth hormone, congenital cardiac diseases, genetic syndromes, or cystic fibrosis) are referred to other health services.

The daily follow-up aims at providing an overall improvement of the nutritional, cognitive, motor, psychological, and social status. The children stay at the center, from Mondays to Fridays, from 7:30 to 17:30. During the day, they engage in educational activities and are divided into groups of approximately 15 children, according to their ages. Pediatricians, nurses, dieticians, social workers, psychologists, and teachers participate in the treatment. The intervention included treatment of all diagnosed infections and other conditions, such as anemia and a diet that rotated daily every 11 weeks, as follows:

7:30–8:30: Patients are admitted. Breakfast (one serving of dairy and carbohydrates, such as bread, biscuits, or cake).
9:00: Snack (one serving of fruit).

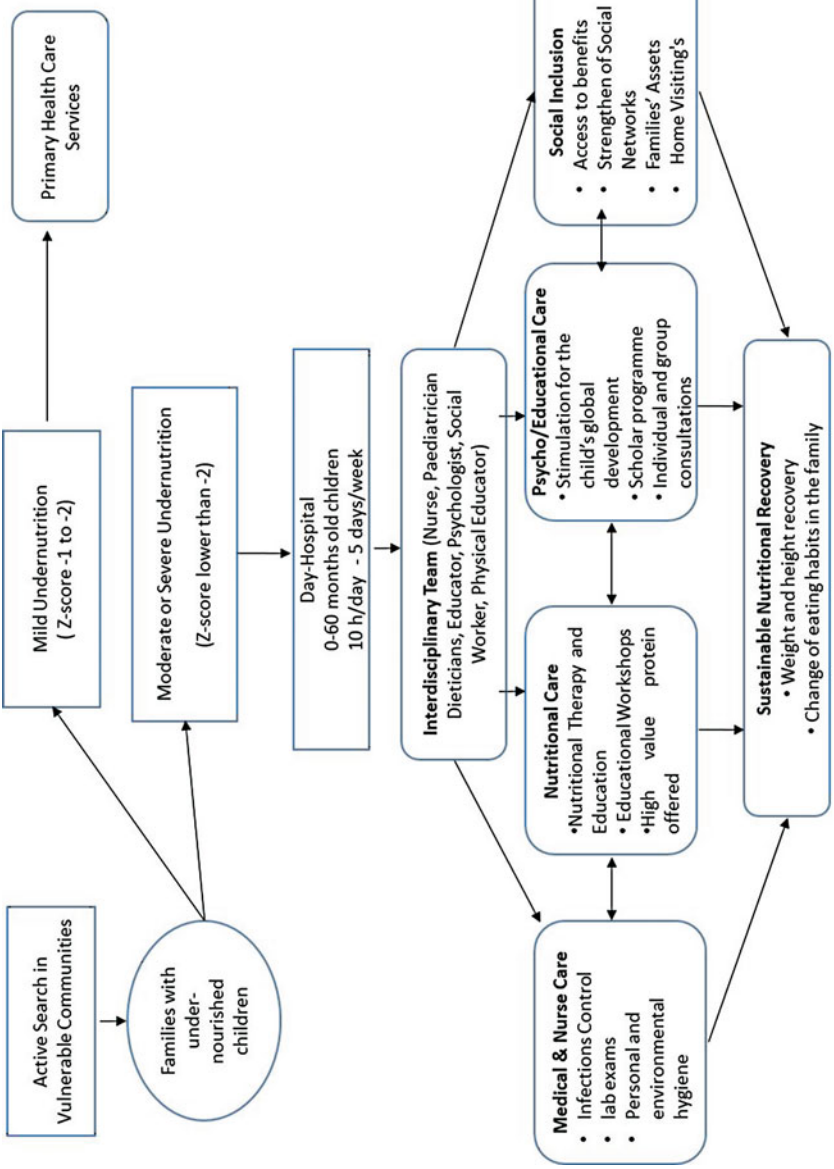

Fig. 5 Flow of day hospital treatment for undernourished children at CREN. For adequate treatment of undernourished children, an interdisciplinary team is necessary

11:30: Lunch: Rice, beans, meat or eggs, salad, and cooked vegetables with a dessert of fruit.
12:00: Nap.
13:30: Afternoon activity period.
14:00: Snack (one serving of dairy).
16:00: Afternoon meal: Rice, beans, protein (fish, beef, chicken, or pork), salad, and cooked vegetables with a dessert of fruit.
17:30: Return to home.

All children receive five meals each weekday using traditional Brazilian food such as: rice, beans, meat, fruit, and vegetables. Ultraprocessed foods are excluded. Meals are provided to meet 70% of daily energy requirements, 100% of daily dietary protein using biological high-value protein (meat, eggs, and milk), and recommended fiber intake according to the Dietary Reference Intake (Trumbo 2002). The meals provided to children supply approximately the following macronutrient composition as percent of total calories for children 6–12 months, 1–3, and 4–8 years of age, respectively: 45% carbohydrates, 15% protein, and 40% fat; 55% carbohydrates, 15% protein, and 20% fat; and 57% carbohydrates, 17% protein, and 18% fat. The family of each child is instructed to offer two more meals at home. Infant formulas are used for children less than 1 year of age who are no longer breastfed. Food supplements or special formulas are not used.

Micronutrient supplements like iron (Wayhs et al. 2012), zinc (Trumbo et al. 2001), and vitamins are used in prophylactic doses. Higher doses are used in cases of deficiency, with clinical or laboratory evidence, according to the recommendations of the Brazilian Pediatric Society (Wayhs et al. 2012; de Paula et al. 2016).

A pediatrician monitored the clinical status, laboratory results, and anthropometric progress of each child on a daily basis during their treatment at CREN as follows:

7:30–8:30: Patients are admitted and undergo a preliminary exam by nurses and are referred to the attending pediatrician for a physical exam. When health problems are detected, antibiotics, bronchodilators, and/or other necessary medications are prescribed.
9:00: Micronutrient supplementation: Vitamin complexes (A, B, C, and D) and Zn are provided to all children. Iron is provided according to age and laboratory test results. Administration of medications to patients as needed.
12:00: Oral hygiene and nap.
13:00: Monitoring of vital signs during sleep (i.e., temperature, pulse, and respiratory rate) and bathroom break (monitoring and recording of bowel movements and urination).
15:00: Administration of medications to patients as needed.
16:45: Oral hygiene and bathroom break (monitoring and recording of bowel movements and urination).
17:15: Consulting period with nurses to determine follow-up of medication protocol and continuation of basic health care at home for patients receiving medications.

Nutritional Education

An important aspect of CREN's intervention is nutritional education. The children participate in nutrition education workshops according to the psychomotor and cognitive readiness with the objective of facing the feeding problems. The contents of these workshops aim at the knowledge of the varieties of fruits and vegetables, to promote the neuropsychomotor development, improving the relationship between child and food, enhancing palatability, and promoting the development of good eating habits. Parents are also involved in treatment in frequent activities through the participation in regular nutrition education workshops for reinforcement of nutrition, for the expansion of its social networks and for health advice. Novel foods are also displayed during these meetings.

Monitoring and Treating Infectious Diseases

Children are monitored for infections on daily basis. Parents and caregivers are required as to the presence of symptoms such as fever, cough, runny nose, dyspnea, vomiting, diarrhea, or presence of worms in the stool. Positive responses are recorded. Diagnosis is recorded along with notation of medications prescribed. Intestinal parasites are confirmed by testing of stool sample or by maternal report of occurrence of intense anal itching or elimination of worms in feces. All children admitted at the Day Hospital receive deworming medication.

Discharge

Discharge from the day-hospital occur when the child reaches the weight and height for age greater than −1.64 Z scores or when they reach the age to enter regular school (6 years). Following discharge, the child continues to receive treatment using an established outpatient regimen.

A description of the nutritional and health outcomes of a sample of children treated at CREN showed that 92.5% of the children recovered at least one anthropometric index and 67.9% recovered weight and height (Alves Vieira et al. 2010). Almost half of the children presented nutritional recovery of more than 0.50 Z score in height for age (46.2%) and about 40% in weight for age (38.7%). The mean age at admission was 23.7 months, with an equal proportion of boys and girls. The mean duration of treatment was 16.4 months for all children, and the longer treatment time was associated with higher weight for age and height for age increases. The mean birth weight was 2563 g, and approximately 40% of the children were classified as low birth weight. The gain in stature was statistically different according to the birth weight, being greater among those who were born smaller. The most prevalent diseases during treatment were upper respiratory infections, and 82% of children showed at least one episode, 44% had diarrhea, and 18% had lower respiratory tract infections.

Recovered children at CREN present increase in height for age greater than weight for age, in general. Past studies showed normalization of body composition

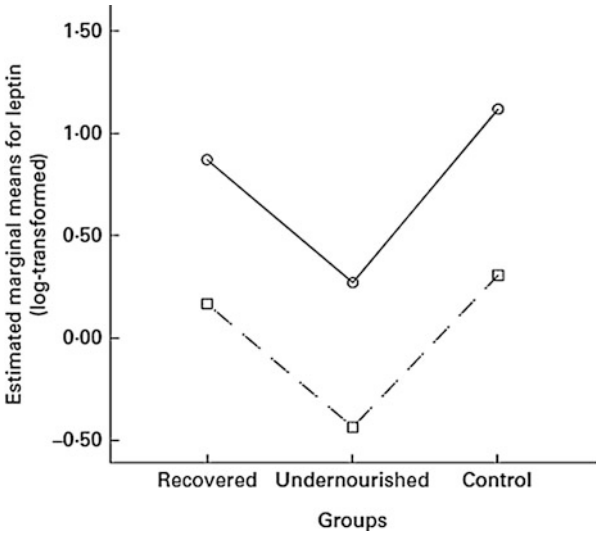

Fig. 6 Serum leptin concentrations in children recovered from undernutrition, undernourished, and well-nourished children. Boys (*dotted line*) have significantly lower leptin concentrations than girls (*continuous line*). Leptin concentrations in both sexes in the undernourished group were significantly lower than those in the other two groups. The scale is in logarithms (Reprinted with permission from British Journal of Nutrition (2014), 112, 937–944)

and bone mass (das Neves et al. 2006). In terms of food consumption, a study found higher protein intake in recovered children, compared to a control nontreated group of children living in the same poor communities, even after 6 years of discharge. Moreover, recovered children demonstrated normalization of insulin and glucose metabolism (Martins et al. 2008), and normal leptin concentrations in both sexes (Fig. 6) (Martins et al. 2014).

A study performed with the objective of determining cortisol activity found lower cortisol response after recovery comparing to undernourished children, but similar to that of well-nourished controls, indicating a normal HPA response after treatment (Martins et al. 2016). The daily cortisol response was also measured after an unpleasant stimulus (immersing the right hand in cold water for 1 min, at 10:00 h) (Fig. 7) and a pleasant stimulus (watching a video with pictures of nature at 14:00 h) (Fig. 8). After the application of the unpleasant stimulus, there was an increase in cortisol for all children (controls, stunting, and underweight) with exception of the recovered ones. No significant differences were found between groups in terms of response to the pleasant stimulus, with exception of a slight elevation in cortisol concentrations among undernourished children.

Another interesting result was lower free T4 concentrations in the recovered children in comparison to controls (Martins et al. 2016). This can indicate a programming effect that may lead to future accumulation of body fat, and this justifies the maintenance of a continuous observation of anthropometric and clinical indicators as well as encouragement for healthy lifestyles in these children.

In conclusion, programs and policies should be designed to prevent undernutrition taking into account the findings on its long-term effects on the health of the world's low-income population.

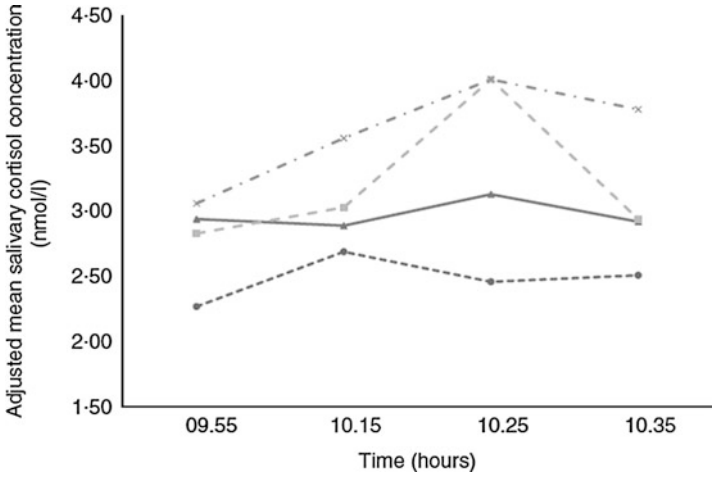

Fig. 7 Salivary cortisol concentrations in children recovered from undernutrition, undernourished, and well-nourished children submitted to unpleasant stimulus (cold pressor test). Salivary cortisol concentrations were similar in all groups before the application of the stimulus and increased after the unpleasant stimulus in the control, stunted, and underweight groups but not in the recovered group. Control; stunted; underweight; recovered (Reprinted with permission from Br J Nutr. 2016 14;115(1):14–23)

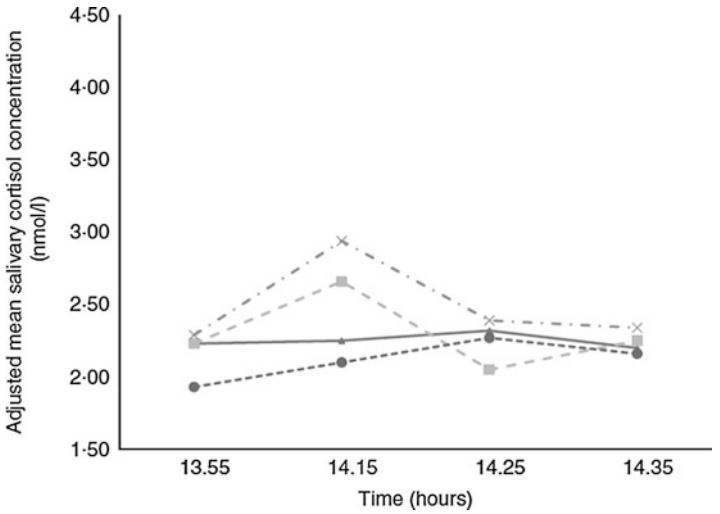

Fig. 8 Salivary cortisol concentrations in children recovered from undernutrition, undernourished, and well-nourished children submitted to pleasant stimulus (pictures of nature). No differences were found between groups in terms of response to the pleasant stimulus; however, the undernourished groups showed an increase of salivary cortisol after the pleasant stimulus in comparison with the recovered and control groups. Control; stunted; underweight; recovered (Reprinted with permission from Br J Nutr. 2016 14;115(1):14–23)

Dictionary of Terms

- **Undernutrition** – Refers to children with low weight for age, short stature for age or stunting.
- **Basal metabolic rate** – Oxygen consumption in total rest, refers to basal energy expenditure (after 12 h of fasting and 8 h of sleep).
- **Resting metabolic rate** – Oxygen consumption measured in recumbent position. This value is higher than basal metabolic rate.
- **Programming** – Adaptation to any kind of biological or psychological insult (low supply of nutrients and energy, for example) that occurs during critical periods of body development (intrauterine or postnatal period). This metabolic adaptation, at one hand, allows the individual to survive, but at the cost of permanent changes in the morphology and physiology of organs.
- **Z-score** – Can be positive or negative, with a positive value indicating the score is above the mean and a negative score indicating it is below the mean. Positive and negative scores reveal the number of standard deviations; the score is either above or below the mean.

Summary Points

- Undernutrition in early life promotes morphological and physiological changes associated with programming effects and noncommunicable diseases in adulthood.
- Undernourished children, adolescents, and adults show lower thyroid hormone activity.
- There is a marked decrease in IGF-1 in undernutrition, although higher GH concentrations can be observed.
- Undernutrition is a major form of stress and, therefore, shows higher cortisol concentrations.
- Undernourished children have normal/low glucose concentrations, lower insulin production, and higher insulin sensibility. This condition is associated with the development of insulin resistance in adulthood and diabetes.
- Lower concentrations of leptin can be observed in undernourished children.
- Undernourished children have delayed puberty and lower concentrations of FSH and LH.
- Undernutrition in early life is associated with the development of hypertension in adult life.
- Undernutrition in children promote changes in body composition. Higher fat mass and lower lean mass can be observed in stunted children.
- Adequate treatment is important to ensure recovery of height and weight. Recovered children show normal insulin, leptin, and cortisol concentrations.

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Thyroid Axis and Energy Balance: Focus on Animals and Implications for Humankind

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Patricia Joseph-Bravo, Mariana Gutiérrez-Mariscal, Lorraine Jaimes-Hoy, and Jean-Louis Charli

Contents

Abstract

Research on animals has revealed multiple mechanisms, brain circuits, and peripheral signals that coordinate energy homeostasis. This review summarizes information relevant to the hypothalamic-pituitary-thyroid axis, one of the outputs that the central nervous system uses to control energy utilization. It is hierarchically organized and controlled by paraventricular nucleus hypophysiotropic thyrotropin-releasing hormone neurons integrating central and

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peripheral information. These neurons regulate thyrotropin secretion from anterior pituitary and thyroid hormone production. Tissue concentrations of thyroid hormones depend in addition on transporters and deiodinases expressed by target tissues. Thyroid hormones control basal metabolic rate, thermogenesis, lipolysis, and glycolysis, as well as the development and performance of immune and nervous systems; they exert feedback control on the axis at multiple levels. Fasting, food restriction, malnutrition, stress, and disease downregulate the activity of the thyroid axis, an adaptation that minimizes energy utilization. In contrast, diet-induced obesity activates the axis, although deiodinase activities limit its capacity to compensate for energy excess. Maternal nutritional status or stress during gestation, and/or lactation, programs offspring's body weight, neuroendocrine axes, and energy metabolism in the adult. Studies in animals contributed to identify pathophysiological events of the thyroid axis associated with human diseases.

Keywords

Thyroid axis · Energy balance · Thyrotropin · Thyroid hormone · Thyrotropin-releasing hormone · Arcuate nucleus · Paraventricular nucleus · Adrenal axis · Stress · Deiodinase · TRH-degrading ectoenzyme · Programming · Diet · Tanycytes

List of Abbreviations

Note	Italics are used for gene or mRNA names, i.e., *Trh* for animals and *TRH* for humans, and capital letters, i.e., TRH, for peptides/proteins (HUGO Gene nomenclature Committee; Mouse genome informatics)
11-β-HSD	11-β hydroxysterol dehydrogenase
3V	third ventricle
Abd	abdominal
AC	adenylyl cyclase
Act	activity
ACTH	adrenocorticotropin hormone
AgRP	agouti-related peptide
Apit	anterior pituitary
AR	adrenergic receptor
Arc	hypothalamic arcuate nucleus
ATP	adenosine triphosphate
Avp	arginine vasopressin
BAT	brown adipose tissue
BBB	blood–brain barrier
BDNF	brain–derived neurotrophic factor
BP	blood pressure
BW	body weight
CART	cocaine- and amphetamine-activated transcript

CAs	catecholamines
Ch	cholesterol
Cort	corticosterone
CREB	cAMP-response element-binding protein
CRH	corticotropin-releasing hormone
Cx	cortex
d	days
db	diabetes mice
Dio1	deiodinase type 1
Dio2	deiodinase type 2
Dio3	deiodinase type 3
DMN	hypothalamic dorsomedial nucleus
E	embryonic
F	female
FA	fatty acids
FFA	free fatty acids
FR	food restriction
FT3	free triiodo-L-thyronine
FT4	free thyroxine
G	gestation
GABA	γ-aminobutyric acid
GC	glucocorticoids
GH	growth hormone
GR	glucocorticoid receptor
Gs	signal-transducing G protein
Hc	hippocampus
HFD	high-fat diet
HOMA-IR	homeostatic model assessment for insulin resistance
HPA	hypothalamic-pituitary adrenal axis
HPT	hypothalamic-pituitary-thyroid axis
Ht	hypothalamus
I− excess + LP	iodine excess plus a low protein diet
I	iodine
Ins	insulin
InsR	insulin receptor
JAK2	janus kinase 2
KO	knockout
L	lactation
LC	locus coeruleus
LDL-c	low-density lipoprotein cholesterol
LH	lateral hypothalamus
LP	low protein diet
Lpl	lipoprotein lipase
LPS	lipopolysaccharide

M	male
MBH	mediobasal hypothalamus
Mc4R	melanocortin 4 receptor
Mct10	monocarboxylate transporter 10
Mct8	monocarboxylate transporter 8
ME	median eminence
mo	months old
Mr	mineralocorticoid receptor
MS	maternal separation
NE	norepinephrine
NEFA	non-esterified fatty acids
NPY	neuropeptide Y
Nr3c1	GR gene
NTIS	non-thyroidal illness syndrome
NTS	nucleus tractus solitarius
OATP1C1	organic anion transporter family member 1C1
Ob	obese mice
ObRb	leptin's receptor b isoform
PD	postnatal day
Pepck	phosphoenolpyruvate carboxykinase
Pit	pituitary
PKA	protein kinase A
POMC	proopiomelanocortin
Ppargc1a	peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PPit	posterior pituitary
PTU	propylthiouracil
PVN	hypothalamic-paraventricular nucleus
RMR	resting metabolic rate
SCh	suprachiasmatic nucleus
SOCS3	suppressor of cytokine signaling 3
STAT3	signal transducer and activator of transcription 3
T-^{131}I uptake	thyroid ^{131}iodine uptake
T3	triiodo-L-thyronine
T4	thyroxine
TBG	thyroxine-binding globulin
Tg	thyroglobulin
TG	triglyceride
TH	thyroid hormone
TPO	thyroid peroxidase
TR or Thr	thyroid hormone receptor
TRE	thyroid response element
TRH	thyrotropin-releasing hormone
TRHDE	TRH-degrading ectoenzyme
TRHR1	TRH receptor-1

TSH	thyrotropin
TSH-R Tshr	TSH-receptor
TT3	total triiodo-L-thyronine
TT4	total thyroxine
UCP1	uncoupling protein 1
VMN	hypothalamic ventromedial nucleus
W	weight
WAT	white adipose tissue
wk	week
Y1/Y5R	NPY receptor-1/5
yrs	years old
αMSH	α-melanocyte-stimulating hormone

Introduction

Most anatomical and functional studies on the relationship between HPT axis and energy balance have been performed in rodents, due to their short gestation time and half-life. Molecular mechanisms are dilucidated in cell cultures or in mice with modified genomes. Research on ovine is also included as many temporal patterns of fetal tissue and organ development are similar to humans (Johnsen et al. 2013).

Energy balance relies on the adequate access of fuel, for metabolic and physical activity, provided by food intake or metabolism and mobilization of endogenous reserves (glycogen, adipose tissue, and protein in pathological circumstances) and modulated by the sympathetic system, GC, and TH. TH controls basal metabolic rate regulating enzymes of metabolic pathways in almost all cells and tissues; they are crucial for thermogenesis, mitochondrial biogenesis, various aspects of metabolism, development and performance of immune and nervous systems, central regulation of sympathetic outflow, and expression of adrenergic receptors at target organs. Symptoms caused by dysfunctions of the thyroid axis overlap with many found in the metabolic syndrome (Fliers et al. 2014; Mullur et al. 2014).

The hypothalamus is recognized as the "site" of homeostasis regulation. Circulating metabolites and hormones are sensed by hypothalamic structures involved in sensing energy status such as the Arc, ventromedial, dorsomedial, and PVN nuclei, as well as the LH (Fig. 1). Among them, the Arc, localized at the base of the brain with a loose blood-brain barrier, acts as an integrator of peripheral and central cues involved in feeding and energy status (Sutton et al. 2016). Two groups of neurons control food intake, and one synthesizes POMC and CART; POMC is processed to several active neuropeptides including αMSH, which acts through the Mc4R. The other is formed by neurons expressing *Agrp*, *Npy*, and GABA. AgRP binds to Mc4R opposing the actions of αMSH. This set of neurons, and their targets, form the arcuate-melanocortin system as their actions converge on the Mc4R receptor. POMC/CART neurons are activated in conditions of energy surfeit by leptin (hormone synthesized and secreted by adipocytes), insulin, and a variety of metabolites; they have anorexic actions and stimulate energy expenditure (Webber

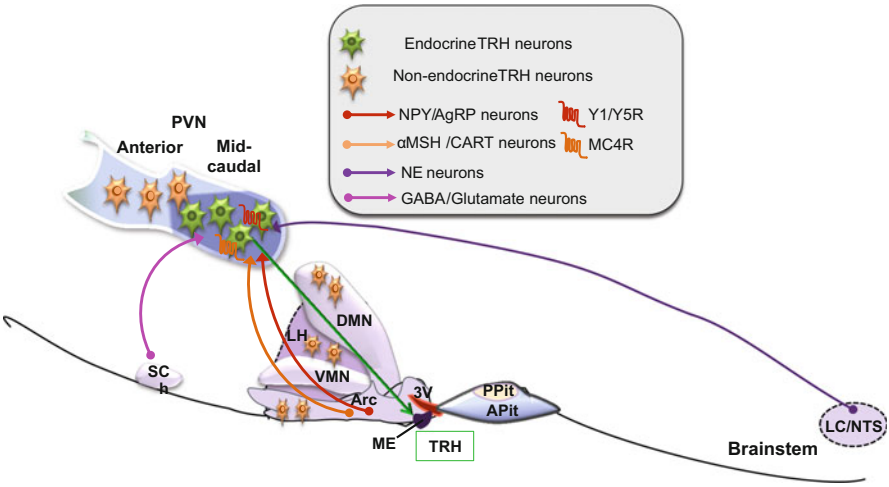

Fig. 1 Major hypothalamic nuclei involved in energy homeostasis and PVN-TRH neurons innervation. Innervations of mid-caudal paraventricular nucleus hypophysiotropic TRHergic neurons arising from the arcuate nucleus, suprachiasmatic nucleus, and brainstem (Modified from Joseph-Bravo et al. (2015a). Copyright © 2015, Society for Endocrinology)

et al. 2015). AgRP/NPY/GABA neurons promote hunger and lower energy expenditure and are activated by ghrelin, a hormone secreted by empty stomach. *Mc4r* KO mice are hyperphagic and obese (Sutton et al. 2016). Another crucial nucleus is the PVN that controls the HPT and HPA axes and is the principal hypothalamic output to brain stem nuclei controlling the sympathetic and parasympathetic nervous system (Fliers et al. 2014; Joseph-Bravo et al. 2015a).

The Hypothalamic-Pituitary-Thyroid Axis

The HPT axis is coordinated by PVN hypophysiotropic TRH neurons that project to the median eminence where TRH is released in the vicinity of portal vessels and β2-tanycytes (Fig. 2). Tanycytes express *Dio2* that transforms T4 to T3 and the *Trhde*, which inactivates released TRH. Together with TRH secretion rate, TRHDE sets the levels of TRH that enter the portal vessels communicating with the pars distalis of the pituitary. When TRH reaches thyrotrophs, it binds to TRHR1 and stimulates the synthesis of TSH, its glycosylation, and release to the systemic circulation; TSH secretion is also regulated by other hypothalamic and peripheral signals, including TH and GC (Mullur et al. 2014). TSH binds to TSH-R in the thyroid inducing the synthesis and secretion of T4 (Fekete and Lechan 2014; Joseph-Bravo et al. 2016). T4 is transformed to T3 in thyroid but mainly in target tissues by Dio1 and Dio2 (Fig. 2). TH travels in the bloodstream bound to proteins (transthyretin, thyroxine-binding protein, and albumin), leaving a low proportion of free hormones. The concentration of T3 is exquisitely modulated by deiodinases

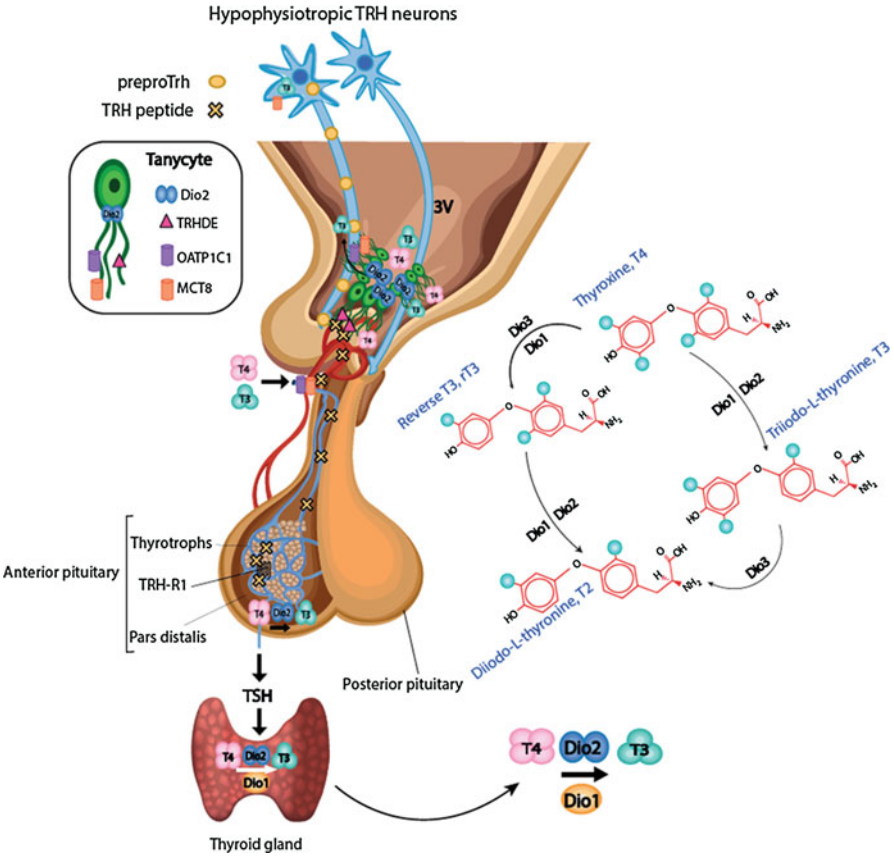

Fig. 2 Hierarchical organization of the HPT axis. Critical cells of the axis include the TRH neurons of the paraventricular nucleus, tanycytes, thyrotrophs, and follicular cells of the thyroid. Deiodinases 1, 2, and 3 can transform T4 to other thyronines in many cell types (Modified from Joseph-Bravo et al. (2016))

differentially regulated by the sympathetic system and by several hormones including TH, in a tissue-specific manner (Gereben et al. 2015). TH enters the cell through MCT8 (higher affinity for T3 than T4), MCT10, and OATP1C1 (more selective for T4 than T3) (Mendoza and Hollenberg 2017). The availability of TH also varies due to modulation of their clearance (Bianco et al. 2014).

In rodent hypothalamus, circulating T4 passes from blood vessels into tanycytes through OATP1C1, where it is deiodinated to T3, which is then transported to the extracellular space by MCT8. It has been hypothesized that T3 is then taken up by TRHergic nerve terminals that contain MCT8, retrogradely transported to the nucleus where it negatively regulates *Trh* transcription (Fekete and Lechan 2014). T3 in anterior pituitary inhibits transcription of *Tshb*. T3 binds to TR-α, TR-β1, or TR-β2 that recognizes TH-response elements in target genes; tissue and gene

specificity is provided by the type of TR and by the set of coregulators required for T3-regulated transcription. T3 or T4 acts also through membrane receptors (integrin αυβ3) and kinase transduction pathways (Mullur et al. 2014).

The activity of TRHergic hypophysiotropic neurons and, hence, of the HPT axis coincides with its active role in metabolism; they are regulated at different levels and in multiple conditions that drive acute or chronic metabolic changes. Understanding this multifactorial regulation aids to the comprehension of the role this axis plays in energy homeostasis, in the development and function of different organs, and in related diseases. HPT activity is modulated by circadian and nutritional status. PVN-TRH neurons are stimulated by αMSH through the Mc4R receptor and by leptin whereas inhibited by AgRP and NPY (Fekete and Lechan 2014). PVN-TRHergic neurons receive neuronal inputs from the suprachiasmatic nucleus which is the central sensor of light changes and by hormones modulated by day-night cycles which directly or indirectly influence HPT activity; plasma concentrations of TSH and TH are highest during the inactive period of animals (Fliers et al. 2014).

Finally, albeit a major role of TSH is to control TH secretion, TSH-R is also present in other organs where TSH activates Dio2 in BAT and affects membrane potential of cardiac cells or development of T cells (Fröhlich and Wahl 2016).

Hypothyroidism

In hypothyroid situations, TSH and TRH concentrations are elevated by lack of T3-negative feedback and the opposite occurs in hyperthyroidism. T3 feedback occurs at different levels of HPT axis (Fig. 3). Central hypothyroidism is produced in *Trh* or *Trhr1*-KO mice, whereas hyperthyroidism in *Tr*-KO mice (Mendoza and Hollenberg 2017).

Inhibition of the HPT axis leads to decreased metabolic rate, impaired thermogenesis, and altered lipid metabolism and cardio and muscle-skeletal systems. The activity of HPT axis is decreased in chronic situations as fasting, food restriction, malnutrition (Table 1), stress, and disease. Fasting or food restriction diminishes serum TH levels but, contrary to primary hypothyroidism when TSH levels are high, TSH and TRH (protein and mRNA) are decreased causing tertiary hypothyroidism. Fasting-induced inhibition of *Trh* expression involves decreased circulating leptin concentration, stimulation of AgRP/NPY/GABA and inhibition of POMC/CART neurons, upregulation in tanycytes of *Dio2* and of *Trhde* (Fekete and Lechan 2014), as well as inhibition of *Dio3*, the enzyme that degrades T3 (Fig. 2).

Stress activates HPA axis, which functions similarly to HPT axis: PVN neurons that synthesize and release CRH activate pituitary adrenocorticotropin synthesis and release which in turn regulates that of GC from the adrenal cortex (cortisol in humans; Cort in rodents). The HPA axis is activated by either physical or psychological stress-releasing GC within minutes. Physical stress (infection, cold, heat, pain) activates neurons in brain stem nuclei that connect directly with the PVN, whereas psychological stress activates limbic areas (amygdala, hippocampus, frontal cortex) that influence directly or indirectly PVN-CRHergic neurons (Herman and

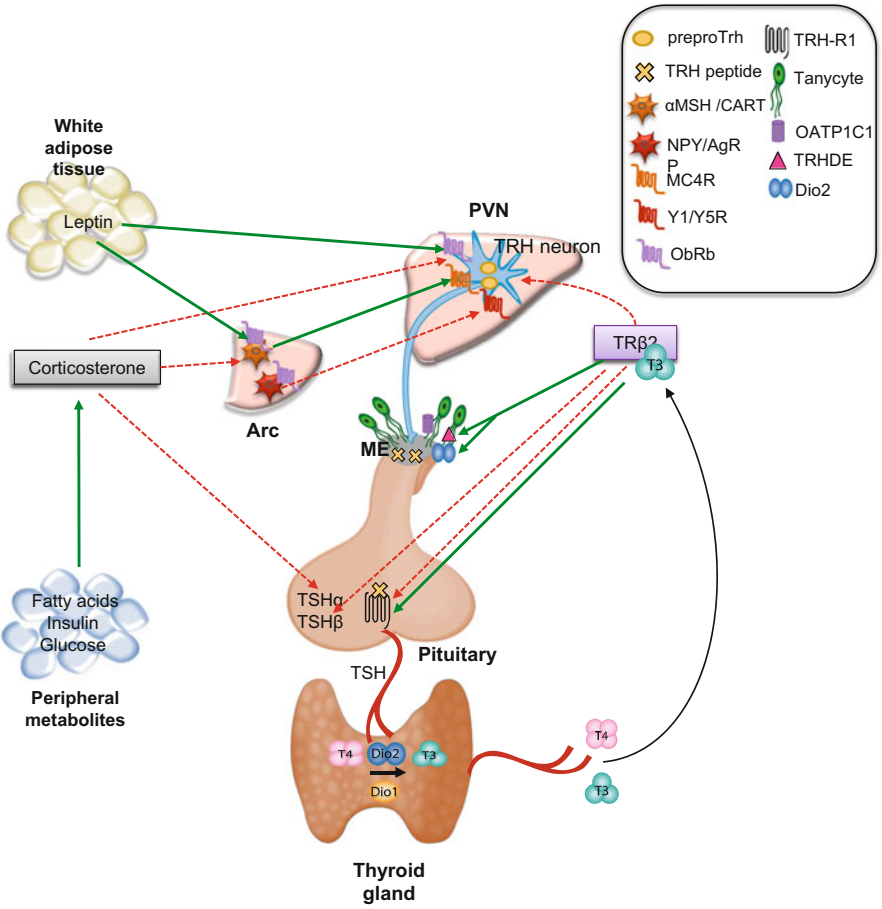

Fig. 3 Regulation of the central arm of the HPT axis by thyroid hormone feedback and energy balance signals. Thyroid hormones inhibit *Trh* and *Tsh* synthesis through TR-β2, stimulate the expression of *Trhde* and *Dio*2 in tanycytes, inhibit that of *Trhr1* in pituitary, and regulate either positively or negatively TRs and deiodinases, acting thus at many levels to set the activity of the HPT axis. Hormones and metabolites control the activity of TRH neurons directly or indirectly through Arc neurons (Modified from Joseph-Bravo et al. (2015b). Copyright © 2015, Society for Endocrinology)

Tasker 2016; van Bodegom et al. 2017). Several rat models of chronic psychological stress demonstrate HPT axis inhibition (decreased expression of PVN-*Trh*, pituitary *Tsh,* and serum concentrations of TSH and of TH). Administration of Cort for several days decreases PVN-*Trh* expression in male rats, as well as circulating TSH and TH concentrations, whereas the activity of Dio2 increases in tanycytes (Joseph-Bravo et al. 2015a; Moog et al. 2015). Stress affects HPT axis activity likely indirectly because chronic stress or Cort administration alters neuronal plasticity, dynamics of HPA responses, food intake, adiposity (high leptin serum levels), and increase Arc-*Npy*

Table 1 Effects of nutritional status on HPT axis in adult animals. The nutritional status of adult animals exerts profound effects on the hypothalamic-pituitary-thyroid axis; effects vary depending on the intensity and/or duration of the energy or micronutrient deficiency

Model	Duration and sex	Body W	HPT axis	Metabolic parameters
Fasting rat	36 h (M)	↓	Serum: ↓TT4, TT3 Liver: ↓TT3, *Dio1,* ↑*Dio3* mRNA and act, ↑*Mct10* (1)	Liver: T3 responsive genes: ↑*Pepck*; ↓*Fas*, *Spot14*
Fasting rat	36–72 h (M)	↓	PVN: ↓*Trh* (48 h) MBH: ↑*Trhde, Dio2* (48 h) ME: ↑TRHDE (72 h) Serum: ↓TSH (60, 72 h), ↓TT3 (36, 72 h), ↓TT4 (36, 48, 60 h) (Lazcano 2015 in [2])	Serum: ↑Cort (36, 60, 72 h)
Fasting rat	72 h (M and F)	↓	M: Ht: ↑Dio2 act; ME: ↑*Dio2*; serum: ↓FT4, FT3, TT4, TT3 F: PVN: ↓*Trh*; Ht: ↓TRH content/release; pit: ↓*Tshb*; Serum: ↓FT4, FT3, TT4, TT3 (3)	Arc: ↑*Agrp, Npy*; ↓*Pomc* Serum: ↑Cort; ↓leptin (M and F)
18% FR rat	14 days (M)	↓	PVN: ↓*Trh*; MBH: ↑Dio2 act; BAT: ↓*Ucp1*, Dio2 act; Liver: ↓Dio1 act Serum: ↓TSH, TT3 (Uribe 2014 in [4])	Serum: ↑Cort
33% FR rat	21 days (M and F)		M: Pit: ↓TSH; serum: ↓TSH, TT3, TT4, FT4, FT3, rT3 F: ME: ↑TRH; Pit: ↑*Tshb,* ↓TSH; serum: ↓TSH, TT3, TT4, FT4, FT3, rT3 (van Haasteren 1996 in [4])	Serum: ↑Cort (M and F)
50% FR rat	21 days (M)	↓	Serum: ↓TT4 Liver: ↓TT4, TT3, ↑*Dio3* mRNA and act, *Mct10* (1)	

FR bulls	125 days (M)	↓	Serum: ↓FT3, FT4, TT3, TT4 (5)	Serum: ↓insulin, glucose, leptin, TG; ↑NEFA, creatinine
Rat iodine deficient	3 months (F)		Serum, liver, heart, muscle, BAT, brain: ↓TT4; liver, heart, BAT, brain: ↓TT3; thyroid, pit, heart, muscle: ↑Dio1 act; thyroid, BAT, pit, cortex, heart: ↑Dio2 act; thyroid: ↑W (6)	
Rat: deficient: iron Selenium	4–5 weeks 6–8 weeks (M)	↓	Serum: ↓TT4, TT3; liver: ↓Dio1 act, TPO; blunted thermoregulation Serum: ↑TT4, ↓TT3; liver ↓Dio1 act; brain, pit, BAT: ↓Dio2 act (7)	BAT: ↓W
Rat: I^- excess + LP	6 months (M)		Serum: ↓FT4, TT4, FT3, TT3; thyroid: apoptotic cells (8)	

Numbers in parenthesis correspond to reference list
(1) de Vries et al. (2015b)
(2) Joseph-Bravo et al. (2016)
(3) Boelen et al. (2008)
(4) Jaimes-Hoy et al. (2016)
(5) Keogh et al. (2015)
(6) Lavado-Autric et al. (2013)
(7) Hess and Zimmermann (2004)
(8) Gao et al. (2013)
Symbols: ↓ reduced, ↑ increased versus control animals

and *Agrp* expression (Morris et al. 2015), which by themselves affect HPT activity (Joseph-Bravo et al. 2015a).

Infection induces, as chronic illness, a NTIS in humans characterized by low TH and low or normal TSH. LPS injection to rats, which mocks infection, diminishes *Trh* and *Tshb* expression, TSH and TH, while *Dio2* expression and activity in tanycytes rapidly increases even after interfering with variations in serum concentrations of T4 or Cort (Fekete and Lechan 2014). LPS also affects TH levels at the periphery altering hepatic and muscle metabolism. LPS injection to steers depresses TSH, T3, and T4 serum levels, and the effect is exacerbated if animals are kept at high ambient temperatures (32–40 °C vs. 19 °C) that also decrease HPT activity (Kahl et al. 2015). A rabbit model of NTIS caused by chronic inflammation of the limb reduces *Trh* expression in the PVN and T3 circulating levels; these animals have increased expression of *Dio2* (but not activity), of *Mct10* and *Oatp1c1*, and of T4 concentration in hypothalamus, but local T3 does not increase. As rabbits are parenterally fed, these effects are not due to negative energy balance (Fliers et al. 2014). Cytokines, secreted in inflammatory conditions, regulate pituitary expression and activity of *Dio1*, *Dio2*, and *Thrb* (depending on the species) liver *Dio1*, thyroid iodide transport, thyroglobulin, and peroxidase (de Vries et al. 2015a).

Hyperthyroidism

Hyperthyroidism and thyrotoxicosis are characterized by high TH and low TSH serum concentrations, causing symptoms such as palpitations, tremor, fatigue, anxiety, heat intolerance, polydipsia, risk of osteoporosis, and cardiac failure; these are accompanied by increased energy expenditure, loss of body weight, decreased gluconeogenesis, lipolysis, and cholesterol, many of which are reproduced in experimental animals (Mullur et al. 2014).

In male rodents, HFD starting at weaning, or in young adults, promotes central activation of HPT axis, as demonstrated by increased PVN-*Trh* mRNA and TRH levels, serum TSH levels, thyroid iodide uptake, and TH levels; however, small changes in Dio1 and Dio2 activities in the periphery limit the capacity of the HPT axis to compensate for energy excess (Araujo et al. 2010; Perello et al. 2010). Enhanced circulating leptin levels, induced by HFD, are likely causative for upregulation of TRH neurons (Perello et al. 2010), but leptin also regulates other aspects of TH metabolism, including deiodinases activity in various tissues, and TH hormone production (Fontenelle et al. 2016). The critical role of leptin is consistent with the hypothyroid phenotype of Ob/Ob or db/db mice (Myers and Leibel 2015). It should be noted that HFD duration, diet composition, development of leptin resistance, production of inflammatory cytokines, and sex and genetic propensity to obesity likely impact on HPT axis adjustments (Xia et al. 2015; Fontenelle et al. 2016).

Response of the HPT Axis to Acute Stimuli

Homeostasis requires rapid and efficient neuroendocrine responses to a threat. HPT axis is activated by energy demanding situations as cold exposure and physical activity. Within minutes, TRH and TSH are released followed by T4. Simultaneously, synthesis of TRH and of TSH increases transiently, contributing to replenish the depleted pools provoked by release. T4 and the sympathetic system activate BAT, an active thermogenic organ (Fig. 4); hypothyroid mice may die at low temperatures (Mullur et al. 2014; Joseph-Bravo et al. 2015a). Moderate exercise, or increased physical activity, activates the HPT axis at all levels: PVN-*Trh* and serum T4 concentrations increase proportionally to exercise performed. TH regulates mitochondrial muscle biogenesis, cardiac and respiratory fitness, as well as

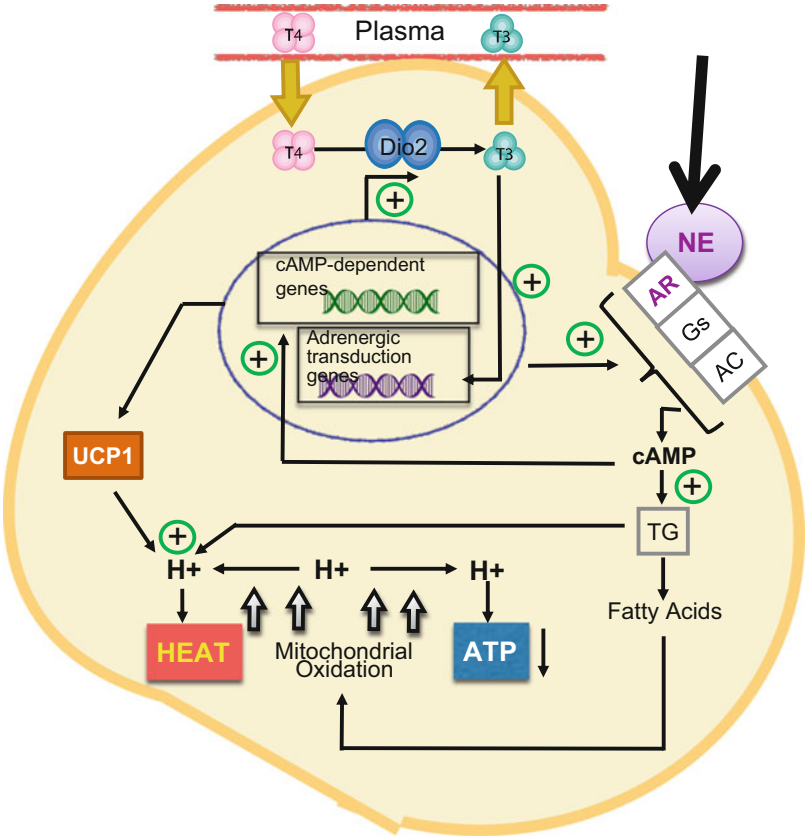

Fig. 4 Control of thermogenesis in brown adipose tissue. Adrenergic inputs activate Dio2 that transforms T4 to T3; T3 binds TR-β2 and stimulates synthesis of UCP1 and Dio2. UCP1 is activated in the mitochondrial membrane and uncouples the respiratory chain, resulting in heat production instead of ATP

lipid and glucose metabolism, mobilizing energy substrates to active tissues. Hypothyroid rats decrease their physical performance and show signs of fatigue. Exercise done to exhaustion or at high intensity decreases circulating TH serum concentrations as the organism detects negative energy balance (Joseph-Bravo et al. 2015a).

Acute stress inhibits the response of the HPT axis, but the magnitude of this inhibition depends on how strong or how long the stressor is perceived (Joseph-Bravo et al. 2015a; Moog et al. 2015). Stress or Cort injection blunts activation induced by cold or physical exercise: neither TRH, TSH, nor TH shows the cold-induced increase, nor Dio2 activation in BAT. The characterization of *Trh* gene promoter helped to unravel mechanisms by which hormones, neurotransmitters, and transduction pathways modulate transcription (Joseph-Bravo et al. 2015a, b, 2016). *Trh* transcription is activated by noradrenaline (via PKA), an effect inhibited by cotreatment with GC; the mechanism involves interaction between activated GR and PKA impeding phosphorylation of CREB and its binding to *Trh* promoter. This interaction (also shown for *Crh* transcriptional regulation) explains the fast interference of GC on the neuronal activation produced by cold that constrains the activation of HPT axis (Sotelo-Rivera et al. 2017 and references therein), although other unidentified mechanisms by which stress exerts inhibitory effects, at diverse levels and dynamics, likely exist. Fast inhibitory Cort effects on TRH neurons may contribute to the negative effects of stress on metabolism. Glucocorticoids also inhibit TSH release, as well as the activities of deiodinases.

Programming of the HPT Axis

HPA and HPT axes activities depend on the history of the animal. Interferences during development can produce deleterious outcomes that prevail until adulthood. The HPT axis of altricial mammals, as rodents, matures during the first 10 postnatal days, whereas in precocial species as lamb or human, most elements are fully expressed at birth (Forhead and Fowden 2014; Préau et al. 2015). The temporal expression profile of the elements involved in HPT activity varies in a tissue-dependent manner. Transporters, deiodinases, and TR appear early in development, but thyroid maturation is attained at midterm in sheep and human, whereas in rat it is delayed to the last gestation quarter. Maternal TH hormones are important for human and rat development during the first two thirds of fetal life; sheep, pigs, and horses are, in contrast, independent of maternal thyroid status (Moog et al. 2015; Forhead and Fowden 2014). TH bioavailability is controlled during gestation in rodents and humans, by regulating the amount of T4, T3, and iodine; for example, Dio3 presence in placenta prevents excess of T3 passing to the fetus (Moog et al. 2015). PVN-*Trh* expression increases to adult levels by the end of lactation, and feedback regulation of HPT axis appears postnatal; connections between Arc and PVN that regulate the activity of HPT axis are formed between the eighth and tenth PD in rat (Dearden and Ozanne 2015; Joseph-Bravo et al. 2016). Long-term effects of perturbations depend on the time window of development of the various participants (Préau et al. 2015).

THs are essential for adequate fetal metabolism (oxygen and glucose consumption), mitochondrial biogenesis, and differentiation and maturation of many organs and systems; timing of TH deficit or excess affects adequate development at various levels. Brain development involves TH-regulated genes for cell migration, proliferation, and maturation of neurons and glia (Préau et al. 2015). Deleterious effects on cognition in children from hypothyroid mothers have been long recognized and now characterized in several animal models; rats show deficiency in spatial learning tasks and decreased expression of nerve growth factor in hippocampus. TH effects on neuroplasticity occur even during adulthood (Moog et al. 2015; Raymaekers and Darras 2017). Congenital hypothyroidism in humans occurs with a single nucleotide polymorphic mutation in various elements of HPT axis; for example, MCT8 mutations produce psychomotor and brain retardation (Allan-Herndon-Dudley syndrome). Similar defects occur in double mutant *Mct8/Oatp1c1* (mice compensate MCT8 defect with OATP1C1, expressed lightly in humans and primates) (Mendoza and Hollenberg 2017). Offsprings from hyperthyroid dams show thyroid and brain developmental defects, increase in oxidative damage, and deformed neurons and glia in various brain regions of rats (Ahmed et al. 2012). Cardiac performance is impaired after an ischemic insult in adults whose mothers became hyperthyroid during the last half of gestation (Lino et al. 2015).

Mother Nutritional Status

Maternal protein or calorie restriction during gestation and/or lactation programs the offspring's body weight, causes dysfunction to various neuroendocrine axes, and alters energy metabolism and parameters regulating energy homeostasis as the melanocortin system in the adult; effects on thyroid function of the offspring vary depending on the severity of the restriction, the timing of gestation and/or during lactation, and the offspring's age and sex. As malnutrition evokes a hypothyroid condition in the mother, this probably adds up to the response of the offspring to nutrient deficiencies or other insults. Table 2 depicts the principal effects reported on HPT axis and associated metabolic variables. Food restriction decreases HPT activity, whereas protein restriction induces high TH levels in rat offspring due to higher transfer by milk (de Moura et al. 2008). Offsprings from 50% energy/protein-restricted mothers, raised on a high-carbohydrate/high-fat diet, are predisposed for hyperphagia and obesity (Wattez et al. 2013).

Maternal protein restriction during gestation and/or lactation and early weaning affects the structure of hypothalamic nuclei, energy expenditure, and body composition in the offspring, becoming susceptible to develop obesity and metabolic syndrome as adult; HPA development is affected, as well as adipocyte metabolism leading to fat accumulation later in life. Maternal undernutrition increases Cort secretion which reduces placental 11-β-HSD. Fetal Cort exposure affects vasculature and maturation of brain cells, while HPA tone is hyperactivated until adulthood, and GR methylation patterns are disturbed depending on the time of nutrient deficiencies (Correira-Branco et al. 2015; Wattez et al. 2013). As discussed below, stress affects HPT function, and

Table 2 Maternal nutritional status programs offspring's body weight, HPT axis, and energy homeostasis. Maternal caloric or protein restriction during gestation and/or lactation programs the offspring's body weight, energy metabolism, and hypothalamic and thyroid axis parameters regulating energy homeostasis. Effects are dependent on type, duration, and time period during which dams are exposed to the restriction, as well as on offspring age

Model	Programming period	Offspring sex and analysis time	Body W	WAT W	Food intake	Hypothalamus	HPT axis	HPA axis	Metabolic parameters
24 h fast rat	G: d14-21 L: d1-14	(M) PD14	↓				Serum: ↓TSH, FT3, FT4 ↑T-^{131}I uptake. Changes in serum are reversed in pups when mothers are refed for 10 days during the L period (1)		
20% FR rat	G: d1-14	(M) PD25-180	↑ PD180	↑ PD180	↑ PD25, PD180	PD25: Arc: ↓#NPY neurons Ht: ↓*Pomc*, *InsR* (García 2010 in [2])	PD25: Serum: ↓TT3 BAT: ↓UCP1, *Dio2* ↓ body temperature and BAT UCP1 in response to cold (3)		PD25: Serum: ↓leptin WAT: ↓*InsR* BAT: ↓*Lpl* PD180: ↑HOMA-IR
		(F) PD25-180	≠	≠	↑ PD25, PD180	PD25: ↓*Npy, Pomc, InsR, ObRb* ↓*Pomc, ObRb* (García 2010 in [2])	PD25: BAT: ↓UCP1 ↓ body temperature and BAT UCP1 in response to cold (3)		PD25: Serum: ↓leptin PD180: WAT: ↓*ObRb*

40% FR rat	G and L	(F) PD21-90	↓ PD21-90	↓ PD90	≠		Serum: ↓TT3 (PD21); ↑TSH and ↓FT3 (PD90) (Ayala-Moreno 2013 in [4])		PD90: ↓RMR
50% FR rat	G: d14-21 and L	(M) E21 PD7-70	↓ E21, PD7-21			↑BDNF (E21, PD7-14) ↑*Bdnf* receptor (PD14) ↓*Pomc* (PD14, 30) PVN, VMN, Arc: ↑cell proliferation (E21-PD15) ME: ↑cell proliferation (E21-PD8) (Coupé 2009 in [2])	Brain: ↓TT4 (PD23), TT3 (PD14), ↓Dio2 act (PD8, 14) Pit: ↓TSH (PD14-70) Heart: ↓TT4 (PD14), ↓TT3 (PD8-14) Liver: ↓TT4, TT3 (E21, PD14); ↓Dio1 act (PD8-70) Serum: ↓TT4, TT3 (PD14-70) (Aláez 1992 in [5])	E21: Hc: ↓*Mr*, *Gr*; Ht: ↓*Crh*; Serum: ↓ACTH, Cort PD120: Hc: ↑*Mr*, ↓*Gr*; Pit: ↑*Pomc*; PD320: Hc: ↑*Mr*, ↓*Gr*; Ht: ↑*Avp*; Pit: ↑*Pomc*; Serum: ↑Cort (Vieau 2007 in [6])	E21, PD21: Serum: ↓leptin
50% FR sheep	G: d105-147	(M) 6 months (F) 2 years					M: Heart: ↑*Dio2*, *Thrb* F: Thyroid: ↑*Tshr,* TPO; liver: ↑*Thrb*; heart: ↑*Dio2*, *Thrb;* WAT: ↓*Thr, Dio2*; serum: ↑TT3, TT4 (7)		

(*continued*)

Table 2 (continued)

Model	Programming period	Offspring sex and analysis time	Body W	WAT W	Food intake	Hypothalamus	HPT axis	HPA axis	Metabolic parameters
LP (8%) rat	L	(M) PD12-180	↓ PD25-180	↓ PD180	≠	↓αMSH in LH (PD16) ↑*Npy, Pomc, Agrp* (PD12) (Coupé 2010 in [2])	PD21: ↓T-^{131}I uptake; serum: ↓TT3, ↑TSH PD30: ↑T-^{131}I uptake; serum: ↑TT4 PD60: ↑T-^{131}I uptake PD180: ↑T-^{131}I uptake; pit: ↑Dio2 act; liver: ↑Dio1 act; muscle: ↑Dio1 act, ↓Dio2 act; serum: ↑TT3, TT4, TSH (8)	↑Cort, adrenal CAs (PD180) (8)	PD5-16: serum: ↑leptin PD21: Serum: ↓ins, TG; ↑LDL-c, adiponectin, leptin PD150: leptin resistance PD180: serum: ↓insulin, glucose
FR pair-fed to LP (8%) rat	L	(M) PD150	↓ PD8-21 ↑ PD 90-150		≠		Serum: ↑TT3, ↓TSH (PD150) (8)		PD21: serum: ↑leptin PD150: leptin resistance; pit: ↑cells expressing ObRb

Rat deficient	G	(M) PD12					TT4	TT3	TSH	Cx/Hc TT3	
Cupper			↓					↓			
Iron			↓				↓	↓	↑	/↓	
PTU			↓				↓	↓	↑	↓/↓ (9)	
Rat iodine deficient	G	(M)					TT4	TT3	TSH	Thyroid W	
		PD14	≠	≠							
		PD21	≠	≠			↓		↑	↑	
		PD60	≠	≠			↓			(10)	

Note: Italics in the table is used for gene or mRNA names and capital letters for peptides/proteins
Numbers in parenthesis correspond to reference list
(1) Fetoui et al. (2006)
(2) Wattez et al. (2013)
(3) Palou et al. (2015)
(4) Joseph-Bravo et al. (2015a)
(5) Joseph-Bravo et al. (2016)
(6) Yam et al. (2015)
(7) Johnsen et al. (2013)
(8) de Moura et al. (2008)
(9) Bastian et al. (2014)
(10) Gilbert et al. (2013)
Symbols: ↓ reduced, ↑ increased, ≠ no difference versus control animals

therefore, the effects of malnutrition on HPA axis may contribute to altered HPT development.

In rodents and nonhuman primates, maternal overnutrition before and/or during gestation and/or lactation can modify hypothalamic sensing and HPT axis activity at birth or weaning and leads to adult obesity and adaptations of the HPT axis that differ from those observed earlier (Table 3).

The thyroid axis is very susceptible to alcohol and nicotine that cause long-term HPT inhibition in the offspring and metabolic alterations (Portolés et al. 1988; Ramadoss et al. 2008; Lisboa et al. 2015). Research during the last decade has demonstrated the danger of endocrine disruptors, which are present in plastics, insecticides, flame retardants, and cosmetics to name a few; due to space limitations, it is not possible to cover this issue but comprehensive reviews are available (Duntas 2015; Préau et al. 2015).

Stress

The HPA develops in rodents during the first 2 weeks of gestation. During pregnancy, GC participate in the development and maturation of multiple organs, including the brain, pituitary, and thyroid. This occurs at defined time windows, and administration of high doses of GC at other times, as used in many therapies, may alter normal development. Furthermore, mother stress is sensed by the fetus that shows, in several species, similar changes in GC concentration as the mother (Moisiadis and Matthews 2014; van Bodegom et al. 2017).

GC administration, at an equivalent anti-inflammatory dose, at day 16 of gestation (when the thyroid is rapidly growing and maturing) diminishes the number of thyrotrophs in pituitary and inhibits thyroid growth but promotes its maturation which may affect their response as adults (Manojlović-Stojanoski et al. 2014). Dexamethasone injection to pregnant rats from day 18 to 21 decreases core body temperature as well as *Trh* mRNA levels, number of expressing cells, and stained fibers in the PVN of adult rat females, although males have normal to reduced postnatal TH levels with a tendency to remain low in adulthood (Carbone et al. 2012). In sheep, GC treatment near term modulates activities of Dio1 and Dio3 increasing T3 serum concentrations; variations in TH concentrations also affect the HPA axis supporting a dual regulation of both hormones for adequate development (Forhead and Fowden 2014; Moog et al. 2015).

Perturbations at postnatal critical periods like lactation or adolescence affect programming with long-term consequences in the adult. Meaney and his group elucidated the mechanism involved in the effect of inadequate caring by the mother during lactation that induces in pups an overreactive HPA axis when exposed to stress as adults. These animals present diminished hippocampal expression of GR (important for negative feedback of HPA), due to epigenetic changes on the promoter of *Nr3c1* which has been detected in several paradigms of early-life stress in animals and humans (Turecki and Meaney 2016). A model of MS of the pups for a few hours a day during the first 2 or 3 weeks of lactation reproduces these findings;

Table 3 Effects of maternal overnutrition on the offspring's HPT axis and energy homeostasis. Maternal overnutrition before and/or during gestation and/or during lactation programs the offspring's body and white adipose tissue weights, hypothalamic-pituitary-thyroid axis, and energy homeostasis

Model	Period	Offspring sex, analysis age	Body weight	WAT weight	Food intake	Hypothalamus	HPT axis	Metabolic parameters
Rats, HFD	8 weeks before mating + G, L	(M) PD21	↑	↑		Arc: ↓SOCS3, pSTAT3/ STAT3	PVN: ↑*Trh* Serum: ↑TT3, FT4 (Franco 2012 in [1])	Serum: ↑leptin, glycaemia Adrenal: ↓catecholamine WAT (inguinal): ↓β3-AR, ↑leptin. Liver: ↑β2-AR glycogen
Rats, 3 versus 10 pups/litter	L	(M) PD21, PD180	PD21, PD180 ↑	PD21, PD180 ↑	PD21, PD180 ↑	PD180: ↓JAK2, pSTAT3/ STAT3	Serum: PD21: ↑TT3, FT4, TSH. PD180: ↓TT3, FT4 (Rodríguez 2009 in [2])	PD21: serum: ↑leptin
Rats, 3 versus 10 pups/litter	L, from 3rd day	(M) Adult		↑			Ht: ↓*Trh*, ↑Dio2 act Pit: ↑Dio2 act. Pit explant: ↓TSH content, basal or TRH-induced release Thyroid: ↓Dio1 act; muscle: ↓Dio1 act WAT: ↓Dio1 act, ↑Dio2 act, ↓TRb; BAT: ↑Dio2 act; plasma: ↓TT3, FT4 (Lisboa 2015 in [2])	BAT: ↓UCP1

(*continued*)

Table 3 (continued)

Model	Period	Offspring sex, analysis age	Body weight	WAT weight	Food intake	Hypothalamus	HPT axis	Metabolic parameters
Rats, 2–3 versus 8 pups/ litter	L, from 3rd day	(M) PD70	↑	↑	↑	Arc: ↑*ObRb*, ↓*Npy* to fasting, ↑*Socs3* PVN: ↓*Crh*	Medial PVN: ↓*Trh*; unchanged by fasting Serum: ↓TT3 change by fasting, ↓TT4, ↓TT4 change by fasting (Aréchiga-Ceballos in [2])	BW: ↓change with fasting Abd WAT: ↓with fasting, Cort not changed. Serum: ↑leptin
Japanese macaque, HFD/treats	Successive gestations	3rd trimester fetus					Anterior Ht: ↓*Trh, Dio3, Thra1* Thyroid: ↑*Dio2, Dio3*, ↓*Tshr*, *Tg*, *Tpo*, *Slc5a5, Ppargc1a* Liver: ↓*Dio2, Dio3*, ↑*Thra1*, *Thrb*, *Med1*, *Gata2*, *Ppargc1a*, *Spot1*, *Acta1* TRE of *Thrb*: ↑diacetylation of H3K9,14ac, ↑MED1, NCOA1 occupancies Serum: ↓FT4 (Suter 2002 in [1])	Serum: ↑FFA, TG

Note: Italics in the table is used for gene or mRNA names and capital letters for peptides/proteins
Numbers in parenthesis correspond to reference list
(1) Dearden and Ozanne (2015)
(2) Joseph-Bravo et al. (2016)
Symbols: ↓ reduced, ↑ increased versus control animals

this and other models of early-life stress allowed identification of several hypo- or hyper-methylated gene promoters pertaining to HPA axis and other brain regions (van Bodegom et al. 2017).

MS causes gender-specific changes in HPT, and male rats have increased basal Cort levels as adults and secondary hypothyroidism (low TSH and T3 serum concentrations); *Trh* expression is not modified but that of *Trhde* increased, which explains HPT inhibition only at pituitary and thyroid levels. In contrast, female rats have increased expression of *Trh* and high white adipose mass, which points for a subclinical hypothyroidism. MS blunts the response of HPT axis to threats as fasting only in males (Jaimes-Hoy et al. 2016) or to cold exposure (unpublished). Males seem to be more susceptible to the stress induced during lactation, and, since stress diminishes the HPT response of adults, the effects of MS could be combined with the higher stress response of MS males, together with possible effects on *Trhde* programming.

Conclusion

The HPT axis is submitted to multilevel regulation and integrates environmental, emotional, endocrine, and metabolic signals. It is programmed by the effects of maternal care, early-life stress, and nutrition. Aberrant programming or inappropriate nutrition, as well as stress and diseases, can alter HPT activity and contribute to dysfunctional metabolic status in adults.

Policies and Protocols

The status of the HPT axis is primarily evaluated based on determination of the serum or plasma concentrations of TSH, total and free T4, and T3. Quantitative assays utilize specific antibodies that bind the hormone. In radioimmunoassay, hormone in sample competes in solution with a fixed quantity of radioactive hormone for antibody binding; the antibody-hormone complex is separated from the free hormone and signal quantified. In enzyme-linked immunosorbent assay, hormone in sample is immobilized onto a plate and detected by the specific antibody coupled to an enzyme that generates either a colored or fluorescent signal. The intermolecular interactions of antibody and ligand are susceptible to interferences from substances present in the sample; thus, it is extremely important to evaluate parallelism between standard curve and sample dilutions. It is also critical to choose an assay that is optimized for the species under study, since even if the chemical structure of TH is species independent, the concentration and type of serum-binding proteins differ between humans and rodents, making assay conditions optimized for human samples not applicable for rodent samples and vice versa. The knowledge of the factors that modulate the activity of the

HPT axis should also be considered when defining sampling time and not only fasting duration; for interpretation of clinical results, careful consideration of previous stress, body weight, sex, and age is necessary. Given the importance of accuracy, reliability, and reproducibility in measurements and evaluation of normal values, attempts have been made to set international standards (Vesper et al. 2016). Work with experimental animals allows measurements of TH-sensitive gene expression in multiple tissues, which complements information given by circulating hormone concentrations. A comprehensive manual for standardization of measurement of markers of HPT axis function has been recently published (Bianco et al. 2014).

Dictionary of Terms

- **Deiodinase** – An enzyme that removes an iodo group from one of the rings of thyroid hormone.
- **Energy balance** – Balance between energy intake and expenditure through basal metabolism, thermogenesis, and physical activity.
- **Hypophysiotropic thyrotropin-releasing hormone neurons** – Neurons of the hypothalamic-paraventricular nucleus, expressing thyrotropin-releasing hormone, that project to the median eminence and regulate thyrotropin secretion from the anterior pituitary.
- **Melanocortin system** – Arcuate nucleus neurons that synthesize α-melanocyte-stimulating hormone and Agouti-related peptide and target neurons expressing the melanocortin receptors 3 and 4, which are critically involved in energy homeostasis.
- **Programming** – Early-life conditions that set adult functions.
- **Tanycytes** – Modified ependymal cells localized at the base and ventral portion of the lateral wall of the third ventricle.
- **Thermogenesis** – Production of heat in response to changes in environmental temperature or diet.
- **Thyrotropin-releasing hormone-degrading ecto-enzyme** – A membrane-bound peptidase that inactivates thyrotropin-releasing hormone in the extracellular space.

Summary Points

- Animal research provides knowledge about energy balance and thyroid axis relationship.
- Thyroid axis is hierarchically organized; paraventricular hypophysiotropic thyrotropin-releasing hormone neurons integrate central and peripheral information to control thyrotropin secretion from pituitary; thyrotropin controls thyroid hormone production by the thyroid.

- Tissue concentrations of thyroid hormones depend on transporters and deiodinases, and their actions on thyroid hormone receptors.
- Thyroid hormones control basal metabolic rate, thermogenesis, lipolysis and glycolysis, and development and performance of immune and nervous systems; they exert feedback control on the hypothalamic-pituitary-thyroid axis at multiple levels.
- Thyrotropin-releasing hormone neurons in the paraventricular nucleus are target of proopiomelanocortin hormone/cocaine- and amphetamine-activated transcript, and neuropeptide Y/agouti-related peptide/γ-aminobutyric acid arcuate neurons, a major hub for sensing and relaying information related to energy balance.
- Acute stimuli, such as cold or exercise, transiently activate the hypothalamic-pituitary-thyroid axis.
- Chronic events as fasting, food restriction, malnutrition, stress, and disease downregulate the activity of the thyroid axis.
- Fasting or food restriction effects on the hypothalamic-pituitary-thyroid axis are mediated by a reduction in circulating leptin concentration.
- Interactions between stress and the thyroid axis activity occur through many mechanisms, including glucocorticoid receptor-protein kinase A-dependent interference with thyrotropin-releasing hormone transcription.
- Diet-induced obesity activates the thyroid axis, although deiodinases activities limit the capacity of this axis to compensate for energy excess.
- Inadequate activity of the thyroid axis leads to deleterious outcomes.
- Maternal under- or mal-nutrition, stress, infection, alcoholism, nicotine, and toxics including endocrine disruptors affect thyroid hormone signaling in the fetus, leading to defects in differentiation and maturation of many organs and systems, including the brain.
- Maternal nutritional status or stress during gestation, and/or lactation, programs the offspring's body weight, neuroendocrine axes, and energy metabolism in the adult.
- Mechanisms involved in the programming of adult energy balance include brain structural alterations, epigenetic regulation of key genes, and altered thyroid and adrenal axes.

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Calorie Restriction and Insulin Sensitivity in Obesity

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Meera Shah

Contents

Abstract

Caloric restriction has long been shown to improve insulin action and glucose control. In this chapter, we review the evidence behind different strategies to restrict calories, its impact on insulin sensitivity, putative mechanisms by which it improves insulin sensitivity, and the longevity of these methods in improving glucose metabolism where such evidence exists. Coverage includes sections on caloric restriction versus weight loss, techniques, macronutrients, the role of the liver and skeletal muscle after caloric restriction, and the gut microbiome.

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Caloric restriction · Insulin sensitivity · Glucose control · Weight loss

Introduction

The global incidence of obesity is rising bringing with it increased risk of weight-related medical complications including impairments in glucose metabolism. Weight loss can help slow down the progression of prediabetes to type 2 diabetes (Hamman et al. 2006), and caloric restriction is the cornerstone of any successful weight management program. In this chapter, we review the evidence behind different strategies to restrict calories, its impact on insulin sensitivity, putative mechanisms by which it improves insulin sensitivity, and the longevity of these methods in improving glucose metabolism where such evidence exists.

Caloric Restriction or Weight Loss?

Traditional caloric restriction is defined as a decrease in calorie consumption by 20–50% of needs (Omodei and Fontana 2011). In human intervention studies on people with or without type 2 diabetes (T2DM), reducing caloric intake has universally been shown to reduce insulin resistance and improve insulin action. Towards the end of the last century, the study of very low calorie diets (VLCD) was of great interest in the treatment of obese patients with type 2 diabetes. With this diet, patients were allowed to consume 400–800 kcals/ day of high-quality protein and carbohydrate, typically in liquid form, supported by an aggressive vitamin and mineral supplementation program. Hepatic glucose output decreased in conjunction with a decrease in fasting plasma glucose. Studies using the euglycemic-hyperinsulinemic clamp showed that VLCD enhanced the ability of insulin to suppress hepatic glucose production, and glucose disposal rates increased severalfold after weight loss with a VLCD. Therefore, it was concluded that VLCDs improved peripheral and hepatic insulin sensitivity in obese patients with T2DM, independent of weight loss (Henry et al. 1986). Although these diets resulted in improved metabolic parameters and an eventual reduction in body weight, there were several concerns regarding their generalizability and safety. Patients would often experience significant side effects including orthostatic hypotension, nausea, headache, and dehydration. There was a need for careful supervision of electrolytes due to early reports of death from cardiac arrhythmias and an increased risk of refeeding syndrome when calorie consumption was liberated (Frank et al. 1981). However, probably most relevant to the patient was the significant difficulty in adhering to the diet in the long term, which would often result in resumption of previous dietary habits and ultimately weight gain.

However, recent human data suggests that the initial improvements in glycemic control and insulin sensitivity that occur as a result of a VLCD may have sustained benefits in some people as long as the weight is not regained. Steven et al. studied

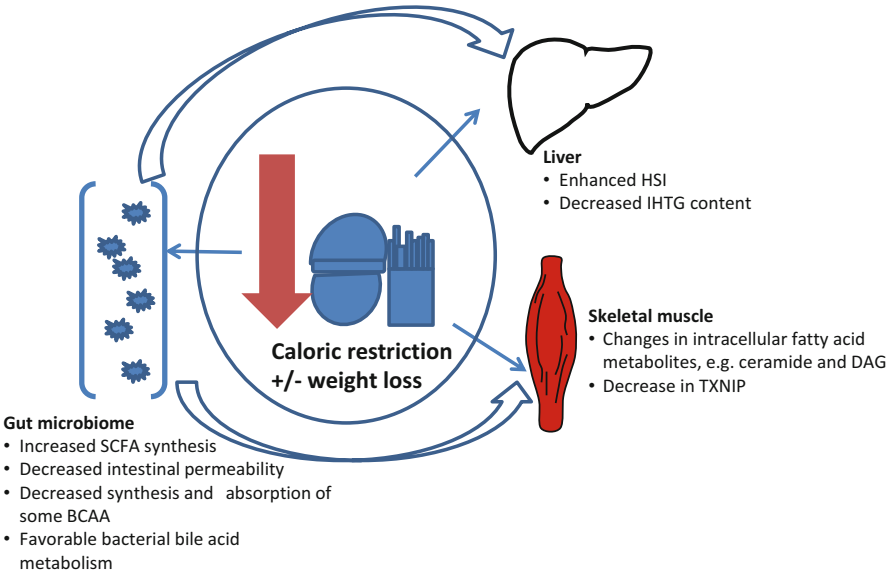

Fig. 1 The roles of the liver, skeletal muscle, and gut microbiome after caloric restriction in improving insulin sensitivity. Legend: *SCFA* short chain fatty acids, *BCAA* branched-chain amino acids, *HIS* hepatic insulin sensitivity, *IHTG* intrahepatic triglyceride content, *DAG* diacylglycerol, *TXNIP* thioredoxin-interacting protein

29 participants with type 2 diabetes who consumed 624–700 kcals/day for 8 weeks. Mean weight loss was 14% during this phase, after which there was gradual introduction of solid food to promote weight maintenance. Approximately 40% of participants ("responders") had normalization of fasting glucose which stayed normal off hypoglycemic agents at 6 months follow-up, suggesting that diabetes remission was achievable through an initial brief VLCD followed by a sustainable weight-management program. Hepatic insulin resistance improved in both responders and nonresponders based on results from euglycemic-hyperinsulinemic clamp studies. However, the primary driver of the robust improvement in glucose metabolism in responders was improved beta call responsivity, specifically first phase insulin response. Important to note that at baseline, responders were younger (52 years old vs. 60 years old), had lower HbA1c (7.1% vs. 8.4%) and shorter duration of diabetes (3.8 years vs. 9.8 years) versus nonresponders, emphasizing the importance of beta cell reserve in the improvements seen in glycemic control after VLCD and weight maintenance (Steven et al. 2016). Whether remission of type 2 diabetes through early VLCD has longevity beyond the study duration is not yet known, although data from bariatric surgery is certainly compelling in this regard (Schauer et al. 2014).

In recent years, bariatric surgery has offered an interesting in vivo model to address the question of which reigns supreme in improvements in glycemic control: caloric restriction or weight loss. Patients with type 2 diabetes who undergo Roux-en-Y gastric bypass (RYGB) surgery for weight loss often show impressive

improvements in glycemic control within the first few days of surgery, prior to any significant weight loss. Jackness et al. studied matched cohorts of obese patients with similar duration and control of diabetes to compare the effect of caloric restriction (CR) alone vs. caloric restriction in the setting of RYGB. Patients were subject to a 500 kcal/day diet for between 14 and 24 days and subsequently studied with an intravenous glucose tolerance test (IV GTT). The degree of weight loss after intervention (diet alone or diet plus surgery) was similar. The acute insulin response rate, sensitivity of glucose elimination to insulin and disposition index (measure of insulin secretion in relation to prevailing insulin sensitivity) as well as the homeostasis model assessment of insulin resistance (HOMA-IR) improved to a similar degree after equivalent caloric restriction in both groups (Jackness et al. 2013). However, the use of an intravenous GTT excluded the contribution of gut incretins, which are known to be elevated in the postprandial setting after RYGB and which influence glucose metabolism (Laferrere et al. 2008). Nevertheless, the conclusion of the authors regarding the fundamental role of caloric restriction in the metabolic changes observed after bariatric surgery is important and continues to be the subject of ongoing studies (Fig. 1).

Does Technique Matter?

The typical calorie restriction program advocates global calorie restriction of either a certain amount of calories (e.g., 500 kcals/day) or a certain percentage of calories (e.g., 25% of needs/day), and is usually based on the goals and abilities of the individual. However, this method works best when combined with rigorous self-monitoring (Das et al. 2007) which can be difficult to adhere to in the long term. Additionally, patients also report that restricting calories daily is frustrating and curtails their freedom to choose freely. Therefore an alternative method, intermittent fasting (IF), whereby calories are only restricted on a certain day or days of the week has been studied to assess its efficacy as a weight loss tool, and to determine the effects of this type of dietary intervention on markers of glucose metabolism including insulin sensitivity. A significant limitation of many CR and IF studies is the short duration of intervention and of subsequent follow-up. Nevertheless, certain themes have emerged as important in helping enhance our understanding of how calorie restriction improves insulin sensitivity.

Foremost, the degree of calorie restriction seems proportional to the improvements seen in insulin sensitivity. Varady et al. studied 16 patients with prediabetes and prescribed a supervised IF program consisting of 75% CR on 1 day a week followed by ad libitum intake for the remainder of the week. The average weight loss was 6%, with a corresponding decrease in HOMA-IR by 19% (Varady et al. 2009). When CR was 80% on 1 day followed by ad libitum feeding for the remainder of the week, an 8-week intervention in a population with prediabetes led to 8% weight loss, 6% improvement in fasting glucose, and 33% decrease in

HOMA-IR (Johnson et al. 2007). In both studies, participants were not provided advice on exercise and therefore the effects on body weight were assumed to be purely a result of caloric restriction. No relationship could be established between insulin sensitivity and visceral fat but this may be a limitation of the small number of participants in these studies. Similarly, studies using daily CR show a linear relationship between degree of caloric restriction and improvements in insulin sensitivity as measured by HOMA-IR. For example, a 6 week study using 50% CR resulted in a 70% decrease in HOMA-IR (Xydakis et al. 2004), while studies using 25% CR showed a decrease in HOMA-IR by 15–20% from baseline (Harvie et al. 2011; Trussardi et al. 2013). Also noteworthy is that improvements in insulin sensitivity were seen as early as 3 weeks (and 3% weight loss) after the intervention (Eshghinia and Mohammadzadeh 2013), providing further evidence that CR in any form, be it global reduction, intermittent fasting or alternate day fasting, may be utilized as a short-term strategy to improve insulin sensitivity.

Do Macronutrients Matter?

Several studies in humans have shown that improvements in insulin sensitivity are seen early during dietary restriction before any weight loss occurs (Kelley et al. 1993; Assali et al. 2001), highlighting the role of tissue sensitivity to nutritional cues; the natural follow-up question therefore is whether the type of macronutrient exposure determines tissue response. The macronutrient composition of CR diets has been studied to elicit if differences exist due to the relative amount of protein or fat in iso-caloric conditions. When weight loss was the primary goal of the study, the answer seems to be no as shown in the next few studies discussed. One group looked at two different alternate day fasting diets (25% CR) in obese individuals, consisting of 45% fat/40% carbohydrate and 25% fat/ 60% carbohydrate for 8 weeks (Klempel et al. 2013). At the end of the study period, both sets of participants lost an equivalent amount of weight and had preservation of fat free mass to a similar degree. There was a 7 cm decrease in waist circumference in both groups; waist circumference is an indirect marker for visceral fat mass. Also, participants who followed the high fat diet reported better adherence likely due to better palatability of the food.

Another group looked at how portion control, energy density, and glycemic index compared as weight loss methods. Participants were advised to restrict calories based on an individual weight loss goal of 0.5–1 kg/week and given individualized nutrition guidelines per their intervention group, i.e., information on low energy density or low glycemic index foods or portion control. After 12 weeks there was no significant difference in body weight and percentage body fat between groups. There was a significant improvement in HOMA-IR from baseline in all three groups, and no between-group difference was observed (Melanson et al. 2012).

Other groups however have shown that in the setting of weight neutrality, the quality of macronutrients may be important in determining effect on glucose metabolism. When overweight and obese volunteers were placed on an iso-caloric, weight-maintaining diet that differed only by the amount of whole grains (dietary fiber) consumed for 6 weeks, the group on the whole grain diet had a significant improvement in HOMA-IR and other insulin sensitivity indices as measured by the euglycemic-hyperinsulinemic clamp, compared to individuals on the refined grain diet (Pereira et al. 2002). The authors concluded that dietary fiber from whole grains modulated glucose metabolism and hypothesized that changes in gut microbial composition and the production of short chain fatty acids (SCFA) in particular explained some of the observed differences. The role of the gut microbiome in modulating glucose metabolism will be briefly discussed in this chapter.

The Role of the Liver After Caloric Restriction in Improving Insulin Sensitivity

The liver and skeletal muscles are the main organs where insulin action results in changes in glucose concentration. As mentioned previously, in the short term VLCDs have been shown to improve hepatic insulin sensitivity; however, there have also been reports of short-term fasting causing insulin resistance in humans (Duska et al. 2005; Bergman et al. 2007). The hypothesis put forward for this observation was that differences in carbohydrate consumption were responsible; such that acute insulin resistance was seen when total carbohydrate consumption was <50 g/day but not when it was >100 g/day, possibly due to increased lipolysis in the former with resultant higher free fatty acid concentrations (Jensen et al. 1987; Kelley et al. 1993). To address the short- and long-term effects of equivalent caloric restriction with a high carbohydrate (>180 g/day, "HC") and low carbohydrate (<50 g/day, "LC") diet in obese insulin-resistant subjects, 22 subjects were studied after 48 h of the intervention and again after a 7% weight loss was achieved (Kirk et al. 2009). Both groups achieved equivalent weight loss at the end of the intervention and there were no between group differences seen in body-fat and fat-free mass. In general and in both groups, short-term caloric restriction led to a decrease in intrahepatic triglyceride (IHTG) content, increase in hepatic insulin sensitivity, and decrease in endogenous glucose production rate, whereas 7% weight loss improved skeletal muscle insulin sensitivity and improved in cellular insulin signaling. However, acutely, the LC diet led to a greater reduction in the rate of appearance of glucose (Ra) and greater improvement in the hepatic insulin sensitivity index compared to the HC diet. The authors hypothesized that the decline in circulating insulin levels with the LC diet led to enhanced lipolysis of IHTG and hepatic fatty acid oxidation and a decline in endogenous glucose production, similar to the physiologic adaptations that occur during the early response to starvation (Klein and Wolfe 1992). They also propose this to be one mechanism by which improvements in glucose metabolism seen early after RYGB occur.

The Role of Skeletal Muscle After Caloric Restriction in Improving Insulin Sensitivity

The cellular mechanisms that modulate insulin sensitivity have been studied extensively and are well understood. In this context, there are several pathways that are able to self-regulate in a nutrient-rich environment, i.e., excess caloric consumption. For example, the p85 regulatory subunit in skeletal muscle can be induced after as little as 3 days of positive energy balance (Cornier et al. 2006), while caloric restriction in rodents improves glucose transport within the skeletal muscle in conjunction with decreasing the abundance of p50 and p55 (McCurdy et al. 2005). Human studies have shown an increase in the expression of another regulatory protein, the deacetylase sirtuin 1 (SIRT1) during caloric restriction, which leads to improvements in insulin sensitivity (Civitarese et al. 2007). Furthermore, weight loss does decrease fatty acid flux, but to what extent this contributes to the improvements seen in insulin sensitivity is unknown (Kelley et al. 1993; Assali et al. 2001).

Several groups have studied overweight or obese subjects to further elicit underlying mechanisms in humans, with inconsistent results. In overweight subjects, induction of acute muscle insulin resistance was associated with a transient increase in total and cytosolic diacylglycerol (DAG) content (an intermediate product of fatty acid metabolism) (Szendroedi et al. 2014), but there was no association observed between insulin resistance and alterations in muscle ceramide, another intermediate product of fatty acid metabolism. Conversely, another group showed that muscle ceramide accumulation in in vitro culture inhibited muscle insulin sensitivity (Chavez et al. 2005). Yet another group showed that a 16 week CR program resulting in 9% weight loss decreased the concentrations of bioactive lipids including ceramide and DAG and improved insulin sensitivity (Dube et al. 2011). In contrast, the reductions in these intramyocellular lipids were not observed by a group that studied obese participants under similar conditions. The average weight loss achieved was 10% body weight. The group did, however, uncover the potential role of lower thioredoxin-interacting protein (TXNIP) levels in enhancing nonoxidative glucose disposal, thus supporting the role of TXNIP in mediating the improvement in peripheral insulin sensitivity after CR (Johnson et al. 2016). TXNIP impairs insulin signaling, and skeletal muscle TXNIP deletion has been shown to be protective against high-fat diet–induced insulin resistance in rodents (Hui et al. 2008). The differences observed in these studies may be due to the type of participants studied and the methodology used, but does in sum support the role of caloric restriction in modulating intracellular insulin signaling, and thus insulin action at the tissue level.

The Role of the Gut Microbiome After Caloric Restriction in Improving Insulin Sensitivity

The human host has ~10^{14} microbes in the colon which contain approximately 10-fold more genes than the human genome. These genes have diverse functions including energy harvest, neurohormonal function, gut barrier function, and bile

acid modification. The genetic material that encodes enzymes (e.g., to break down dietary polysaccharides or utilize glycans) can affect fatty acid concentrations including SCFA, an example of which is butyrate. The biggest influence of the composition of the gut microbiome is diet, and therefore a compelling relationship between caloric restriction, diet, and the gut microbiome composition may exist to explain improvements seen in insulin sensitivity with dietary change.

Even when subjects are exposed to the same sort of diet, there is a range of clinical effects observed and one explanation for the heterogeneity of response to caloric restriction may be differences in microbial gene richness, defined as the number of bacterial genes in an individual's stool. Cottilard and colleagues (Cotillard et al. 2013) studied a matched European cohort of overweight and obese individuals stratified by gene richness. Interestingly, subjects with lower gene richness had greater prevalence of metabolic dysregulation at baseline. With 6 weeks of caloric restriction, this group showed an increase in gene number but had less of a robust response in terms of improvement in HOMA-IR and hsCRP when compared to the group with higher gene richness. Conversely, higher gene richness at baseline was associated with a more marked improvement of adipose tissue and systemic inflammation following equivalent caloric restriction. The authors concluded that gene richness may help predict the efficacy of dietary intervention on inflammatory variables in overweight or obese individuals. It is estimated that up to 40% of the European population may have low gene richness, and therefore the clinical implication of this finding is considerable (Cotillard et al. 2013).

The gut microbiome also has an important role in maintaining gut barrier function and intestinal permeability, and there may be a correlation between systemic inflammation and insulin resistance. Studies in mice have shown that fluorescently labeled *E. Coli* mixed in chow were able to be detected in the circulation particularly after a high-fat diet, providing evidence for intestinal translocation. These mice exhibited higher levels of lipopolysaccharide (LPS), new insulin resistance, and changes in the composition of gut microbiota. When the cell surface receptor for LPS, CD 14, was knocked out, the mice had less weight gain, were more insulin sensitive, and had less systemic and tissue inflammation. Mice that were given probiotic supplementation for 1 month showed decreased bacterial translocation and improved insulin sensitivity suggesting that an intervention designed to manipulate gut microbial composition could reverse the adverse metabolic phenotype induced by high-fat diet (Amar et al. 2011a). Taken together, this outlines the interplay between endotoxins, intestinal permeability, and insulin sensitivity, at least in rodents. In humans, there is indirect evidence that bacterial translocation may contribute to insulin resistance and dysregulation of glucose homeostasis. In a small study, a group of patients were followed for 9 years and then stratified according to the presence or absence of type 2 diabetes (T2DM). The level of circulating bacterial genetic material (16SrDNA) in the blood at baseline moderately predicted the onset of T2DM (OR = 1.29 after adjustment for age, sex, and fasting blood glucose) (Amar et al. 2011b). Although preliminary, this observation potentially provides an alternative mechanistic explanation for how dietary change improves parameters of glucose metabolism.

In one of only a handful of human intervention studies looking at changes in the gut microbiome and associations with insulin sensitivity, investigators were able to show improvements in insulin sensitivity by infusing intestinal microbiota from lean healthy donors to male recipients with metabolic syndrome. This improvement was associated with increased levels of butyrate-producing intestinal microbiota (Vrieze et al. 2012). Interestingly, the authors also found increased gut microbiota diversity was associated with improved insulin sensitivity.

Other putative mechanisms by which the gut microbiome may improve glucose metabolism include decreased synthesis and absorption of branched-chain amino acids that lead to insulin resistance (Derrien et al. 2004) and bacterial metabolism of bile acids, e.g., stimulation of FGF-19 which has metabolic effects on the farsenoid X receptor (FXR) in the β cell and liver (Gerhard et al. 2013). Whether dietary manipulation can recreate the microbial profile associated with improved insulin sensitivity is still unknown but may offer a novel mechanistic insight and potential therapeutic approach to address the problem in humans.

Conclusion

Obesity and insulin resistance share the common risk factor of excess calorie consumption, and calorie restriction in various forms has been shown to ameliorate this process. The effect of caloric restriction on cellular signaling, fatty acid metabolism, and potentially favorable changes in the composition of the gut microbiome are mechanisms by which these improvements occur, and offer novel insights into potential therapeutic strategies for the treatment of insulin resistance in humans.

Dictionary of Terms

- **Calorie** – A unit used to measure the energy value of food.
- **Insulin sensitivity** – The ability of insulin to change glucose concentrations. When insulin sensitivity is low, this is often termed insulin resistance.
- **Prediabetes** – The state of either having impaired glucose tolerance or impaired fasting glucose.
- **Very low calorie diet (VLCD)** – Traditionally, this describes an intake of less than 800 kcals/day.
- **Refeeding syndrome** – A physiologic response to the reintroduction of calories after a prolonged period of calorie deprivation, characterized by significant intracellular electrolyte shifts and manifests as low phosphorus, potassium, and magnesium concentrations in the blood.
- **Euglycemic-hyperinsulinemic clamp** – A research method used to quantify insulin secretion and action, i.e., insulin sensitivity.
- **Homeostasis model assessment of insulin resistance (HOMA-IR)** – A mathematical formula for assessing β-cell function and insulin resistance from basal (fasting) glucose and insulin or C-peptide concentrations. Another method used to measure insulin sensitivity.

Summary Points

- Caloric restriction in any form improves insulin sensitivity, although the durability of effect is yet unknown.
- Calorie restriction improves insulin sensitivity before weight loss occurs.
- Caloric restriction improves insulin sensitivity by enhancing skeletal muscle insulin signaling and hepatic insulin action.
- Macronutrient composition during caloric restriction may have an impact on the degree of improvement of insulin sensitivity.
- The gut microbiome is a novel player in the improvements seen in insulin sensitivity during caloric restriction.

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The Ketone Body Beta-Hydroxybutyrate in Starvation

58

P. Rojas-Morales and J. Pedraza-Chaverri

Contents

Abstract

The ketone body β-hydroxybutyrate (BHB) is a central molecule that plays pivotal roles in whole-body energy metabolism by fueling most tissues in conditions of nutrient deprivation, but its function is not limited to a metabolic intermediate during nutritional challenge. Emerging studies have positioned BHB as a signaling metabolite regulating a broad range of processes – from

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inflammation to longevity – through cellular receptors, epigenetic modifications, and by yet unknown mechanisms. In addition, several pieces of evidence point out that BHB might function as a signal controlling energy metabolism in the particular scenario of negative energy balance. This chapter provides an overview of how BHB, apart from helping to solve energy crisis by directly fueling oxidative metabolism, has the potential ability to reduce energy expenditure and promote autophagy, thus contributing to fine-tune systemic energy balance in the face of food limitation.

Keywords

Starvation · Beta-hydroxybutyrate · Ketone bodies · Signaling · Autophagy · Energy expenditure · Fuel metabolism · Gene expression · Inflammation · Insulin resistance

List of Abbreviations

AcAc	Acetoacetate
AcAc-CoA	Acetoacetyl-coenzyme A
acetyl-CoA	Acetyl-coenzyme A
AKT	Protein kinase B
AMPK	AMP-activated protein kinase
BDH1	BHB dehydrogenase
BHB	β-Hydroxybutyrate
CoA	Coenzyme A
$FADH_2$	Flavin adenin mononucleotide
FFA	Free fatty acids
FFAR3	Free fatty acid receptors 3
FOXO3	Forkhead box O3
GPCRs	G Protein-coupled receptors
HCAR2	Hydroxycarboxylic acid receptor 2
HDACs	Histone deacetylases
HMGCL	3-Hydroxy-3-methylglutaryl-CoA lyase
HMG-CoA	3-Hydroxy-3-methylglutaryl-CoA
HMGCS2	3-Hydroxy-3-methylglutaryl-CoA synthase 2
mTOR	Mechanistic target of rapamycin
NAD^+	Oxidized nicotinamide adenine dinucleotide
NADH	Reduced nicotinamide adenine dinucleotide
NF-kB	Nuclear factor kappa B
NLRP3	Nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3
PI3K	Phosphatidylinositol 3-kinase
SCOT	Succinyl-CoA:3-oxoacid CoA transferase
Suc-CoA	Succinyl-CoA
TCA	Tricarboxylic acid cycle
TG	Triglycerides

Introduction

Prolonged starvation is a common occurrence in the wild and has profound metabolic consequences that may result in tissue dysfunction and ultimately death. Thus, the ability to cope with fluctuating nutritional resources is paramount for life. One decisive strategy to survive starvation is the mobilization and utilization of fat depots. The ketone body β-hydroxybutyrate (BHB) is a fat-derived molecule that contribute to maintain basal energy metabolism by fueling most tissues during food limitation (Robinson and Williamson 1980). In this regard, BHB functions as a "superfuel" by yielding more ATP molecules per oxygen consumed than the oxidation of other fuels such as glucose and pyruvate (Cahill and Veech 2003). However, because it has become evident that BHB has the ability to regulate energy metabolism by acting at the central nervous system and peripheral tissues through elaborated signaling pathways, it is tempting to say the term "superfuel" would fall short of what BHB can actually do. This chapter is aimed to describe ketone body metabolism and the signaling functions of BHB along with its several distinct metabolic consequences that might be important in determining the successful adaptation to starvation.

Metabolic Adaptation to Starvation

Food deprivation imposes a tremendous metabolic challenge to eukaryotic organisms. In order to face nutritional stress and survive, organisms reduce energy expenditure by eliminating unnecessary activities and temporally avoiding growth and reproduction to allocate nutritional resources only for tissue maintenance and function (Wang et al. 2006). In this sense, at both cellular and systemic levels, several metabolic processes and biochemical pathways are activated (or enhanced) while other are suppressed. For example, in mammals, when nutrient supply is limited, the highly conserved "self-eating" process autophagy is enhanced in most, if not all, tissues to degrade cytoplasmic components and to provide nutrients (lipids, amino acids, and carbohydrates) to cells for recycling and energy production. Importantly, metabolic pathways such as glycogen breakdown, gluconeogenesis, lipolysis, fatty acid oxidation, and ketogenesis parallel autophagy to coordinately satisfy systemic energy requirements and to provide glucose mainly for neurons, the highest energy-demanding cells in the brain. Thus, as a result of reduced glucose levels, occurring at the onset of fasting, blood concentrations of both fatty acids and ketone bodies rise (Fig. 1a).

Virtually, all organs in the body exhibit particular metabolic profile and require specific metabolic fuels to meet their energy needs. The brain is one of the most energy-demanding tissues that, in optimum nutritional conditions, continuously consumes large amounts of glucose to support synaptic activity and related processes (Mergenthaler et al. 2013); however, during food shortage, in order to maintain cognitive function essentially constant over time, the brain relies on ketone bodies as major metabolic fuels. In this regard, ketone bodies provide more than half of the

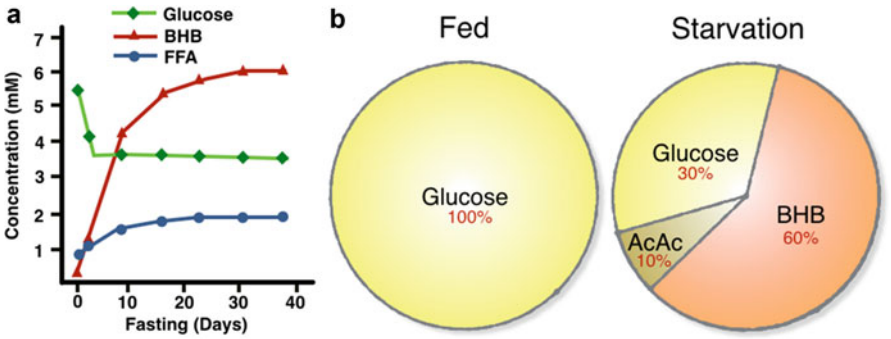

Fig. 1 Metabolic changes and fuel partitioning in the brain during starvation. (**a**) Blood concentrations of glucose, free fatty acids (*FFA*), and β-hydroxybutyrate (*BHB*) during fasting in humans. (**b**) The ketone bodies BHB and acetoacetate (*AcAc*) displace the limiting glucose in supplying the energy that brain requires to function correctly during periods of food limitation

energy that brain requires during starvation (Fig. 1b). Since a continuous glucose supply to the brain is a major goal of the metabolic rearrangements occurring during starvation, glycogen breakdown and gluconeogenesis in the liver directly contribute to maintain blood glucose at a suboptimal level. On the other hand, the adipose tissue and the liver significantly contribute to spare glucose by providing fatty acids, through lipolysis, and ketone bodies, through fatty acid oxidation and ketogenesis, respectively, as alternative fuels for most tissues (Fig. 2).

Because proteins have a broad range of important cellular functions (in the form of structural proteins, enzymes, and signaling hubs) and they constitute neither a real energy storage nor an efficient metabolic fuel and because the waste product ammonia that result from oxidative deamination of protein-derived amino acids might lead to toxicity, during fasting protein catabolism becomes a tightly regulated process: in the first hours of fasting, muscle protein breakdown increases to support gluconeogenesis, but then decline allowing the fat-derived carbon backbones to supply almost all the energy that tissues (including the brain) need to maintain essential functions for extended periods of time (days, weeks, or months depending on the species). However, in extreme cases of prolonged nutrient scarcity, when fat depots are exhausted and no food is ingested, muscle proteins are used as the ultimate resource until death.

Because of the ability of fat to spare glucose along with vital proteins during both acute (fasting) and long-term (starvation) food shortage, it comes to no surprise that the survival of an organism to famine will depend on how much fat can store during periods of plenty. For example, laboratory rats having more fat due to high-fat diet feeding survive food deprivation twice as long as the lean, chow-diet fed, age-matched controls (Goodman et al. 1984). In the same sense, while a lean human being can barely survive a few months with only water (with mineral and vitamin supplements), obese humans can survive for up to 1 year without any bite of food (Stewart and Fleming 1973). In other cases, it is known that both naturally

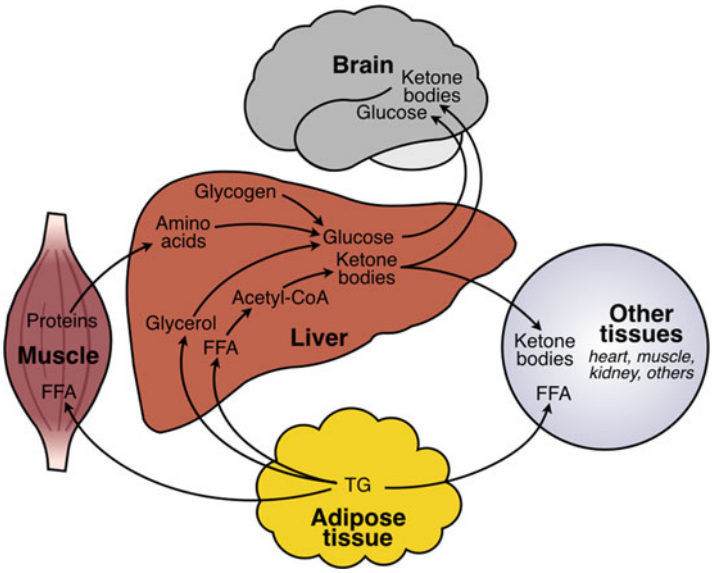

Fig. 2 Fuel metabolism in starvation. During starvation, the energy stored in the form of triglycerides (*TG*) in the adipose tissue is used to sustain whole-body energy requirements. This energy travels through the bloodstream to distant tissues in the form of free fatty acids (*FFA*) or ketone bodies (produced mainly in the liver). Also, in this situation, muscle proteins are coordinately degraded to synthesize glucose for the brain

occurring king (*Aptenodytes patagonicus*) and emperor (*Aptenodytes forsteri*) penguins repeatedly survive long-lasting breeding cycles without feeding at the expense of fat depots, resulting in reproductive success but also in severe weight loss (Groscolas and Robin 2001). Similarly, in insects such as flies and ants, starvation resistance is positively correlated with adiposity (Ballard et al. 2008; Dussutour et al. 2016). Thus, relying on endogenous, large energy stores, particularly as fats, is remarkable advantageous during starvation.

Ketone Body Metabolism

In order to utilize the energy stored in the form of fats, activation of tissue-specific metabolic programs occurs to break down triglycerides, to oxidize the resulting fatty acids, and to produce ketone bodies, mainly BHB. In the fed state, insulin stands out as the principal hormone inhibiting lipolysis, but during fasting, along with reduced insulin signaling, glucagon becomes a major hormone triggering the activation of adipocyte lipases, thus promoting the mobilization of free fatty acids (FFA). Circulating FFA are avidly taken up by peripheral tissues, such as the heart and skeletal muscles, and then oxidized in a multistep pathway (β-oxidation) to produce acetyl-coenzyme A (acetyl-CoA), which can be further oxidized in the tricarboxylic

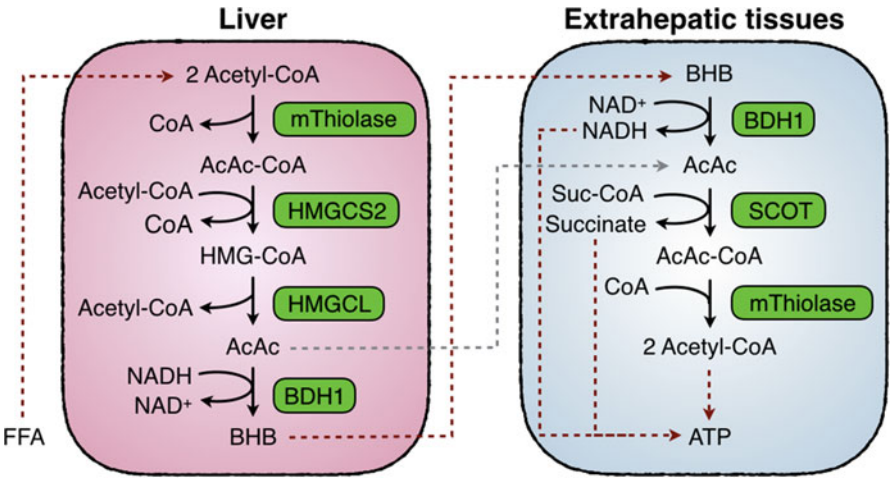

Fig. 3 Ketone body metabolism. Oxidation of free fatty acids (*FFA*) in liver mitocondria produces acetyl-coenzyme A (*acetyl-CoA*). Accumulating acetyl-CoA is preferentially directed to enzymatically produce first acetoacetate (*AcAc*) and then β-hydroxybutyrate (*BHB*). Both ketone bodies are exported out of the liver to produce acetyl-CoA back again and then ATP in distant tissues. Key: *CoA*, coenzyme A; *AcAc-CoA*, acetoacetyl-CoA; *HMG-CoA*, 3-hydroxy-3-methylglutaryl-CoA; *HMGCS2*, 3-hydroxy-3-methylglutaryl-CoA synthase 2; *HMGCL*, 3-hydroxy-3-methylglutaryl-CoA lyase; *BDH1*, BHB dehydrogenase; *SCOT*, succinyl-CoA:3-oxoacid CoA transferase; *Suc-CoA*, succinyl-CoA

acid cycle to produce reducing equivalents (NADH and $FADH_2$) for ATP synthesis. However, in the liver, FFA-derived acetyl-CoA is diverted to produce ketone bodies, first acetoacetate (AcAc) and then BHB, through a series of sequential enzymatic reactions within the process called ketogenesis, the synthesis of ketone bodies (Fig. 3). The rate-limiting enzyme in this process is 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2) which condenses acetoacetyl-CoA (AcAc-CoA) and acetyl-CoA into 3-hidroxy-3-methylglutaryl-CoA (HMG-CoA). This tightly regulated (transcriptionally and posttranslationally) enzyme, almost exclusively expressed in the liver, is decisive in channeling increasing amounts of acetyl-CoA into the ketogenic pathway. As such, HMGCS2 is particularly important in preventing liver injury during fatty acid overload (Cotter et al. 2014). Once synthesized, HMG-CoA is cleaved into one molecule of AcAc and one of acetyl-CoA by the action of the enzyme HMG-CoA lyase. To finally terminate ketogenic reactions, the inner mitochondrial membrane-bound enzyme BHB deshidrogenase 1 (BDH1) produces BHB from AcAc. By using the $NADH/NAD^+$ redox potential, BDH1 facilitates the interconversion between AcAc and BHB, allowing the liver to synthesize and peripheral tissues to oxidize BHB.

Once synthesized, BHB is exported out of the liver to be oxidized into AcAc within extrahepatic mitochondria. Because most tissues except the liver exhibit high protein levels of the enzyme succinyl-CoA:3-oxoacid-CoA transferase (SCOT), AcAc is rapidly activated into AcAc-CoA. Then, mitochondrial thiolases

can oxidize AcAc-CoA to produce two acetyl-CoA molecules. Additionally, the oxidation of AcAc also results in succinate production that together with acetyl-CoA and NADH (initially produced by BDH1) contribute to ATP synthesis through oxidative phosphorylation.

The ketone body AcAc is also exported out of the liver, but the actual BHB blood concentrations are typically threefold higher than those of AcAc. Because AcAc is efficiently oxidized within extrahepatic mitochondria, it is unknown why BHB is produced as a metabolic intermediate that connects fat stores in adipocytes to ATP production in most tissues. Nonetheless, two major potential explanations exist. The first one is related to the high stability of BHB: as AcAc is spontaneously decarboxylated into acetone, which does not contribute to ATP production, and lead only to energy wasting in times of energy crisis, synthesizing BHB may be key to bypass this futile route. Second, as the coenzyme NADH is required for AcAc reduction into BHB, it is likely that the BHB synthesis is a strategy to regenerate the NAD^+ pool that sustains the β-oxidation flux, since every round of the oxidation cycle of the acyl chain of FFA requires NAD^+. Nonetheless, since BHB is an ancient energy source (from bacteria to humans) (Cahill 2006) plus the fact that it is now part of a growing list of metabolites having signaling functions (as discussed later) (Newman and Verdin 2014), it would be simplistic to say that BHB is synthesized just to fulfill these two metabolic requirements.

The balance between ketone body synthesis and degradation is of special importance, so that, in extreme cases, high blood ketone body concentrations might result in the death of the organisms. Many conditions that favor reduced blood glucose levels also promote the activation of hepatic ketogenesis. Prolonged fasting and endurance exercise are the main physiological situations that drastically reduce blood glucose levels and also promote an elevated glucagon-to-insulin ratio, thus rapidly increasing ketone body levels (from micromolar to millimolar concentrations). However, in pathological situations such as diabetes, not only enhanced ketogenesis but also reduced ketolysis is observed (the utilization of ketone bodies), thus promoting an accumulation of these molecules, which is associated with oxidative stress and aberrant activation of signaling pathways that together might contribute to tissue dysfunction and diabetes complications (Kanikarla-Marie and Jain 2016).

There is no doubt that the liver is the principal contributor of blood ketone bodies when nutritional stress occurs, but some studies point out that other tissues and cell types have the ability to provide ketone bodies at least locally. Some of them are the kidney, skeletal muscle, astrocytes, and the gut and retinal epithelial cells (Puchalska and Crawford 2017). However, more studies are needed in order to reveal the physiological consequences of ketogenesis in extrahepatic tissues.

Requirement of β-Hydroxybutyrate During Starvation

It is a general thought that ketone bodies are essential molecules in the metabolic adaptation to starvation. However, despite this longstanding dogma that has remained for almost a century, it is still unknown whether ketone bodies are really

necessary to survive starvation. Contrary to what intuition would indicate, it turns out that disruption of ketone body oxidation in specific ketolytic tissues does not compromise survival in mice during moderate starvation (Cotter et al. 2013). This finding suggests, at a first glance, that ketone bodies are dispensable under nutritional challenge. Intriguingly, previous experiments have shown that newborn mice having higher levels of ketone bodies due to impaired ketolysis die perinatally as a result of energy imbalance but also by ketoacidosis (Cotter et al. 2011), suggesting a prominent role of ketone body metabolism in neonatal metabolic homeostasis. However, experimental approaches designed to finally delineate the role of ketone bodies in starvation physiology in the adulthood – as such with whole-body conditional disruption of ketone body oxidation – are still lacking. Moreover, the fact that many tissues such as the kidney and especially the heart and skeletal muscle can utilize both FFA and ketone bodies during fasting complicates even harder the scenario for researchers in elucidating the net contribution of ketone body in fueling starved organisms. Thus, the real biological significance of ketone bodies, and of BHB, in metabolic homeostasis during negative energy balance, until now, is far from clear.

Signaling Functions of β-Hydroxybutyrate

Many metabolites have the ability to regulate signaling transduction pathway, and BHB is one of them. Although the signaling functions of ketone bodies were recognized more than three decades ago (Robinson and Williamson 1980), it was only recently that the in vitro and animal experiments showing that (a) BHB regulates lipolysis and sympathetic activity through G protein-coupled receptors (GPCRs), the hydroxycarboxylic acid receptor 2 (HCAR2), and the free fatty acid receptor 3 (FFAR3), respectively (Taggart et al. 2005; Kimura et al. 2011) and that (b) physiological concentrations of BHB inhibit class I and class II histone deacetylases (HDACs) – as similar as its structurally related molecule butyrate (which only differs in the hydroxyl group that is present in the BHB molecule) – to affect the expression of a discrete set of genes (Shimazu et al. 2013) represented a breakthrough in the field of metabolic control and gene expression regulation by metabolites and paved the way for more studies positioning BHB as a signaling molecule that links nutritional status to stress response pathways.

For example, it is known up to date that BHB possesses anti-inflammatory activity in part by inhibiting the nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 (NLRP3) inflammasome and the nuclear factor kappa B (NF-kB) signaling (Youm et al. 2015). Also, BHB induces the AMP-activated protein kinase (AMPK) signaling to reduces endoplasmic reticulum stress (Bae et al. 2016) and reduces the mechanistic target of rapamycin (mTOR) pathway to promote cellular differentiation (Wang et al. 2017). In addition, BHB extents lifespan in the nematode *Caenorhabditis elegans* though several putative signaling pathways (Edwards et al. 2014). Furthermore, recent studies have found β-hydroxybutyrilation – a form of posttranslational modification – as

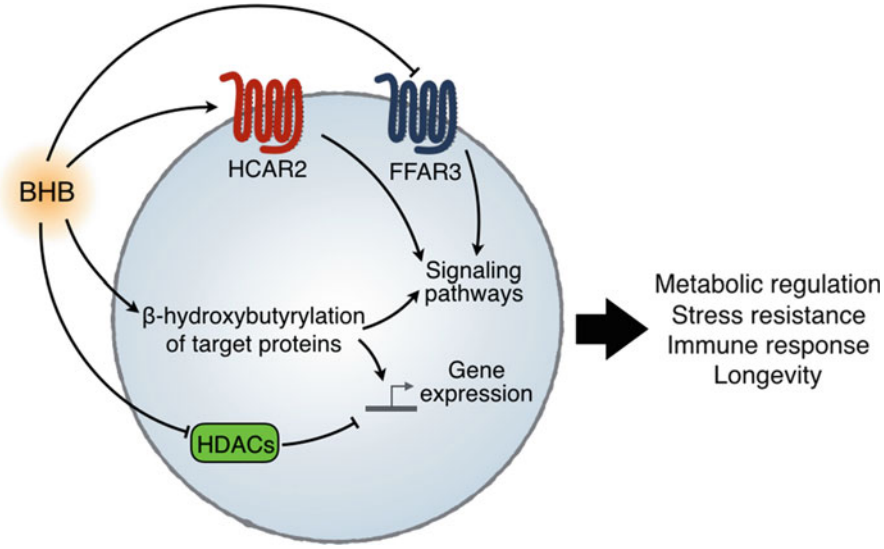

Fig. 4 Regulatory functions of β-hydroxybutyrate (*BHB*). BHB regulates several processes through G protein-coupled receptors, posttranslational modification of proteins, or by controlling gene expression by epigenetic mechanisms. Key: *HCAR2*, hydroxycarboxylic acid receptor 2; *FFAR3*; free fatty acid receptor 3; *HDACs*, histone deacetylases

another level of regulation by BHB (Xie et al. 2016). Taken together, these findings suggest that BHB has profound effects in a broad range of processes by acting as a signaling molecule (Fig. 4).

β-Hydroxybutyrate as a Starvation Signal

As explained above, BHB regulates several processes through receptors, posttranslational and epigenetic modifications, and by yet unknown mechanisms. Additionally, cumulating evidence reveals that BHB overlaps starvation in different ways (Fig. 5). Furthermore, BHB might function even as a signal fine-tuning metabolic pathways and processes to successfully cope with nutritional stress imposed by starvation (Rojas-Morales et al. 2016).

For example, it is believed that, by activating HCAR2, BHB inhibits adipocyte lipolysis to reduce FFA overload and possible premature death during starvation (Taggart et al. 2005). In addition, by acting through the same receptor, it is likely that BHB promotes adiponectin release to control feeding behavior at the central nervous system (Plaisance et al. 2009). On the other hand, by inhibiting FFAR3, BHB lowers sympathetic activity and overall metabolic rate (Kimura et al. 2011). Also, through partially unknown mechanisms, BHB reduces norepinephrine signaling, thus contributing to reduce catecholamine-induced thermogenesis (Cañas et al. 1998) and possibly energy expenditure as seen during starvation.

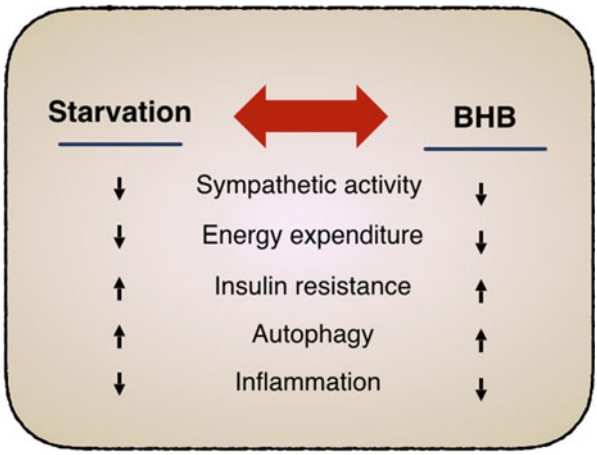

Fig. 5 Similar effects of starvation and β-hydroxybutyrate (*BHB*). BHB replicates the effect of starvation on several processes

During nutrient deprivation, tissues become insulin resistant in order to spare glucose for the brain, a process called starvation-induced insulin resistance. It has been shown that BHB reduces insulin-mediated glucose uptake in both skeletal muscle and cardiomyocytes through a mechanism likely dependent on PI3K/AKT signaling down-regulation (Yamada et al. 2010; Pelletier et al. 2007), suggesting that during starvation, by targeting insulin signaling in peripheral tissues, BHB contributes to fuel brain energy metabolism partly by its own oxidation in neuronal cells and also by providing glucose.

By inhibiting HDACs, BHB epigenetically controls gene expression. Among the most strongly unregulated stress response genes in the kidney of mice exposed to BHB was the transcription factor FOXO3, which orchestrates an antioxidant program to counteract oxidative stress (Shimazu et al. 2013). Notwithstanding, the function of FOXO3 is not limited to keeping redox balance, but this protein is also a transcriptional activator that upregulates many genes participating in autophagy (Mammucari et al. 2007). In this sense, owing to the fact that autophagy serves as one of the most ubiquitous survival mechanisms to nutrient deprivation, it is likely that BHB also regulates autophagy during starvation. Recently, this has been shown to be true in neurons deprived of glucose, where BHB promoted autophagy flux and survival (Camberos-Luna et al. 2016). Currently, in addition to FOXO3, it has been enumerated several potential, yet untested signaling routes regulated by BHB that might promote autophagy during nutrient deprivation (Rojas-Morales et al. 2016).

Finally, BHB might also be engaged in reducing inflammation during starvation. Since organisms require large amounts of both energy and building blocks to mount an appropriate inflammatory response, reducing inflammation is the most plausible thing to do when nutrient resources are scarce. By inhibiting inflammatory intermediates participating in the onset of inflammation (as noted previously), BHB might tip the balance toward saving energy in response to energy crisis (Rojas-Morales et al. 2016). Taken together, although incomplete information still exists, all these findings indicate BHB serves important regulatory role during starvation.

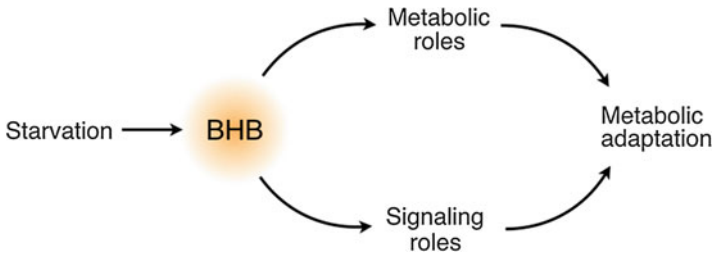

Fig. 6 The dual nature of β-hydroxybutyrate (*BHB*) during starvation. BHB can function as a substrate and a signal during nutritional challenge. By doing this, BHB might contribute to fine-tune metabolic adaptation to starvation

Concluding Remarks

Today, it is well recognized that BHB is not simply a metabolic intermediate because of its ability to engage both cell-surface and intracellular receptors and to coordinately regulate major cellular function and metabolism through its action on gene expression and signaling transduction pathways. Processes spanning from immunity to longevity are regulated by BHB. In this chapter, we have reviewed both the metabolic and signaling properties of BHB in the particular scenario of nutrient deprivation. We believe that BHB mediates metabolic response to nutrient availability through a complex set of mechanisms that still await further elucidation. Essentially, this response involves the dual nature of BHB: as a substrate and a signal (Fig. 6). Although it can take quite a long time to reveal the molecular underpinnings defining the net contribution of BHB to the biology of starvation, the functions of BHB in many aspects of biomedical research are increasingly rising.

Policies and Protocols

In this chapter, we have described that blood β-hydroxybutyrate (BHB) level rises during starvation. In the following, we describe how to measure plasma BHB in a 96-Well microtiter plate.

Principle

The assay is based on the oxidation of BHB into acetoacetate by β-hydroxybutyrate dehydrogenase that uses NAD^+ as a cofactor. Thus, as a result of BHB oxidation, NADH levels increase. In experimental settings, NADH can be easily detected spectrophotometrically at 340 nm. The addition of hydrazine to the reaction ensures complete conversion of BHB into acetoacetate.

Reagents

1. Tris-HCl buffer 100 mM, pH 8.5
2. D-β-hydroxybutyrate stock solution (Cayman Chemical, 700,192) 1 mM, suspended in Tris-HCl buffer.
3. BHB standards (0 to 0.5 mM) prepared from BHB stock solution.
4. β-Nicotinamide adenine dinucleotide (NAD^+) 4 mM, suspended in Tris-HCl buffer.
5. β-hydroxybutyrate dehydrogenase (Sigma, H9408) 4 U/ml, suspended in Tris-HCl buffer.
6. Hydrazyne hydrate (Sigma, 225,819) 4 mM, suspended in Tris-HCl buffer.

Procedure

7. Add 50 μl of each standard or plasma sample to wells.
8. Add 50 μl of hydrazyne hydrate.
9. Add 50 μl of NAD^+.
10. Add 50 μl of D-β-hydroxybutyrate dehydrogenase.
11. Incubate the plate at 25 °C for 10 min.
12. Read the absorbance at 340 nm using a plate reader.

Calculation

To calculate the amount of BHB in samples, plot the absorbance values of each standard as a function of BHB concentration, and then calculate the BHB concentration in samples from the standard curve.

Dictionary of Terms

- **Autophagy** – Autophagy is a process enhanced during stressful situations such as starvation that degrade cytoplasmic components within membranous structures called autolysosomes. This process is activated with the purpose of delivering essential molecules (lipids, amino acids, and carbohydrates) for recycling and energy production in most cells.
- **Histone deacetylases (HDACs)** – A family of proteins that remove the acetyl moiety of acetylated proteins (mainly histones). These enzymes are associated with transcriptional repression.
- **Ketoacidosis** – Pathological situation that results from both elevated production and reduced oxidation of ketone body characterized by a drop in blood pH. This pathological state is commonly observed in diabetic patients.

- **Ketogenesis** – Set of enzymatic reactions acting in concert to convert acetyl-CoA – produced during the oxidation of lipids and some amino acids – to the ketone bodies acetoacetate and β-hydroxybutyrate, occurring mainly in the liver.
- **Ketolysis** – The process of utilization/oxidation of ketone bodies to produce acetyl-CoA and then ATP, occurring in most tissues but not in the liver.
- **β-hydroxybutyrylation** – A newly identified type of posttranslational modification of lysine residues of proteins with β-hydroxybutyrate.

Summary Points

- This chapter describes the functions of ketone bodies, specially β-hydroxybutyrate (BHB), in starvation.
- BHB is a metabolic intermediate that connects fat stores in adipose tissue to ATP synthesis in most tissues in the body.
- By functioning as a metabolic intermediate, BHB sustains energetic requirements and spares both glucose and proteins during starvation.
- Recent studies have shown that BHB stands out as a signaling metabolite controlling signaling transduction pathways and gene expression.
- BHB functions as a substrate and a signal.
- BHB contributes to starvation-induced insulin resistance.
- BHB contributes to reduce energy expenditure and inflammation during starvation.
- BHB regulates autophagy through several putative signaling pathways.
- We propose that β-hydroxybutyrate regulates metabolic pathways contributing to fine-tune metabolic adaptation to starvation.

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Health Impacts of Omega-3 Fatty Acid Deficiency

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F. D. Russell and L. T. Meital

Contents

Abstract

Humans lack the capacity to synthesize omega-3 polyunsaturated fatty acids (n-3 PUFAs); thus, these essential nutrients must be obtained from the diet. Pelagic fish are a rich source of the long chain n-3 PUFAs, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA), while oil derived from plants such as flaxseed and soybean are excellent sources of the shorter chain n-3 PUFA, α-linolenic acid (ALA). Although national and international health and government organizations provide recommendations for dietary intake of n-3 PUFAs, targets are rarely met by most populations. Furthermore, segments

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within these populations may have particularly low intake and/or bioavailability of n-3 PUFAs that correlate with actual or increased risk of disease. Specific examples include babies that are fed nonfortified infant milk formula, individuals with polymorphic genes encoding enzymes with low efficacy for transport of fatty acids across membranes, and vegan populations with negligible EPA and DHA intake. The relationship between n-3 PUFA intake and n-3 PUFA bioavailability is complex and influenced by many factors, including genetics, smoking status, age, gender, and ratio of n-6 to n-3 PUFAs in the diet of the individual. The omega-3 index is a measure of the combined EPA plus DHA content of red blood cell membranes, expressed as a percentage of total membrane fatty acids, and serves as a better indicator of an individual's n-3 PUFA status than measurements of dietary intake. This chapter examines evidence highlighting associations between n-3 PUFA deficiency and adverse impacts on human mental and physical health. While the focus is on human n-3 PUFA deficiency, findings from cell and animal-based research have been included where this contributes toward improved understanding of human deficiency. The chapter also explores new and developing methods for production of n-3 PUFAs that promise clean and sustainable alternatives to fish, to address n-3 PUFA deficiency in human populations.

Keywords

Omega-3 polyunsaturated fatty acids · Omega-3 index · Docosahexaenoic acid · Eicosapentaenoic acid · Alpha-linolenic acid · Deficiency · Vegan · Vegetarian · Visual acuity · Fish intake

List of Abbreviations

ALA	Alpha-linolenic acid
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic
EPA	Eicosapentaenoic acid
GC-MS	Gas chromatography-mass spectrometry
LPC	Lysophosphatidylcholine
Mfsd2a	Major facilitator superfamily domain 2a
mRNA	Messenger ribonucleic acid
n-3 index	Omega-3 index
n-3 PUFA	Omega-3 polyunsaturated fatty acid

Introduction

The beneficial, pleiotropic effects of omega-3 polyunsaturated fatty acids (n-3 PUFAs), whether acquired through the diet or consumed as a nutrient supplement, have been widely reported by an extensive research literature. Consequent to this,

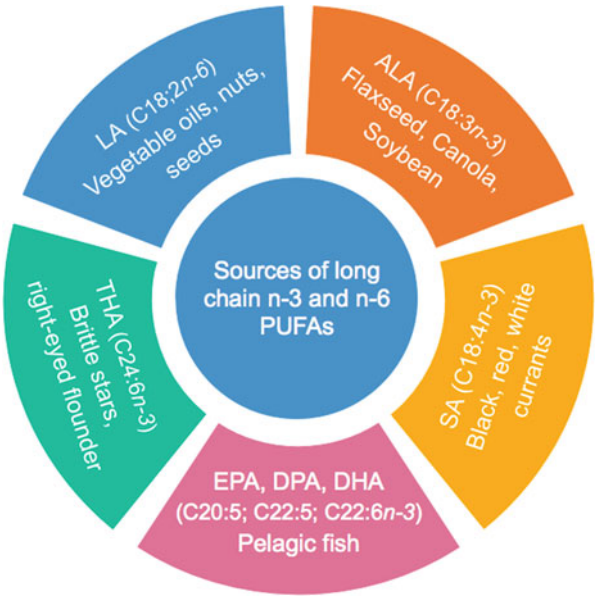

Fig. 1 Primary dietary sources of n-3 and n-6 polyunsaturated fatty acids. Linoleic acid (LA); α-linolenic acid (ALA); stearidonic acid (SA); eicosapentaenoic acid (EPA); docosapentaenoic (DPA); docosahexaenoic acid (DHA); tetracosahexaenoic acid (THA)

national and international health and government organizations have provided recommendations regarding dietary intake of n-3 PUFAs, primarily α-linolenic acid (ALA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA), as a means of maintaining good health or providing protection against disease (Russell and Bürgin-Maunder 2012). However, for many individuals basal intake of DHA and EPA falls well below recommended levels (Meyer 2016). Notable exceptions are Japanese and Inuit populations, the diets of which include high intakes of oily, pelagic fish, seafood or marine mammal fat, all rich sources of DHA and EPA (Fig. 1). The Codex Alimentarius Commission develops international food standards as part of the Food and Agriculture Organization of the United Nations and the World Health Organisation. An electronic Working Group of the Commission is currently exploring whether a nutrient reference value of 250 mg/day for EPA + DHA can be recommended for risk reduction of fatal coronary heart disease events (Codex Alimentarius Commission CX/NFSDU 17/39/6). Opinion within the electronic Working Group is divided, and deliberations on this matter are ongoing. A related consideration to identifying if, and how much EPA and DHA is needed to provide health benefit, is whether dietary deficiency of these fatty acids may lead to adverse health outcomes. In this chapter, n-3 PUFA deficiency is defined as suboptimal levels of n-3 PUFA that correlate with actual or increased risk of disease that can be corrected with dietary or nutraceutical supplementation. The aim of this chapter is to examine evidence highlighting associations between n-3 PUFA deficiency and adverse impacts on human mental and physical health. Findings from studies that have used cell or animal models of n-3 PUFA deficiency have been included where this provides insight into n-3 PUFA deficiency in humans.

Impact of Diet on Erythrocyte Membrane Phospholipid n-3 PUFA Content

The omega-3 index (n-3 index) provides a quantitative measure of EPA + DHA, expressed as a proportion of total fatty acids incorporated into erythrocyte membrane phospholipids. Table 1 compares the n-3 index for groups of people consuming vegan, vegetarian, omnivore, or high-fish diets. The table includes several studies that have used nonstandardized protocols to calculate the n-3 index as evidenced by some differences in fatty acid selection for determination of total fatty acids. With this limitation in mind, the n-3 index was typically high for individuals with greater intakes of fish or marine mammal fat. A study by Kawabata et al. (2011) found that the mean daily intake of fish in a cohort of Japanese men aged 67.1 ± 4.3 years was 111.2 g (905 mg/day EPA + DHA), providing an n-3 index of 9.0%. In the same study, the mean daily intake of fish in a cohort of Japanese women aged 63.5 ± 4.3 years was 77.9 g (751 mg/day EPA + DHA), providing an n-3 index of 8.3% (Table 1). While populations with a high-fish diet had a correspondingly high n-3 index (>8%), those who adhered to a long-term vegan diet had a correspondingly low n-3 index (<4%), with omnivores somewhere in between these two extremes. These findings are not surprising and are consistent with the n-3 PUFA content of foods included in the respective diets. The EPA intake of vegan men and women has been reported to be ~14.4-fold and ~50-fold lower than a population who consumed fish and meat (Welch et al. 2010). In the same populations, vegan men and women were shown to consume no DHA in their diet whilst the cohort that consumed fish and meat had a daily intake of 0.18 g/day (men) and 0.15 g/day (women).

Daily dietary intake of ALA has been reported to be between 8.8- to 37.5-fold higher than EPA and 6.6- to 18.7-fold higher than DHA in surveys of fatty acid intake in Western populations (Welch et al. 2010; Tressou et al. 2016; Raatz et al. 2017). Despite this, the level of ALA in plasma and membrane phospholipids of most cell types is lower than that of EPA and DHA, suggesting a metabolic precursor role of ALA (Baker et al. 2016). When supplemented with an ALA-enriched diet, subjects were observed to have a significantly increased plasma EPA concentration with little or no change in DHA concentration (Brenna et al. 2009). Although soybean, chia seed, flaxseed, and canola oil are abundant sources of ALA, efficiency of conversion of ALA to EPA and DHA is estimated to be only ~5% and < 0.5%, respectively (Welch et al. 2010) (Fig. 2), raising the possibility that adherence to a nonfatty acid supplemented vegan diet may lead to n-3 PUFA deficiency. However, this conclusion requires some qualification. (i) Higher conversion rates are reported for women than for men (Proust et al. 2014) and for smokers compared to nonsmokers (Pawlosky et al. 2007). (ii) Genetic variability in genes encoding Δ^6 and Δ^5 desaturases, two rate-limiting enzymes involved in the biosynthesis of n-3 and n-6 PUFAs, may affect rate of fatty acid conversion. Two haplotypes have been identified, one having significantly greater efficiency for conversion of ALA to DHA than the other (Ameur et al. 2012). (iii) In rats, liver Δ^6 desaturase mRNA expression is increased when animals are fed an ALA-depleted diet (Hofacer et al. 2011). However, conversion efficiency is also influenced by the ratio of n-6 to n-3 PUFAs. In a

Table 1 n-3 Index in selected populations adhering to vegan, vegetarian, omnivore, or high fish/marine fat diets. The types of fatty acids used in the determination of the n-3 index are shown

Diet	Population	n-3 Index	Fatty acids	Reference
Vegan (duration ≥1 year and no fatty acid supplements)	22–85 years; $n = 166$; men/women (1:1.09 ratio)	3.73%	14:0, 16:0, 18:0, 18:1 t, 18:1n-9, 18:2 t, 18:2n-6, 18:3n-3, 20:3n-6, 20:4n-6, 20:5n-3, 22:4n-6, 22:5n-3, 22:6n-3	(Sarter et al. 2015)
Vegan (duration ≥1 year and no fatty acid supplements)	20–54 years; $n = 40$; men	3.48%	14:0, 16:0, 18:0, 18:1 t, 18:1n-9, 18:2 t, 18:2n-6, 18:3n-3, 20:3n-6, 20:4n-6, 20:5n-3, 22:4n-6, 22:5n-3, 22:6n-3	(Sarter et al. 2015)
Vegan (duration ≥2 years)	Mean 49 years (40–70 yr); $n = 23$; men/women (1:1.88 ratio)	2.71 wt%	16:0, 16:1n-7, 18:0, 18:1n-9, 18:2n-6, 18:3n-3, 20:3n-6, 20:4n-6, 20:5n-3, 22:4n-6, 22:5n-6, 22:5n-3, 22:6n-3	(Pinto et al. 2017)
Vegan (duration ≥1 year)	21–66 years; $n = 22$; men/women (1:0.833 ratio)	2.1 wt%	16:0,18:0, 22:0, 24:0, 18:1, 24:1, 18:2n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6, 20:5n-3, 22:5n-3, 22:6n-3	(Sanders et al. 1978)
Vegetarian (≤1 fish meal/month for ≥1 year)	18–43 years; $n = 103$; men/women (1:3.22 ratio)	4.6–4.8 wt%	16:0, 18:0, 16:1n-7, 18:1n-9, 20:3n-9, 18:2n-6, 18:3n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6, 18:3n-3, 20:5n-3, 22:5n-3, 22:6n-3	(Geppert et al. 2005)
Omnivore	21–66 years; $n = 22$; men/women (1:0.833 ratio)	7.0 wt%	16:0,18:0, 22:0, 24:0, 18:1, 24:1, 18:2n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6, 20:5n-3, 22:5n-3, 22:6n-3	(Sanders et al. 1978)
Omnivore	Mean 54 years, $n = 24$; men/women (1:1 ratio)	5.42 wt%	16:0, 16:1n-7, 18:0, 18:1n-9, 18:2n-6, 18:3n-3, 20:3n-6, 20:4n-6, 20:5n-3, 22:4n-6, 22:5n-6, 22:5n-3, 22:6n-3	(Pinto et al. 2017)
High fish diet in a Japanese population (mean daily intake, 77.9–111.2 g)	Men, 60–75 years, $n = 22$; women, 56–73 years, $n = 32$	9.0% (men); 8.3% (women)	Full list of fatty acids not provided, but included: 18:3n-3, 20:5n-3; 22:5n-3, 22:6n-3; 18:n-6; 20:3n-6; 20:4n-6	(Kawabata et al. 2011)

(*continued*)

Table 1 (continued)

Diet	Population	n-3 Index	Fatty acids	Reference
High marine mammal fat diet in an Inuit population (≥5 g/day)	45–74 years, $n = 232$; men/women (1:1.34 ratio)	9.35%	C18:3n-3, C20:5n-3, C22:5n-3, C22:6n-3, C20:4n-3, C22:3n-3, C182n-6, C20:4n-6, C20:3n-6, C22:4,n-6, C18:1n-7, C18:1n-9, C22:1n-9, C24:1n-9, C16:0, C18:0, C22:0, C24:0	(Proust et al. 2014)

The n-3 index is the EPA + DHA peak areas as a percentage, or mole percentage of total peak areas of all identified fatty acids within erythrocyte membranes. Vegan; no meat, fish, eggs, or dairy products. Vegetarian; typically no meat products. Omnivore; mixed diet, including meat, fish, eggs, and dairy products. High fish diet, as indicated

cell-based assay tracking [^{13}C]-labeled fatty acids, conversion of ALA to EPA and DHA was much higher when cells were exposed to medium containing a low (e.g., 0:1, 1:1) compared to a high (e.g., 4:1, 9:1) n-6 to n-3 PUFA ratio (Harnack et al. 2009). This is of interest because the ratio of n-6 to n-3 PUFAs in the diet of vegan and vegetarian populations is greater than omnivorous populations (Davis and Kris-Etherton 2003) and may therefore contribute to a low conversion of ALA to EPA and DHA in these cohorts.

Animal Models of n-3 PUFA Deficiency

The impact of n-3 PUFA deficiency has been investigated in animal models through mechanisms involving gene deletion and through dietary modification.

Δ^6 Desaturase catalyzes the metabolism of linoleic acid (LA) to fatty acid precursors of arachidonic acid and the metabolism of ALA to fatty acid precursors of EPA and DHA (Leonard et al. 2002) (Fig. 2). The deletion of the gene encoding for Δ^6 desaturase leads to deficiency of EPA and DHA if the diet is not supplemented with these fatty acids. The effect of n-3 PUFA deficiency on motor coordination was investigated in Δ^6 *desaturase* knockout and wild-type mice that were bottle-fed a diet comprising LA + ALA with or without DHA supplementation (Harauma et al. 2017). Cerebellar DHA composition in Δ^6 *desaturase* knockout mice was only 39.6% of that in wild-type mice, consistent with substantial DHA deficiency within the brain. Evaluation of cerebellar function was measured indirectly using a motor coordination ability test. Motor function was significantly impaired in Δ^6 *desaturase* knockout mice compared to wild-type mice, suggesting a causal effect of DHA deprivation. Consistent with this hypothesis, motor function was rescued by addition of DHA to the diet of the Δ^6 *desaturase* knockout animals (Harauma et al. 2017).

An unexpected and unique opportunity to examine the effect of n-3 PUFA deficiency within the brain was presented following the discovery that lysophosphatidylcholine-esterified DHA (LPC-DHA) is transported across the blood-

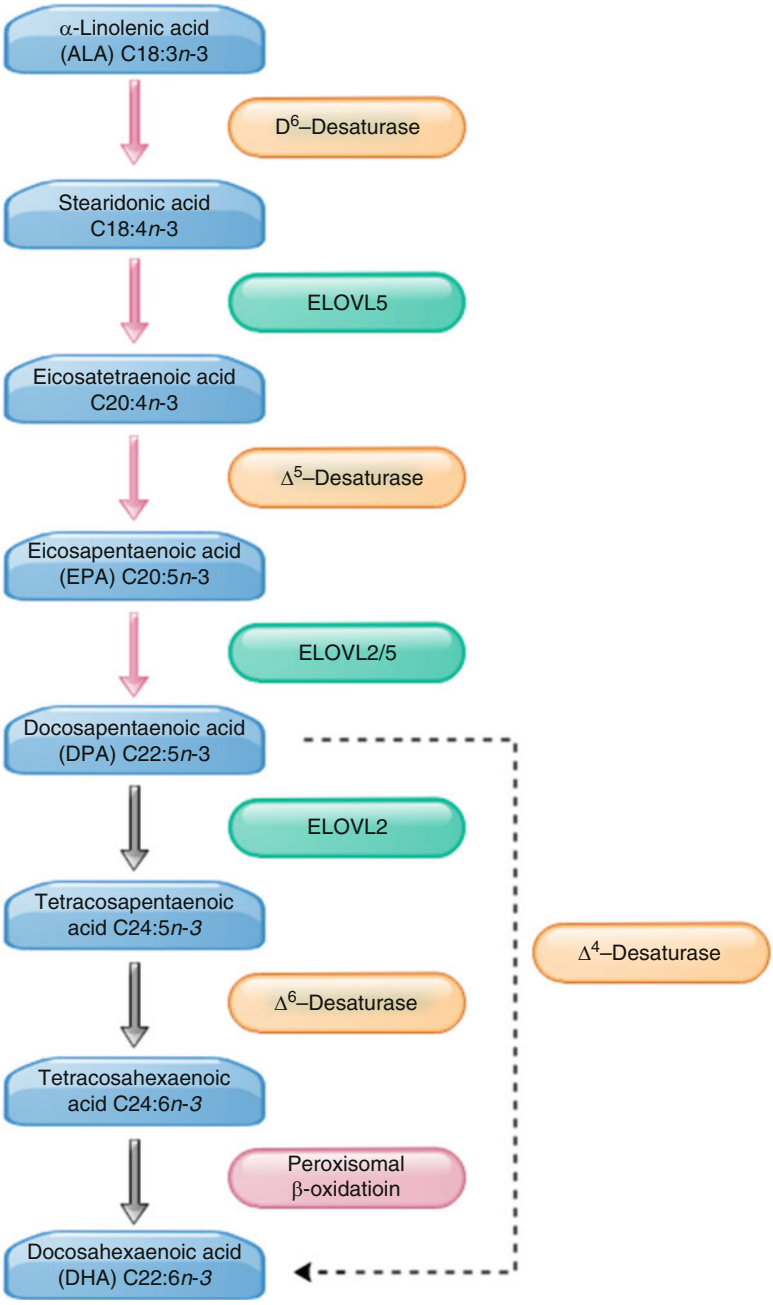

Fig. 2 Biosynthesis pathway for production of n-3 PUFAs from plant-derived ALA. Fatty acids are metabolized by activity of desaturase and elongase enzymes. DPA may be processed via a coupled microsomal-peroxisomal pathway (grey arrows), or activity of Δ^4 desaturase (dashed arrow), which was recently reported in human cells (Park et al. 2015)

brain barrier by the sodium-dependent symporter, major facilitator superfamily domain 2a (Mfsd2a) (Nguyen et al. 2014; Wong et al. 2016). Mfsd2a, which is expressed in cerebral microvessel endothelial cells, is needed to maintain a high DHA composition within brain phospholipids. Deletion of the *Mfsd2a* gene in mice leads to a 60–77% lower level of DHA in brain phospholipids compared to wild-type mice (Nguyen et al. 2014; Wong et al. 2016), and this was associated with loss of Purkinje cells in the cerebellum and mature neurons in the hippocampus, microcephaly, motor dysfunction, anxiety, and deficits in learning and memory (Nguyen et al. 2014). While *Mfsd2a* knockout mice were reported to have reduced blood-brain barrier function (Ben-Zvi et al. 2014), blood-brain barrier integrity was found to be preserved in a later study (Wong et al. 2016). Although deficiency of other fatty acids in *Mfsd2a* knockout mice may contribute to structural and functional abnormalities, it is noteworthy that arachidonic acid levels were *higher* in *Mfsd2a* knockout mice compared to wild-type mice (Nguyen et al. 2014; Wong et al. 2016) and that Mfsd2a has greater capacity to transport LPC-DHA than other LPC-conjugated fatty acids such as oleate and palmitate (Nguyen et al. 2014).

DHA is incorporated into glycerophospholipids of the retina of the eye and is the most abundant n-3 PUFA within photoreceptor outer segment membrane discs (Wong et al. 2016). In addition to its expression within the blood-brain barrier, Mfsd2a is also expressed in retinal capillary endothelium that forms the blood-retinal barrier and in retinal pigment epithelium. Deletion of the *Mfsd2a* gene in mice leads to a 50% reduction in DHA uptake in the eyes, with levels of DHA-containing phospholipids in the eyes declining by 40% compared to wild-type mice (Wong et al. 2016). The deficiency of DHA in the eyes was associated with disorganization and atrophy of the outer segment, formation of gaps between the outer segment and retinal pigment epithelium, and edema within the basal membrane of the retinal pigment epithelium (Wong et al. 2016). Selective knockout of *Mfsd2a* gene in the capillary endothelium (but not retinal pigment epithelium) leads to preserved structural integrity of the eye, suggesting that transport of DHA within the retinal pigment epithelium is critical to structural integrity and development within the eye (Wong et al. 2016).

Health Effects of n-3 PUFA Deficiency in Humans

Investigations into the effect of DHA deficiency on visual acuity in humans have primarily used newborn term or preterm babies as subjects, with the babies receiving infant formula as the sole source of nutrition. Advantages of this approach are firstly that the fatty acid composition of the infant formula can be easily manipulated and secondly that the baby does not receive additional fatty acids, including DHA that is present in human breast milk. In humans, the photoreceptor outer segment begins to form at about 25 weeks of gestation (Martinez et al. 1988). Martinez and colleagues examined postmortem tissue of a baby born prematurely at 25 weeks gestation and who died at 4 months from viral pneumonia. The baby, who had been fed milk formula that was deficient in ALA and DHA, had a retinal DHA level that was only

50% of that expected for a baby receiving these fatty acids in the diet. While the study supports the importance of diet on DHA incorporation in retinal membranes in the early stages of life, the impact of this on eye function was not investigated. This has since been examined in several studies. The effect of DHA deficiency on visual evoked potential visual acuity in humans was examined in 244 term babies that were randomized to receive infant milk formula (infant formula) containing an absence of DHA, or 0.32%, 0.64%, or 0.96% DHA (Birch et al. 2010). Each formula was matched for levels of arachidonic acid, LA and ALA. Visual acuity was significantly poorer in babies that were deprived of DHA compared to those receiving DHA-fortified formula, at ages 1.5, 4, 6, 9, and 12 months. Interestingly, the magnitude of response was the same for the five DHA doses, consistent with a maximal effect with 0.32% DHA.

In addition to visual acuity deficits, a substantial research literature suggests lower levels of n-3 PUFAs correlate with a heightened predisposition toward developmental and mental disorders, cancer and cardiovascular, inflammatory, and neurodegenerative diseases (Table 2).

Factors Leading to n-3 PUFA Deficiency in Humans

Deficiencies of n-3 PUAFs in human populations can be traced to restricted dietary intake of EPA and DHA in foods or nutritional supplements. Indeed, many countries have population data indicating low erythrocyte membrane fatty acid composition that can be ascribed to low consumption of foods that are enriched in EPA and DHA (Stark et al. 2016). Other factors leading to n-3 PUFA deficiency include genetic abnormalities that reduce the efficiency of movement of n-3 PUFAs across non-aqueous barriers, and genetic variation that affects the efficiency of n-3 PUFA metabolism.

Dietary Restriction in Infants

Young children represent a segment of the population at particular risk of n-3 PUFA deficiency, with vulnerability spanning the developmental stages from conception through infancy. During prenatal growth and development, n-3 PUFAs are delivered to the fetus via placental transfer. The *in utero* accretion of DHA in the last trimester of pregnancy is estimated to be 43 mg/kg/day (Lapillonne and Jensen 2009). Taking into account the amount of DHA that is obtained through enteral and parenteral routes and through synthesis, the amount of DHA available to preterm infants (gestational age of $\leq$28 weeks) for accretion after the first week of life is only 16% of that normally accumulated *in utero* (Lapillonne et al. 2010). Although the amount of DHA available for accretion in the preterm infant increases over the first 4 weeks of life, the cumulative amount of DHA at gestational age of 32 weeks is estimated to be only 43–73% of that which is accumulated *in utero* (Lapillonne et al. 2010). Notwithstanding several limitations reported by the authors in the

Table 2 Meta-analyses examining low fish intake and/or low n-3 PUFA levels and association with developmental disorders, mental disorders, inflammatory disease, cardiovascular disease, neurodegenerative disease, or cancer

Disorder		Population characteristics	Summary of inclusion criteria	Main finding	Effect size or relative risk (95% CI)	Heterogeneity	Source
Developmental disorders	Autism spectrum disorder (ASD)	Children (≤12 years; $n = 825$)	Case ($n = 423$); control group with typical development ($n = 402$) Measurement of erythrocyte, serum, plasma, plasma phospholipid or whole blood n-3 PUFAs	Lower n-3 PUFAs in case compared to control group	DHA SMD -2.14^{***} (-3.22 to -1.07) EPA SMD -0.72^{**} (-1.25 to -0.18)	DHA; $I^2 = 97\%$ EPA; $I^2 = 88\%$	(Mazahery et al. 2017)
	Attention deficit hyperactivity disorder (ADHD)	Children and adults (8–35 years; $n = 587$)	Case ($n = 311$); control group with typical development Measurement of erythrocyte and/or plasma n-3 PUFAs	Lower n-3 PUFAs in case compared to control group	EPA, DHA, ALA, and DPA; $g = 0.42^{***}$ (0.26 to 0.59)	EPA, DHA, ALA, and DPA; $I^2 = 0\%$	(Hawkey and Nigg 2014)
		Children and adolescents (4–17 years; $n = 396$)	Case ($n = 200$); control group with typical development ($n = 196$) Measurement of erythrocyte, blood phospholipid, cholesteryl ester, or buccal cell n-3 PUFAs	Lower n-3 PUFAs in case compared to control group	Total n-3 PUFAs $g = -0.58^{***}$ (-0.87 to -0.29)	Total n-3 PUFAs $I^2 = 46\%$	(Chang et al. 2018)

Mental disorders	Depression	General population and community dwellers ($n = 107{,}098$)	Fish consumption assessed by validated food frequency questionnaires	Populations with the highest fish consumption had a 22% lower risk of depression when compared with the lowest fish consumption	RR = 0.78 (0.69 to 0.89)	$I^2 = 61\%$	(Grosso et al. 2016)
		Adolescents and adults from the general population (150,278)	Studies in which fish consumption was assessed Depression, excluding postpartum depression and depression in pregnancy	Populations with the highest fish consumption had a 17% lower risk of depression when compared with the lowest fish consumption. Subgroup analysis revealed reduced risk for studies conducted in Europe only	RR = 0.83^{***} (0.74 to 0.93)	$I^2 = 64.5\%$	(Li et al. 2016)
	Bipolar disorder	Adolescents and adults ($n = 265$)	Case ($n = 118$); control group with typical development ($n = 147$) Erythrocyte n-3 PUFAs	Lower DHA levels in case compared to control group	$d = -0.98^{***}$ (-1.33 to -0.63)	$I^2 = 0.0\%$	(Mcnamara and Welge 2016)
	Schizophrenia	Adults ($n = 1222$)	DPA: (i) medication naïve ($n = 104$), (ii) nonmedicated, previous neuroleptic use ($n = 61$); (iii) neuroleptics ($n = 286$) DHA: (i) medication naïve ($n = 104$); (ii) nonmedicated, previous neuroleptic use ($n = 74$); (iii) neuroleptics ($n = 389$) Erythrocyte n-3 PUFAs	Antipsychotic-naïve and treated patients with schizophrenia were found to have decreased erythrocyte levels of both DPA and DHA when compared to a healthy control cohort	DPA $d = 1.14^{***}$ (0.72 to 1.57) DHA $d = 0.67^{***}$ (0.26 to 1.07)	Overall DPA $I^2 = 87.4\%$ Overall DHA $I^2 = 90.5\%$	(Hoen et al. 2013)

(*continued*)

Table 2 (continued)

Disorder		Population characteristics	Summary of inclusion criteria	Main finding	Effect size or relative risk (95% CI)	Heterogeneity	Source
Inflammatory disease	Rheumatoid arthritis (RA)	Adults (18–89 years; $n = 174{,}701$) and cases ($n = 3346$)	Case ($n = 3346$); control ($n = 174{,}701$) Fish consumption assessed by food frequency questionnaires	A nonstatistically significant inverse association was detected between fish consumption and RA (linear response model) Two servings of fish/week were associated with a 24% reduction in the risk of RA compared to no fish/week (spline model)	Total fish consumption RR = 0.96 (0.92 to 1.01) (linear response model) RR = 0.76 (0.57 to 1.02) (spline model)	Total fish consumption $I^2 = 0.00\%$ (linear response model) $I^2 = 0.00\%$ (spline model)	(Di Giuseppe et al. 2014)
Cardiovascular disease	Coronary heart disease (CHD)	Adults, population-based (18–97 years; $n = 45{,}637$)	(i) Total CHD, case ($n = 7973$); (ii) fatal CHD, case ($n = 2781$); (iii) nonfatal MI events, case ($n = 7157$) Measurement of n-3 PUFAs in total plasma, phospholipids, cholesterol esters or adipose tissue	(i) Lower DPA in case (total CHD) compared to control group (ii) lower DPA and DHA in case (fatal CHD) compared to control group (iii) lower EPA in case (nonfatal MI events) compared to control group	Risk ratios for event DPA (i) RR = 0.94** (0.90 to 0.99) Ii) RR = 0.90 (0.85 to 0.96) DHA (ii) RR = 0.90** (0.84 to 0.96) EPA (iii) RR = 0.71 (0.56 to 0.90)	Overall DPA i) $I^2 = NA$ Ii) $I^2 = 0.0\%$ Overall DHA Ii) $I^2 = 31.2\%$ Overall EPA Iii) $I^2 = NA$	(Del Gobbo et al. 2016)

		Population-based subjects (n = 315,812)	Prospective cohort studies Case (n = 4472) Fish consumption assessed by self-administered questionnaires or interview	Low and moderate fish consumption produced 16 and 21% decreases in CHD mortality risk when compared with lowest levels of fish consumption. Dose-response analysis indicated a 6% decrease in risk of CHD mortality with each 15 g/day increment of fish intake	Low RR = 0.84 (0.75 to 0.95) Moderate RR = 0.79 (0.67 to 0.92) Dose-response RR = 0.94 (0.90 to 0.98)	Low I^2 = 20.1% Moderate I^2 = 56.7% Dose-response I^2 = 63.1%	(Zheng et al. 2012)
	Heart failure (HF)	Population-based subjects (n = 176,441)	Case (n = 5480) prospective studies Fish consumption assessed by food frequency questionnaires; dietary or plasma phospholipid EPA/DHA	Populations with the highest fish consumption had a 15% lower risk of HF when compared with the lowest fish consumption. Highest EPA/DHA intake was associated with a nonsignificant 14% reduction in HF compared to lowest EPA/DHA intake	Fish intake RR = 0.85 (0.73 to 0.99)* EPA/DHA intake RR = 0.86 (0.74 to 1.00)	Fish intake I^2 = 8% EPA/DHA intake I^2 = 44%	(Djoussé et al. 2012)

(*continued*)

Table 2 (continued)

Disorder		Population characteristics	Summary of inclusion criteria	Main finding	Effect size or relative risk (95% CI)	Heterogeneity	Source
	Elevated blood pressure (BP)	Population-based adults (≥18 years; $n = 76{,}701$)	Prospective cohort studies Assessment of fish consumption (food frequency questionnaires): case, ($n = 3590$); participants, $n = 17{,}710$) Assessment of n-3 PUFA intake (serum, plasma, whole blood, or erythrocyte n-3 PUFAs): case, ($n = 16{,}907$); participants ($n = 38{,}494$)	Highest versus lowest fish consumption associated with reduced risk of elevated BP Highest versus lowest level of circulating n-3 PUFAs was associated with a 27% reduction in the incidence of elevated BP	Fish consumption RR = 0.96 (0.81 to 1.14) Circulating n-3 RR = 0.73** (0.60 to 0.89)	Fish consumption $I^2 = 44.7\%$ Circulating n-3 $I^2 = 75\%$	(Yang et al. 2016)
	Stroke	Population-based adults (35–79 years; $n = 20{,}460$)	Prospective cohort studies Stroke events ($n = 2836$) Ischemic stroke events ($n = 2476$) Hemorrhagic stroke events ($n = 252$) Measurement of serum, plasma, whole blood or erythrocyte n-3 PUFAs	Highest levels of circulating n-3 PUFAs were associated with a 14% reduction in stroke risk. Stratification by stroke subtype indicated a 19% reduction in the risk of ischemic stroke. No significant difference was found for hemorrhagic stroke	Stroke RR = 0.86 (0.76 to 0.98) Ischemic stroke RR = 0.81 (0.68 to 0.96) Hemorrhagic stroke RR = 0.95 (0.60 to 1.49)	Stroke $I^2 = 0.00\%$ Ischemic stroke $I^2 = 6.5\%$ Hemorrhagic stroke $I^2 = 0.00\%$	(Yang et al. 2017)

	Metabolic syndrome (MetS)	Population-based participants ($n = 7860$)	Prospective cohort studies Incident cases ($n = 1671$) *Note: analysis of seven cross-sectional studies produced a nonsignificant inverse association between fish or n-3 PUFA intake and risk of MetS*	Highest versus lowest fish consumption was associated with a 29% reduction in the incidence of MetS. Highest versus lowest intake of n-3 PUFA was associated with a 42% reduction in the incidence of MetS	Fish consumption RR = 0.71 (0.58 to 0.87) n-3 PUFA intake RR = 0.58 (0.48 to 0.70)	Fish consumption $I^2 = 60.7\%$ n-3 PUFA intake $I^2 = 63.6\%$	(Kim et al. 2015)
Neuro-degenerative disease	Dementia and Alzheimer's disease (AD)	Population-based participants ($n = 22{,}402$)	Prospective cohort studies in which n-3 fatty acids or fish intake was examined in relation to dementia or AD Fish and/or n-3 fatty acid intake evaluated by semi-quantitative FFQ or FFQ	No statistically significant association was found for n-3 fatty acids and risk of dementia or AD or for fish intake and risk of dementia. Highest versus lowest fish intake was associated with a 36% lower risk of AD	Fish intake RR = 0.64* (0.44 to 0.92)	Fish intake $I^2 = 59\%$	(Wu et al. 2015)
		AD patients and cognitively intact elderly controls	Circulatory DHA and EPA status, case ($n = 488$); control ($n = 1245$) Brain DHA status, case ($n = 237$); control ($n = 220$) Circulatory n-3 PUFA status assessed by measurement of DHA and EPA in plasma, serum or erythrocytes Brain DHA status assessed by measurement	Circulatory DHA and EPA levels were significantly lower in AD patients compared to control. Brain levels of DHA were significantly lower in AD patients compared to control. Results were corrected for age differences between AD patients and controls	Circulatory DHA $g = -1.23^{**}$ (-2.08 to -0.38) Circulatory EPA $g = -0.28^{*}$ (-0.53 to 0.034) Brain DHA $g = -0.52^{**}$ (-0.81 to -0.23)	NR	(De Wilde et al. 2017)

(*continued*)

Table 2 (continued)

Disorder		Population characteristics	Summary of inclusion criteria	Main finding	Effect size or relative risk (95% CI)	Heterogeneity	Source
			of DHA in brain tissue or CSF				
	Amyotrophic lateral sclerosis (ALS)	Population-based participants ($n = 100{,}2082$)	Case ($n = 995$), prospective cohort studies Total n-3 PUFA intake assessed by semi-quantitative FFQ Multivariable adjusted RR	Highest quintile of total n-3 PUFA intake was associated with a 34% reduction in risk of ALS when compared with lowest quintile	Total n-3 PUFAs RR = 0.66*** (0.53 to 0.81)	NR	(Fitzgerald et al. 2014)
Cancer	Brain	Population- and hospital-based participants ($n = 501{,}617$)	Case ($n = 4428$), observational studies (cohort or case-control) Total fish or fresh fish intake assessed by FFQ	Highest versus lowest fish consumption was associated with a 17% reduction in the risk of brain cancer	Fish intake RR = 0.83 (0.70 to 0.99)	Fish intake $I^2 = 37.5\%$	(Lian et al. 2017)
	Breast	Population-based female participants ($n = 883{,}585$)	Case ($n = 20{,}905$), prospective cohort studies Fish intake, marine n-3 PUFA or ALA measured as dietary intake or tissue biomarkers	Highest versus lowest marine n-3 PUFA level was associated with a 14% reduction in the risk of breast cancer. No significant association was observed for fish intake or ALA exposure	Marine n-3 PUFA RR = 0.86** (0.78 to 0.94)	Marine n-3 PUFA $I^2 = 54\%$	(Zheng et al. 2013)
	Colorectal	Population-based participants ($n = 731{,}555$)	Case ($n = 8775$), prospective studies Total n-3 fatty acids or marine fatty acids assessed by FFQ	No significant association was observed for total n-3 fatty acids and colorectal cancer risk or for marine fatty acids and colorectal cancer risk	n-3 Fatty acids RR = 0.99 (0.92 to 1.06) Marine fatty acids RR = 1.00 (0.93 to 1.07)	n-3 Fatty acids $I^2 = 10.5\%$ Marine fatty acids $I^2 = 0.00\%$	(Chen et al. 2015b)

	Liver	Hospital- and population-based participants	Case ($n = 3624$), retrospective case-control studies and prospective studies Total fish intake assessed by interview or self-reported FFQ	Highest versus lowest fish consumption was associated with an 18% reduction in the risk of liver cancer Dose-response analysis indicated a 6% lower risk of liver cancer with each 1 serving/week increase in fish intake	Total fish intake RR = 0.82 (0.71 to 0.94) Dose-response RR = 0.94 (0.91 to 0.98)	Total fish intake $I^2 = 12.8\%$ Dose-response $I^2 = 0.00\%$	(Huang et al. 2015)
	Prostate	Adults ($n = 461{,}050$)	Prospective cohorts; dietary intake ($n = 446{,}243$) and biomarker studies ($n = 14{,}807$) Dietary intake assessed by FFQ and biomarker studies assessed by measurement of serum or plasma phospholipids	No statistically significant association was found for dietary intake of n-3 PUFAs and risk of prostate cancer or for biomarkers of n-3 PUFAs and risk of prostate cancer	Dietary intake of n-3 PUFAs RR = 1.00 (0.93 to 1.09 Biomarkers of n-3 PUFAs RR = 1.07 (0.94 to 1.20)	Dietary intake of n-3 PUFAs $I^2 = 50.4\%$ Biomarkers of n-3 PUFAs $I^2 =$ NR	(Alexander et al. 2015)

Abbreviations: *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid, *DPA* docosapentaenoic acid, *n-3 PUFA* omega-3 polyunsaturated fatty acid, *ADHD* attention deficit hyperactivity disorder, *FFQ* food frequency questionnaire, *SMD* standardized mean deviation, *g* Hedges' g, *d Cohen's d*, *RR* relative risk, *NR* not reported

*p < 0.05; **p < 0.01; ***p < 0.001

determination of the above estimates, the conclusion of the studies is that infants born prematurely are particularly susceptible to DHA-deficiency and may benefit from additional dietary DHA supplementation.

In breastfed infants, n-3 PUFAs are delivered across the blood-milk barrier from mother to infant. Whilst breastfeeding is endorsed as the preferred method for provision of n-3 PUFAs to infants (Koletzko et al. 2008), this is not always adopted for a variety of reasons (Zhang et al. 2015). In these circumstances, infant formula is often the only source of nutrition to be received by the infant. The Food and Agriculture Organization and the World Health Organisation (FAO/WHO) and Food Standards Australia New Zealand set no mandatory minimum requirement for DHA or EPA in infant formula (FSANZ, Consultation Paper, 2016; FSANZ SD1 Attachment A1.1 – Nutrition Assessment: Proposal P1028; CODEX STAN 72-1981). Inclusion of DHA in infant formula is optional for manufacturers, and if it is present, its level must be no greater than the amount of added arachidonic acid and no less than the amount of added EPA to reflect fatty acid ratios naturally present in breast milk (CODEX STAN 72-1981). Some manufacturers add DHA to infant formula, providing consumers with choice of product. Not surprisingly, the lipid composition of formula has a marked influence on plasma lipid composition. Infants who were fed formula that contained no added arachidonic acid or DHA for 4 months had a very low total plasma concentration of DHA-containing lipids (Uhl et al. 2016). In contrast, infants who were fed formula containing added arachidonic acid and DHA (each at 72 mg/mL) for 4 months had a total plasma concentration of DHA that was not different to breast-fed infants (Uhl et al. 2016). An assessment by FSANZ was that "the addition of LCPUFAs to infant formula does appear to have some positive, albeit minor influence on the development of visual acuity in infants compared to standard formulations" (FSANZ Final Assessment Report, 2007). There is now a growing body of evidence showing that severe deprivation of DHA during development has considerable negative impact on brain function. With concerns for detriment to brain and eye function, a consensus recommendation was issued by a group of pediatric medicine researchers for the addition of DHA to infant formula at levels between 0.2% and 0.5% of total fatty acids, arachidonic acid at levels at least that of DHA, and EPA at levels that do not exceed that of DHA (Koletzko et al. 2008).

Restricted Consumption of n-3 PUFAs in Vegan and Vegetarian Populations

Oily fish are a particularly rich source of n-3 PUFAs with key health organizations recommending consumption of 2 to 3 fish meals per week, providing 250–500 mg of EPA + DHA per day (NHF Position Statement, 2015; European Food Safety Authority). Although meat and poultry have a more moderate n-3 PUFA composition than fish, high-level consumption of these foods can contribute substantially to total dietary intake (Howe et al. 2006). While plant-derived foods provide large amounts of ALA, they tend to be relatively deficient in DHA and EPA and, as mentioned above, are converted to DHA and EPA with poor efficiency. Thus,

individuals favoring vegan and vegetarian (lacto-, ovo-, or lacto-ovo) eating styles may become deficient in DHA and EPA if their diets do not include n-3 PUFA supplementation (refer to section "Impact of Diet on Erythrocyte Membrane Phospholipid n-3 PUFA Content" and Table 1).

Genetic Abnormalities Leading to n-3 PUFA Deficiency

When HEK293 cells were made to express Mfsd2a with p.Thr159Met and p.Ser166Leu substitutions, esterification of exogenous LPC containing DHA into phosphatidylcholine was significantly lower than in cells expressing wild type Mfsd2a (Guemez-Gamboa et al. 2015). The findings raise the question as to whether individuals who express a gene encoding an aberrant Mfsd2a protein might also exhibit cerebral DHA deficiency. A recent study identified two families with genetic abnormalities in the *Mfsd2a* gene: one presenting with a p.Thr159Met amino acid substitution and another with a p.Ser166Leu amino acid substitution (Guemez-Gamboa et al. 2015). Individuals with the aberrant genes exhibited microcephaly and intellectual disability, suggesting that loss of fatty acid transport function across the blood-brain barrier contributes to cerebral DHA deficiency and that this is associated with severe structural and functional abnormalities. It is important to note that LPC-DHA is not the only plasma source of DHA for the brain. The rate of uptake of nonesterified DHA from the plasma to the brain is ten-fold higher than the rate of LPC-DHA uptake (Chen et al. 2015a). This level of redundancy in DHA supply would be expected to temper any diminution in supply of LPC-DHA by aberrant Mfsd2a protein expression.

Processes That Affect Omega-3 Fatty Acid Content and Quality

Food production (e.g., aquaculture) and food processing procedures (e.g., cooking methods) can impact on the quantity and quality of n-3 PUFAs that are consumed in the diet.

Aquaculture contributes 26.5% of production and comprises 38.4% of the monetary value of the New South Wales seafood industry (Hussain et al. 2017). Fish are unable to synthesize n-3 PUFAs and so must obtain these from ingestion of supplied feed. Studies investigating fatty acid profiles of wild and farmed fish report higher levels of n-3 PUFAs in wild compared to farmed fish (Strobel et al. 2012). A fall in n-3 PUFA content of farmed fish was attributed to changes to aquaculture practice in which feed of marine origin was replaced with plant protein sources (Strobel et al. 2012). Strategies to provide new sources of EPA and DHA in aqua-feed have been reviewed recently (Sprague et al. 2017).

The quality of fatty acids in dietary products such as fish and infant formula may be affected by thermal treatment. For example, pan frying of freshwater fish caused a significant decrease in n-3 PUFA content, while the n-3 PUFA content of baked fish was similar to that of the raw fish (Schneedorferová et al. 2015). A

decrease in n-3 PUFA content was observed in Pacific saury, a pelagic fish, following pan frying, grilling, and deep frying, with the greatest loss observed after deep frying (42% loss) (Cheung et al. 2016). The reduction in n-3 PUFA content was attributed to spillage of liquefied subdermal fat during grilling or leaching of fatty acids into the frying oil during deep-frying (Cheung et al. 2016). Lipid oxidation was also reported in grilled and pan-fried saury, and this may have contributed to the observed loss of fatty acid content.

Where infant formula is given to babies, this product is usually prewarmed prior to feeding. In a study examining the amount of fatty acids in ready-to-feed infant formula, the DHA content was reported to be unaffected by warming milk in preboiled water or warming using a microwave (Loughrill and Zand 2016). In contrast, a 22.2% decrease in ALA content was observed in milk formula that was microwave-treated compared to nonheated milk formula. In view of these findings, consideration may need to be given to the type of thermal treatment of dietary products when striving to meet recommended intake levels of n-3 PUFAs.

Strategies to Increase Plasma, Cell, and Tissue n-3 PUFA Levels

Dietary modification is a commonly employed method of increasing n-3 PUFA intake. The Heart Foundation of Australia recommends consumption of food that can deliver levels of EPA + DHA between 250 to 500 mg/day and ALA at 1 g/day (Heart Foundation Position Statement). Oily fish and seeds and nuts are foods with particularly high levels of these fatty acids (Fig. 1). However, despite reports identifying associations between good health and dietary intake of n-3 PUFAs, daily intake of n-3 PUFAs is below recommended levels in most Western populations (Meyer 2016). Potential reasons for this are many and include high cost of fish, undesirable taste or smell of fish, concerns for sustainability in fishing practices, and perceived risk of heavy metal toxicity (Grieger et al. 2012). The consumption of capsules containing fish, krill or flaxseed oil, consumption of red meat, or fortified foods such as eggs, yoghurt, and milk, and for babies, n-3 PUFA-fortified infant formula, may provide satisfactory alternative methods for increasing plasma and cell membrane levels of n-3 PUFAs.

As described above, vegan and vegetarian populations may be deficient in n-3 PUFAs; thus, interest exists in strategies that increase n-3 PUFA levels while avoiding consumption of fish or other animal products. Although ALA is a plant source of n-3 PUFAs, efficiency in conversion of ALA to EPA and DHA is low in humans, particularly when the n-6 to n-3 PUFA ratio is high (see above) (Harnack et al. 2009). A working group of the Italian Society of Human Nutrition recommended that vegetarians should regularly consume foods that are enriched in ALA while limiting intake of foods that have a high LA content (Agnoli et al. 2017). The advice is underpinned by evidence that ALA and LA are competitive substrates for Δ^6 desaturase and that an increase in dietary intake of ALA and concomitant decrease in LA will favor ALA as a Δ^6 desaturase substrate. Consistent with this, erythrocyte EPA composition increased by 51.3% and erythrocyte

DHA composition increased by 18.8% in subjects who reduced their consumption of LA from 7.4% to 2.4% of energy, for 12 weeks, without altering their intake of n-3 PUFAs (Macintosh et al. 2013). The working group also recommended that vegan and vegetarian populations who have an increased requirement for n-3 PUFAs (e.g., pregnant and breastfeeding women) should include an algal-derived supplement that contains EPA + DHA.

Algal sources of n-3 PUFAs, such as marine phytoplankton, contain preformed EPA and DHA. In the food-web, certain species of marine phytoplankton are consumed by filter-feeding zooplankton, which are in turn consumed by zooplanktonivorous fish (Sargent 1997). While these fish are a primary dietary source of n-3 PUFA for humans, the fatty acids can also be obtained from the algal source. Marine phytoplankton-derived oil contains high levels of DHA and EPA, and this was used as a source of n-3 PUFAs to investigate its effect on the n-3 index in a cohort of 48 vegans. Participants consumed algal-derived oil (172 mg DHA, 82 mg EPA per day) for 4 months, during which time the mean n-3 index rose from 3.1% to 4.8% (Sarter et al. 2015).

Transgenic plants are also being developed that can improve the efficiency of long chain n-3 PUFA production. For example, transgenic *Camelina sativa* has been engineered with improved desaturase and elongase enzyme activities. The plants were found to accumulate 12.4% DHA and 3.3% EPA within the seed oil, rivaling levels that are accumulated in pelagic fish (Petrie et al. 2014). Importantly, the increased n-3 PUFA content of the seed oil was not accompanied by new production of n-6 PUFAs, leading to the expectation that consumption of the seed oil would maintain a low n-6 to n-3 PUFA ratio.

Policies and Protocols

Determination of the n-3 index involves extraction of phospholipids from erythrocytes using chloroform and KCl solutions. Extracted lipids are hydrolyzed using a HCl/H_20/acetonitrile solution and exposed to a derivatizing agent (e.g., 1-tert-butyldimethylsilylimidazole), prior to GC-MS analysis. The n-3 index is the combined EPA and DHA peak areas, calculated as a proportion of total area of fatty acids, within the sample. The HS-Omega-3 Index® is a proprietary technology that was developed to standardize the n-3 status of clinical subjects using erythrocytes or whole blood, dried blood spot samples (Köhler et al. 2010). The premise of the application is that the n-3 index is most meaningful when the same group of fatty acids are used to assess "total" erythrocyte membrane fatty acid composition. This is important because the relative abundance of EPA + DHA in membranes is affected by selection of fatty acids included in the assay. The HS-Omega-3 Index® method evaluates the fatty acids esterified in membrane glycerophospholipids, not sphingolipids, and uses a mathematical correction factor to convert dried blood spot data to an equivalent erythrocyte n-3 index. A series of 24 fatty acids used to determine the HS-Omega-3 Index® has been reported previously (Köhler et al. 2010).

Dictionary of Terms

- **Omnivore** – A person with a mixed diet, consuming both meat, and plant products.
- **Pelagic fish** – Oily fish that inhabit deep water, not in contact with either shore or ocean-floor.
- **Phytoplankton** – Unicellular algae belonging to the taxonomic kingdom Protista.
- **Polyunsaturated Fatty Acids** – Fatty acids that include two or more double bonds in their chemical structure.
- **Vegan** – A person who adheres to a plant-based dietary style involving complete avoidance of animal-derived food, including meat, fish, eggs, honey, and dairy products.
- **Vegetarian** – A person who adheres to a plant-based dietary style that typically excludes meat, fish and other marine animals but may include animal by-products in the form of eggs (ovo-vegetarian), milk (lacto-vegetarian), or both (ovo-lacto-vegetarian).
- **Zooplankton** – Aquatic organisms that feed on phytoplankton.

Summary Points

- With the exception of island nations such as Iceland and Japan, basal intake levels of DHA and EPA fall well below recommended guidelines.
- The relationship between the amount of n-3 PUFAs consumed in the diet and n-3 PUFA bioavailability is influenced by a range of factors including genetic make-up, smoking status, age, gender, and ratio of n-6 to n-3 PUFA consumption.
- Transgenic and animal models of restricted n-3 PUFA supply have revealed an important role for DHA in normal brain growth and cognition and have highlighted associations between DHA deprivation and motor dysfunction, visual impairment, and cognitive deficits.
- Although ALA features prominently in modern human diets, conversion of this plant-based n-3 fatty acid to EPA and DHA is reported to be highly inefficient (~5% and $< 0.5\%$, respectively). Individuals favoring plant-based eating styles are thus vulnerable to n-3 PUFA deficiency with adherence to a long-term vegan diet being reported to correlate with an n-3 index $<4\%$. The corresponding value in populations consuming high fish diets is typically $>8\%$.
- Young infants represent an additional at-risk segment of the population with n-3 PUFA deficiency presaging adverse impacts on brain and eye function.
- Meta-analytical studies involving large cohorts of adolescents and/or adults have reported a correlation between low n-3 PUFA levels and a heightened risk of developmental impairments, mental, inflammatory and neurodegenerative disorders, cardiovascular diseases, and neoplasms.
- Strategies to address low n-3 PUFA levels in vulnerable segments of the population include dietary or nutraceutical intervention and provision of alternative n-3 PUFA-rich products in the form of marine phytoplankton or transgenic plants.

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Fatty Acid Uptake by the Heart During Fasting

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Abstract

The heart consumes the highest energy substrates per weight in the entire body. The heart preferentially uses fatty acids (FAs) as energy-providing substrates; 60–90% of cardiac adenosine triphosphate (ATP) is derived from FA oxidation (FAO), with the remaining derived from glucose, lactate, ketone bodies, and amino acids. During fasting, FAO is increased, whereas glucose utilization is reduced. Sources of FAs in circulation are free FA (FFA) bound to albumin and two forms of triglyceride-rich lipoproteins (TGRLP), chylomicrons (CM), and very-low-density lipoproteins (VLDL). Following lipolysis of TG (triglyceride) of TGRLP on the cell surface of endothelial cells, FAs traverse the muscle-type continuous capillary layer via protein-mediated and/or nonprotein-mediated pathways. In turn, FAs bound to albumin in the interstitial space are taken up by the heart via the flip-flop mechanism, which is likely facilitated by several plasma-membrane proteins, including FAT (FA translocase)/CD36. Most FAs are utilized in the heart to generate ATP and the remaining FAs are used as TG storage, membrane phospholipid components, and lipid mediators. During fasting, FA uptake and oxidation are enhanced via transcriptional activation by peroxisome proliferator-activated receptor γ (PPARγ) in capillary endothelial cells and by PPARα and PPARγ coactivator-1α (PGC-1α) in cardiomyocytes. Inherited disorders that affect FAO compromise the function of the heart in catabolic states, such as fasting, which causes sudden infant death syndrome. This section describes the mechanisms that regulate FA uptake by the heart in the fed or fasted state as well as the effects of fasting on cardiac function.

Keywords

FFA · TGRLP · CM · VLDL · Lipolysis · LPL · GPIHBP1 · FAT/CD36 · Flip-flop · Trans-endothelial fatty acid transport · FABP3/4/5 · FAO · PPARα · PPARγ · PGC-1α · Inherited disorders

List of Abbreviations

ATP	Adenosine triphosphate
CM	Chylomicrons
ECs	Endothelial cells
ETC	Electron transport chain
FABP	Fatty acid binding protein
FAD	Flavin adenine dinucleotide

FAO	Fatty acid oxidation
FAT	Fatty acid translocase
FATP	Fatty acid transport protein
FFA	Free fatty acid
GPIHBP1	Glycosylphosphatidylinositol-anchored protein 1
LPL	Lipolysis, lipoprotein lipase
Meox2	Mesodermal homeobox-2
NAD	Nicotinamide adenine dinucleotide
PDH	Pyruvate dehydrogenase
PDK4	Pyruvate dehydrogenase kinase 4
PGC-1α	PPARγ coactivator-1α
PPAR	Peroxisome proliferator-activated receptor
PPRE	PPAR response elements
REE	Resting energy expenditure
RXR	Retinoid X receptor
TCA cycle	Tricarboxylic acid cycle
Tcf15	Transcription factors 15
TG	Triglyceride
TGRLP	TG-rich lipoproteins
VEGF-B	Vascular endothelial growth factor-B
VLDL	Very-low-density lipoproteins
VLDLR	VLDL receptor

Introduction

Energy Expenditure of the Entire Body and Heart

The heart weight is less than 0.5% of the total body weight, but its resting energy expenditure (REE) is the highest among all organs (440 kcal/kg/day, Table 1). As a result, the heart accounts for approximately 9% of the total REE despite its small size (Gallagher et al. 1998). Although muscle preferentially takes up and metabolizes FAs in the fasting state, all of the muscle energy requirements cannot be met by β-oxidation. By contrast, the liver has a greater capacity for β-oxidation than it requires and produces a large amount of acetyl-CoA as the length of fasting increases (Marray 2009). This acetyl-CoA in the liver is used to synthesize ketone bodies, which are major metabolic fuel for skeletal and cardiac muscle as well as the brain. During prolonged starvation, glucose represents less than 10% of the total body energy consumption.

Energy Substrates and ATP Production in the Heart

The normal adult heart uses two main energy substrates, FA and glucose, to generate adenosine triphosphate (ATP), the high-energy phosphate, for cardiac work (Doenst et al. 2013; Huss and Kelly 2004; Kolwicz et al. 2013). The amount of ATP

Table 1 Mass-specific organ metabolic rate at rest in humans (kcal/kg/day). Heart weight is less than 0.5% of the total body weight, but its resting energy expenditure is the highest among all organs (Data are cited from the study by Gallagher et al. (1998) with permission from the American Physiological Society)

Brain	Heart	Liver	Kidney	Skeletal muscle	Adipose tissue	Residual
240	440	200	440	13	5	12

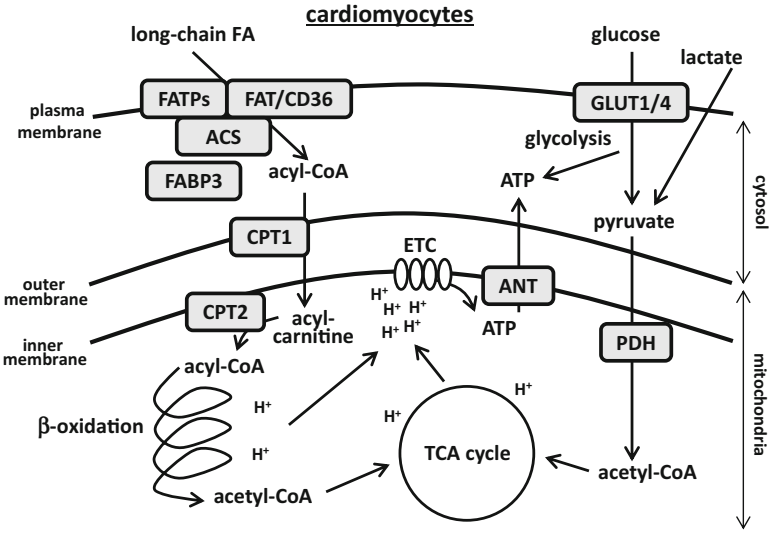

Fig. 1 Uptake of FAs and glucose and their oxidation pathways in the heart. FAs are catabolized in the mitochondrial matrix by β-oxidation, whereas pyruvate derived from glucose and lactate is oxidized by the PDH complex. Acetyl-CoA derived from both pathways is oxidized by the TCA cycle to generate NADH (supply of H^+; proton). A proton gradient established by the ETC drives ATP formation when coupled to oxidative phosphorylation. *ACS* acyl-CoA synthetase, *ANT* adenine nucleotide translocator, *CPT* carnitine palmitoyltransferase, *ETC* electron transport chain, *FABP* fatty acid binding protein, *FAT* fatty acid translocase, *FATP* fatty acid transport protein, *GLUT* glucose transporter, *PDH* pyruvate dehydrogenase (Original figure)

combusted by the heart during a 1-day period is 15–20 times its own weight. The major functions of cardiomyocytes in cardiac work are contraction (pump function), Ca^{2+} uptake into the sarcoplasmic reticulum, and maintenance of the sarcolemmal ion gradients. Under normal conditions, nearly all ATP is generated from mitochondrial oxidation of FA and glucose; 2% or less is derived from anaerobic glycolysis. FA oxidation (FAO) supplies 60–90% of myocardial ATP in the healthy adult heart, whereas the remaining (10–40%) comes from glucose, lactate, ketone bodies, and certain amino acids (Fig. 1). FAs are catabolized in the mitochondrial matrix during β-oxidation, which generates NADH, FADH2 (supply of H^+; proton), and acetyl-CoA. Pyruvate is derived from glucose, and lactate is oxidized by the pyruvate dehydrogenase (PDH) complex to generate acetyl-CoA. Acetyl-CoA derived from both pathways is oxidized by the tricarboxylic acid (TCA) cycle to generate NADH.

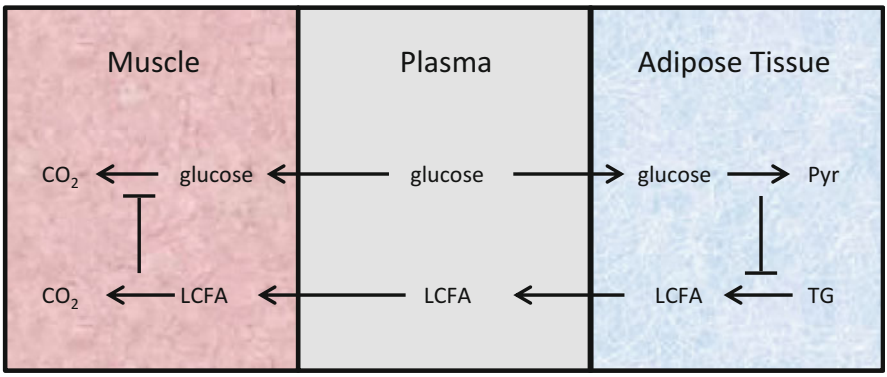

Fig. 2 The "glucose-fatty acid cycle," a homeostatic mechanism to control circulating concentrations of glucose and FAs. The effect of glucose is mediated by insulin. Hormones that control adipose tissue lipolysis affect circulating concentrations of FAs, and FAs, in turn, control fuel selection in muscle. *LCFA* long chain FA, *TG* triacylglycerol, *Pyr* pyruvate (This figure is from the review by Hue and Taegtmeyer (2009) with permission from the American Physiological Society)

A proton gradient established by the electron transport chain (ETC) drives ATP formation when coupled to oxidative phosphorylation (Fig. 1). During fasting, FAO serves as the primary myocardial ATP generating pathway, whereas glucose utilization is minimized. Prolonged fasting results in an (paradoxical) increase in cardiac glycogen and lipid stores (Goldberg et al. 2012; Suzuki et al. 2002; Taegtmeyer et al. 2016), but their storage capacity is very limited for cardiac work. Therefore, the pathways of cellular FA uptake are a major source of energy supply during fasting and FA uptake and oxidation must be tightly coupled to generate ATP.

Competition Between FA and Glucose (Randle Cycle)

The effects of hormones on fuel metabolism are well known. The high insulin/glucagon ratio of the postprandial state promotes lipid and carbohydrate storage. A high glucagon/insulin ratio, characteristic of the fasted state, stimulates adipose tissue lipolysis, and hepatic glucose production to preserve the glucose supply to tissues that exclusively rely on glucose. The glucose-fatty acid cycle, generally referred to as the Randle cycle (Hue and Taegtmeyer 2009), represents nutrient-mediated fine-tuning that works on top of the more coarse hormonal control (Figs. 2 and 3). This dynamic adaptation to nutrient availability applies to the interaction between adipose tissue and muscle (Fig. 2). Hormones that control adipose tissue lipolysis affect the circulating concentrations of FAs, and FAs, in turn, control fuel selection in muscle. Although the biochemical mechanism by which FAO inhibits glucose utilization in muscle seems to be clear, the reciprocal aspect, namely, inhibition of FAO by glucose, only has a limited plausible mechanistic explanation (Fig. 3). Impairment of glucose metabolism by FA (or ketone body) oxidation is mediated by short-term inhibition of several glycolytic steps, namely,

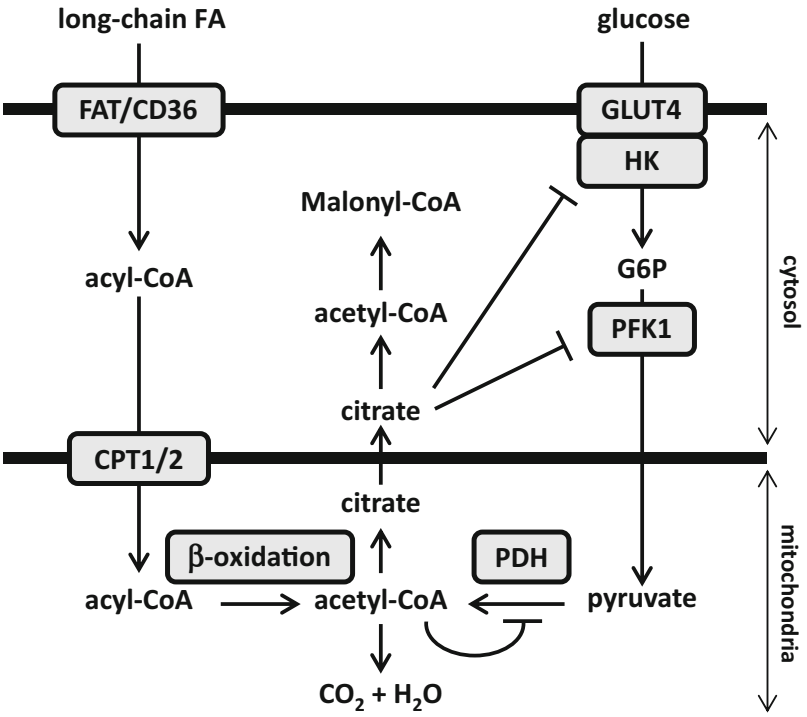

Fig. 3 Mechanism of inhibition of glucose utilization by FA oxidation in cells. PDH inhibition is caused by acetyl-CoA and NADH accumulation resulting from FA oxidation, whereas PFK inhibition results from citrate accumulation in the cytosol. The mechanism of inhibition of glucose uptake is not clear. *CPT* carnitine palmitoyltransferase, *FAT* fatty acid translocase, *GLUT* glucose transporter, *G6P* glucose-6-phosphate, *HK* hexokinase, *PFK* phosphofructo-1-kinase, *F6P* fructose-6-phosphate, *PDH* pyruvate dehydrogenase (Original figure)

glucose transport (Glut4 and hexokinase: HK) as well as phosphorylation of phosphofructo-1-kinase (PFK-1) and pyruvate dehydrogenase (PDH). The extent of inhibition is most severe at the level of PDH and less severe at the level of PFK and glucose uptake. Allosteric inhibition of PDH is caused by acetyl-CoA and NADH accumulation resulting from FAO, whereas PFK inhibition results from citrate accumulation in the cytosol. PDH is also inactivated by phosphorylation via pyruvate dehydrogenase kinase 4 (PDK4), which is induced by the nuclear receptor PPARα during fasting. The mechanism of inhibition of glucose uptake is not clear.

Competition Between FA and Ketone Bodies

The heart also readily oxidizes ketone bodies (β-hydroxybutyrate and acetoacetate) in a concentration-dependent manner (Cotter et al. 2013; Stanley et al. 2003). Whereas plasma ketone body concentrations are normally very low, the concentrations become

elevated during fasting (Marray 2009). A moderate elevation of the plasma ketone body concentration inhibits FAO in the normal heart in vivo. Suppression of FAO by ketone bodies might be mediated through inhibition of FA uptake by the myocardium, independent of changes in myocardial malonyl-CoA concentrations, and not through effects on mitochondrial oxidation. The mechanism underlying inhibition of the cellular FA transport system by ketone bodies remains unclear.

Fatty Acid Uptake by the Heart

Complexity of the Machinery Used in FA Metabolism in the Heart

During the past two decades, there have been many major advances toward understanding FA metabolism in the heart. For example, recent findings have demonstrated that capillary vessels in the heart are not only conduits for plasma and blood cells but also have a significant role in FA metabolism, which dramatically affects FA uptake. Lipoprotein lipase (LPL) produced by cardiomyocytes is transported and anchored by a newly found protein produced by endothelial cells, in which the complex works. Interestingly, it is likely that FAs released from CM and VLDL are taken up via different routes. Several key molecules in the endothelium have been found to function in unique fashions. This main text describes several topics, including the source of FAs, mechanism of capillary endothelial FA uptake, mechanism of myocardial FA uptake, transcriptional regulation of key molecules for FA metabolism, and human genetic disorders associated with cardiac FA metabolism.

Source of FAs

FAs are supplied to the heart as either free FA (FFA) bound to albumin or as FAs released from TG contained in TGRLP: CM, synthesized in the intestine from exogenous dietary fat and VLDL, or synthesized by the liver from endogenous lipid (Evans and Hauton 2016; Lopaschuk et al. 2010). FFAs bound to albumin originate from adipose tissue lipolysis, with some derived from "spillover" through the action of LPL. Both circulating FFA and TGRLP significantly contribute to the overall FA supply to cardiac myocytes. Normal circulating FFA concentrations range between 0.2 and 0.6 mM. These levels can vary dramatically from very low concentrations in fetal circulation to over 2 mM during severe stress, such as myocardial ischemia and uncontrolled diabetes. Prolonged fasting also increases the release of FFA from adipose tissue. Chronic or acute increases in circulating FFA have a major impact on the rates of cardiac FA uptake and β-oxidation, as the arterial FA concentration is the primary determinant of the rate of myocardial FA uptake and oxidation. The plasma TG concentration is also highly variable: fasting plasma TG is typically 0.6–0.7 mM, increasing to 1.5–3.0 mM following a mixed meal. Because TG yields 3×FA

upon complete hydrolysis, availability of plasma TG-FA greatly (90%) exceeds the availability of circulating FFA. However, defining the myocardial preference for lipids has proved difficult.

Mechanism of Capillary Endothelial FA Uptake

Among the complicated machinery that regulates cardiac energy metabolism, FA uptake from circulation and its further transfer to cardiac myocytes through capillary endothelial cells (ECs) are the first critical components (van der Vusse et al. 2000). FAs are supplied to tissues as FFA bound to albumin or TGRLP. In contrast to sinusoidal ECs, which have a large fenestrate (100–200 nm) that allows for the passage of small particles, including albumin (5–10 nm) and chylomicron remnants (30–80 nm), capillary ECs in the heart and skeletal muscle (i.e., muscle-type continuous capillary) do not allow these processes. Instead, theoretically, trans-endothelial FA transport is the most likely mechanism that supplies FA to cardiac myocytes (Wagenmakers et al. 2016). In terms of trans-endothelial FA transport, there are several critical steps: (1) lipolysis on the cell surface, (2) FA uptake by capillary ECs, and (3) FA transport across endothelial cells, each of which has complex mechanisms.

Lipolysis of TGRLP by LPL on the Surface of Capillary ECs

LPL is an essential enzyme that hydrolyses TG contained in TGRLP (Evans and Hauton 2016). Despite contributing less than 0.5% of the total body mass, the myocardium possesses 5% of the total heparin-releasable LPL. Given that more than 90% of plasma FA is contained within TGRLP and that the heart has the highest amount of expressed LPL, lipolysis of TGRLP mediated by LPL is likely to be a principal source of FA for cardiomyocyte metabolism (Chiu et al. 2016). Indeed, cardiac-specific LPL knockout mice exhibited postprandial triglyceridemia (Augustus et al. 2004), whereas cardiac-specific LPL expression in systemic LPL knockout mice improved triglyceridemia (Levak-Frank et al. 1999). These findings support the notions that lipolytic activity by LPL in the heart is a major determinant in the regulation of the plasma TG level, and therefore, FAs derived from TGRLP are a major energy substrate for the heart. Importantly, in the heart, LPL is produced in cardiomyocytes and is transferred to the luminal side of the ECs, where the enzyme functions (Fig. 4). For a long time, it was assumed that LPL was bound to heparin sulfate proteoglycans on the surface of capillary ECs (Adeyo et al. 2012). Recently, it was reported that GPIHBP1, a glycosylphosphatidylinositol-anchored protein of capillary ECs, is the principal binding site for LPL on endothelial cells and that it is responsible for transporting LPL to the capillary lumen (Fig. 4). GPIHBP1 binds to LPL from interstitial spaces and shuttles it across endothelial cells to the capillary lumen. At the luminal side, its ability to bind to both TGRLP and LPL allows it to serve as a platform for TG lipolysis along the luminal surface of capillaries (Adeyo et al. 2012). Mice lacking LPL as well as GPIHBP1 develop severe chylomicronemia, even when fed a low-fat diet. Likewise, patients with genetic mutations of LPL as well as GPIHBP1 have severe chylomicronemia (Adeyo et al. 2012; Merkel et al. 2002).

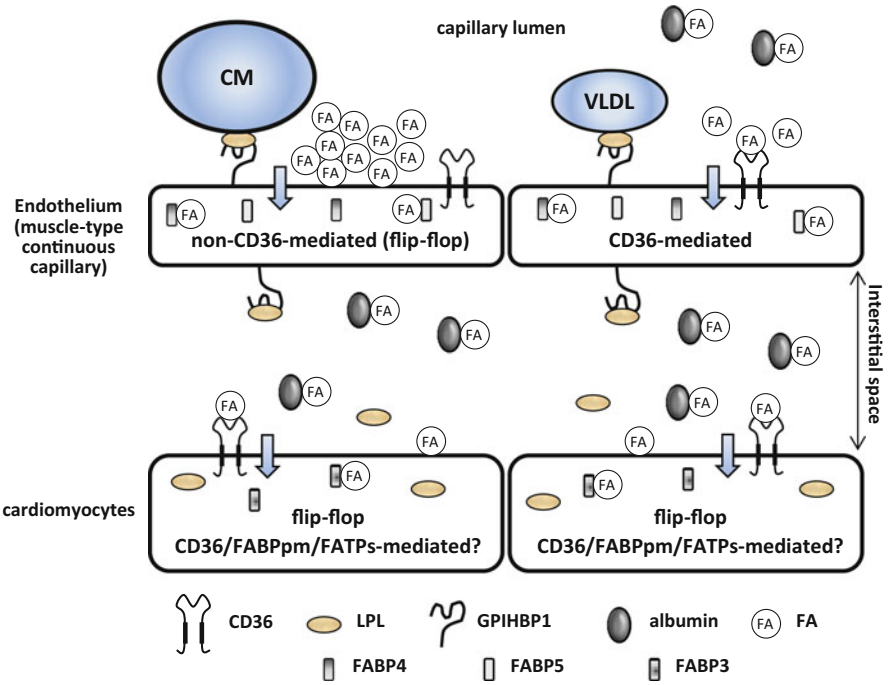

Fig. 4 Lipolysis of TG contained in TGRLP on the luminal side of the capillary endothelium, trans-endothelial FA transport, and FA uptake by cardiomyocytes. This figure has a long and essential story in this section. See the text for details (Original figure)

FA Uptake by Capillary ECs

Studies in many mammalian cell types using FA bound to albumin at increasing molecular ratios yielded evidence of two distinct pathways of FA uptake (Abumrad and Goldberg 2016), a low-capacity saturable pathway and a high-capacity non-saturable pathway (Fig. 4). The saturable pathway has kinetics that are consistent with protein facilitation, with a high affinity for long-chain FAs (Km approximately 10 nM). The nonsaturable pathway operates at high ratios of FA, presumably via the flip-flop mechanism. Indeed, CD36, also known as fatty acid translocase (FAT), is a high-affinity receptor for long-chain FAs (Km of 5–10 nM) and is suitable for the low levels of FFAs in circulation. In the heart, CD36 is more abundant on capillary ECs compared to cardiomyocytes. Bharadwaj et al. demonstrated by using heart-specific LPL-KO and CD36-KO mice that VLDL-derived TG-FA (low local release of FAs) entered the cell through a CD36-mediated channel (high affinity, low capacity, saturable), whereas CM-derived TG-FA (high local release of FAs) entered through a non-CD36-mediated route (low affinity, high capacity, nonsaturable, presumably by the flip-flop mechanism) (Bharadwaj et al. 2010). Unlike deficiencies for LPL as well as GPIHBP1, CD36 deficiency in humans and mice does not cause hyperchylomicronemia, supporting the notion that FAs released from CM are taken up by free diffusion in the absence of CD36. However, the details of TG-FA channeling are still uncertain.

Fatty acid transport proteins 3 and 4 (FATP3/4), which are induced in capillary ECs in response to an increase in vascular endothelial growth factor-B (VEGF-B) secreted from cardiomyocytes, are also required for FA transport of capillary ECs (Hagberg et al. 2010). However, the contribution of these molecules, including CD36 and FATP3/4, to FA uptake is still uncertain because endothelial-specific knockout models for them have not yet been established.

FA Transport Across Capillary ECs

Following FA uptake on the luminal side of ECs through several possible routes, intracellular FAs need to be delivered to the abluminal side of capillary ECs (van der Vusse et al. 2000). Although there are several options for this process, trans-endothelial FA transport mediated by fatty acid binding proteins 4 and 5 (FABP4/5) is a possible pathway that allows FAs to be shuttled in the cytoplasm (Fig. 4). Cytoplasmic FABPs are a family of 14–15 kDa proteins that bind to long-chain FA with high affinity. As lipid chaperones, FABPs may actively facilitate the transport of lipids to specific compartments in cells. FABP4/5 are exclusively expressed in capillary ECs in the heart (Iso et al. 2013). In double knockout mice for FABP4/5, cardiac uptake of FA was substantially reduced, whereas the uptake of glucose was remarkably increased. Importantly, FA uptake was not altered in isolated cardiomyocytes ex vivo, suggesting that capillary ECs can transport FAs via cytoplasmic FABP4/5 to cardiomyocytes (Fig. 4). The hypothesis of trans-endothelial FA transport by FABP4/5 needs to be addressed using endothelial-specific knockout mice.

Mechanism of Myocardial FA Uptake

Following FA transport through the muscle-type capillary endothelial layer, FAs are bound by albumin (300 μM) in the interstitial space of the heart (Fig. 4). After being taken up by cardiomyocytes, FAs are bound by cytoplasmic FABP3 (also designated as H-FABP; 150–300 μM), which acts as the intracellular counterpart of albumin (Glatz et al. 2016). The total FA concentration is 100–400 μM in the interstitial space, whereas it is 50 μM in the cytoplasm. Albumin and FABP3 each provides a buffer for FAs, whereby each FA molecule is immediately replenished by the release of another FA molecule from the protein binding site. The direction and overall rate of FA uptake are determined by the transsarcolemmal gradient of FAs. Trans-sarcolemmal uptake of FA by cardiomyocytes comprises three separate steps: adsorption, flip-flop and desorption.

1. **Adsorption**: Entry of FAs into the outer leaflet of the lipid bilayer, which is facilitated by membrane-associated proteins.

Similar to capillary ECs, the main membrane-associated protein facilitating FA uptake is CD36 in cardiomyocytes (Figs. 1 and 4). Recently, the crystal structure of LIMP2 (Lysosomal Integral Membrane Protein 2), a member of the CD36

superfamily of scavenger receptors, was determined (Neculai et al. 2013). The putative structure of CD36 according to homology modelling suggests that it has a large cavity that serves as a tunnel through which lipids are delivered to the outer leaflet of the plasma membrane. Another protein, plasma membrane FABP (FABPpm; also known as GOT2), is expressed on the extracellular side of cardiomyocytes, myocytes, and ECs. FABPpm associates with CD36 at the plasma membrane, where its primary role seems to be to assist CD36 in the binding and transport of FAs. All FATPs (FATP1–6) have been shown to enhance cellular FA uptake. However, there has been much debate regarding the localization of FATPs and whether they are true FA transporters or whether they simply drive cellular FA influx by intracellular acylation of FAs. The contributions of FABPpm and FATPs to FA uptake are unlikely to be as much as that of CD36.

2. **Flip-flop**: Flip-flop is a transbilayer lipid motion that occurs in the phospholipid bilayer membrane and is independent of receptor-mediated fatty acid uptake.

Biophysical studies have shown that the "flip-flop" of FA in the phospholipid bilayer is very fast for virtually all FA types. Therefore, the possible role for membrane-associated proteins in the adsorption step is most likely restricted to determining the membrane domain where "flip-flop" takes place, rather than the rate of FA translocation from the outer to the inner leaflet.

3. **Desorption**: Movement of FA into the intracellular aqueous phase and hydration of FAs.

This step appears to be rate-limiting for all transmembrane transport and is strongly dependent on the chain length and degree of unsaturation of the FA. There is some evidence that transmembrane proteins, particularly CD36, provide a docking site for cytoplasmic FABP3 (Figs. 1 and 4) or for enzymes that act on FAs, such as acyl-CoA synthetase (Fig. 1). These protein-protein interactions may facilitate the desorption step.

Lipoprotein Receptor-Mediated TGRLP Uptake by the Heart

Direct uptake of CM and VLDL particles or remnant particles via lipoprotein receptors has also been proposed, although their contributions to TG uptake remain disputed (Evans and Hauton 2016). Receptor-mediated assimilation of TGRLP has been reported for many tissues, including the heart; the principal cardiac lipoprotein receptor is believed to be the VLDL receptor (VLDLR). VLDLR binds and internalizes VLDL as well as other apo-E containing lipoproteins, in concert with LPL and apo-E, suggesting that VLDLR has a LPL-facilitating function. VLDLR knockout mice fed high fat diets show decreased cardiac uptake of [^{3}H]TG without altered plasma lipoprotein levels, despite decreased LPL expression. The mechanism of transport of TGRLP across capillary ECs also remains elusive.

The PPAR Family Transcriptionally Regulates Myocardial Energy Metabolism

Acute changes in flux through metabolic pathways are mediated by changes in substrate concentrations and allosteric modification of enzymes catalyzing these reactions (e.g., Randle cycle). Chronic changes in mitochondrial oxidative capacity and substrate selection are also mediated at the gene transcriptional level. Cardiac metabolism is transcriptionally regulated by the three members of the PPAR family (PPARα, β/δ, and γ) of ligand-activated transcription factors (Finck 2007; Huss and Kelly 2004; Rowe et al. 2010). PPARs do not act on a single target, but rather orchestrate several pathways whereby nutrients regulate their own metabolism. The expression of PPARα is high in tissues with an elevated capacity for FAO, such as the liver, heart, brown fat, and kidneys. PPARα regulates FA homeostasis via transcriptional activation of genes encoding key enzymes in FA catabolism, such as FA uptake and FAO. PPARβ/δ is almost ubiquitously expressed and transcriptionally regulates FAO, but these metabolic effects are probably not as essential as those of PPARα. PPARγ is adipose tissue-enriched and thought to play a vital role in regulating fat storage.

Transcriptional Regulation by PPARs and Cofactors

PPAR family members regulate the expression of target genes by binding to direct repeat response elements (PPAR response elements; PPRE) in the promoter region of target genes with their obligate heterodimeric partner, retinoid X receptor (RXR) (Fig. 5). The activity of the PPAR/RXR complex is modulated by the availability of ligands for PPAR and RXR (Finck 2007; Huss and Kelly 2004; Rowe et al. 2010). Potentially, the most relevant endogenous ligands for PPARs are long-chain FAs and their metabolites. However, the specific species of FA metabolites that serve as endogenous natural ligands for PPARs have yet to be fully established. High-affinity pharmacological agents that can modulate the activity of PPARs exist, including fibrates for PPARα and thiazolidinediones for PPARγ. When engaged by a ligand, PPARs recruit transcriptional coactivators that are necessary to initiate target gene transcription. Several coactivators interact with PPARα, including steroid receptor coactivators (SRC) and p300. The best-characterized coactivator of PPARs in the heart is cardiac-enriched PPARγ coactivator-1α (PGC-1α) (Fig. 5). In cardiac myocytes, the activation of PGC-1α drives a strong induction of PPARα target genes encoding FAO enzymes. PGC-1α also coactivates other transcription factors, including estrogen-related receptors (ERRα and γ) and the nuclear respiratory factor 1 (NRF-1), to stimulate mitochondrial biogenesis and enhance expression of components of the electron transport chain. These findings suggest that PGC-1α acts to augment the capacity for ATP production in a global manner by inducing expression of enzymes that are involved in multiple components of these catabolic pathways.

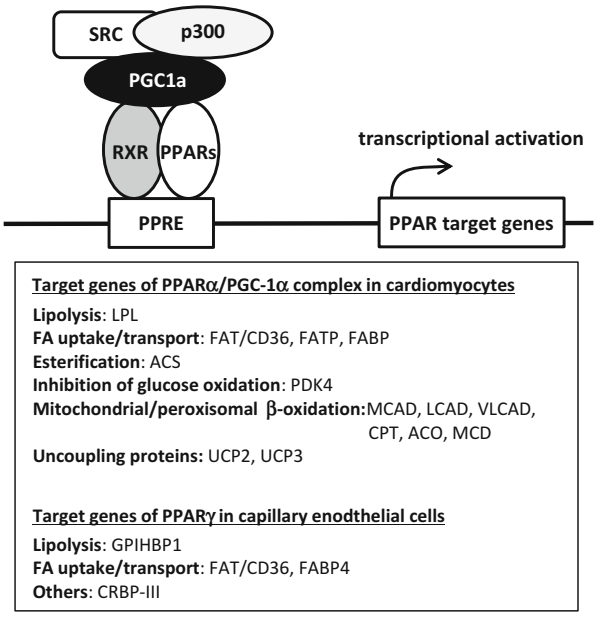

Fig. 5 Transcriptional regulatory complex by PPARs and their cofactors and PPAR-regulated genes involved in cardiac energy metabolism. Many key players for cardiac metabolism are transcriptionally regulated by PPARγ in capillary endothelial cells and by PPARα in cardiomyocytes. *PPRE* PPAR response elements, *RXR* retinoid X receptor, *SRC* steroid receptor coactivator (Original figure)

Gene Expression Regulated by PPARα and PGC-1α in the Heart During Fasting

PGC-1α activity and expression levels are induced by cold exposure, fasting, and exercise, stimuli that are known to promote oxidative metabolism (Rowe et al. 2010). PGC-1α binds to and co-activates PPARα in cardiomyocytes, thereby inducing numerous genes that are critical for FA handling (Fig. 5), including CD36 (import into cell), CPT1b (import into mitochondria), PDK4 (reciprocal inhibition of pyruvate entry into mitochondria), and MCAD (rate-limiting step in medium chain FA β-oxidation). The expression of several PPARα target genes and the rates of FAO are diminished in the hearts of PPARα null mice as well as PGC-1α at baseline and fail to be induced in response to fasting. Thus, PPARα and PGC-1α are essential transcription factors for controlling FA oxidation in the heart during fasting.

Gene Expression Regulated by PPARγ in Capillary ECs During Fasting

Unlike cardiomyocytes, critical genes for FA handling in capillary ECs are regulated by PPARγ (Fig. 5). Expression of PPARγ is induced in capillary ECs by fasting, leading to induction of its target genes, such as GPIHBP1, CD36, and FABP4 (Goto et al. 2013; Kanda et al. 2009). Importantly, endothelial-specific PPARγ knockout mice exhibited hyperchylomicronemia after olive oil gavage as well as higher levels of circulating FFA during fasting, which are consistent with defective function of GPIHBP1 (via LPL) and defective function of FABP4/CD36, respectively. Thus,

endothelial PPARγ in the heart facilitates FA uptake via both a LPL-mediated low affinity/high capacity/nonsaturable pathway and a CD36-midiated high affinity/low capacity/saturable pathway, both of which are enhanced during fasting.

Gene Expression Regulated by Other Factors

Meox2/Tcf15 Heterodimer

Mesodermal homeobox-2 (Meox2) is a homeobox gene that is expressed in EC. Meox2 forms a heterodimer with a basic helix-loop-helix transcription factors 15 (Tcf15), which is highly expressed in capillary ECs. The Meox2/Tcf15 heterodimer drives endothelial expression of genes associated with FA metabolism, including PPARγ, FABP4/5, CD36 and GPIHBP1, to facilitate FA uptake and transport across the capillary endothelium (Chiu et al. 2016; Coppiello et al. 2015). Given that the haplodeficiency of Meox2/Tcf15 in mice impairs cardiomyocyte contractility, this heterodimer not only plays a role in endothelial regulation of FA but may also be involved in altering cardiac energy substrates. The involvement of Meox2/Tcf15 in fasting-induced gene expression remains to be addressed.

VEGF-B/FATPs Axis

Paracrine signaling by VEGF-B from tissues also regulates trans-endothelial FA transport (Hagberg et al. 2010). VEGF-B is expressed in most adult tissues, with the highest expression found in the myocardium, skeletal muscle, and BAT. VEGF-B secreted from myocardium specifically binds to VEGF receptor-1 and to the common co-receptor neuropilin-1 expressed in capillary ECs, which in turn induces the expression of two FA transport proteins, FATP3 and FATP4. Endothelial FATP3/4 are required for FA transport across the vascular endothelial layer. In sharp contrast to the induction of PPARα target genes during fasting, the expression level of VEGF-B is suppressed by fasting and is enhanced by a high fat diet. These findings suggest that the VEGF-B/FATPs axis and cardiac PPARα plus endothelial PPARγ axis function in different nutritional states.

Cardiac Function During Starvation

The effect of starvation on the heart may not be well recognized because it rarely results in heart failure (Taegtmeyer et al. 2016; Webb et al. 1986). Starvation causes a decreased rate of protein synthesis without a change in the rate of protein degradation, leading to a reduction in contractile protein subunits, resulting in cardiac atrophy (Samarel et al. 1987). As the myocardial mass decreases during starvation, the stroke volume and cardiac output decrease proportionately. However, the stroke-volume index and cardiac index remain normal or rise slightly because of the decrease in body size. Additionally, starvation is also associated with a fall in heart rate, blood pressure, and blood volume. Cardiac function in patients with

anorexia nervosa is also known to be maintained. Thus, cardiac atrophy during starvation is unlikely to cause heart failure because the demand of cardiac work is simultaneously reduced.

Refeeding Syndrome

Although cardiac failure is rare in malnutrition, it is common that fatal responses occur by the refeeding of starved people, which is known as refeeding syndrome (Webb et al. 1986). Potential complications of refeeding syndrome include heart failure, fatal arrhythmia, respiratory insufficiency, and hematological derangements. Refeeding is associated with a sudden reversal of compensatory factors, including catecholamines, renin-angiotensin-aldosterone system, salt intake, and sodium retention. These changes increase blood volume and pressure. Furthermore, catecholamines augment the shift of potassium, magnesium, and phosphate into cells, with potentially deleterious effects on cardiac function and rhythm.

Association Between Defective FA Utilization and Cardiac Dysfunction in Humans

Inherited disorders that affect FA oxidation (FAO) seriously compromise the function of the heart and other highly energy-dependent tissues, such as the brain, skeletal muscle, kidney, and liver (Houten and Wanders 2010; Spiekerkoetter and Wood 2010). For almost every enzyme involved in FAO, including CACT (carnitine-acylcarnitine translocase), CPT1a, CPT2, MCAD (medium-chain acyl-CoA dehydrogenase), MTP (mitochondrial trifunctional protein), and VLCAD (very long-chain acyl-CoA dehydrogenase), inherited defects have been reported. Such defects encompass a wide spectrum of clinical disease, presenting in the neonatal period or infancy with recurrent hypoketotic hypoglycaemia, liver dysfunction, myopathy/rhabdomyolysis, and cardiac diseases, such as dilated or hypertrophic cardiomyopathy and/or arrhythmias. These functional disturbances can cause sudden infant death syndrome. This disease is triggered by a catabolic state, such as during fasting, sustained aerobic exercise, surgery, and infections. Thus, a decrease in metabolic flexibility (FAO defects + hypoketotic hypoglycaemia) may lead to acute cardiac dysfunction in a catabolic state. Alternatively, accumulating metabolites, such as acylcarnitines and FAs, due to defective FAO might be toxic to the heart, which may contribute to arrhythmias.

Other gene mutations with defective FA utilization in human are CD36, LPL, and GPIHBP1. CD36 deficiency results in a reduction of myocardial FA uptake and a marked increase in glucose uptake (Abumrad and Goldberg 2016; Glatz et al. 2016). Although CD36 mutations are known to be associated with an increase in the left ventricular mass and cardiovascular events, there are no obvious symptoms and physical findings due to these mutations throughout their life (Love-Gregory and Abumrad 2011). Genetic mutations for LPL and GPIHBP1 cause type I

hyperlipoproteinemia, or familial chylomicronemia (Patni et al. 2000). Often, patients with deficiencies in LPL and GPIHBP1 are not diagnosed until they develop pancreatitis, at which time the serum levels of TG are severely elevated. In contrast to FAO deficiency, genetic mutations of CD36, LPL, and GPIHBP1 do not result in cardiomyopathy, even in a catabolic state. In these genetic disorders, the partial FA uptake pathway should function, as suggested by animal models. Additionally, gluconeogenesis and ketogenesis by the liver are maintained. These findings suggest that energy supply by partial uptake of FAs and by other compensatory energy substrates is sufficient for cardiac work, even under a situation in which the cardiac energy demand is increased.

Protocol

Methods to Estimate Fatty Acid Uptake by the Heart

The traditional methods of organ balance measurements of fuel metabolism require measurements of the rates and amounts of fuel delivery and uptake by using invasive tools to collect blood samples. Imaging studies using radiolabelled tracers can provide accessible, accurate, and quantitative measurements of fatty acid uptake safely and non-invasively. ^{123}I-BMIPP (15-(p-iodophenyl)-3-(R,S)-methyl pentadecanoic acid) has been most extensively used in humans. The 3-methyl group in the side chain of BMIPP inhibits β-oxidation, resulting in prolonged retention in the myocardium. Gamma rays from ^{123}I are imaged by SPECT (single photon emission CT). The uptake of ^{123}I-BMIPP appears to reflect the uptake of circulating free fatty acid, but not fatty acid derived from TGRLP through lipolysis. Instead of ^{123}I-BMIPP, ^{125}I-BMIPP is frequently used for animal studies because of its longer half-life. Fatty acid oxidation is not estivated by radiolabelled BMIPP. To estimate fatty acid uptake from TGRLP, radiolabelled chylomicron and VLDL are necessary. These studies have been performed using animal models, but not in humans. The preparation of radiolabelled chylomicron and VLDL requires a complicated special process, which limits extended studies regarding the uptake of fatty acid derived from TGRLP.

Dictionary of Terms

- **Triglyceride-rich lipoprotein (TGRLP)** – A collective term for chylomicron and VLDL. TGRLP is a major source of fatty acid supply to the heart.
- **Free fatty acid (FFA)** – Most FFA is produced by lipolysis of triglyceride in adipocytes and bound to albumin in plasma. Some are derived from the spillover of those of TGRLP through the action of lipoprotein lipase.
- **Chylomicron** – Chylomicron is the largest TGRLP and is synthesized in the intestine from exogenous dietary fat.

- **Very low density lipoprotein (VLDL)** – VLDL is the second largest TGRLP and is synthesized by the liver from endogenous lipid.
- **Lipolysis** – Triglycerides in TGRLP or adipocytes are hydrolyzed by several types of lipases, resulting in the generation of glycerol and FFA. This process is termed lipolysis.
- **Flip-flop** – Flip-flop is a transbilayer lipid motion that occurs in the phospholipid bilayer of membranes and is independent of receptor-mediated fatty acid uptake.
- **Trans-endothelial fatty acid transport** – Muscle-type nonfenestrated capillary functions as a barrier between circulation and the interstitial space for the movement of FFA. FFA is transported across the endothelial layer by several steps of protein-mediated mechanisms, not by passive diffusion.
- **Peroxisome proliferator-activated receptor (PPAR)** – Three members of the PPAR family (PPARα, β/δ, and γ) are ligand-activated transcription factors that regulate metabolism in many organs, including cardiomyocytes and capillary endothelial cells.

Summary Points

- FA is a central energy substrate for the heart, both in the fed and fasted state. FA oxidation supplies 60–90% of myocardial ATP, whereas the remaining ATP (10–40%) comes from glucose, lactate, ketone bodies, and certain amino acids.
- FA uptake through capillary endothelial cells in the heart comprises three separate steps: lipolysis on the cell surface, FA uptake by the plasma membrane, and FA transport in the cytoplasm.
  1. Lipolysis: Circulating TG contained in TG-rich lipoproteins (CM and VLDL) is hydrolyzed by LPL located on the luminal surface of the capillary endothelial cell. In the heart, LPL is produced by cardiomyocytes. GPIHBP1, expressed in capillary endothelial cells, is indispensable as a binding site for LPL on endothelial cells and for transporting LPL to the capillary lumen.
  2. FA uptake: Low levels of FAs derived from VLDL are taken up by a CD36-mediated channel (high affinity, low capacity, saturable), whereas high levels of FAs from CM are taken up by free diffusion through the plasma membrane via the flip-flop mechanism (low affinity, high capacity, nonsaturable).
  3. FA transport: Capillary endothelial cells can transport FAs via cytoplasmic FABP4/5 to the interstitial space of cardiomyocytes.
- FA uptake by cardiomyocytes comprises three separate steps: adsorption, flip-flop, and desorption.
  1. Adsorption of FAs bound to albumin in the interstitial space is facilitated by plasma-membrane proteins, such as CD36 and others, which enhances the next step, flip-flop.
  2. Flip-flop of FA in a phospholipid bilayer of the plasma membrane is through free diffusion and is very fast for virtually all FA types.
  3. The desorption step is rate-limiting and is facilitated by cytoplasmic FABP3 as well as some enzymes that have binding sites for CD36.

- Direct uptake of CM and VLDL particles or remnant particles via lipoprotein receptors has also been proposed, although their contributions to TG uptake remain disputed.
- The expression of genes associated with FA catabolism in cardiomyocytes is enhanced by PPARα and its coactivator, PGC-1α, during fasting, which facilitates FA uptake and oxidation.
- Expression of FA handling genes in capillary endothelial cells is mainly enhanced by PPARγ, which facilitates lipolysis and trans-endothelial FA transport.
- Cardiac atrophy during starvation does not cause heart failure because the demand of cardiac work is simultaneously reduced.
- Uncontrolled refeeding often causes potential complications, including heart failure and fatal arrhythmia, in the starved people, which is known as refeeding syndrome.
- Inherited disorders that affect mitochondrial FA oxidation cause acute cardiac dysfunction in a catabolic state, such as fasting, which is observed in patients with sudden infant death syndrome.
- Cardiac dysfunction does not occur in patients with genetic mutations of genes associated with FA uptake (CD36) and lipolysis of TG contained in TG-rich lipoproteins (LPL and GPIHBP1), even in a catabolic state.

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Cardiometabolic Risk in Marasmus and Kwashiorkor Survivors

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Abstract

Infants with marasmus have lower mortality rates than infants with kwashiorkor during the acute phase of severe acute malnutrition. The intermediary metabolism of marasmic infants during this acute phase is more "thrifty" than kwashiorkor infants, and thus they appear better adapted to survive starvation/famine. The lower birth weight of marasmus infants also suggests that they previously experienced intrauterine growth restriction, which may account for their altered metabolism. While this provides a survival effect during severe acute malnutrition, it becomes maladaptive if they are in more obesogenic environments. Adult marasmus survivors are smaller, more stunted, more likely to have beta-cell dysfunction and glucose intolerance, and more liver fat than kwashiorkor survivors. Both adult survivors of marasmus and kwashiorkor are at increased risk of hypertension and other cardiac outcomes. Preliminary epigenetic studies have highlighted differentially methylated genes that can explain the observed phenotypes in the marasmus survivors. These data indicate that survivors of severe acute malnutrition, especially marasmus survivors, are at high cardiometabolic risk in later life. There is a lack of data on effective interventions in these individuals.

Keywords

Marasmus · Kwashiorkor · Birth weight · Body composition · Glucose · Beta-cell function · Fatty liver · Blood pressure · Cardiometabolic · Appetite · Epigenetic · Survival

List of Abbreviations

ChroSAM study	Chronic disease outcomes after severe acute malnutrition in Malawian children study
DEXA	Dual energy X-ray absorptiometry
IGI	Insulinogenic index
NAFLD	Nonalcoholic fatty liver disease
oDI	Oral disposition index
OR	Odds ratio
SAM	Severe acute malnutrition
WBISI	Whole body insulin sensitivity index
WFH	Weight-for-height

Introduction: Defining Marasmus and Kwashiorkor, and Their Clinical Differences

Since 1999, the World Health Organization identifies severe acute malnutrition (SAM) in children ages 6–60 months by severe wasting and/or the presence of bilateral nutritional edema. In 2006, the criteria were modified and wasting was defined as the weight-for-height (WFH) being less than −3 SD (or z-scores) of the WHO growth standards, or alternatively, if the mid-upper arm circumference is less than 115 mm. The rationale for the WFH cutoff was that infant mortality was increased below −3 z-scores. Also, at that cutoff, there is little harm using therapeutic feeding in the currently recommended protocols and therapeutic feeds. The presence of bilateral nutritional edema is a clinical indicator that facility-based, rather than community-based, care is urgently needed.

Historically though, many countries used a more qualitative classification of SAM where they described the syndromes of marasmus and kwashiorkor. The clinical features and outcomes of these syndromes were very distinctive. Children with nonedematous severe wasting (marasmus) had a better prognosis than edematous children wasted (kwashiorkor), even though kwashiorkor children often had less severe wasting. These clinical syndromes were reinforced by the 1970 Wellcome classification system, which classified marasmus as wasting using a weight-for-age criterion <60% in comparison to the National Center for Health Statistics (NCHS) standard growth curves. Kwashiorkor was defined as wasting of 60–80% weight-for-age and the presence of edema (Wellcome Trust Working Party 1970). Kwashiorkor also had other clinical signs such as skin and hair changes (dermatoses), hypopigmentation, fatty liver (hepatic steatosis) and hypoalbuminemia. An intermediate syndrome called marasmic-kwashiorkor was defined as weight-for-age <60% and the presence of nutritional edema. At present, less clinicians use the Wellcome classification and instead use the WHO criteria. However, the older classification may have utility if the clinical syndromes have different short-term prognoses, pathophysiologic origins, or long-term outcomes. As such, kwashiorkor was associated with much higher mortality rates than marasmus. In more recent times, marasmus mortality rates have increased mostly due to concomitant infections with HIV and respiratory pathogens (Trehan et al. 2013; Munthali et al. 2015).

Differences in Intermediary Metabolism

The origins of the distinct differences in the two clinical syndromes have not been clearly elucidated and remain mysterious. To date, no reliable differences have been shown in premorbid diets, exposure to environmental toxins, pattern of infections, or genetic polymorphisms. Notably though, there are differences in their intermediary metabolism. During the acute phases of SAM, both syndromes show the appropriate changes in fat, protein, and carbohydrate metabolism as seen in fasting states. That

Table 1 Intermediary metabolism of children with severe acute malnutrition

Metabolism during acute phase	Marasmus	Kwashiorkor
Protein turnover	↑↑	↑
Lipid turnover	↑↑	↑
Gluconeogenesis	↑↑	↑
Glycogenolysis	↑↑	↑
Glutathione levels	↓	↓↓
Salvage of urea-N	↑↑	↑

The relative differences in the intermediary metabolism of children during the acute phase of severe acute malnutrition are shown and these may give a survival advantage of marasmus children compared to kwashiorkor children. The table summarizes some of the data from (Reid et al. 2000, 2002; Badaloo et al. 2002; Jahoor et al. 2005, 2008; Badaloo et al. 2006a, b)

is, protein and lipid turnover are increased to provide sufficient amino acids, glycerol, and nonesterified fatty acids to support energy metabolism and to maintain plasma glucose concentrations close to 5 mmol/L for proper brain function. However, marasmus infants have higher rates (Table 1) of lipolysis, whole body protein turnover, gluconeogenesis, glycogenolysis, salvage of urinary urea nitrogen, and better concentrations of the intercellular antioxidant glutathione (Reid et al. 2000, 2002; Badaloo et al. 2002, Badaloo et al. 2006a, b; Jahoor et al. 2005, 2008). This differential response would improve the ability of marasmic children to sustain amino acid and lipid supply for intermediary metabolism during the period of starvation. Infants with kwashiorkor are less likely to compensate metabolically even though they have greater tissue stores than marasmus infants. Following recovery, protein turnover is approximately 30% faster and lipid oxidation is approximately 35% higher in kwashiorkor survivors than in marasmus. Metabolomic studies confirm that their metabolic signatures are different (albeit with some degree of overlap) as there are significant differences in certain amino acids, biogenic amines, sphingomyelins, phosphatidylcholines, and acylcarnitines even after clinical recovery (Di Giovanni et al. 2016).

This metabolic pattern suggests that marasmus infants are better adapted to fasting and famine compared to kwashiorkor infants. As such, during starvation, infants with kwashiorkor decompensate clinically as they are unable to provide metabolic fuel fluxes and thus have higher mortality rates.

Does Developmental Biology Explain Their Metabolic Differences?

If this teleological concept is correct, the metabolic adaptations would have occurred in earlier life, that is, before birth. In support, individuals who develop marasmus in infancy have lower birth weights than their kwashiorkor counterparts (Coulter et al. 1988; Forrester et al. 2012). In Jamaica, marasmus infants were 333 g (95% CI: 217–449 g) lighter at birth than kwashiorkor infants (Forrester et al. 2012). The survival of the marasmus infants was also better, as the mortality rate for

kwashiorkor infants was higher (odds ratio for death 3.7 [95% CI 1.5–9.2]). This demonstrates a fitness-advantage for marasmus infants in a nutritionally constrained, postnatal environment.

One possible explanation is a *predictive adaptive response* is operating (Gluckman et al. 2008). In other words, fetuses may experience intrauterine growth retardation if the prepregnancy nutritional state of the mother is poor, or if maternal nutrition during pregnancy is inadequate. These offspring undergo metabolic adaptions during this plastic period similar to that seen in marasmus so as to enable survival in utero (Barker 1995). These changes would increase their fitness and odds of survival if the postnatal environment is also a situation of famine (see chapter ▶ "The Effects of Prenatal Exposure to the Dutch Famine 1944–1945 on Health Across the Lifecourse").

If individuals who survive marasmus are demonstrating a predictive adaptive response and their nutritional plane subsequently improves, they will become "mismatched" for their environment, that is, adapted for famine, but now live in a calorie-surfeit environment. Such individuals would be predisposed to cardiometabolic risk in later life, such as increased ectopic and intra-abdominal adiposity, less lean mass with a tendency toward sarcopenia and osteopenia, abnormal appetite, obesity, glucose intolerance, insulin resistance, beta-cell dysfunction, elevated blood pressure, low-HDL-cholesterol, increased serum triacylglycerols, accelerated atherosclerosis, nonalcoholic fatty liver disease, and polycystic ovary disease. There are several studies that have examined the effect of starvation and famine per se on the development of cardiometabolic changes in various retrospectively assembled cohorts. However, there are sparse data specifically examining the differences in survivors of marasmus and kwashiorkor. If both syndromes also have different long-term clinical outcomes (e.g., mortality, chronic noncommunicable diseases, neurocognitive outcomes), this would support identifying the different clinical phenotypes and after recovery, individuals should be marked as high-risk and deserving of more aggressive interventions. Also, it is unclear if malnutrition in infancy per se increases their cardiometabolic risk. Infancy (birth to age 2 years) is a plastic period and thus postnatal malnutrition such as in SAM (where there is macronutrient deficiency, as well as micronutrient deficiencies) may induce a predictive adaptive response.

Cardiometabolic Risk in Later Life

Body Composition in Later Childhood and Adulthood

In many lower and middle income countries, there is the coexistence of malnutrition and obesity – the so-called double nutritional burden. Children with SAM are more prone than children who never experienced malnutrition to develop obesity in later life if they live in an obesogenic environment (Tzioumis and Adair 2014). One hypothesis that can connect both planes of nutrition is that malnutrition induces persistently lower growth levels, lower levels of serum growth factors (e.g., IGF-I), and possibly higher cortisol levels and lower insulin levels. This endocrine milieu is

conducive to stunting, less muscle mass as well as a greater propensity to adiposity during calorie-surfeit periods (Sawaya et al. 2004).

In the ChroSAM study, Malawian children who were admitted for SAM before age 2 years, and were reviewed at age 9 years, had less lean mass, more stunting, smaller mid-upper arm circumference and less handgrip strength than their sibling or community controls despite evidence of catch-up growth (Lelijveld et al. 2016). Survival bias and probable persistence of a lower socioeconomic status may have impacted their findings, but other studies show that survivors of SAM in general may have persistent growth deficits and increased risk of obesity and the metabolic syndrome in later life (Hult et al. 2010; Vaiserman 2017). However, in many of these studies, the data were not analyzed by SAM syndrome. One study in South African adolescents found that survivors of kwashiorkor had longer growth spurts and were heavier and possibly taller than their community controls (Cameron et al. 1986). However, they were not different to their siblings (Sauniere et al. 1986). There is little information on marasmus survivors and even less that compare marasmus and kwashiorkor survivors.

In Jamaica, the mortality rate of SAM during infancy is low (4.1%) (Forrester et al. 2012), so survival bias is less of a confounder. In a retrospectively assembled cohort study of Jamaican survivors of SAM (86 had marasmus, 77 had kwashiorkor, and 78 had marasmic-kwashiorkor) who were treated in hospital as infants within the previous 50 years (Soares-Wynter et al. 2011), there were differences in anthropometry and body composition by DEXA imaging. After adjusting for age and sex, adult kwashiorkor survivors were 2.6 cm taller, 8.9 kg heavier, and had greater BMI (~ 2.6 kg/m^2) than marasmus survivors (Fig. 1).

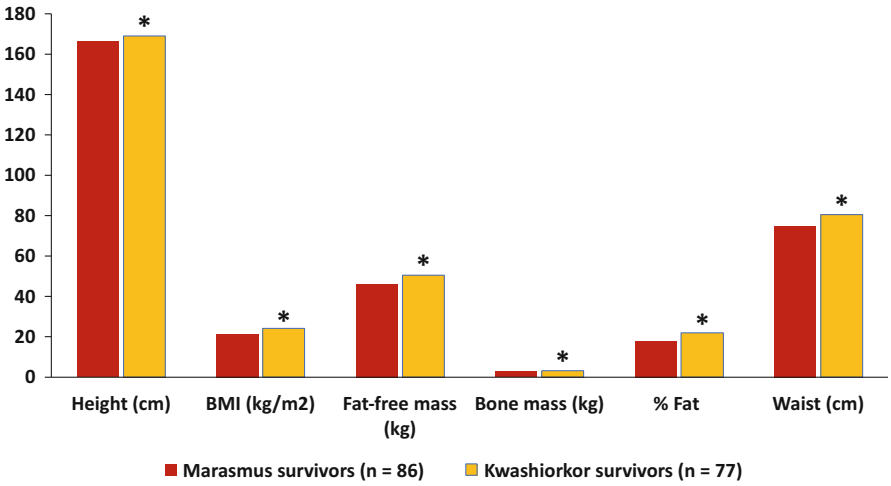

Fig. 1 Anthropometry and body composition in adult survivors of severe acute malnutrition. This graph shows the anthropometry and body composition measured by dual x-ray absorptiometry in adult marasmus and kwashiorkor survivors in Jamaica. In general, marasmus survivors were significantly shorter, smaller and had less fat-free, bone and fat mass. *$P < 0.01$ (Data adapted from Soares-Wynter et al. 2011)

They had significantly more fat-free mass (50.5 vs. 46.2 kg), bone mass (3.15 vs. 2.79 kg), fat mass (15.2 vs. 10.7 kg), % fat (21.9 vs. 17.8%), and waist circumference (80.5 vs. 74.8 cm) compared to marasmus survivors. Even after adjusting for adult height, these differences remained. When matched for BMI, the differences for fat-free mass and bone mass remained significant. Survivors of marasmus-kwashiorkor had intermediate values.

The kwashiorkor survivors weighed more at birth than the marasmus survivors (3.06 vs. 2.60 kg; $P < 0.001$), and marasmus-kwashiorkor were intermediate (2.85 kg). Multiple regression analyses adjusting for stunting (i.e., height-for-age), wasting (i.e., weight-for-height), and birth weight showed that the presence of nutritional edema was associated with adult fat-free mass and fat mass. Birth weight was positively associated with fat-free mass but not with fat mass. So, these data indicate that adult survivors of marasmus are smaller, more stunted, have less lean mass, and that birth weight is a predictor of their fat-free mass. The adult kwashiorkor survivors were similar in size to community controls who never experienced SAM (unpublished data). The implications are that marasmus survivors are more prone to sarcopenia in later life. If they are later exposed to an obesogenic environment, they are also at risk of sarcopenic obesity, since they have limited capacity to store fat in subcutaneous depots and a greater tendency to accumulate in visceral compartments.

Glucose Metabolism in Later Childhood and Adulthood

During the acute phase of SAM, there is increased secretion of counter-regulatory hormones, increased lipolysis and higher concentrations of nonesterified fatty acids leading to increased peripheral insulin resistance in many (Cook 1967; Becker 1983), although not all studies (Spoelstra et al. 2012). Infants with kwashiorkor and marasmus are glucose intolerant to a similar degree, but some studies suggest that this is due mostly to impaired insulin secretion (Robinson and Picou 1977; Spoelstra et al. 2012) rather than insulin resistance. Intestinal absorption of glucose is more impaired in kwashiorkor children, although it is also seen in marasmus children with hypoalbuminemia (Bandsma et al. 2011; Kvissberg et al. 2016).

In general, these changes in glucose metabolism resolve after nutritional rehabilitation (i.e., restoring WFH to >85%), especially in kwashiorkor patients (Robinson and Picou 1977), (Soliman et al. 1986). Marasmic children have delayed recovery of glucose-stimulated insulin responses up to 3 months after recovery despite normalization of glucose utilization and lipolysis (James and Coore 1970), (Graham et al. 1976). However, there is very little data on metabolic studies of marasmus survivors in later childhood. Survivors of kwashiorkor during childhood probably have normal glucose metabolism. In support of this, Ugandan children had normal insulin responses and glucose tolerance at ages 11–19 years (Kajubi 1972), while South African adolescents had normal insulin responses during intravenous glucose tolerance testing 10 years after experiencing SAM (Becker et al. 1971). There is one report of survivors having

more impaired glucose tolerance 6–12 years after their in-patient admission for kwashiorkor (Cook 1967). It is possible that these survivors experienced further postnatal undernutrition after their hospital discharge (James and Coore 1970), which could explain this observation. In the ChroSAM study (Lelijveld et al. 2016), glucose tolerance was normal in the entire Malawian cohort, but the data were not segregated by SAM syndrome.

Data on adults are few, but in general, the experience of famine in infancy and childhood can increase the risk of diabetes in later life. Epidemiological studies show adult survivors of famine have more glucose intolerance than those not exposed (Vaiserman 2017). Dutch and Chinese women who underwent famine/starvation during childhood (i.e., during ages 0–17 years) (Li et al. 2010; van Abeelen et al. 2012a) had an increased risk of type 2 diabetes as adults after adjusting for potential confounders (such as smoking and socioeconomic status). The risk was more in persons who self-reported exposure to severe famine compared to those with moderate exposure. Famine during young adulthood (i.e., age $\geq$18 years) was not associated with increased risk. This age-specific effect of famine is supported by animal models. In undernourished rats, reduced dietary protein in early life (i.e., gestation, during lactation and as a weanling) had persistent effects into older life, but not if the insults occurred in adult life (Minana-Solis Mdel and Escobar 2008). The effect of famine was increased in Chinese women who adopted a Western diet or became overweight (Li et al. 2010). In a meta-analysis of 11 published articles, early-life famine exposure was associated with an increased relative risk of 1.36 (95% CI 1.12–1.65) for those exposed during fetal-infant life, and there was a trend for childhood exposure (RR 1.40 [95% CI 0.98–1.99]) (Liu et al. 2016) (Fig. 2). However, these famine studies did not differentiate by malnutrition phenotype. Also, the mechanism of glucose intolerance was not clear, that is, if it was due to more insulin resistance or beta-cell dysfunction. Young Mexican men who had SAM in infancy were more glucose intolerant and hyperinsulinemic compared to controls (Gonzalez-Barranco et al. 2003), but they were not less insulin sensitive (as measured by euglycemic hyperinsulinemic clamp) than controls except when they were matched for high levels

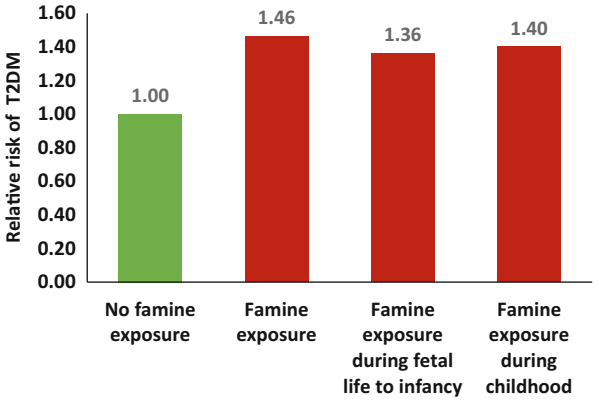

Fig. 2 Meta-analysis of the studies examining the exposure to famine in early life and the risk of type 2 diabetes in adulthood. In this 2016 meta-analysis of 11 cohort studies, famine exposure during early life, especially fetal-infant exposure, was associated with increased risk of type 2 diabetes (*T2DM*) in adulthood (Data are from Liu et al. 2016)

of intra-abdominal fat (Boule et al. 2003). Notably, in that study they did not report any measure of beta-cell function, neither did they differentiate the participants by nutritional edema.

In a cohort of adult Jamaican survivors (age 17–50 years), glucose metabolism was studied in 43 marasmus survivors, 38 kwashiorkor survivors, 70 age, sex, and BMI-matched community controls; and 40 age- and birth weight matched controls using a five-point oral glucose tolerance test (Francis-Emmanuel et al. 2014). Marasmus survivors had a 10.9-fold increased prevalence of glucose intolerance (95% CI: 2.1–55) and more fasting hyperinsulinemia compared to kwashiorkor survivors after adjusting for age, sex, and BMI. The degree of fasting during infancy also correlated with the 2-h postchallenge glucose levels as adults. Birth weight was correlated with the fasting glucose levels. The insulin sensitivity (calculated by the Matsuda index) in marasmus survivors tended to be less but insulin secretion (as estimated by the insulinogenic index and oral disposition index) was significantly reduced (Fig. 3a). In a subset (20 marasmus survivors, 20 kwashiorkor survivors) who also had euglycemic hyperinsulinemic clamps performed, there was no significant difference in insulin sensitivity, insulin clearance, adiponectin, or lipids (Fig. 3b) (Thompson et al. 2011). Also, their insulin sensitivity was not significantly different from community controls who never experienced SAM.

These results suggest that the endocrine pancreas is plastic in early life. Marasmus survivors have significantly lower birth weight probably due to intrauterine growth restriction which caused beta-cell dysfunction and then glucose intolerance in later life. Thus, persons with marasmus may have two nutritional insults - the first during intrauterine life making the beta-cells more sensitive to a second postnatal insult during an episode of SAM in infancy. A single nutritional insult in infancy, as seen in kwashiorkor, may not be sufficient to permanently alter glucose metabolism. It is not clear if the defect in glucose-stimulated insulin secretion is due to altered beta-cell function and/or mass. Possible mechanisms include decreased beta-cell neogenesis, differentiation, apoptosis, necrosis, or even increased alpha-cell function/mass. Subsequent sedentary lifestyle and increased food consumption in later life can then increase the prevalence of diabetes by inducing insulin resistance or glucolipotoxicity. It is likely that this phenotype increases the risk of developing the variant of diabetes associated with malnutrition (also called atypical diabetes, ketosis-prone diabetes, J-type diabetes, malnutrition-related diabetes) (Morrison et al. 1995; Rao and Yajnik 1996).

Cardiovascular System in Adulthood

During the acute phase of SAM in infants and children, the mean arterial pressure is about 8.6 mm Hg lower than those without severe malnutrition (Silverman et al. 2016). The systemic vascular resistance index is also lower, but there are no

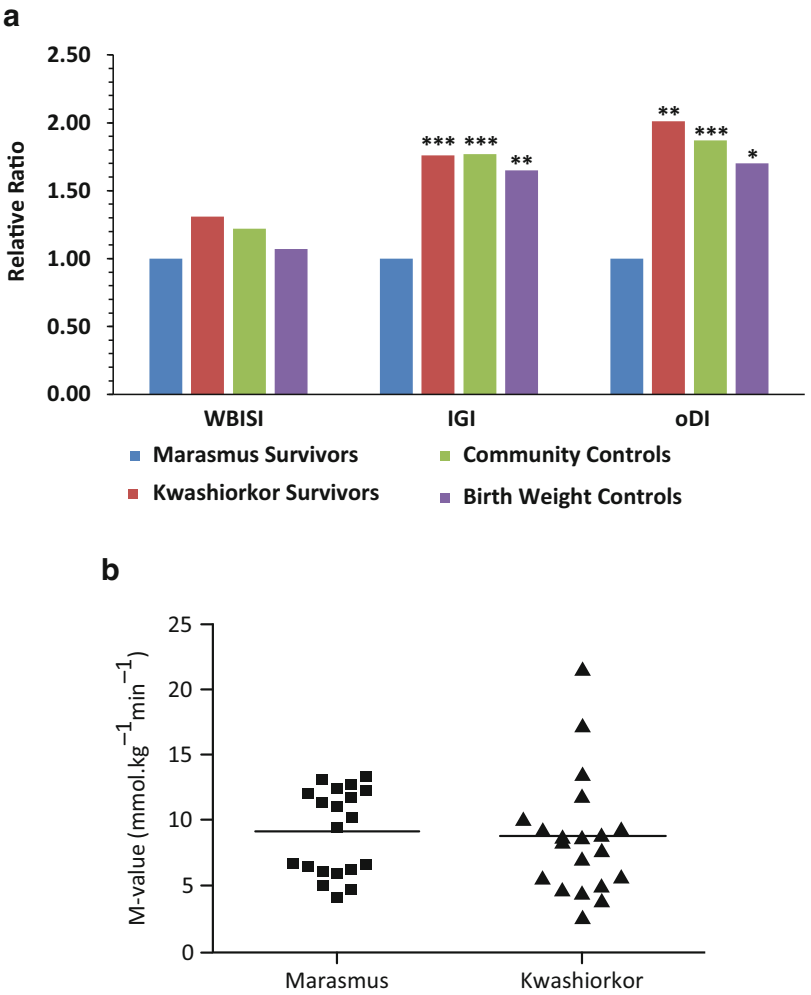

Fig. 3 (**a**) and (**b**) Glucose metabolism in adult survivors of severe acute malnutrition. The relative ratios after adjustment for age, sex, and BMI for measures of glucose metabolism were calculated during a five-point oral glucose tolerance test in adult survivors of marasmus (43) and kwashiorkor (38), as well as 70 community controls and 40 birth weight controls. Insulin sensitivity was calculated by the whole body insulin sensitivity index (*WBISI*) and was not significantly different among the groups using marasmus survivors as the comparator group. Marasmus survivors had significantly lower insulin secretion as measured by the insulinogenic index (*IGI*) and the oral disposition index (*oDI*). Notes: $*P < 0.05$, $**P < 0.01$, $***P \leq 0.001$ (Data are from Francis-Emmanuel et al. 2014). Insulin sensitivity measured during a euglycemic hyperinsulinemic clamp in adult marasmus and kwashiorkor survivors. There was no difference in their insulin sensitivity expressed as M-value (the glucose disposal rate during the last 30 min of the clamp). These data on insulin sensitivity are consistent with insulin sensitivity as measured by WBISI in 3A (Data are from Thompson et al. 2011)

differences in the cardiac index, heart rate, or stroke volume index. This is despite the wasting effects of SAM on the heart muscle (Alleyne 1966) and the cardiovascular changes were thought to fully recover after nutritional rehabilitation.

Irrespectively, adult survivors of SAM do have pervasive alterations in their cardiovascular system. Exposure to famine is associated with increased risk of hypertension in Chinese adults after adjustment for age and other potential confounders. There was at least a 24% increased risk in people if the exposure to the famine was in fetal life, 83% in infancy, 44% in early childhood, 67% in mid-childhood, and more than twofold in late childhood (Wang et al. 2012; Chen et al. 2014; Yu et al. 2017). The risk of hypertension was higher for persons who were overweight and who were at the upper and lower economic strata after experiencing severe famine in infancy (Wang et al. 2016b) giving a "U"-shaped association. This indicates that the risk is further exacerbated by the postfamine nutritional environment. The Biafran famine during the Nigerian Civil War also emphasized this fact, as exposure during the fetal-infant period was associated with 2.49 increased odds of having systolic blood pressures $\geq$140 mm Hg in lean adults, but there was an almost sevenfold increased odds in overweight/obese adults (Hult et al. 2010). Arterial calcification is seen in hypertensive adults who may be at risk of coronary artery disease and this may be affected by exposure to famine. Hence, postmenopausal Dutch women, who were exposed to famine as adolescents (ages 10–17 years) in the Second World War, had more coronary artery calcification (OR 3.5–4.6), but there was no apparent increase in those exposed at younger ages (Idris et al. 2013).

There is one study that evaluated whether the risk of hypertension is different by clinical phenotype of SAM during infancy. So, 62 adults who survived kwashiorkor, 54 marasmus survivors, and 45 age, sex, and BMI-matched community controls (mean age 28.8 $\pm$ 7.8 years) were evaluated using echocardiography and tonometry (Tennant et al. 2014). After adjusting for age, sex, weight, and height, major differences were found between former SAM cases and controls but not between marasmus and kwashiorkor survivors. Systolic blood pressure and heart rate were normal and not significantly different between the groups. However, diastolic blood pressure was significantly increased in all SAM survivors compared to controls (4.3 mm Hg; 95% CI: 1.2–7.3 mmHg). SAM survivors had smaller left ventricular outflow tract diameters and hence decreased stroke volume, cardiac output and higher systemic vascular resistance than controls (Table 2). The difference in systemic vascular resistance between SAM survivors and controls was large – about 5.5 (95% CI: 2.8–8.4) mmHg*min/L (approximately half a standard deviation).

Since there was a marked inverse correlation between left ventricular outflow tract diameter and systemic vascular resistance, SAM survivors are potentially at higher risk of developing hypertension than controls. Their reduced cardiac output may also put them at risk of heart failure. It is not clear what the pathophysiological mechanisms of these observed changes are, but theoretically they include:

Table 2 Differences in cardiovascular measures (SD scores and confidence intervals) between controls and all severe acute malnutrition (*SAM*) survivors

Measurement	Difference in SD scores of controls minus all SAM survivors*	*P*
Cardiac output	0.53 (0.21–0.85)	0.001
Stroke volume	0.44 (0.11–0.77)	0.009
Systemic vascular resistance	−0.67 (−1.01 to −0.33)	<0.001
Left ventricular outflow tract diameter	0.67 (0.36–0.99)	<0.001
Ejection fraction	−0.42 (−0.77 to −0.07)	0.02
Carotid intimal media thickness	0.25 (−0.08–0.58)	0.1
Visceral fat mass	−0.07 (−0.38–0.24)	0.6

Measurements of cardiac anatomy and function are shown as the differences in SD scores between participants with SAM and community controls.
*The SD scores are controlled for age, sex, height, and weight (Data are from Tennant et al. 2014)

- Higher sympathetic drive leading to arteriolar constriction, secondary to enhanced adrenergic and hypothalamic-pituitary-adrenal axes (as seen in adults who had a lower birth weight).
- Underdevelopment of the arterial tree which could lead to the abnormal systemic vascular resistance.
- The increased systemic vascular resistance may be a compensatory mechanism to preserve perfusion of some organs in the face of reduced cardiac output.

Nonalcoholic Fatty Liver Disease (NAFLD) in Childhood and Adulthood

Infants with SAM have hepatic steatosis, or fatty liver, by ultrasonography or necropsy, although kwashiorkor infants have more than marasmus infants (Doherty et al. 1992). While the pathogenesis is not clear, it is unlikely to be related to the export of triacylglycerols from the liver in very low density lipoproteins (VLDL). Both marasmus and kwashiorkor infants synthesize VLDL-apo B-100 at a faster rate as the degree of hepatic steatosis increases (Badaloo et al. 2005). Since the rates of lipolysis in kwashiorkor are lower than in marasmus, persons with kwashiorkor may have less beta-oxidation of fatty acids leading to the accumulation of intrahepatic triacylglycerols. The degree of this hepatic steatosis slowly reduces with successful nutritional rehabilitation. Resolution occurs by later childhood as was shown in Ugandan children (mean age 8.8 years) who had liver biopsies 7 years after having kwashiorkor, although a few had stellate fibrosis radiating from the portal area (Cook and Hutt 1967).

Despite this resolution by childhood, early life exposure to food restriction is associated with fatty liver disease later in life. Children with NAFLD were four times more likely to have experienced intrauterine growth restriction (Nobili et al. 2007).

Persons exposed to the Chinese Famine (1959–1961) during fetal life and infancy had more NAFLD as adults (Chen et al. 2016). However, these associations were sex specific. That is, women, but not men, who were exposed during fetal life and childhood had a significantly higher prevalence of moderate-severe steatosis (OR 1.8 after adjusting for age, rural/urban residence, economic status, BMI, diabetes, dyslipidemia, and hypertension) as diagnosed by ultrasonography compared to those who were not exposed (Wang et al. 2016a). Women who were exposed during adolescence did not have a change in their risk. However, this sex dimorphism was not seen in another group of individuals who were also exposed to the Chinese famine in the Chongqing district. Both men and women born during the famine had increased risk (OR 1.38–1.55) and this risk was still elevated in those born after the famine (OR 1.14 vs. 1.24 for men and women after adjustment of age). The men also had increased risk of obesity and hyperglycemia (Chen et al. 2016).

There is a dearth of data of NAFLD in survivors of marasmus and kwashiorkor, but based on the predictive adaptive response hypothesis, one would expect that marasmus survivors to have more liver fat, despite kwashiorkor survivors having more fatty liver as infants. In a Jamaican study of 45 marasmus survivors, 43 kwashiorkor survivors, and 84 community controls (age 29.0 ± 8.4 years; BMI 23.5 ± 5.0 kg/m^2) liver attenuation and liver: spleen ratio was measured using single slice computerized tomography at T12/L1 intervertebral disc space (Thompson et al. 2015). Both measurements are indices of hepatic steatosis and lower values indicate more fat. Malnutrition survivors had less liver fat than controls (1.3 ± 0.2 vs. 1.2 ± 0.9, $P = 0.03$). Marasmus survivors had lower birth weight than kwashiorkor survivors (−0.51 kg; $P = 0.02$), had smaller waists, and were significantly thinner with less body fat. After adjusting for age, sex, and birth weight, marasmus survivors had a lower mean liver attenuation than kwashiorkor survivors indicating they had more hepatic steatosis (Fig. 4). Interestingly, they did not did not have changes in alanine transaminase, adiponectin, fetuin A or insulin resistance (measured by HOMA-IR) as would be expected in steato-hepatitis. Neither were there any significant changes in serum triglycerides, HDL-cholesterol or LDL-cholesterol.

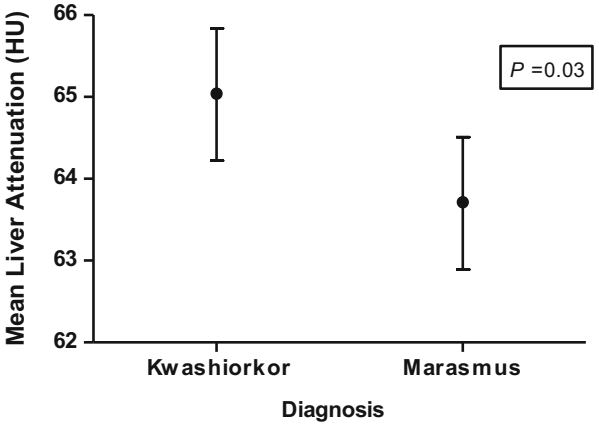

Fig. 4 Regression of mean liver attenuation against diagnosis of severe acute malnutrition. The mean attenuation of the liver and SD (as *bars*) in Hounsfield units is shown for adult survivors of marasmus and kwashiorkor after adjustment for age, sex, and birth weight. Marasmus adults had a significantly lower liver attenuation indicating that they have more liver fat (Data are from Thompson et al. 2015)

Based on these data, it is conceivable that the pathogenesis of fatty liver in individuals exposed to SAM is different along the life course. So, marasmus infants may have less liver fat accumulation as they are more effective in utilizing nonesterified fatty acids as a metabolic fuel (i.e., more efficient beta-oxidation) than kwashiorkor infants. In later life, marasmus survivors can accumulate more liver fat as during weight gain (especially if it is rapid during catch-up growth or later childhood), more fat is partitioned into abdominal and ectopic depots rather than subcutaneous compartment ("the fat overflow hypothesis"). In the Jamaican adult cohort, the pathogenesis apparently did not involve insulin resistance or inflammation. However, it is also possible that as the participants were relatively young and lean, they have more liver fat but have not yet developed inflammation. It remains to be seen if markers of inflammation would emerge with aging or weight gain.

Changes in Appetite Control in Later Life

Increased appetite induced by an episode of SAM may be one mechanism to promote obesity and cardiometabolic risk in later life. In animals, restriction of protein or calorie during gestation and/or suckling induces hyperphagia in postnatal life, probably through changes in the orexigenic peptides agouti-related peptide and neuropeptide Y, with simultaneous decreased hypothalamic levels of the anorexigenic peptide pro-opiomelanocortin (Orozco-Solis et al. 2009). It is unknown if prenatal and/or postnatal malnutrition causes similar changes in humans. Ghrelin, an orixogenic peptide, is higher in the serum of children in the acute stages of SAM, especially in those with marasmus, compared to healthy children (Altinkaynak et al. 2008). Leptin levels are correspondingly lower and are similar in kwashiorkor and marasmus children (Kilic et al. 2004). There are no data, to our knowledge, if these changes are persistent through the life course.

In a feeding trial of adult SAM survivors, there were no changes in appetite control (for protein and total energy) or satiety regulation based on whether they had marasmus or kwashiorkor as infants. However, there was a significant association of lower birth weight with higher protein targeting. This effect of birth weight may be mediated through increasing body weight, which then in turn increases protein leveraging (Campbell et al. 2016).

It is also possible that cognitive changes induced by SAM affect future lifestyle choices. In Dutch women, postnatal exposure to famine during both childhood (ages 0–9 years) and during adolescent years (ages 10–17 years) was associated with more physical inactivity and tobacco use, but there were no changes in diet or alcohol use (Fransen et al. 2016). No changes were seen in the men.

Mortality and Fitness

It is not known if mortality is increased or if reproductive fitness is altered in survivors of SAM and if there is any difference between marasmus and kwashiorkor survivors. It is possible that exposure to famine in utero may increase mortality risk

in future famines experienced in postnatal life (Hayward et al. 2013) although this is controversial. However, one would expect that marasmus survivors living in calorie-surfeit environments would have lower life expectancy (van Abeelen et al. 2012b) and changes in reproductive capacity (Yarde et al. 2013) based on their potential for glucose intolerance, sarcopenia, and fatty liver compared to their kwashiorkor counterparts, but this remains to be proven.

Possible Epigenetic and Genetic Factors

While the mechanism behind these "thrifty phenotype" changes in marasmus survivors is not clear, epigenetic changes are the most likely explanation (Hochberg et al. 2011). In the skeletal muscle of adult marasmus and kwashiorkor survivors, 63 genes were recently found to be differentially methylated (Sheppard et al. 2017). Most of the critical gene nodes were associated with immunity (e.g., HLA-DRB1), musculoskeletal growth (e.g., PITX2), and glucose metabolism (e.g., p21 protein (Cdc42/Rac)-activated kinase PAK1, C-reactive protein CRP1, and hexokinase (HK2)), as well as cardiovascular and neuronal pathways. The variance in methylation in adulthood was related to both prenatal and postnatal nutritional insults. However, the effect, especially for glucose metabolism, was stronger for antenatal insults. In a Bangladeshi study, young adults who were exposed to famine in gestation and/or infancy had abnormal glucose tolerance which was associated with differential methylation at several metastable epialleles in the blood,that is, VTRNA2–1, PAX8, PRDM-9, near ZFP57, near BOLA, and EXD3 (Finer et al. 2016). Most of the variance was also explainable by prenatal exposure, but postnatal nutrition also played an important role.

Obviously, these studies in marasmus and kwashiorkor survivors deserve replication as well as to be expanded to describe the epigenomic profiles in other tissues, since they may be different from muscle. Regardless, combining these data with other peri-conception studies from the Gambia (Dominguez-Salas et al. 2014) and the Dutch Hunger Winter cohort (Tobi et al. 2014), they suggest that maternal diet during pregnancy can cause changes in fetal metastable epialleles that persist into adult life. These epigenetic changes then can account for the observed phenotype of increased cardiometabolic risk in SAM (especially in marasmus survivors). The changes that relate to immunity also imply that the diversity of gut microbiota, which can be different in marasmus and kwashiorkor infants (Smith et al. 2013) may play a lasting role in body composition and cardiometabolic risk.

It is unknown if genetic factors can play a role as there is a significant lack of genetic studies in marasmus and kwashiorkor survivors. Genetic factors could contribute to the development of small for gestational age, edema, and low GSH levels. In a small study of 136 SAM survivors (Marshall et al. 2006), glutathione S-transferase polymorphisms was associated with the risk of developing kwashiorkor. GSTP1 Val(105) homozygotes were significantly more common among the former kwashiorkor patients (OR 3.5; 95%CI: 1.1–10.8) and there was a tendency

for an association between nondeletion GSTT1 genotypes and the presence of nutritional edema (OR 2.4; 95% CI: 1.0–5.9). These polymorphisms may explain some of the variance in glutathione levels, but they do not readily explain the other cardiometabolic changes.

Areas to be Explored

Obviously, the issue of the long-term cardiometabolic risk of SAM survivors requires more study and there are important questions to be answered.

- Is the mortality of adult marasmus survivors definitively increased because of their increased cardiovascular risk?
- Childhood stunting (a more moderate and chronic case of malnutrition) is associated with increased cardiometabolic risk in adults. Do they have the same pathophysiological pathways as those seen in the severe wasting of SAM?
- What is the best nutrient composition for therapeutic feeds for infants with SAM, and what rates of catch-up growth are safe for optimal, long-term cardiometabolic health?
- What happens to these SAM survivors as they gain weight or become more sedentary in their later life?
- Despite epigenetic data giving insight into the pathophysiological pathways, more studies are needed including the role of inflammation, stress responses, metabolic flexibility, growth factors, etc.
- What is the specific role of the gut microbiome on the development of cardiometabolic risk?
- Is there an increased risk of polycystic ovary syndrome in female survivors?
- There are almost no data on effective interventions in adult SAM survivors; whether primary to prevent cardiometabolic risk, or secondary after cardiometabolic disease has occurred.

Policies and Protocols

Oral Glucose Tolerance Test (OGTT)

All subjects were asked to fast for 10–12 h from the night before testing as well as to avoid strenuous exercise and consuming alcohol/caffeine the day before. We measured body composition by dual x-ray absorptiometry using a Lunar Prodigy machine (GE Health Care, USA). Participants then had a 75-g anhydrous OGTT. Five milliliter of blood was taken at 0, 30, 60, 90, and 120 min through an antecubital cannula into fluoridated and heparinized chilled tubes for plasma glucose and insulin measurements.

The whole body insulin sensitivity index (WBISI) was calculated using the method of Matsuda et al. (Matsuda and DeFronzo 1999) as:

$$\text{WBISI} = 10000/\sqrt{(G_0.I_0.G_m.I_m)}$$

where G_0 and I_0 are plasma glucose and insulin concentrations at 0 min, and G_m and I_m are the mean concentrations of glucose and insulin.

Beta-cell function was estimated using the oral disposition index (oDI), which is the ability of the beta cell to compensate for a given level of insulin sensitivity. This was calculated as:

$$\text{oDI} = \text{WBISI} \times \text{insulinogenic index}$$

i.e., $\text{oDI} = \text{WBISI} \times ([I_{30} - I_0]/[G_{30} - G_0])$
where I_{30} and I_0 are insulin concentrations at 30 and 0 min, and G_{30} and G_0 are glucose concentrations at 30 and 0 min.

Cardiac Measurements

Measurements were made using a GE Vivid *i* ultrasound machine with cardiac 3S probes and vascular 8 L probes. Pulse wave velocity was measured from ECG-gated flow waveforms, at 1–2 cm proximal to the carotid bulb and femoral bifurcation. 2D, M-mode and Doppler transthoracic echocardiography was done with the participants lying in the left lateral recumbent position using standard parasternal short and long axis, apical, subcostal, and suprasternal views. All measurements were ECG gated and sweep speeds for M-mode, pulsed wave (PW) or continuous wave (CW), Doppler was set at 100 mm/s. Images and loops were exported in DICOM format. Cardiac output, stroke volume, left ventricular mass, and left ventricular outflow tract internal diameter were then measured using standard techniques. With the neck in slight hyperextension and tilted approximately 30° to the left, the intima-medial thickness was measured in the right carotid and femoral arteries at 1–2 cm proximal to the carotid bulb or femoral bifurcation.

CT Scanning for Liver Fat

Liver fat was assessed using single slice, cross-sectional abdominal CT scanning at T12- L1 intervertebral disc space. Total, subcutaneous and intra-abdominal fat area, volume and mass were measured with one cross-sectional scan at the L4–5 inter-space using a Phillips Brilliance 64-slice scanner and using QCT Pro software. Participants were examined in the supine position with arms outstretched overhead with the scanner set at 120 kV, 100 mA exposure and 5 mm slice thickness. Liver fat data was analyzed using E-Film software. Three regions of interest (ROIs) were placed on the liver (posterior right lobe, anterior right lobe and left lobe, and 1 ROI was placed on the spleen. Each ROI measured a minimum of 1 cm^2 and excluded all major blood vessels. For each region of interest, attenuation was documented (Hounsfield units- HU).

CT images were analyzed with the commercial software package: "Tissue Composition Module" Beta 1.0 (Mindways, Austin, TX, USA). Measurements of total adipose area and visceral adipose tissue were completed by the commercial software package.

Dictionary of Terms

- **Insulin sensitivity** – the ability of insulin to increase glucose uptake in tissues.
- **Beta-cell function** – the ability of the beta-cell in the pancreas to secrete insulin in response to plasma glucose.
- **Disposition index** – a measure of the pancreas' ability to secrete insulin in response to the level of whole body insulin sensitivity.
- **Systemic vascular resistance** – also known as the total peripheral resistance, is the resistance by the body's vasculature to blood flow. This excludes the pulmonary vasculature.
- **Sarcopenia** – the loss of lean tissue as a part of aging.
- **Epigenetics** – the study of changes in gene expression that are not due to alterations in the DNA sequence and which can produce heritable characteristics during development of an organism.
- **Metabolomics** – techniques that identify and quantify the small-molecular metabolic chemicals of a biological system.
- **Orexogenic** – describes any molecule or process that stimulates appetite.
- **Euglycemic hyperinsulinemic clamp** – a procedure to measure the rate of glucose uptake under steady state conditions and therefore is regarded as the gold standard method to calculate insulin sensitivity.

Summary Points

- Children who are born with lower birth weights tend to develop marasmus rather than kwashiorkor if they experience postnatal undernutrition.
- Infants who have marasmus have increased cardiometabolic risk in later life (less lean mass, glucose intolerance, and more fatty liver).
- Both marasmus and kwashiorkor survivors are at risk of cardiovascular changes, including higher systemic vascular resistance and diastolic blood pressure, hypertension and lower cardiac output in adulthood.
- Developmental plasticity is the most likely mechanism in these marasmic children resulting in lower lean mass in later life, beta-cell dysfunction, higher systemic vascular resistance, cardiovascular changes, and fatty liver.
- Preliminary studies indicate that the epigenotype expressed in the skeletal muscle of adult survivors can explain much of the observed phenotype in adult life.
- These epigenetic changes mostly occur in the prenatal period but also extend into the postnatal period.
- There is a lack of intervention studies to ameliorate the increased cardiometabolic risk especially in marasmus survivors.

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Protein Energy Malnutrition and Nutritional Aspect of Heart Disease

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Abstract

Protein energy malnutrition is a prevalent nutrition public health problem affecting mainly developing countries including Iraq. Malnutrition could be a deficiency or an excess nutrient intake, but in this chapter it will refer to deficiency states.

Usually deficiency of energy and protein in the diet is accompanied by mineral and vitamin deficiency simultaneously resulting in both macro- and micronutrient deficiency. Body size and composition will be affected by malnutrition; body organs are affected too. The heart had been thought to be spared from malnutrition ill effects but later on proved to be one of the many organs impacted by this public health problem.

Many types of studies, chronologically, including animal experiments, autopsy for individuals who died of malnutrition, hospital-based observational studies among severely malnourished children, and community-based observational studies among apparently healthy moderately malnourished school children provided evidence of the impact of malnutrition on the heart.

The impact of protein energy malnutrition on heart structure and function is not only seen in severe malnutrition but also in moderate cases as shown in the study of apparently healthy moderately malnourished school children. Structural manifestations as decreased size of the heart, thinner left ventricular wall muscle, and thinner interventricular septum have been documented by different studies. Function of the heart on the other hand has not been affected as measured by ejection fraction and fiber fractional shortening, while stroke volume, blood pressure, and cardiac output have been affected according to the apparently healthy moderately malnourished children study.

Keywords

Protein energy malnutrition · Heart · Cardiovascular · Macronutrients · Micronutrients · Minerals · Vitamins · Starvation · Famine · Malnutrition · Cardiac mass

List of Abbreviations

HAZ	Height for age z-score
IVSD	Interventricular septum thickness during diastole
IVSS	Interventricular septum thickness during systole
LVEDD	Left ventricular end diastolic dimension
LVEDV	Left ventricular end diastolic volume
LVESD	Left ventricular end systolic dimension
LVESV	Left ventricular end systolic volume
PEM	Protein energy malnutrition

PWD	Posterior left ventricular wall during diastole
PWS	Posterior left ventricular wall during systole
WAZ	Weight for age z-score
WHO	World Health Organization

Introduction

Malnutrition

The WHO defines malnutrition as "the cellular imbalance between supply of 'nutrients and energy' and the body's demands for them to ensure growth, maintenance and specific functions." The imbalance could be an excess or deficiency; however this chapter will use malnutrition as deficiency state only. Effects of malnutrition on growth and development of children are extensively investigated and documented. Morbidity and mortality statistics related to malnutrition is huge.

Malnutrition affects the whole body through reduction in the supply of energy, protein, and other nutrients that will alter cell metabolism, structure, and function progressing to organ and system affection (WHO 2003; Suskind et al. 2011).

Muscle mass and bone mass are reduced as a result of chronic PEM, which in turn will predispose individuals to future health problems and alteration in normal body composition. With age people add weight which is mainly adipose tissues. They will develop obesity faster than do individuals with normal muscle and bone mass. Obesity is defined as more adiposity of the body and with altered body composition in terms of low muscle mass, and low bone mass adiposity will be reached faster and at lower increase in body weight.

Malnutrition in Iraq

In developing countries, PEM is one of the major health problems affecting their populations in general and children in particular. Iraq is one of these developing countries where PEM is prevalent. United Nation sanctions imposed on Iraq in 1990 caused progressively increasing numbers of children getting malnourished. A series of nutrition status assessment of under-5 children have been carried thereafter on national and international levels. High prevalence rates for underweight, stunting, and wasting have been documented. Iodine deficiency also showed high prevalence as documented by a survey including Baghdad, Mosul, and Basra in 1992. Total goiter rate and urinary iodine levels were assessed by the first author in collaboration with UNICEF who had sponsored urinary iodine level estimation in Virginia or the USA (Swidan 1994).

Currently millions of people are displaced within Iraq (for years) due to insecurity crisis and invasion by ISIS. Surveys are lacking for many reasons, but malnutrition along with many infectious diseases is affecting those people of course with children on top of victims as reported by the media and seen in hospitals and even in

schools. Currently prevention and correction of malnutrition in Iraq are extremely difficult.

The Heart

The heart is a specialized part of the cardiovascular system. It is the first organ to complete its development during fetal growth.

Heart wall is composed of three layers, pericardium, myocardium, and endocardium. These cover all the chambers except the left atrium which is extra-pericardium (Standring 2008).

The mass of the heart varies in normal adult males from 280 to 340 grams and in normal adult females from 230 to 280 (Standring 2008).

Cardiac muscle differs from skeletal muscle and smooth muscle in cell structure, cell biology, histology, physiology, and metabolism.

Metabolism of the Heart

Cells need energy to carry their specific functions like contraction of muscle cells including cardiac muscle. They capture energy, store it, and release it on need. Fatty acids are the basic energy source for heart contributing (60–100%); in contrast, lactate and glucose contribute much less as energy sources for the heart (0–40%). During fasting, plasma free fatty acid concentrations increase and result in increased myocardial glycogen. Lucien concentration in plasma increases during fasting which contributes to the maintenance of heart mass.

Malnutrition and the Heart

In the first half of the twentieth century, the hypothesis (the heart is spared from the effect of malnutrition) had been well accepted. Earlier doctors used to believe that the effects of malnutrition on the cardiovascular system were nonexistent and the heart was not affected in malnutrition. However, they thought that the cause of this sparing is the special heart metabolic characteristics and body feedback mechanisms that compensate for body needs and heart protection (Webb et al. 1986).

Effects of malnutrition on the heart have been identified following World War II. Keys and colleagues in 1947 reversed this hypothesis and highlighted a new one that the heart is not spared in undernutrition (Webb et al. 1986). Then the study of the effects of malnutrition on the cardiovascular system started.

Those studies indicated that nutrition has a major impact on the heart and in turn affects the way the heart functions and its ability to pump blood efficiently (Kothari et al. 1992). In chronic malnutrition manifested as stunted growth, the heart will never attain normal size. The smaller body will not place sufficient demands on the

heart to induce additional cardiac growth on one hand, and macro- and micronutrients necessary for growth are lacking on the other hand.

Several researchers performed studies to prove this new hypothesis and the type of effect and its extent. At the beginning, researchers depended on postmortem studies of individuals who died of malnutrition by comparing their left ventricle mass with other subjects who died from other causes in Costa Rica study (Regan 1985).

Some researchers used experimental animals to study the effects of malnutrition on mammalian heart especially rats (Pissaia et al. 1980; Freund and Holroyde 1986; Vandewoude 1995; Fioretto et al. 2001) and dogs (Alden et al. 1987) by exposing them to programmed food deficient in protein and energy and matched them with control group with normal nutrition. The hearts of the two groups were studied structurally and functionally by noninvasive and invasive investigations and then postmortem study.

In relation to acute malnutrition, Fabiansen et al. induced acute malnutrition in a group of 5-week-old piglets by feeding them certain poor diet for 7 weeks. Researchers concluded that acute malnutrition is associated with cardiac dysfunction in this pediatric porcine model decided by the increased myocardial performance index (MPI) and pro-atrial natriuretic peptide. Acute malnutrition was also associated with cardiac injury evidenced by elevated levels of cardiac troponin T (Fabiansen et al. 2015).

The above study was stimulated by the fact that severe acute malnutrition (SAM) is responsible for half million mortalities annually among children and that cardiac dysfunction may play a role in these mortalities. Acutely and severely malnourished children have organ atrophy, and their physiology and metabolism are altered. Changes in fluid and electrolytes in addition to impairment of cardiac function among acutely and severely malnourished children are important examples that led World Health Organization (WHO) to develop special guidelines for the management of shock, dehydration, and blood transfusion that differ from standard treatment used for well-nourished children. These WHO recommendations include small volume therapy and low sodium fluids to avoid overload and heart failure in children with severe acute malnutrition. Evidence behind these recommendations is controversial, and WHO called for more research to produce sound evidence-based ones (Fabiansen et al. 2015).

Other researchers studied the effects of malnutrition on hospitalized malnourished children and matched them with the control group (Olivares et al. 2005; Abufaddan et al. 2010). Those hospitalized children were severely malnourished either edematous or non-edematous.

Morphometric data showed that the cardiac muscle mass growth was impaired to the same extent as the body weight, supporting the concept that the heart is not spared in chronic PEM. This concept was first described by Keys et al. in 1947 (Webb et al. 1986), and it was later validated by others (Penpargkul et al. 1980). Cardiac sparing has been observed with shorter-duration PEM protocols limited to hours or days (Drott et al. 1986).

Malnutrition (PEM) Impact on the Heart in Active Children

In hospital malnutrition is seen with very high prevalence in comparison with the general population, and usually these are severe cases with different comorbidities. Mild and moderate cases of malnutrition may pass unnoticed and mistakenly considered as normal children especially in developing countries with poor healthcare systems. For this reason apparently active, primary-school-age children have been targeted for nutrition status assessment using anthropometry and then their heart structure and function assessed to see the impact of PEM. Children have been classified accordingly for the sake of inclusion in the following study done in Iraq in 2011 by Al-Samerraee and Thamer.

A community-based comparative study (retrospective cohort) has been carried by the authors to study some heart structural and functional indicators in malnourished primary school children as exposed and compared them with normally nourished matched non-exposed from the same community.

Children whose height for age (chronic malnutrition indicator) and/or their weight for age were equal to or less than – 2SD have been included as exposed group. Wasting (acute malnutrition) was probably masked by the presence of stunting which reflected chronicity of malnutrition. Non-exposed group had zero or positive z-scores for all anthropometric indicators.

This study differed from the previous ones by selecting physically active school-age children. In this study, anemia as a source of left ventricular morphometric modification has been excluded. Clinical examination, biochemistry, electrocardiographs, and echocardiography have been performed for all participants with the exclusion of children with congenital heart disease (Al-Samerraee and Thamer 2014).

Interventricular septum thickness during diastole (IVSD), interventricular septum thickness during systole (IVSS), posterior left ventricular wall during diastole (PWD), posterior left ventricular wall during systole (PWS), left ventricular end diastolic dimension (LVEDD), and heart mass showed that all of these variables were significantly lower in the exposed group. The following are mean values of the above parameters: exposed group versus non-exposed, respectively, IVSD (0.53 ± 0.05 cm) (0.67 ± 0.08 cm) $\rho < 0.000$, IVSS (0.87 ± 0.10 cm) (0.98 ± 0.16 cm) $\rho = 0.001$, PWD (0.51 ± 0.05 cm) (0.67 ± 0.08 cm) $\rho < 0.000$, PWS (0.78 ± 0.11 cm) (0.97 ± 0.11 cm) $\rho < 0.000$, LVEDD (3.55 ± 0.37 mm) (3.8 ± 0.35 mm) $\rho < 0.000$, and heart mass (44.30 ± 7.24 g) (71.90 ± 17.95 g) $\rho < 0.000$.

These parameters were strongly and positively correlated with anthropometric indicators HAZ and WAZ (Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12).

Heart function was affected too as mean left ventricular end diastolic volume (LVEDV) showed lower values among exposed (malnourished) group (54.43 ± 13.42 ml) than that of the non-exposed (65.24 ± 14.49 ml) $\rho = 0.001$.

Mean left ventricular end systolic volume (LVESV) was lower among exposed (malnourished) group (17.41 ± 4.92 ml) than that of the non-exposed (20.95 ± 5.20 ml) $\rho = 0.003$.

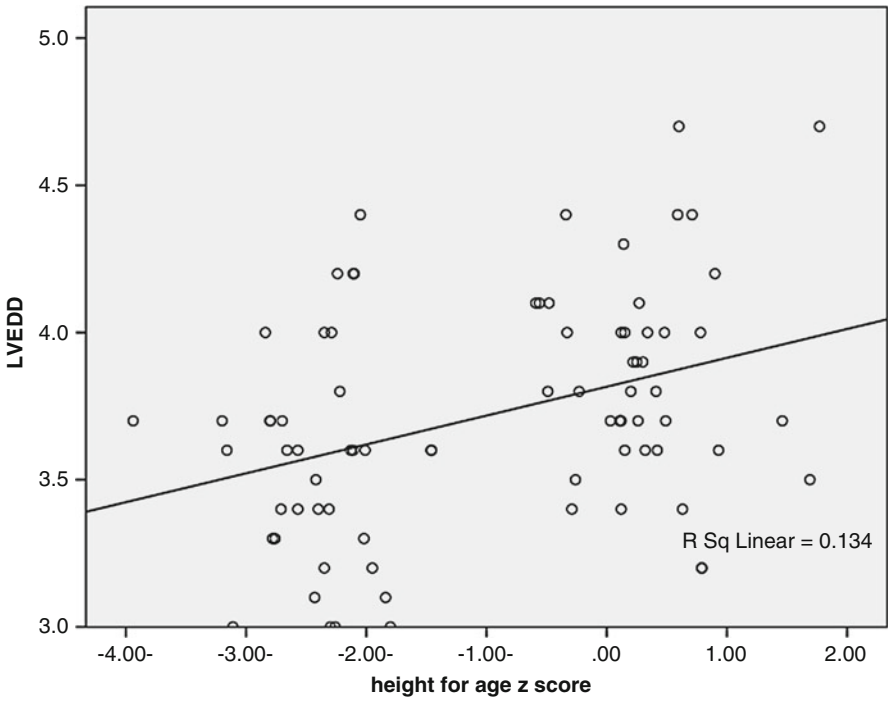

Fig. 1 Correlation between LVEDD and HAZ R = 0.367 $p = 0.001$

Mean left ventricular stroke volume was lower among exposed (malnourished) group (37.62 ± 10.60 ml) than that of the non-exposed (44.55 ± 11.44 ml) $\rho = 0.007$.

Ejection fraction did not differ significantly between the two groups.

Some echocardiography studies in malnourished children report overall preserved pumping function; other studies report systolic dysfunction.

Policies and Protocols

Echocardiography

Echo-Doppler-color Doppler hospital-type apparatus was used. Multi-probe frequency and size included cardiac probe for adult, neonatal, coronary, vascular, M-mode, 2D, and Doppler P and C waves. This was of three frequencies: 2.5, 3.5, and 5 KHz. The new American Society of Echocardiography standards were used as reference values for echocardiographic measurements. The examination was done through all the four major windows, longitudinal long axis, short axis, apical, and epigastric. Starting the examination through the left parasternal long axis is very beneficial because of the major landmark, the mitral valve which will be in the center

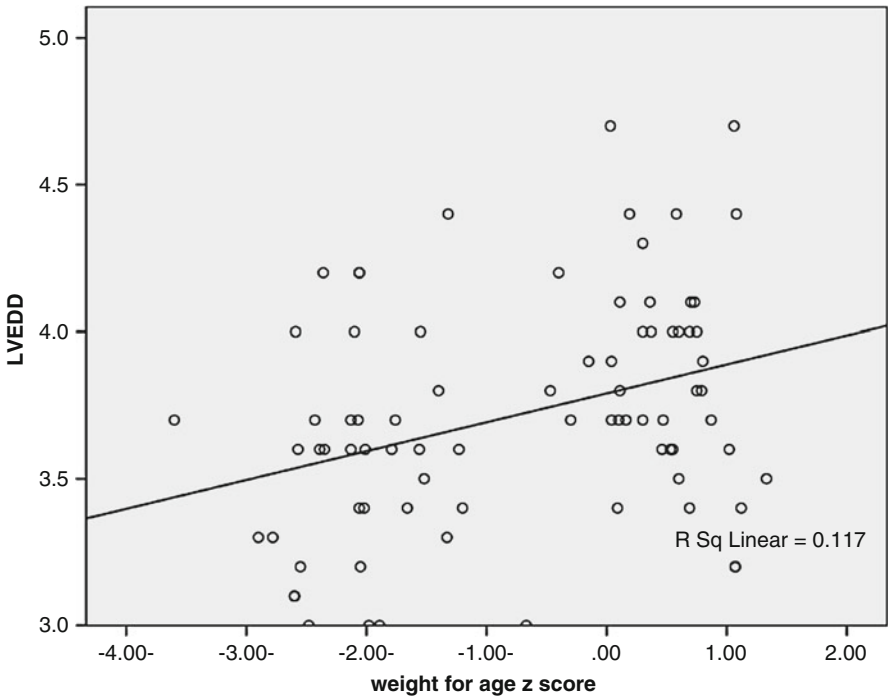

Fig. 2 Correlation between LVEDD and WAZ R = 0.342 $p = 0.002$

of this view. Diagnosis of any organic heart disease and exclusion of such children were done. After that, parasternal long axis was used for obtaining the following measurements:

1. Interventricular septum thickness both systolic and diastolic (IVSS and IVSD)
2. Posterior left ventricular wall both systolic and diastolic (PWS and PWD)
3. Left ventricular end systolic and end diastolic volumes (LVESV and LVEDV)
4. Left ventricular end systolic and end diastolic dimensions (LVESD and LVEDD)
5. Stroke volume
6. Ejection fraction and fiber shortening ratio
7. Mitral valve a wave/E wave ratio

All of the above measurements can be displayed automatically, and from these measurements left ventricular mass for each participant has been calculated.

In South Africa (Bantu population), a common form of heart disease had been based on chronic malnutrition (Higginson et al. 1952). Affected people had poor nutritional background and suffered from recurrent attacks of congestive heart failure. The heart showed gross enlargement, evidenced by chest X-ray. The enlargement affected all chambers by being dilated and hypertrophied. The heart's range of

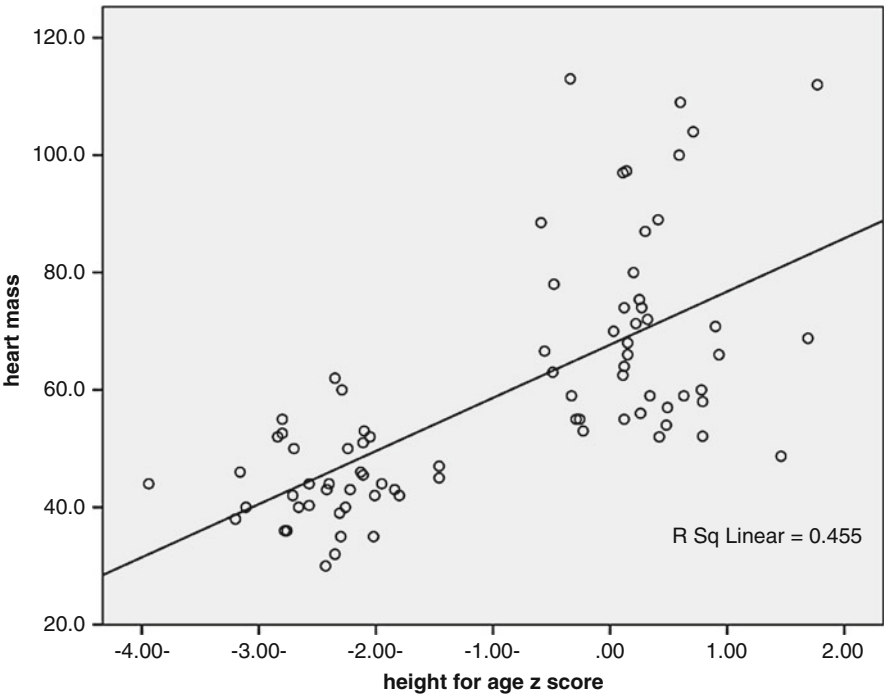

Fig. 3 Correlation between heart mass and HAZ R = 0.675 $p = 0.000$

pulsation was proportionately small, and the circulation was hypokinetic. (Higginson et al. 1952). Postmortem findings were in accord with chronic malnutrition as the etiological background.

Micronutrients and Cardiovascular System

When a diet is deficient in macronutrients (energy and protein), it is also expected to be deficient in micronutrients (minerals and vitamins). Some of micronutrient deficiencies cause specific disease entities. Examples are iron deficiency anemia, iodine deficiency disorders (e.g., goiter, cretinism, and hypothyroidism), rickets and osteomalacia, beriberi, pernicious anemia, pellagra, scurvy, and xerophthalmia.

Deficiencies of specific micronutrients such as iron and vitamin B_1 can cause heart failure. Poor diet may underlie such deficiencies especially in elderly patients in developed countries but mostly children in developing ones. Poor diet is the primary cause of worldwide malnutrition affecting millions of children suffering

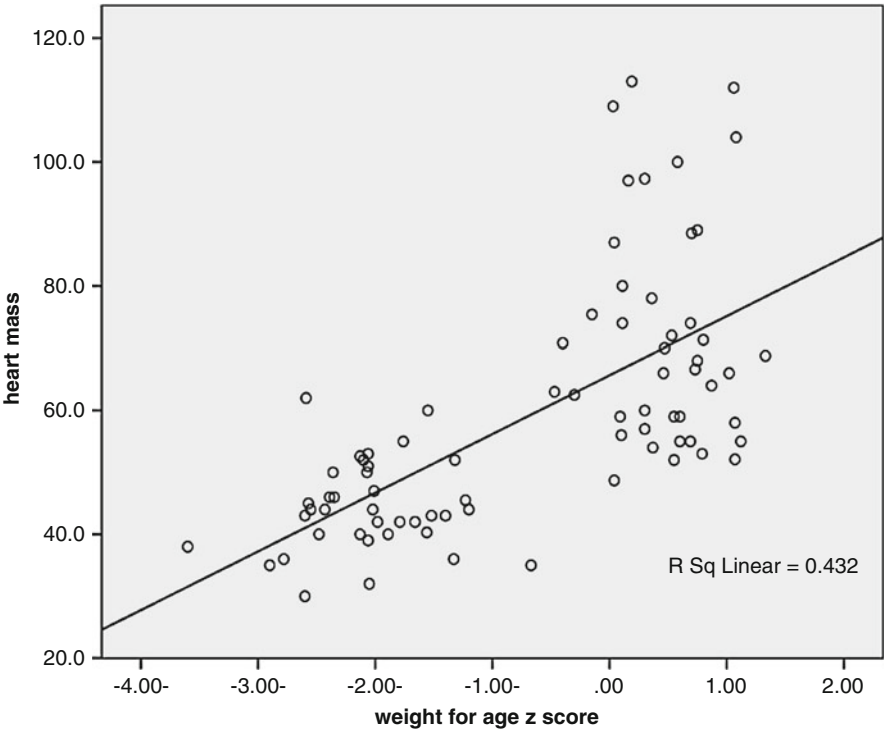

Fig. 4 Correlation between heart mass and WAZ R = 0.657 $p = 0.000$

from both acute (wasting) and chronic (stunting) malnutrition. Studies of specific nutrient levels among such populations are still lacking.

Calcium and Vitamin D

Calcium deficiency may come from lower intestinal absorption after the age of 70 years caused by less calcitriol-sensitive gut and lower renal synthesis of calcitriol.

Low calcium intake has been linked to higher mortality rates from ischemic heart disease in postmenopausal women.

Hypocalcemia-induced cardiomyopathy in children with congenital etiology and hypocalcemia responds dramatically to calcium supplementation. Low calcium levels are proarrhythmic. They have been associated with QT interval prolongation and hypocalcemic ventricular fibrillation (Witte Klauss et al. 2001).

Vitamin D plays a role in cardiovascular system functioning. Experimental studies on rats fed with vitamin D-deficient and calcium-sufficient diets showed

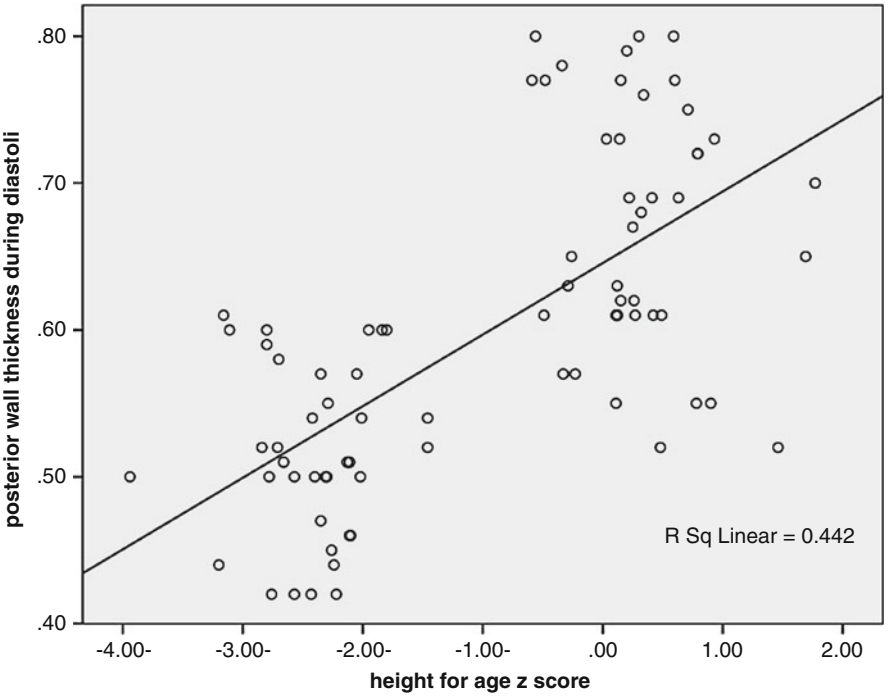

Fig. 5 Correlation between PWD and HAZ R = 0.665 $p = 0.000$

deterioration of myocardial contraction. Supplementation with vitamin D reverted myocardial contraction to normal (Witte Klauss et al. 2001).

Magnesium

Hypomagnesemia is related to worse prognosis for patients with heart failure and an increase in the rate of ventricular ectopics in patients with left ventricular dysfunction and those with normal cardiac function. Replacement with magnesium decreased the rate of ventricular arrhythmias.

Magnesium deficiency can lead to cardiac failure in rats. This can be inhibited by sufficient doses of ascorbate suggesting the possibility of free radical involvement. Heart failure as a consequence to hypomagnesemia has been observed in humans, and left ventricular function has been improved by correcting magnesium low levels. Hypomagnesemia is common in elderly patients with atrial fibrillation and heart failure and can precipitate digoxin toxicity. Ventricular arrhythmias seen in idiopathic cardiomyopathy may respond to magnesium therapy (Witte Klauss et al. 2001).

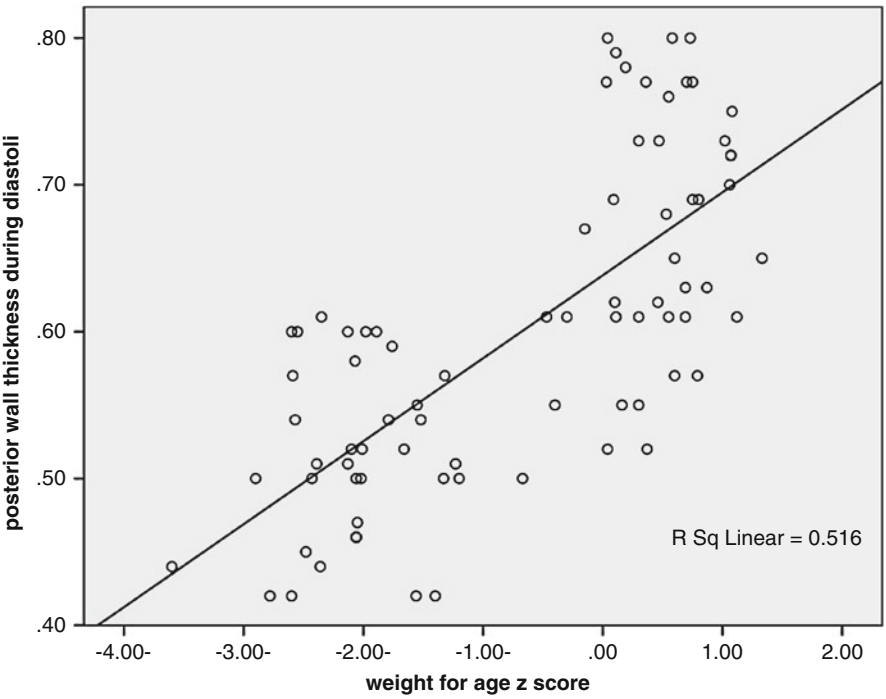

Fig. 6 Correlation between PWD and WAZ R = 0.718 $p = 0.000$

Zinc

Zinc deficiency is common in elderly. It correlates with cardiovascular drug intake and with dietary protein low intake. Low serum zinc with high urinary zinc levels are seen in those with heart failure and may be due to diuretic therapy.

Maternal severe zinc deficiency can cause developmental impairments to the heart. Prenatal zinc status may influence fetal cardiovascular autonomic function through unknown mechanism as stated by a study from Pero. Pregnant women with moderate zinc deficiency were supplemented and fetuses monitored. They had a lower mean heart rate at 20 weeks of gestation and greater heart rate variability and acceleration at 28 weeks, suggestive of greater parasympathetic control of the heart (Christian and Stewart 2010).

Copper

Copper has a role in the regulation of oxidative free radicals; its deficiency increases the susceptibility of lipoprotein peroxidation. Copper deficiency increases the risk of myocyte oxidative damage and may elevate plasma cholesterol concentrations.

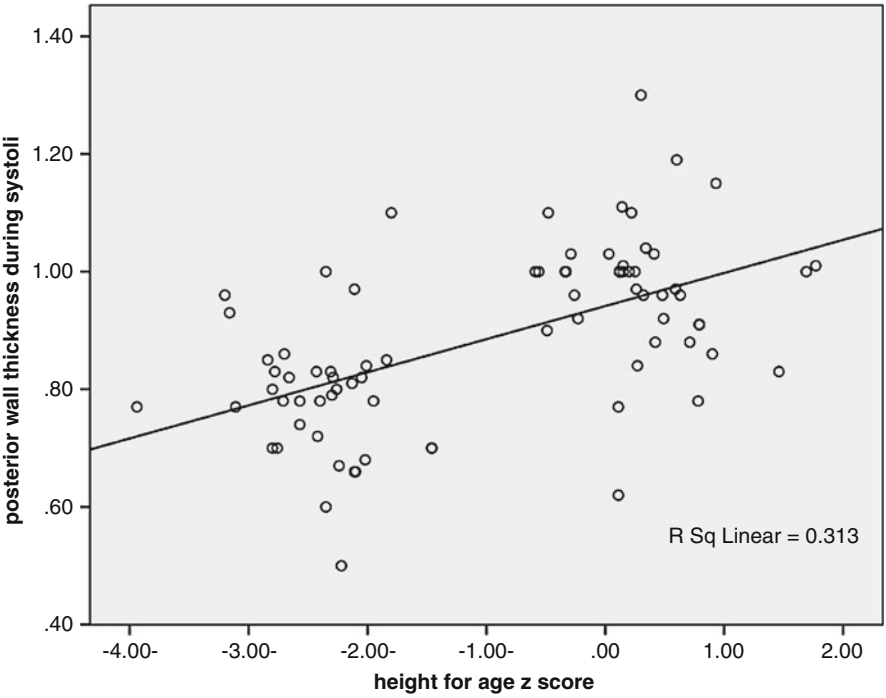

Fig. 7 Correlation between PWS and HAZ R = 0.559 $p = 0.000$

In rats chronic copper deficiency can lead to myofibrillar disarray and mitochondrial fragmentation. Copper deficiency decreases cytochrome C oxidase activity; this may lead to mitochondrial impairment and contribute to cardiac dysfunction. Copper-deficient cardiomyopathy is a recognized entity, and the identification of the genetic basis for defects in the copper-dependent ATPases has highlighted the possibility that copper deficiency has a role in experimental and human cardiomyopathy (Witte Klauss et al. 2001).

Selenium

Selenium is a constituent of the antioxidant enzyme glutathione peroxidase. Pure selenium deficiency is rare, but deficiency symptoms may occur when there is an additional dietary stress such as a vitamin E deficiency. An endemic cardiomyopathy in China, Keshan disease, is a consequence of selenium deficiency, which is also a risk factor for peripartum cardiomyopathy. In western countries, selenium-deficient cardiomyopathy has been described in patients on long-term total parenteral nutrition (Witte Klauss et al. 2001).

Ischemic heart disease and peripheral vascular disease have been linked to low selenium levels. In pig models of myocardial infarction, selenium reduced the

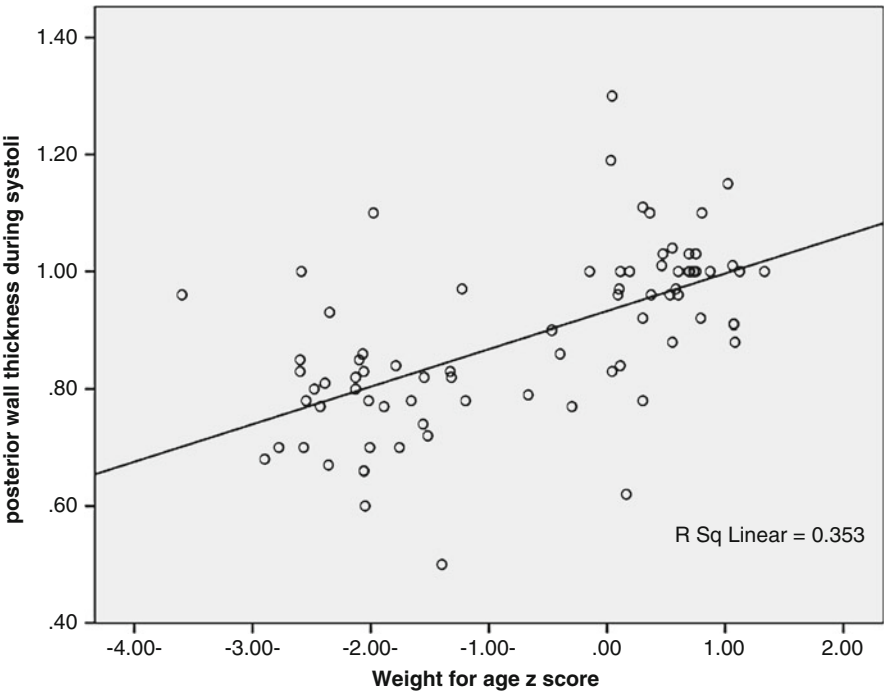

Fig. 8 Correlation between PWS and WAZ R = 0.594 $p = 0.000$

occurrence of late ventricular potentials in the border zone. This may be explained by the fact that selenium deficiency leads to increased levels of lipid peroxidation and, as a consequence, an increase in oxidative stress. Selenium has a role in protecting tissues from oxidative damage, which maintains the ability of cells to produce ubiquinone and preserves it from breakdown by oxidative degeneration. Deficiency leads to mitochondrial ultrastructural changes such as loss of cristae.

Thiamine

Thiamin deficiency occurs in malnourished individuals, in patients with chronic disease, in chronic alcoholics, in intestinal malabsorption, in anorexia, and in cases with high demand for this vitamin. High demand occurs during nutrition therapy with high carbohydrate diets for malnourished patients whose thiamine stores are marginal.

Beriberi, the well-known thiamine deficiency-induced disease, is characterized by cardiac failure. It is a high-output failure, with wide pulse pressure, tachycardia, enlarged heart, vasodilatation, pulmonary congestion, venous distention, and

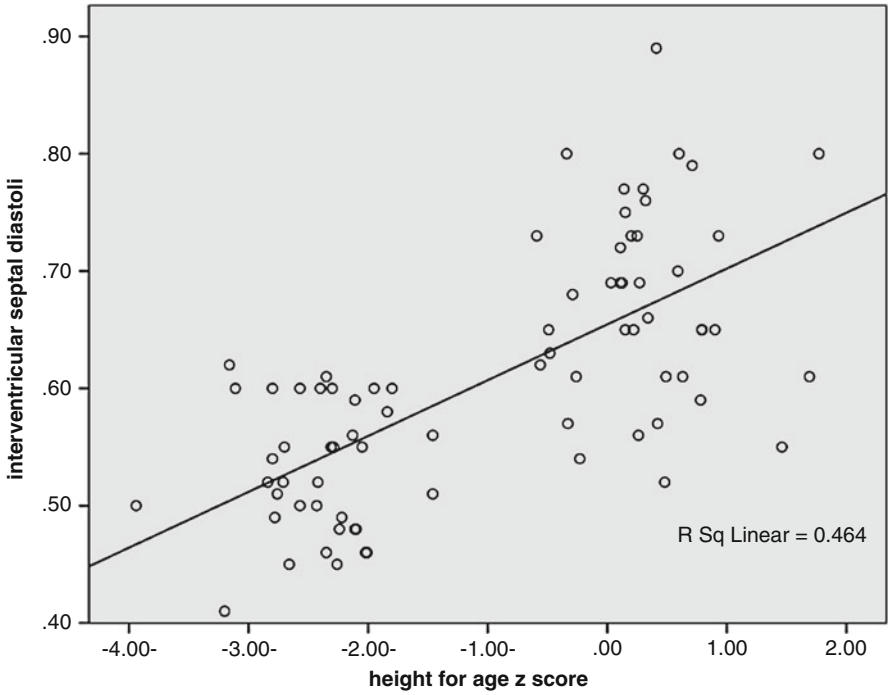

Fig. 9 Correlation between IVSD and HAZ R = 0.681 $p = 0.000$

peripheral edema. Vasodilatation is due to the accumulation of pyruvate and lactate. Response to thiamine is brisk and often with full recovery.

Vitamin (B_1) or thiamine is a coenzyme for decarboxylation in carbohydrate metabolism. Deficiency leads to impaired oxidative metabolism through inhibition of the citric acid cycle and the hexose monophosphate shunt (Witte Klauss et al. 2001).

Vitamins a and E

"Carotenoids, including β-carotene, lycopene, lutein, and α-carotene, are thought to prevent the oxidation of LDL cholesterol and other cell membrane lipids and thus prevent or slow the development of atherosclerosis. However, α-tocopherol (vitamin E) may be more effective than β-carotene in inhibiting LDL oxidation. Supplementation with 800 IU vitamin E, 1000 mg vitamin C, and 24 mg β-carotene in people with coronary artery disease effectively reduces LDL susceptibility to oxidation. Yet the effectiveness of carotenoids alone in preventing or treating heart disease has not been established, and, at present, carotenoid supplementation is not recommended" (Gropper et al. 2009).

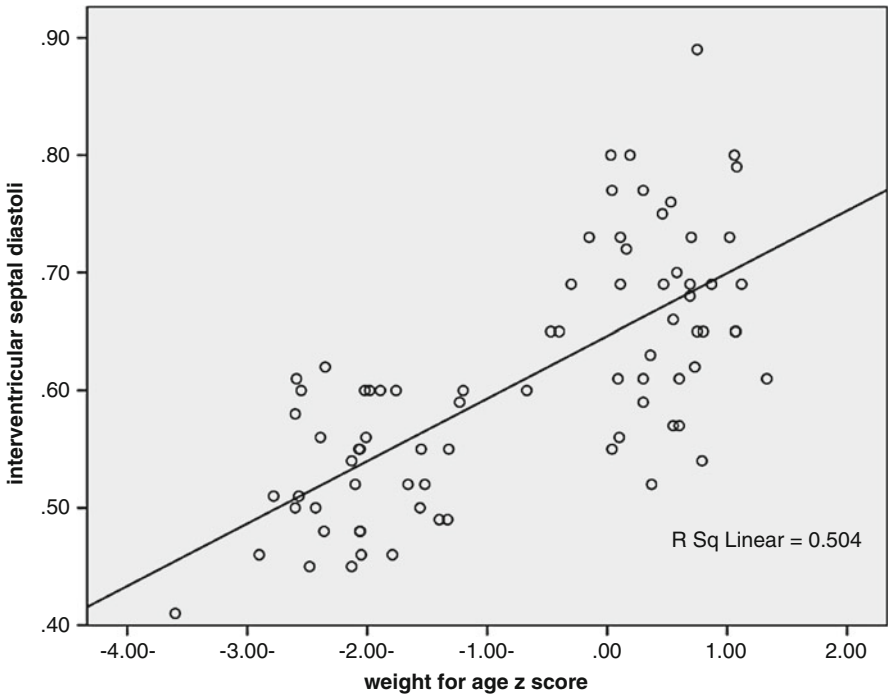

Fig. 10 Correlation between IVSD and WAZ R = 0.710 $p = 0.000$

Riboflavin (B_2)

Experimentally riboflavin deficiency has resulted in abnormal lipid metabolism, with a reduction in the beta-oxidation of fatty acids. It is not known whether riboflavin deficiency has any detrimental effect on cardiac functioning. Children with CHF due to congenital heart disease have an increased risk of riboflavin deficiency.

Vitamin B_6

Low pyridoxal-5′-phosphate is a risk factor for coronary artery disease and extracranial carotid artery disease mediated, in part, by elevated homocysteine levels. However, low B_6 levels are an independent risk factor for coronary artery disease even when homocysteine is taken into account. There are no reports of pyridoxal-5′-phosphate levels in HF.

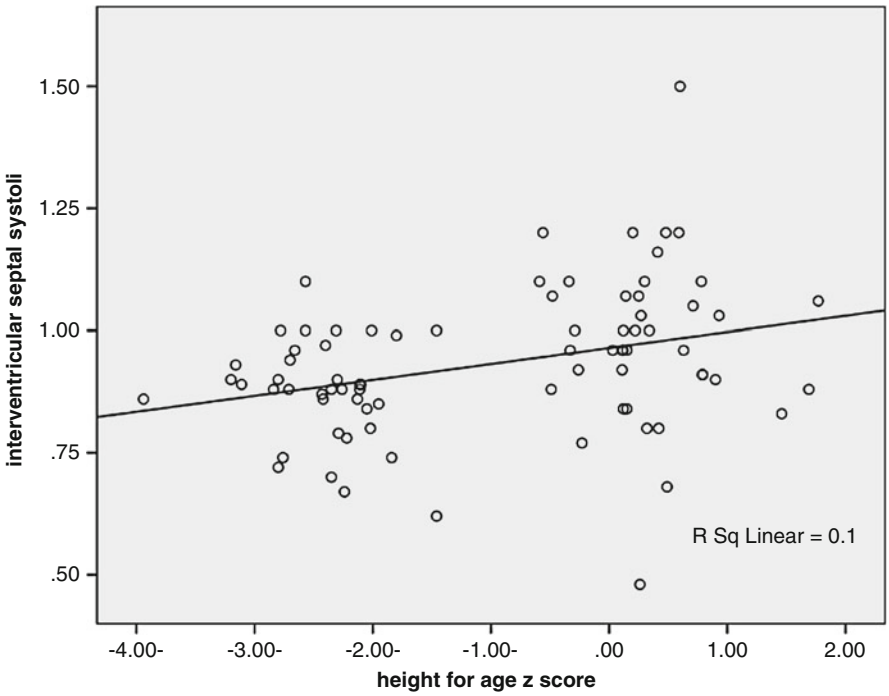

Fig. 11 Correlation between IVSS and HAZ R = 0.316 $p = 0.005$

Folate

The conversion of homocysteine to methionine requires folate, and a strong inverse relationship exists between folate consumption and homocysteine levels among patients with and without hyper-homocysteinemia. Tissue levels of vitamins B_{12}, B_6, and folate are not closely related to blood levels, and many more elderly patients may be deficient than are recognized. There is epidemiological evidence of an inverse link between folate consumption and risk of coronary heart disease (Witte Klauss et al. 2001).

Vitamin B_{12}

Vitamin B_{12} deficiency is associated with elevated levels of homocysteine. High homocysteine means an elevated risk for coronary artery disease. Data about B_{12} status in patients with heart disease will add to scientific knowledge in this regard.

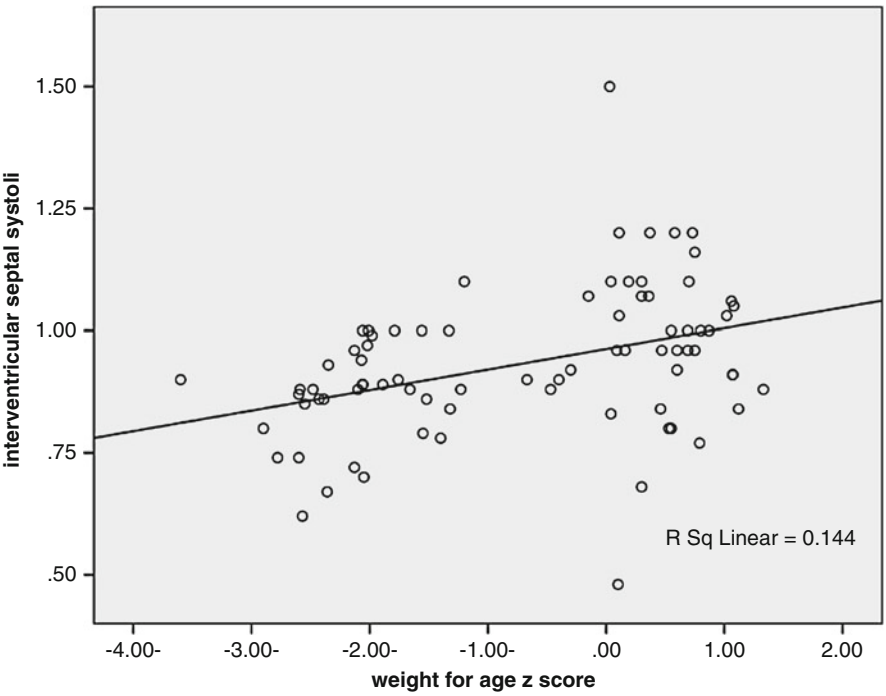

Fig. 12 Correlation between IVSS and WAZ R = 0.379 $p = 0.001$

Vitamin C

High intake of vitamin C has been correlated closely with a reduced risk of death from stroke through long-term follow-up study. The strength of the association was similar to the association of death and diastolic blood pressure. Vitamin C intake has no relation with deaths from heart disease. A study from Finland showed an increased risk of death from coronary heart disease, over 8 years of follow-up on middle-aged men, in those with low plasma ascorbate concentration.

In patients with hypertension, vasodilatory response to acetylcholine is attenuated. This attenuated response is partially reversed by vitamin C, and ascorbic acid supplementation can significantly lower blood pressure in hypertensive patients. Vitamin C improves endothelial function in diabetics and smokers when infused intra-arterially (cigarette smokers have lower plasma and leukocyte levels of vitamin C). Vitamin C infusions improve endothelial dysfunction associated with hypercholesterolemia and possibly caused by oxidative stress. Oral treatment with vitamin C for 30 days improves endothelium-dependent vasodilation in patients with coronary artery disease, and even a single dose of 2 g can improve vasomotor function after 2 h (Witte Klauss et al. 2001).

In heart failure it is unknown whether vitamin C levels are reduced, but improvements of endothelial dysfunction have been seen with vitamin C.

Vitamin E

In middle-aged subjects, high vitamin E intake is associated with a lower incidence of coronary heart disease. Among individuals with high intake of vitamin E, men had a lower risk of developing coronary artery disease by 40% and women by 34%. Similar results are seen in those 65-year-olds and more with additional benefits if they use both vitamin E and vitamin C supplements. Vitamin E for healthy volunteers led to a reduction of platelet stickiness, an effect that has also been seen in diabetics and heart transplant recipients (Witte Klauss et al. 2001).

Dietary Recommendations for Cardiovascular Health

From the above paragraphs, we can conclude that macro- and micronutrient deficiencies affect cardiovascular health in general and the heart structure in particular. On the other hand, high calorie and high fat diets as well as physical inactivity have been considered as causes for obesity and ischemic heart disease and many other chronic noncommunicable diseases. Costs incurred by different societies for the management of these diseases and health problems are expanding continuously. Exploration of future outcomes of protein energy malnutrition on the heart may need more longitudinal studies. Virtual research question may include vulnerability to ischemia, post-myocardial infarction complications, susceptibility to cardiomyopathy, and electrophysiologic abnormalities.

Prevention of cardiovascular diseases and promotion of cardiovascular health are being approached through the following: evaluating the diverse dietary risk pathways rather than just blood lipids or obesity; foods and diet pattern rather than isolated specific nutrients; complex influences of different foods on weight control instead of just counting calories; and implementing strategies for lifestyle change.

Evidence-based dietary recommendations include increase intake of the following food: fruit vegetables (other than starchy ones like potatoes), nuts, legumes, fish, vegetable oils, yogurt, and whole grains. These recommendations also include limiting intake of red meat, processed meats, foods rich in refined grains, starch, added sugars, salt, and trans fat (Mozaffarian 2016).

Health system reforms may be essential to foster health promotion through well-designed policies. Healthy diets as recommended by international associations and increase physical activities should be applied practically in addition to special education programs about their importance in relation to heart and cardiovascular system as a whole.

In our Arab world, promoting cardiovascular health is challenging. Diet rich in calories, refined sugars, starchy food, and fat is preferred. Inactive sedentary life is a prevailing style especially for females. Still health education programs regarding healthy diet and physical activity are developing.

Dictionary of Terms

- Malnutrition means on one hand food intake that exceeds human body needs and results in obesity or increased body weight.
- Malnutrition means on the other hand food intake that does not meet human body needs and results in decreased body size/weight.
- Stunting means short stature in relation to age; it happens when food insufficiency persisted for more than 6 months.
- Wasting is thinness of the human body determined by relating body weight to body height. This is the earliest bad effect of food shortage.
- Echocardiography is an examination and imaging of the heart using ultrasound waves.

Summary Points

- PEM is a public health problem affecting millions of people mainly those in developing countries with children on top of the list.
- Malnutrition may be clinical (severe) or subclinical mild and moderate where children are considered normal with poor healthcare systems.
- PEM causes decrease in body size including internal organs, and the heart is one of them.
- Heart involvement is correlated with severity of PEM determined by anthropometry.
- Studies included laboratory experiments, postmortem studies, hospital-based observational studies, and community-based comparative study.
- Micronutrients like vitamins and minerals are essential for cardiac health as their deficiencies result in many diseases and health impairment.
- Micronutrients can be used to counteract cardiovascular ill health and control degenerative changes of the cardiovascular system.
- The field needs more research that may be longitudinal studies to assess long-term impact of childhood malnutrition on heart health and disease.
- Nutrition as a medical discipline may help, through more focused research work, to control the widespread of heart disease.
- Current knowledge highlighted new areas of concerns for health policies in terms of healthy diet and lifestyle for better cardiovascular health.

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Intermittent Fasting Effects on the Central Nervous System: How Hunger Modulates Brain Function

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Contents

Abstract

Fasting has been present throughout human history and is a regular practice in many cultures and religions. Currently, findings regarding beneficial effects of fasting on body mass control and health have largely stimulated the practice. The number of studies investigating intermittent fasting effects on different pathological states has grown steadily. Evidence suggests that this dietary intervention can delay or even prevent the onset of pathologies, such as neurodegenerative diseases. Indeed, several studies have reported intermittent fasting actions on brain integrity and function. However, fasting may also affect hunger control in less desirable manners. Indeed, the brain is highly sensitive to fasting practice due

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to its pronounced energy demand and its central role in the control of whole body energy balance. In this chapter, the effects of intermittent fasting on brain function are discussed along with a description of the history of human fasting practices.

Keywords
Intermittent fasting · Caloric restriction · Energy homeostasis · Brain function · Hypothalamus · Appetite control · Energy expenditure · Reproductive function · Aging · Cognition · Neurogenerative diseases

List of Abbreviations

AD	Alzheimer's disease
AGRP	Agouti-related peptide
CART	Cocaine- and amphetamine-regulated transcript
GnRH	Gonadotropin-release hormone
HDL	High-density lipoprotein
IF	Intermittent fasting
LDL	Low-density lipoprotein
NPY	Neuropeptide Y
PD	Parkinson's disease
POMC	Pro-opiomelanocortin
VLDL	Very-low-density lipoprotein

Introduction

Fasting is the act of abstaining from food for a specific period of time. It is practiced in many cultures and religions, as well as for weight loss purposes and even political statements. Many types and subtypes of fasting exist (Table 1), with a wide range of changes in net calorie intake and physiological effects in humans and laboratory animals.

In 1994, 14% of US adults had tried fasting as a strategy to control body weight (French and Jeffery 1994), and this number may have risen quite largely in recent years, stimulated by anecdotal beliefs in health benefits of this practice. In fact, overweight and obesity are major global health concerns related to disorders including coronary heart disease and type 2 diabetes. More than 50% of the population in the UK and US, for example, are considered overweight or obese (National Center for Health Statistics 2016; The NHS Information Centre 2012). Caloric deficit is an obvious approach to promote weight loss, achieved by increasing the metabolic demand through physical exercise and/or decreasing calorie intake (Wu et al. 2009). Although a very successful strategy to promote weight loss and reduce disease risk, adherence to caloric restriction decreases over time and is inversely proportional to the degree of restriction (Wing and Phelan 2005). Thus, one to few days a week fasting with ad libitum feeding breaks has become more popular as a supposedly easier way to limit caloric ingestion (Varady 2011).

Table 1 Types and subtypes of fasting

Fasting (types and subtypes)	Description
Dry fasting	Also called absolute fast or complete fast. Consists of total absence of food and liquid intake, including water. Usually practiced for a shorter periods of time. Dry fasting is used by religious practitioners of Hinduism, Judaism, Islam, and Jainism as well as in traditional Chinese Medicine (Fredricks 2013)
Water-only fasting	Allows the ingestion of water, while abstaining from any type of food and other liquids (Fredricks 2013)
Therapeutic fasting	"Therapeutic fasting" is a water-only fast with medical supervision. It was well accepted as an inpatient treatment for morbid obesity during the 1950s and 1960s. More recently, spas and fasting retreats are offering similar activities (Fredricks 2013)
Modified fasting	
- Juice fasting	Juice fasting refers to the consumption of juice only. Juice fasters believe it enhances the body's elimination of metabolic byproducts. It is followed from home or specialized retreats. Certain rules may apply, such as exclusive raw vegetable juice and consumption up to 30 min after production (Fredricks 2013)
- Specific fasting	Specific and exotic types of fasting normally refer to the abstinence or consumption of a given food (Fredricks 2013)
- Intermittent fasting (IF)	Intermittent fasting (IF) is a focus of many recent studies and consists of a repeated pattern of fasting and refeeding (Mattson et al. 2017)
• Every other day fasting	A type of IF, also called alternate day fasting, where 24 h fasts are followed by 24 h ad libitum refeeding
• 5:2 Fasting	Two fast and five feasting days per week (Mattson et al. 2017)
• 20/4	A type of IF where 20 h fasts are followed by 4 h nonfasts
• Time-restricted feeding	Described by Chaix et al. (2014) as limiting daily feeding hours to 9–12 h

Animal models have shown that caloric restriction effects go beyond weight loss and disease prevention. In mammals, caloric restriction modulates several cellular pathways (Table 2) associated with lifespan extension such as insulin signaling, inflammation, hunger, autophagy, gene expression, mitochondrial biogenesis, and function. Ultimately, caloric restriction is a useful model to understand longevity-regulating genes; the National Institute of Aging, Max Planck Institute for Biology of Aging, and other Federal Agencies around the world have heavily invested in research to find drugs which can mimic it (Ingram et al. 2006).

Caloric restriction animal models are, however, labor-intensive. As a result of its relative ease, many studies have substituted caloric restriction (a daily decrease in food intake) for intermittent fasting (IF, often 24 fasting followed by 24 ad libitum feeding, or every other day feeding, Cerqueira and Kowaltowski 2010); unfortunately, both terms are often used interchangeably. Although IF reproduces some of caloric restriction effects, such as decreased body weight (Table 2), criticism has been raised from observations that IF does not necessarily decrease the net caloric

Table 2 Main metabolic and health-related effects of calorie restriction and intermittent fasting (*IF*)

	Calorie restriction	Intermittent fasting
Similar findings in rodents and humans	- Significant and standardized restriction of dietetic calories - Weight loss - Well-documented reduction in blood triglycerides, growth hormone, and insulin (Lee and Longo 2011; Barnosky et al. 2014) - Substantial improvement in insulin sensitivity (Barnosky et al. 2014) - Improved mitochondrial biogenesis (Civitarese et al. 2007) - Regulation of "nutrient-sensing" pathways (Civitarese et al. 2007) - Reduced blood pressure and atherosclerosis risk (Fontana et al. 2004)	- Effects on net calorie consumption depending on ad libitum intake on feasting days - Different reports on body weight: loss, no effect or gain (Lee and Longo 2011; Barnosky et al. 2014) - Differences in short-term and long-term effects (Lee and Longo 2011; Cerqueira et al. 2011) - Marked reduction in blood IGF-1 (Lee and Longo 2011; Fontana et al. 2008) - Reduction in blood insulin and triglycerides (Lee and Longo 2011; Barnosky et al. 2014)
Research findings in rodents (Adapted from Lee and Longo 2011)	- Lifespan extension - Reduced nitrogen excretion - Reduction in body temperature - Extended reproductive span - Reduced cancer incidence; for a subset of malignancies, progression is reduced - Decreased oxidative imbalance markers - Effects observed within weeks or months - Reduction in blood IGF-1 levels	- Arguable effects on lifespan - Divergent effects on diabetes prevention (Dorighello et al. 2013) - Reduced fertility - Increased nitrogen excretion - Varying effects on oxidative markers (Cerqueira et al. 2011) - Reductions in body temperatures - Effects observed after 2–3 days - Use of ketone bodies as energy sources
Research findings in humans	- No reduction in IGF-1 levels (Fontana et al. 2008) - No clear reduction in fasting blood glucose (Barnosky et al. 2014)	- No clear reduction in fasting blood glucose (Barnosky et al. 2014) - May be protective during chemotherapy (Dorff et al. 2016) - Improvement in insulin sensitivity associated with weight loss in obese individuals (Barnosky et al. 2014)

intake in rats (Cerqueira et al. 2011; Chausse et al. 2014). Classical caloric restriction protocols, on the other hand, promote 30–60% reduction in calories, keeping the proportion of macronutrients and correcting the micronutrients levels (Cerqueira and Kowaltowski 2010). During IF, some studies have reported compensation through overeating on the refeeding days (Cerqueira et al. 2011; Chausse et al. 2014).

Importantly, IF effects on rodent lifespan are debatable (Cerqueira and Kowaltowski 2010). Curiously, a parallel can be made with fasting during Ramadan, which promotes more eating during the refeeding period, resulting, in some cases, in weight gain (Bakhotmah 2011).

Indeed, fasting deeply affects brain function promoting neuronal remodeling to counteract nutritional stress. These adaptations are pronounced in the hypothalamus, the brain area that controls whole body energy balance. Hormones and nutrients signaling energy deficits activate compensatory sensor-behavioral responses that are coordinated by hypothalamic neurons (Morton et al. 2014). Therefore, prolonged periods of food deprivation, such as IF, induce marked modulation in hypothalamic function and consequently in feeding behavior. These changes may also be related to beneficial effects of fasting on cognition and brain disease prevention. Indeed, IF has long been recognized to delay cognitive decay and the onset of neurodegenerative diseases (Murphy et al. 2014). Here, the history of fasting in humans will be explored along with discussions about how IF impacts brain function, focusing on its effects on the hypothalamic control of energy balance, cognitive function, and neurodegenerative diseases.

The History of Human Fasting

Fasting has been practiced since the dawn of humankind, when it was imposed by food scarcity. As humanity and food availability evolved, feeding habits shifted from eating when food was available to a limited amount of times per day (main meals) to what recently became a scientifically unsupported nutritinal gold-standard recommendation of eating every 3 h (Mattson et al. 2017). As a result, people in the Western World are constantly in the postprandial state (Lopez-Miranda and Marin 2010), while the human body evolved to endure periods of fasting/starvation, and thus accumulates excess calories, stored mainly as lipids, with ease (Mattson et al. 2017).

Interestingly, throughout history, organized religions and cults embraced periods of fasting as part of their rituals. Indeed, fasting is still a tool for spiritual purification or atonement for the largest known religions (Table 3). Important spiritual leaders such as Gandhi, Jesus, and Buddha fasted for over a month, thus providing evidence that fasting is well within the the capabilities of a healthy adult. Despite the variability in frequency and type, religion-associated fasting has given us hints about the impact of fasting on human health. Muslims undergo long fasts each year throughout the length of Ramadan (and beyond). During the Ramadan month, those practicing fasting present lower total cholesterol, LDL cholesterol, VLDL cholesterol, and triglyceride levels, while HDL levels are markedly increased (Adlouni et al. 1997; Maislos et al. 1993), even without a significant reduction in body weight (Temizhan et al. 2000). During Ramadan, acute coronary heart diseases reduced compared to pre-Ramadan months (Temizhan et al. 1999). One case study of a man undergoing 1 week fast during the Japanese Buddhist ritual Danjiki also showed lower triglycerides levels after fasting (Tanaka et al. 2016).

Table 3 Fasting in the main religions

Religion	Fasting practice
Hinduism	Fasts once a week in addition to other religious observances Fasting before yoga practice is common (Fredricks 2013)
Buddhism	Half-day and 3-day fasts Fasting after noon is a common practice in Tibetan Buddhism (Fredricks 2013)
Judaism	Some holidays involve 25-h fasting periods, while others involve fasts from sunrise to sunset (Fredricks 2013)
Islam	Ramadan involves fasting from sunrise to sunset during a month every year (Fredricks 2013)
Christianity	Many examples of one-day, three-day, and forty-day fasts in the new testament. Abstention from meat on certain holidays. Mormons often fast the first Sunday each month (Fredricks 2013)

Renowned physicians and scientific icons also reinforced this practice due to earlier observations on health and concentration capacity; Socrates, Plato, and Aristoteles fasted for mental clarity, and Pythagoras fasted for 40 days before teaching advanced students. Hippocrates believed fasting assisted the body in healing itself and stated *Our food should be our medicine and our medicine should be our food. But to eat when you are sick is to feed your sickness* (Fredricks 2013). Another Greek Physician, Paracelsus, concluded, nearly 500 years ago, *Fasting is the greatest remedy – the physician within!* (Bragg and Bragg 2004). In fact, fasting is one of the oldest known medical therapies, dating from the Stone Age (Goscienski 2005). Fasting was regularly prescribed to treat and prevent diseases, preserve health, and prolong life. In the 1950s and 1960s, fasting and very-low calorie diets were a well-accepted inpatient treatment for morbid obesity. This is no longer recommended since it has been linked to serious complications such as ventricular fibrillation, lactic acidosis, vitamin and electrolyte deficiency, and sudden death either during fasting or refeeding (Johnstone 2007). Despite this specific criticism, fasting has strong worldwide appeal, and is considered a no cost therapy associated with disease prevention and treatment. It is thus not surprising that healthy individuals follow this practice, without any close monitoring, contributing to a lack of more robust data on the effects of this intervention on human health.

Indeed, IF is becoming an ever more popular strategy to lose weight (Carlson and Hoelzel 1946; Fredricks 2013). IF is actually a pattern of fasting and feeding which alternates between periods of fasting and refeeding (Mattson et al. 2017) which has branched into different subtypes (Table 1). Some studies have demonstrated that calories are often reduced during IF (e.g., weekly calorie intake in an individual on the "5:2" diet is reduced by 25%; Harvie et al. 2013), although the question remains as to what extent physiological responses to IF are mediated by overall caloric restriction.

The earliest IF studies in laboratory models date from the 1880s: The effects on growth and nutrition were tested in chickens (Von Seeland 1887) and salamanders (Morgulis 1913). In 1945, different fasting strategies were tested in rats (fasting to nonfasting days of 1:2, 1:3, and 1:4) and were suggested to impact on lifespan; 1:3

was found to be the most effective. Interestingly, the strongest effects on the reduction of tumor incidence were directly associated with the frequency of fasting days (Carlson and Hoelzel 1946). The shortest window between fasting and non-fasting days (1:2) resulted in a larger number of individuals with higher food intake during the nonfasting days, a result which was reproduced in rats fed every other day (Cerqueira et al. 2011; Chausse et al. 2014). Interestingly, many caloric restriction studies in rodents are, in fact, some form of fasting, as pointed out by Mattson and colleagues (Mattson et al. 2017): rodent caloric restriction models normally involve offering daily allotments of food once or twice a day, or even every 3 days, and the animals consume it all within few hours. Indeed, IF mimics daily caloric restriction in many aspects (Table 2), although there is controversy whether IF and caloric restriction result in similar net calorie intake reduction. While IF effects on lifespan are still arguable, caloric restriction has consistently been shown to extend life spans in rodents since 1935 (McCay 1935; Sohal and Weindruch 1996).

In humans, most caloric restriction and IF interventions are not performed continuously throughout the life, and there is no data regarding the effect on human lifespan. Indeed, most of the research has been focusing on the beneficial aspects of ongoing IF and caloric restriction, or how long the metabolic effects last when they are ceased. Although it is known caloric restriction and fasting affect hunger and other brain and autonomic nervous system characteristics, the behavioral aspects are less explored. These are important, since hunger triggered by caloric restriction or fasting is a central component of weight gain after these interventions cease (Franklin et al. 1948; Stice et al. 2008).

Intermittent Fasting Effects on Brain Function

The brain coordinates complex physiological and behavioral responses to endogenous and environmental stimuli. Indeed, different signals modulate its function inducing neuronal activity remodeling. These changes involve high energy-expending processes such as neurotransmitter synthesis and reuptake, which result in elevated brain energy consumption (Amigo and Kowaltowski 2014); nutrient availability must therefore be accurately regulated to avoid neuronal dysfunction and death. In addition to its high-energy demand, the brain controls whole body nutritional state by regulating energy intake and waste. Fasting-feeding signals are sensed by different neuronal populations that, together, modulate appetite, food reward, locomotor activity, and energy expenditure (Morton et al. 2014). These features make the brain highly sensitive to alterations in feeding patterns such as those introduced by dietary restriction.

Given the brain's susceptibility to fasting, a growing number of studies have been performed to understand how IF affects neuronal function, mostly within an aging perspective. Evidence suggests that fasting cycles can delay brain aging, preventing neurogenerative diseases and cognitive functional decay. Moreover, fasting diets have been related to improvements in brain plasticity, learning, and memory. IF also modulates the central control of energy balance, since this regimen alters the

circadian regulation of the body's nutritional state. The next sections contain discussions about IF effects on neuronal function, focusing on its actions on energy homeostasis control, cognition, and neurodegenerative diseases.

Intermittent Fasting Impacts on Hypothalamic Function

Energy homeostasis is primarily controlled by the hypothalamus. This cerebral area has a permeable blood–brain barrier that allows the entrance of hormones and nutrients signaling body nutritional state. These signals are integrated by distinct neuronal populations which adjust energy balance regulating feeding and energy usage (Morton et al. 2014). In response to energy deficits, hypothalamic orexigenic neurons induce increases in appetite and energy conservation. Anorexigenic neurons, in turn, counteract positive energy balance inhibiting food ingestion and stimulating energy expenditure. The alternating activation of these pathways finely regulates fasting-feeding cycles, ensuring adequate body mass control (Barsh and Schwartz 2002). Changes in feeding patterns should therefore modulate hypothalamic function, adapting physiological responses to the body's nutritional state (Fig. 1).

Dietary regimens that involve fasting have been found to alter hypothalamic control of appetite and body mass. Indeed, IF promotes hyperphagia along with marked activation of hypothalamic orexigenic pathways (Chausse et al. 2014). Several groups have observed increases in the expression of orexigenic neuropeptides neuropeptide Y (NPY) and agouti-related protein (AGRP), using different fasting protocols (Kumar and Kaur 2013; Chausse et al. 2014; Gotthardt et al. 2016; Méquinion et al. 2017). This effect could be related to IF-induced reduction in circulating leptin levels. Minor changes in hypothalamic anorexigenic neuropeptide contents have been reported. Due to enhancements in orexigenic signaling, IF subjects can compensate fasting periods presenting similar cumulative caloric ingestion of nonrestricted groups (Cerqueira et al. 2011; Chausse et al. 2014; Wahl et al. 2016). This suggests that IF outcomes can be a consequence of changes in feeding frequency as well as restrictions in caloric intake, when they occur (Anson et al. 2003). However, it is important to highlight that, in some studies, IF-treated subjects present 10–20% reduction in overall food consumption. In a short-term study, Lauzurica and collaborators observed that IF animals present progressive increases in caloric intake during the first week. Curiously, appetite and NPY neuropeptide levels remained elevated for days after the treatment ended (Lauzurica et al. 2010). This agrees with data showing that orexigenic neurotransmitter expression continues increasing even when IF-treated rats are fed (Chausse et al. 2014). Despite overeating, IF animals display low body mass, indicative of a decrease in energy conversion efficiency, as a result of increases in lipid oxidation and metabolic rates (Chausse et al. 2014). However, the alterations in hypothalamic function underlying IF-induced reduction in feed efficiency are not yet well understood.

The beneficial effects of fasting have been attributed to metabolic and physiological adaptations induced by nutritional stress. These changes include activation of the stress-linked hypothalamic axis that engage sensory-motor responses to food

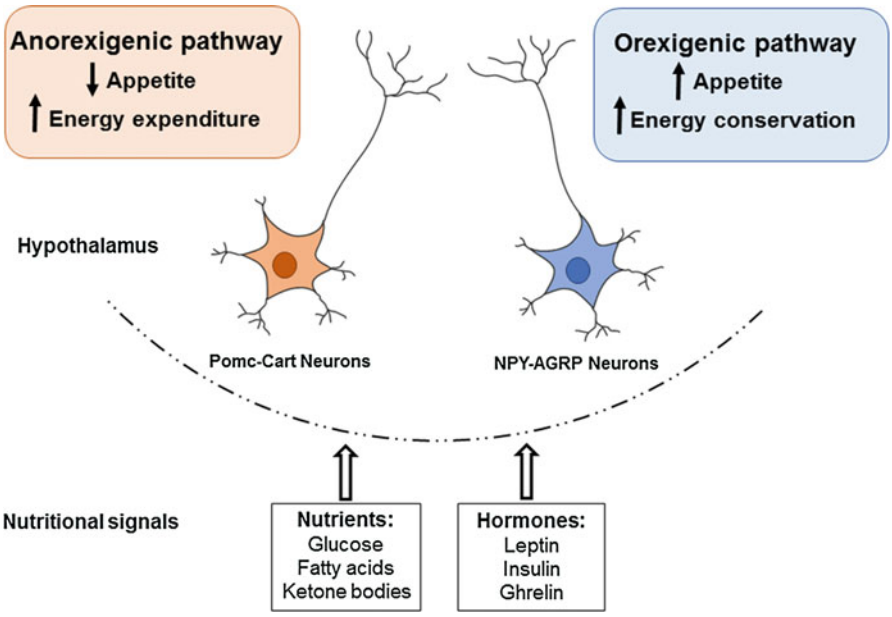

Fig. 1 Hypothalamic pathways controlling body energy balance. Energy balance is controlled by alternating the activation of hypothalamic orexigenic and anorexigenic neurons. Nutritional deficits activate orexigenic neurons (characterized by the expression of NPY and AGRP neuropeptides) inducing increases in appetite and energy conservation. Nutrients and hormones signaling positive energy balance, in turn, activate anorexigenic neurons (characterized by the expression of POMC and CART neuropeptides), promoting decreases in appetite and increases in energy expenditure. Key: *NPY* neuropeptide Y, *AgRP* agouti-related peptide, *POMC* pro-opiomelanocortin, *CART* cocaine- and amphetamine-regulated transcript

scarcity (Mattson 2015). Studies in rodents show that fasting cycles activate the hypothalamic–pituitary–adrenal axis, increasing plasma corticosterone levels (Lauzurica et al. 2010). Moreover, IF promotes an enhancement in norepinephrine content in the medial hypothalamus followed by increases in NPY expression and hyperphagia (Gotthardt et al. 2016). Hypothalamic alterations determining IF-induced voracious feeding should include enhancements in the hedonic and nutritional value of food. Indeed, the hypothalamus orchestrates the activity of different brain areas controlling energetic and sensory aspects of feeding (Morton et al. 2014). Studies in humans suggest that intense dietary restraint is followed by changes in food perception, anxiety, and mood (Green et al. 1994). Nonetheless, the integration between the hypothalamus and other brain areas supporting IF effects on feeding behavior is still incompletely understood.

In parallel to energy balance, the hypothalamus regulates energy-sensitive processes such as reproduction. Fasting-induced alterations in energy availability can promote physiological adaptations or even interrupt this process (Hill et al. 2008). In fact, famine and starvation have long been related to changes in

reproductive cycles and impairments in fertility. For instance, clinical cases of anorexia nervosa comprise nutritional infertility and amenorrhea (Estour et al. 2010). IF impacts on the hypothalamo–hypophysal–gonadal axis have been described in detail in rodents. IF-induced changes in leptin and NPY levels have been linked to reductions in gonadotropin-release hormone (GnRH) expression and its release in the hypothalamus of male and female rats (Kumar and Kaur 2013). Since this neuropeptide is a master regulator of reproduction, changes in GnRH were followed by decreases in testosterone and luteinizing hormone content along with interruption in reproductive function. In a long-term study, approximately 50% of female rats presented alterations in the estrous cycle after 6 months of IF (Martin et al. 2007). These data suggest that the perturbations in energy balance promoted by fasting diets directly impact the neuroendocrine control of reproductive function leading to reductions in fertility. IF effects on hypothalamic function are outlined in Table 4.

Table 4 Intermittent fasting effects on hypothalamic function

Animal model	Dietary intervention	Effects	Reference
Female Sprague-Dawley rats	2 h daily food access (7 days) plus refeeding (14 days)	Hyperphagia ↓ Body mass during restriction time ↑ NPY, corticosterone ↓ POMC, leptin	Lauzurica et al. 2010
Female/male Wistar rats, 3–4 months	24-h fast/24-h fed, 12 weeks	Body mass ↑ NPY ↓ GnRH, testosterone ↓ LH, leptin	Kumar and Kaur 2013
Male Sprague-Dawley rats, 8 weeks old	24-h fast/24-h fed, 3 weeks	Hyperphagia ↓ Body mass ↓ Feed efficiency ↓ Leptin ↑ NPY, AgRP, orexin	Chausse et al. 2014
Male C57BL/6 mice	8 weeks high fat diet followed by 4 weeks 24-h fast/24-h fed	Body mass ↑ Norepinephrine, NPY	Gotthardt et al. 2016
Female C57BL/6 J, 8 weeks old	2 h daily food access, 10 weeks	Hyperphagia ↓ Body mass ↑ NPY, AGRP	Méquinion et al. 2017
Male C57BL/6 J, 4 weeks	22 weeks of high fat diet followed by 12 weeks of mild IF	Hyperphagia ↑ POMC	Seimon et al. 2016

Major effects of intermittent fasting on hypothalamic function are summarized. In general, IF increases hypothalamic orexigenic signaling followed by hyperphagia, even when different fasting protocols were used. Key: *NPY* neuropeptide Y, *POMC* pro-opiomelanocortin, *AGRP* agouti-related peptide, *GnRH* gonadotropin-release hormone, *LH* luteinizing hormone

Intermittent Fasting Effects on Cognition and Neurodegenerative Diseases

Brain function is sustained by the coordinated activity of neurons and nonneuronal cells, which are highly sensitive to perturbations in organism homeostasis. Physiological challenges introduced by alterations in lifestyle and aging, for example, can directly affect brain performance (Mattson 2015). Indeed, aging-related changes in cerebral structure and function are associated with the appearance of neuronal disorders and increased brain vulnerability to diseases. The mechanisms behind brain functional decline have long been investigated and include alterations in neuronal physiology, cell-to-cell interaction, brain volume, and microscopic morphology (Wahl et al. 2016).

Fasting diets delay or even prevent aging-induced neuronal dysfunction. Extensive research regarding IF impacts on the brain have suggested that fasting cycles can delay cognitive decay and neurogenerative diseases (Murphy et al. 2014). IF beneficial effects are supported by reductions in cellular oxidative stress and enhancement in mitochondrial function (Amigo and Kowaltowski 2014; Raefsky and Mattson 2016). IF further maintains brain homeostasis by improving neurogenesis and synaptic plasticity (Murphy et al. 2014). Recent findings about IF effects on cognition and neurodegenerative pathologies are shown in Fig. 2 and will now be discussed.

Changes in behavior and cognition in aged mammals have been associated with a decline in brain function (Anderton 2002). In fact, aging-induced neuronal deterioration in the hippocampus and prefrontal cortex is followed by impairment in memory and learning (Weber et al. 2015). These effects are a consequence of increased neuronal death accompanied by marked decreases in neurogenesis and brain plasticity (Murphy et al. 2014). IF protects the brain against dysfunction and degeneration by increasing neuronal resistance to cellular stress. Findings in rodents suggest that IF enhances neuronal resilience to excitotoxic stress, preventing learning deficits (Qiu et al. 2012). Fasting cycles further induce increments in neurotrophic factors and anti-inflammatory cytokine contents, which prevent hippocampal cell death and stimulate neurogenesis (Arumugam et al. 2010; Marosi and Mattson 2014). Indeed, Lee and colleagues showed that IF can increase the survival of newly generated neurons in the hippocampus (Lee et al. 2000). Learning and memory have been improved by fasting treatment even when the diet is introduced at advanced ages. IF-induced attenuation in spatial learning and motor coordination deficits was observed in old rats at 70% of their lifespan. This effect was a consequence of the increased expression of synaptic proteins regulating calcium homeostasis (Singh et al. 2012). Although beneficial impacts of IF on brain aging have long been demonstrated, some evidences suggest that alternative fasting patterns can compromise cognitive function in humans. Daylight fasting, such as adopted during Ramadan, has been related to impairments in cognitive capability and decision making (Cherif et al. 2016). In addition, long-term food restriction to lose weight promoted self-reported decreases in memory and intellectual function, accompanied by poor sustained attention task results (Green et al. 1994).

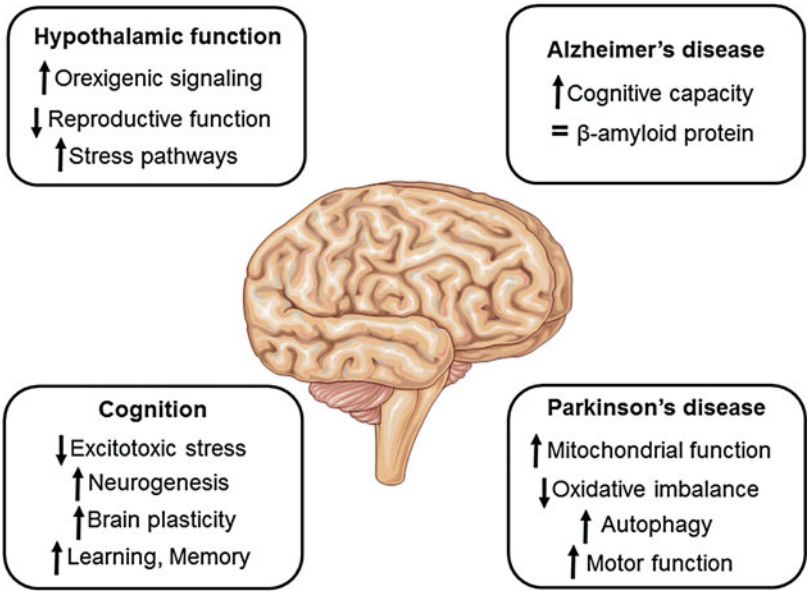

Fig. 2 Intermittent fasting effects on brain function. The main effects of IF on brain function are summarized. IF-induced changes in energy balance are followed by increases in orexigenic signaling in the hypothalamus, in parallel with alterations in hypothalamic axis control of reproduction and stress responses. IF improvements in cognitive capacity are supported by increased neuronal resistance to excitotoxic stress, neurogenesis, and brain plasticity, resulting in preserved learning and memory. IF can delay Alzheimer's disease progression independently of changes in neuronal β-amyloid peptide accumulation. IF also prevents Parkinson's disease development through enhancements in mitochondrial function and autophagy, along with reduction in oxidative imbalance. Of note, IF can hasten the progression of some brain diseases such as amyotrophic lateral sclerosis

Deterioration in cognitive capability is often followed by the onset of neurogenerative diseases. Indeed, Alzheimer's disease (AD) is present in many cases of learning and memory deficits and is considered a major factor causing dementia. AD is associated to neuronal degeneration in brain areas controlling cognition and emotional behaviors (Castellani et al. 2010). This disease is characterized by the aggregation and extracellular accumulation of β-amyloid peptide plaques and by neuronal intracellular aggregation of hyper-phosphorylated forms of the tau protein. AD etiology also includes mitochondrial dysfunction and oxidative imbalance (Mattson 2004). While caloric restriction effects on AD progression have been extensively addressed, only one study has reported IF impact on AD. Using a triple transgenic AD mouse model, Halagappa and collaborators showed that long-term IF treatment could prevent cognitive declines in mice. Differently from caloric restriction, IF effects were not associated with reductions in β-amyloid protein and phospho-tau levels (Halagappa et al. 2007). This suggests that fasting effects on neuronal function are dissociated from cellular morphological alterations present in AD and could be a consequence of metabolic adaptations induced by nutritional stress.

Parkinson's disease (PD) is characterized by alterations in motor function involving rigidity, resting tremors, and walking disability. PD etiology comprises intracellular aggregation of α-synuclein protein followed by marked neurodegeneration in the *substantia nigra* (Jankovic 2008). PD progression is also associated to mitochondrial dysfunction, oxidative imbalance, and accumulation of oxidized and nitrated proteins. This effect is a consequence of decreases in cellular autophagic capacity (Hu and Wang 2016). Different groups have reported IF impacts on the prevention of neuronal dysfunction in PD. These effects include motor function gain along with reduction in neuronal α-synuclein accumulation and death (Duan and Mattson 1999; Holmer et al. 2005; Griffioen et al. 2013). The mechanisms by which fasting cycles attenuate PD symptoms involve enhancements in autophagy and mitochondrial function (Raefsky and Mattson 2016). Notably, IF did not avoid neuronal degeneration in the *substantia nigra* and striatum of rats treated with 6-hydroxidopamine, a chemical-induced model of PD (Armentero et al. 2008). Fasting was also unable to delay the emergence of amyotrophic lateral sclerosis, a neurodegenerative disease promoting motor function decay, and, in fact, hastened its progression (Pedersen and Mattson 1999). IF ineffectiveness in the prevention of some neuronal disorders challenges data collection regarding beneficial fasting effects on brain function. Therefore, further studies should be conducted to uncover IF actions in details, in order to understand and avoid undesirable side effects when used for human treatment.

Conclusion

Fasting has long been practiced by humans, determined by food scarcity or for ritualistic reasons, and is an important selective pressure driving human evolution; cyclic famine episodes introduced positive selection for genes favoring energy storage in human ancestors (Sellayah et al. 2014). Fasting-induced adaptations in neuronal function are further suggested to determine human survival when an individual experiences hunger and is required to obtain food (Mattson 2015). The mechanisms behind these changes are the probable basis for IF effects on brain homeostasis.

Evidence for the beneficial impact of IF on health, along with popular literature supporting fasting cycles to lose weight, has promoted an increase in the practice of diets containing food abstinence. Nevertheless, some concerns arise from studies evaluating IF effects on body homeostasis. First, the activation of hypothalamic orexigenic pathways following IF treatment can induce feeding disturbances or even promote treatment interruption. In fact, the use of IF protocols in humans comprises increases in aversive states on the fasting day, indicating that this diet cannot be tolerated over a prolonged period (Heilbronn et al. 2005). Second, different sets of data suggest that IF can generate deleterious effects. For instance, studies indicate that IF is associated with metabolic impairment in hypercholesterolemic mice (Dorighello et al. 2013). IF has also been related to increases in adiposity accompanied by alterations in insulin sensitivity in rats (Cerqueira et al. 2011). Moreover,

evidence suggests that IF can affect oxidative balance in a tissue-specific manner (Chausse et al. 2015). Third, despite its positive effects on health, IF is not recommended for individuals in physical development, such as young children and pregnant woman, due to their high sensitivity to nutritional deficits (Murphy et al. 2014).

Dictionary of Terms

- **Anorexigenic signaling** – A set of metabolic pathways and physiological processes activated during positive energy balance. Usually comprises increases in energy expenditure and appetite suppression.
- **Autophagy** – Intracellular process by which damaged and dysfunctional organelles and proteins are degraded releasing amino acids for the synthesis of new cell components. It controls cell function and viability.
- **Energy conversion efficiency (feed efficiency)** – Indicates the proportion of ingested calories that is converted into body mass. This index is usually calculated as the ratio between weight gain and total food consumption.
- **Orexigenic signaling** – A set of metabolic pathways and physiological processes activated during energy deficits. Comprises increases in food consumption along with increments in energy conservation.
- **Oxidative imbalance** – Occurs when cellular oxidant production exceeds its removal. Frequently associated with increases in damaged molecules.

Summary Points

- This chapter reviews the effects of intermittent fasting on brain function in parallel to a description of human fasting practice history.
- Fasting has long been practiced by humans and is considered a therapeutic intervention since the Stone Ages.
- Positive effects attributed to intermittent fasting have prompted the practice of diets containing food abstinence as well a number of studies investigating its impacts on health.
- Despite present differences in protocols and in some effects, caloric restriction and intermittent fasting have been recognized to induce improvements in rodents and human health.
- The brain is highly susceptible to fasting diets due to its elevated energy demand and its role in the control of energy homeostasis.
- Intermittent fasting activates hypothalamic orexigenic pathways inducing hyperphagia and disruption in reproduction.
- Intermittent fasting prevents cognitive functional decay and neurodegenerative diseases. These effects are mediated by improvements in neuronal function and in brain plasticity.
- Despite its positive impacts on brain function, intermittent fasting can hasten the progression of some neuronal diseases, such as amyotrophic lateral sclerosis.

- Evidence suggests that fasting cycles can be deleterious in specific genetic backgrounds and affects cell metabolism in a tissue-specific manner.
- The practice of intermittent fasting is not recommended in subjects sensitive to nutritional deficits, such as children and pregnant women and during convalescence.

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Influences of Prolonged Fasting on Behavioral and Brain Patterns

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Silvia Papalini, Mark Berthold-Losleben, and Nils Kohn

Contents

Abstract

Brain functioning requires glucose utilization; however, glucose is a limited resource for the organism, by depending mainly on our food consumption. Prolonged fasting can inevitably reduce the amount of glucose necessary to maintain neuronal activities and therefore, can negatively affect cognitive

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processes. To date, fasting for esthetical reasons or for unhealthy habits are the common behaviors that lead to low blood glucose levels; however, their effects on brain functioning, such as memory processes, attention levels, and self-control are still poorly investigated. The present work wants to summarize some of the most recent evidences on prolonged fasting effects on brain functioning and attempts to integrate these evidences in a recent model of self-regulation. Additionally, the consequences of low blood glucose levels on neuronal activities (fMRI) are described and discussed from the practical and clinical point of view. Overall, prolonged fasting and subsequent low blood glucose levels seem to decrease self-regulation abilities and negatively affect the attentional system. These results suggest that glucose levels need to be taken in account in fMRI protocols and monitored in circumstances where brain functioning is already compromised, such as in dementia and psychiatric conditions.

Keywords

Blood glucose · Cognitive functions · Fasting · Functional connectivity · Mood induction · Resting state · Visual stimulation · Working memory

List of Abbreviations

ATP	Adenosin triphosphate
BGL	Blood glucose levels
BOLD	Blood-oxygenation level dependent
CBF	Cerebral blood flow
CBV	Cerebral blood volume
CMRglu	Cerebral glucose consumption
CMRO2	Cerebral metabolic rate of oxygen
CPT	Continuous performance test
DMN	Default mode network
fMRI	Functional magnetic resonance imaging
HRF	Hemodynamic response function
ICA	Independent component analysis
pACC	Pregenual anterior cingulate cortex
PCC	Posterior cingulate cortex
PET	Positron emission tomography
V1	Primary visual cortex

Introduction

Glucose is the principal fuel of each single neuron of our brain. Its metabolism is indispensable for brain functioning: production of neurotransmitters, information processing, maintenance of homeostatic plasticity, and other vital neuronal and glial activities (Mergenthaler et al. 2013). Additionally on a cognitive level, optimal blood glucose levels are essential for virtually any cognitive process, such as for example memory and attention. Human physiology has evolved an elaborate system aimed to

maintain homoeostasis of the glucose system. Normal blood glucose levels are kept by daily food consumption; logically, our eating behaviors but also specific diseases such as diabetes can affect blood glucose levels and our cognitive processes.

For instance, disequilibrium between food intake and energy expenditure or prolonged fasting, often precipitate hypoglycemic states with consequent drastic effects on physiology (Roh et al. 2016). Indeed, recurring hypoglycemia has been linked to structural and functional brain changes (Warren and Frier 2005).

From the perspective of a cognitive neuroscientist, two aspects are important regarding glucose metabolism and eating behavior. Firstly from a psychological perspective, fasting or simply irregular eating behavior may have an influence on blood glucose levels, which in turn affects brain functioning but also cognitive, affective, and motivational processes. From this point of view, low blood glucose levels might have additional impairing effects in circumstances where cognition is already compromised, such as in case of brain diseases (i.e., Parkinson, Alzheimer). Secondly from a methodological perspective, whether low glucose levels due to prolonged fasting have influences on the signal most often applied in functional neuroimaging examinations in the MRI (BOLD-signal) is a problem which has so far not sufficiently been investigated (Anderson et al. 2006). From this latter point of view, providing evidence and understanding the relation between glucose levels and brain activations may indeed increase the reliability of the research practice, especially in functional magnetic resonance imaging (fMRI) designs.

Consequently, the present chapter aims to contribute to a better comprehensive understanding of these psychological and methodological considerations. The first part of the chapter provides an overview on how glucose levels are regulated at central level and the relation between glucose metabolism and BOLD signal. Subsequently, the second part describes a series of studies that focus on the effects of prolonged fasting on vision, memory, and emotional processes to elucidate the psychological and methodological issues from a cognitive neuroscientists' perspective.

The last part of the chapter summarizes the most important findings and outlines future directions and considerations.

Glucose Regulation and Distribution in the Brain

Blood glucose level homeostasis, with a normal range between 70 and 110 mg/dl, is maintained mainly via two hormones, insulin and glucagon, and by the parasympathetic nervous systems activities (Sprague and Arbelaez 2011). Both, the activation and deactivation of the autonomic nervous system are centrally regulated by complex mechanisms at the level of the hypothalamus and brain stem (Thorens 2011).

For instance, recent studies report how neuronal cells of specific hypothalamus nuclei and vagal complex show different glucose sensitivities: neurons inhibited by the decrease of extracellular levels of glucose, and neurons excited by the increase of extracellular levels of glucose (Burdakov et al. 2005; Routh 2010). In return,

glucose-sensitive neurons activities would modulate neurotransmitters and autonomic nervous system activity, in a series of complex mechanisms (Burdakov et al. 2005; Routh 2010; Grayson et al. 2013). Thus, neurons effectively have the leverage to very substantially modulate whole brain functioning.

Critically, our physiological system ensures the optimal distribution of glucose to all the brain regions under a broad variety of conditions: motor and cognitive activity and tasks, affective and motivational processes, resting attentional and meta-cognitive processes, and also switch and interaction between these instances. Neuroimaging studies have shown that during cognitive processes, our organism maintains the optimal supply of glucose for task-related brain activations by several means, for instance by withdrawing glucose from other task-unrelated regions (Sokoloff 1999). Additionally, cerebral glucose consumption (CMRglu) increases or decreases differently across functionally connected sets of brain regions when switching from rest conditions to task-dependent activity and *vice versa.* This means that the functional relations within areas of the same networks (and probably also between brain areas of different networks) are regulated in a complex and engagement-associated way (Sokoloff 1999; Riedl et al. 2014).

The principal implication from this evidence is that the amount of glucose in our brain is quantitatively limited and has to be optimally distributed across brain circuits. Given that the brain cannot generate or store glucose (in sufficient quantities), the peripheral blood glucose circulation, peripheral energy storage, and our eating behavior are essential for an optimal brain functioning (Cryer 2007).

Glucose and Its Relation with the Most Common Functional Neuroimaging Signal

Glucose metabolism requires 20% of our daily amount of calories. Aerobic oxidative phosphorylation and glycolysis are the main metabolic processes for glucose synthesis. The principal outcome of these processes is represented by Adenosin Triphosphate (ATP), the energy necessary for neuronal and glia functioning (Magistretti and Allaman 2015). Neuronal functioning, mainly action potentials and synaptic potentials (both pre and post), together with the preservation of the ion gradients, need 75–80% of the total amount of ATP available (Hyder et al. 2013). Neuronal functioning, during for example different kinds of cognitive processes, is investigated via neuroimaging techniques that take advantage of the relation between neuronal functioning and glucose necessity. Neuronal groups involved in definite tasks or states increase their demand of ATP in order to activate and maintain the cognitive processes necessary to the organism to obtain optimal functioning.

The vast majority of fMRI studies rely on measurement of the blood-oxygenation level dependent (BOLD) signal. The BOLD-signal is dependent upon several physiological changes: rate of blood oxygenation, cerebral metabolic rate of oxygen (CMRO2), cerebral blood flow (CBF), and cerebral blood volume (CBV) (Buxton

2010). These changes are all linked to ATP (Buxton et al. 2014). However, the relation between glucose request and BOLD-signal is not direct and causal.

Nevertheless, the recruitment of certain brain regions in particular cognitive processes usually is related to higher glucose metabolism levels and elevated BOLD signal. Yet, how the two relate under various conditions is up to now unclear.

Low Blood Glucose Levels and Brain Functioning

Blood circulation ensures sustained supply of glucose to the brain. Changes in our diet can affect the amount of glucose necessary and available in time to maintain brain functioning (Cryer 2007).

Low glucose states in the brain only occur under pathological conditions or due to prolonged nutrient deficiency, because a stable provision of glucose to the brain is maintained as long as possible (Paulson et al. 2010). In case of prolonged fasting and persistent hypoglycemic state, impairment on cognitive processes in healthy adults has been shown at below 55 mg/dl (Warren and Frier 2005). However, when low BGL persists, compensatory processes are triggered in order to preserve a normal cognitive ability, but how the brain can adapt in low BGL circumstances is still unclear (Warren and Frier 2005). In extreme situations, between a range 41–49 mg/dl, hypoglycemia can cause coma and death (Ben-Ami et al. 1999).

It is now well established that hypoglycemic state negatively affects cognitive processes and also brain functions (Warren and Frier 2005). For instance, hypoglycemic effects have been investigated with several neuroimaging techniques like functional Magnetic Resonance Imaging (fMRI), Positron Emission Tomography (PET) (Rooijackers et al. 2016), and their combination (Wehrl et al. 2013). Especially fMRI experiments are effective in detecting cognitive impairment during hypoglycemic states given the link between glucose levels of specific task-related regions and magnitude of the BOLD-signal (Driesen et al. 2007).

Nevertheless, much less is known about the effects of low but still normal blood glucose levels on brain functioning in the healthy population. Even in nonclinical conditions prolonged fasting such as fasting or being on a diet for aesthetic reasons could lead to a cognitive impairment that has the potential to interfere with the normal functioning and safety of the individual (for instance during driving a motorized vehicle and working in professions that require high levels of concentration).

Effects of Fasting During Task-Related Brain Activity

The following section mainly describes results from a set of studies investigating the influence of prolonged low normal blood glucose levels in healthy individuals in a naturalistic setting. The focus is on brain functioning, cognition and affect, as well as basal attention processes (Chechko et al. 2015; Kohn et al. 2015, 2016).

The conceptual aim of the reported studies was to elucidate the interaction of mood regulation and variations in energy availability as are apparent in fasting and which are operationalized in this context as a manipulation of blood glucose levels. All studies are conducted in a double-blind, placebo-controlled, cross-over design on 40 subjects (20 male and 20 females). Each participant was tested twice with 4 weeks between the two measurements. Measurements took place between 12 a.m. and 1 p. m. All subjects were studied after overnight fasting (>16 h. In one condition, subjects received an infusion of glucagon (0.5 mg/h) to stabilize blood glucose levels and simulate a re-feeding situation. In the second condition subjects received an infusion of sodium chlorite (50 ml) as a placebo control. Both conditions were administered in counter-balanced order. The infusion in both conditions in total lasted for an hour during which several experiments were conducted. Blood was additionally drawn at several time-points during the experiment to investigate a set of food intake and glucose metabolism related blood parameters (epinephrine, norepinephrine, insulin, and cortisol) to better characterize potential effects of the manipulation (Fig. 1). Importantly, the condition in which the placebo was administered is the actual condition of interest as this is the low BGL condition. The project included three fMRI experiments per condition started after 6 mins after the injections, plus two resting state measurements at the beginning and the end of each measurement. The resting state measurements were implemented to investigate changes in the functional connectivity of the brain at rest. The experiments were not only designed to investigate mood regulation, but the project included two levels of control experiments. One level was a basal visual stimulation experiment as a means of basic visual control, one working memory experiment (CPT, 2-back) as a means of higher level cognitive control, and a mood regulation experiment with a mood induction task (Fig. 1). Briefly, mood induction tasks are usually used in research, and especially fMRI experiments, to evaluate the neuro-correlates that reflect positive or negative effects (Westermann et al. 1996) while the continuous performance test (CPT) with a 2-back condition is a well-validated working memory task (Beck et al. 1956). The experiments are described in detail in the respective publications. Manipulation of blood glucose levels was successful in the conditions (high BGL: 114.5 mg/dl; low BGL: 73.9 mg/dl) and hormonal state reflected a re-feeding situation in the glucagon condition.

Effects of Low Blood Glucose Levels on Visual Processing

In the first experiment the basal visual stimulation in a fasting situation was studied. It was investigated whether the presentation of a lateralized, flickering checkboard is associated to differential brain activation patterns in the elevated blood glucose versus the non-modulated fasting condition. Small but reliable differences in the prolonged fasting condition compared to the elevated glucose condition in higher order occipital brain areas were found. The modulation of brain activity in higher order occipital areas in comparison to early visual processing areas such as V1 can be seen as indication that low normal blood glucose levels after overnight fasting do

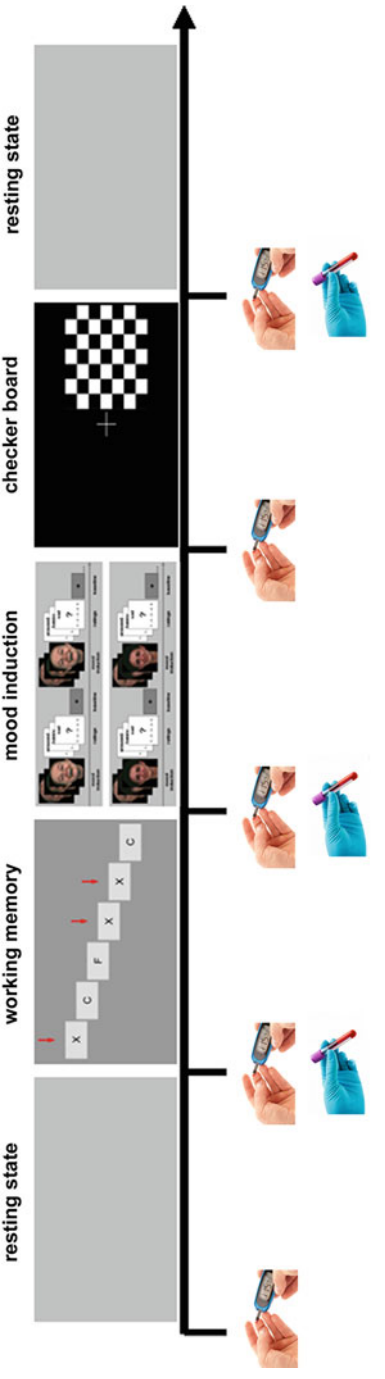

Fig. 1 Design of the study. This figure displays one randomization of the experiments conducted after the start of the infusion at the time of the first blood glucose measurement. The experiments took about one hour. After the final resting state an anatomical scan was measured. The order of working memory and mood induction experiments was counter balanced between participants

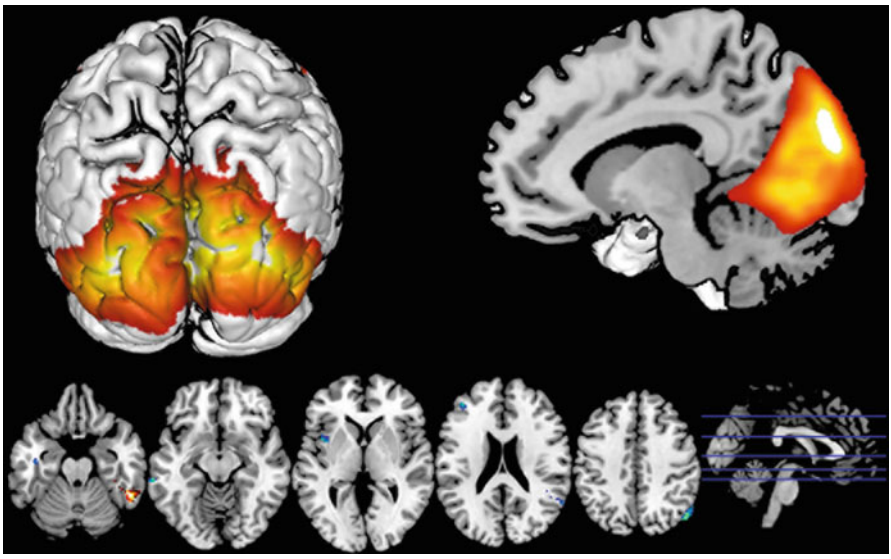

Fig. 2 Blood glucose levels effects on brain activity during visual processing. In high blood glucose levels conditions the visual component is strongly coupled with parietal, frontal, temporal gyrus, and Insula (in hot). Subjects with lower blood glucose levels showed stronger activity/connectivity in the ventral attentional pathway (*lower panel*, hot and *blue*) (Adapted from figure in. Kohn et al. (2016). Brain Struct Funct. 221:147–158, Springer-Verlag Berlin Heidelberg 2014)

not impact the blood-oxygenation level dependent (BOLD) signal that is measured in fMRI. Such a modulation would invariably (or at least with unsystematic variation) affect voxels with the strongest signal the most; the strongest signal is measured in this case in V1. As there was no difference in these voxels, yet differences were observed in brain areas that subserve higher-order visual processes, which are also known to covary with attentional processes (Fig. 2), an influence of overnight fasting on attentional processes was proposed. For instance, during right-sided stimulation, higher normal BGL were associated to higher activity in the left middle occipital gyrus. During left-sided stimulation higher normal BGL were associated to higher activity in high order visual areas too, like the right middle occipital gyrus, right temporal gyrus, left cuneus, and right lingual gyrus (Fig. 2).

To analyze if fasting leads to a general modulation of visual processing, additionally functional connectivity patterns at rest were investigated via Independent Component Analysis (ICA, (Beckmann and Smith 2004)). Here we analyzed large scale brain networks that can be reliably extracted in a data driven fashion and which have previously been well characterized (Smith et al. 2009). Template maps of visual networks were projected into the experimental data and tested. Interestingly, differences between the two conditions were found supporting the findings from the task activity study. The observed modulation by fasting was partially overlapping with the dorsal and ventral stream of attention as proposed by Goodale and Milner (Goodale and Milner 1992; Corbetta and Shulman 2002). Both the streams originate

from the primary visual cortex (V1), but while the dorsal path passes through the occipital lobe in the direction of the parietal lobe, the ventral stream follows a different path, by passing through the occipital towards the inferior temporal lobe. Briefly, the ventral pathway is involved in identification and recognition of objects while the faster dorsal pathway is involved in spatial vision, space-based attention and seems responsible for attentional deployment (Itti and Koch 2001). In low BGL conditions, subjects showed stronger activity and connectivity to areas that can be assigned to the ventral attentional pathway. This stronger connectivity could reflect a switch in a more basal (conservative) functioning of the brain, where an internal focus (or object-based focus) would be preserved at the face of general attentive processes (Goodale and Milner 1992). This switch may arguably be more ideal in terms of energy consumption and/or subserves the identification of potential food sources more goal-orientated.

Conversely, in the high BGL condition the visual component was found as strongly coupled with parietal, frontal, temporal gyrus and the insula. It might be speculated that the strong functional connectivity between visual areas and the dorsal stream and regions belonging to the salience network (i.e., the insula) (Menon and Uddin 2010) may reflect an enhanced general attentional processing in the high BGL condition. Additionally, the high BGL condition was associated with higher arousal, which generally suggested a possible indirect and beneficial effect of higher epinephrine levels on attentional processes.

In summary, low BGL was associated to a decreased activity in brain regions involved in general attentional processes but not to a general decreased activity at the level of the primary visual input into the brain. These results speak against direct influences of low normal BGL on the intensity of the BOLD signal and also against a systematic impact of low normal BGL on the latency and temporal propriety of the HRF.

Effects of Low Blood Glucose Levels on Working Memory and Mood

The next task was a continuous performance task in which subjects saw a succession of letters on a computer screen and were in two different conditions required to respond to a target letter with a button press. In one condition, subjects were asked to press every time they saw an X on the screen and in the second condition subjects were required to respond every time they saw a letter that had been displayed two letters before. This task is also commonly referred to as n-back task (Riccio et al. 2002; Rottschy et al. 2012). In our experiment we had a 0-back and a 2-back condition.

The working memory performance between the low BGL and the high BGL condition did not significantly differ. Yet differences in brain activation in the thalamus and hypothalamus were observed. Specifically, the low BGL group was associated to a decreased recruitment of the bilateral thalamus, the bilateral basal ganglia, the posterior cingulate cortex (PCC), and the rostral prefrontal cortex (Fig. 3).

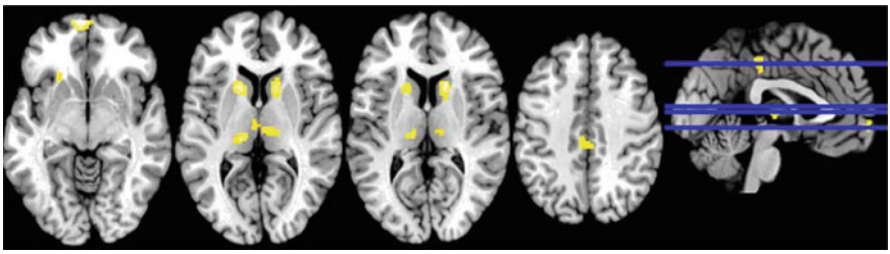

Fig. 3 Blood glucose levels effects on brain activity during working memory task. During the two-back task (working memory task) the low blood glucose levels group showed decreased activation of the bilateral thalamus, the bilateral basal ganglia, the posterior cingulate cortex (*PCC*), and the rostral prefrontal cortex (in *yellow*) (From: Chechko et al. 2015. Copyright: 2014 Wiley Periodicals, Inc.)

Thalamus nuclei are one of the regulators of BGL (Cho et al. 2011) and together with the basal ganglia are involved in working memory processes (McNab and Klingberg 2008), and transmission of information to the prefrontal cortex (McNab and Klingberg 2008; Chechko et al. 2015). The posterior cingulate cortex is, however, a region with a higher glucose uptake at rest, by showing a decrease in glucose consumption during demanding cognitive tasks (Greicius et al. 2007). Thus, decreased activation in these areas might indicate an impaired ability of the brain to switch from resting conditions to working memory-related activations in low BGL conditions compared to high BGL conditions. These brain areas thus seem to respond to small changes in peripheral blood glucose, which are still in the normal range.

Interestingly, additional analyses looking into the effect of order of the working memory experiment and the mood regulation showed that prior cognitive effort (having completed the mood regulation task before the working memory task) lead to further decreased activation of the abovementioned brain regions. Nevertheless, the changes in working memory-related brain activations were not associated with changes in working memory performances. This suggests neurobiological changes (e.g., compensatory increase of brain activity) might precede behavioral impairments. That is, the brain's activity may show alterations before differences in behavioral parameters are observable.

Yet, as the previous study related brain activation changes to changes in catecholamines rather than blood glucose itself and no direct relation to blood glucose was observable in this sample, the results could also hint at effects that relate to self-regulation failure (Heatherton and Wagner 2011). A relatively recent theory that links glucose brain metabolism to cognitive effort (such as performing a working memory test) and mood regulation is represented by the "strength model" of self-regulation (Gailliot et al. 2007). In detail, this model sustains that every action that we take, every thought or external information that we need to elaborate, requires energy, which in terms of the model very literally means glucose. Glucose as the central resource for self-regulation underlies every act of regulation according to this theory and depletion of glucose is identified as the process behind failures in self-

regulation (Gailliot and Baumeister 2007). Recent meta-analyses question the reliability of the strength model itself and show that self-regulation failure empirically is not very strongly associated to glucose (Carter and McCullough 2014; Dang 2016). Yet, the importance of glucose for self-regulation *per se* has been supported empirically (Kurzban 2010; Beedie and Lane 2012; Orquin and Kurzban 2016). Glucose allocation and hormonal processes that are triggered by glucose rather than general and physical glucose depletion has been proposed to underlie self-regulation failure (Hagger and Chatzisarantis 2013; Kohn et al. 2016). That is, a crucial determinant of self-regulation failure may be the cognitive resources available in a particular state or in other terms, the situational "challenge" level. Self-regulation failure may only be apparent when we are challenged to a sufficient degree. In terms of the current publication, one could argue that low BGL following prolonged fasting might negatively affect working memory processes in the brain, especially after previous cognitive efforts. However, the 2-back paradigm before or after a cognitive demanding task did not challenge the subjects sufficiently to show a detrimental behavioral effect.

To determine influences of low blood glucose on mood regulation, a combined mood induction procedure with facial expressions and recruitment of autobiographical memories have been used. Thus, the subjects were asked to try to generate a happy and a sad mood state. Results show that also for the mood induction procedure, behavioral differences represented by the subjective affective state of the participants could not be observed when collapsing over the order. Similar as in the working memory task, brain activation differences are nevertheless observable. In this experiment, high BGL were associated to stronger brain activation in the pregenual anterior cingulate cortex, the posterior cingulate cortex, and the thalamus. These brain regions are involved in emotional processing (Vogt 2009; Lindquist et al. 2012).

Comparison of the order of the experiments revealed that subjects, who had to work on the working memory paradigm before the mood induction procedure, were less good at generating a sad state and this was only apparent in the non-modulated fasting condition (Fig. 4). Brain activity in this condition was also in comparison to the elevated condition strongly increased (Fig. 5). For instance, strong brain activity was found in the orbitofrontal and frontal cortex, temporal pole, and the dorsal anterior cingulate gyrus, brain areas usually involved in emotional processing and emotion regulation (Fitzgerald et al. 2008; Dyck et al. 2011; Lindquist et al. 2012) and often found to be linked to depressive symptoms and sad mood state (Habel et al. 2005; Killgore et al. 2007) (Fig. 5). One explanation of this effect relates to effort. Similar to the explanation in the working memory task, stronger brain activity is observed in the absence of behavioral differences; the effort account assumes that the stronger activity patterns in emotional processing areas of the brain observed in the non-modulated fasting condition are caused by higher effort to reach a similar level of performance. In line with the assumption that blood glucose leads to failure of regulation only in a sufficiently challenged state, behavioral differences (e.g., regulation failure) can only be observed in the un-modulated fasting condition in which stronger recruitment of brain areas supporting the task fail to balance performance.

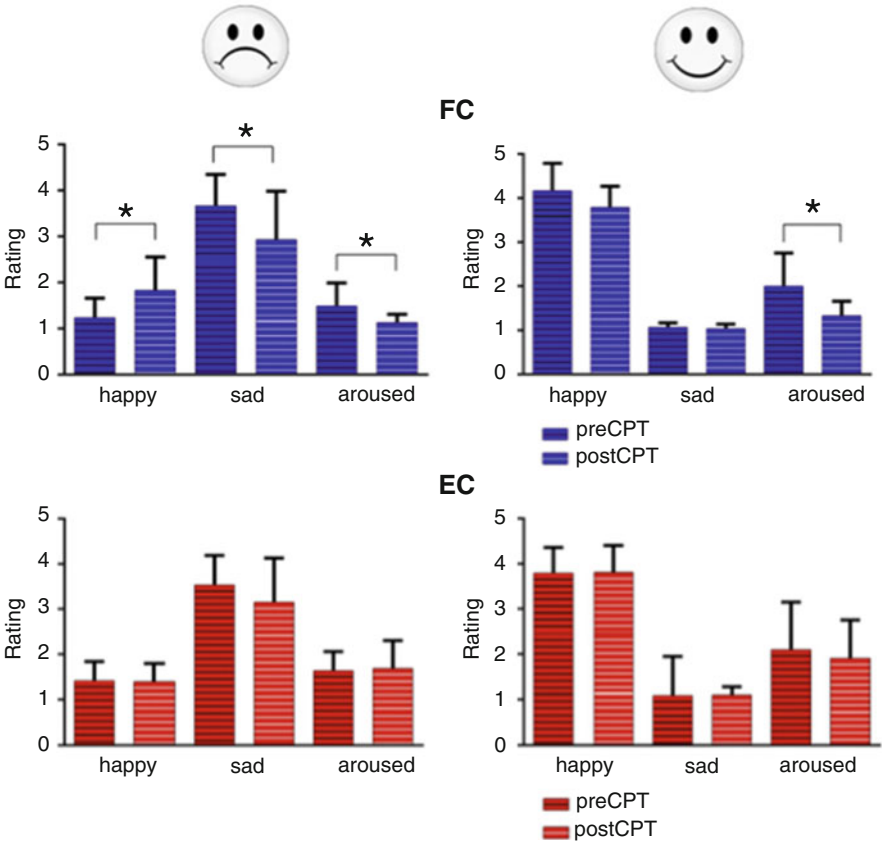

Fig. 4 Mood induction tasks. The figure shows the performance of the two groups (low blood glucose levels group –FC- in *blue*, and high blood glucose levels group –EC- in *red*), at the mood induction task, before (*darker bars*) and after the working memory task CPT (*lighter bars*). The results showed FC group was less able to induce a sad mood (*left side* of the panel) compared with the EC group, after the working memory task. The FC group did show lower arousal rating after CPT in both the sad and happy conditions of the mood induction task (From: Kohn et al. 2015. Neuroimage 113: 246–256. Copyright: Elsevier Inc.)

Thus, the results partially support the strength model of self-regulation. However, given that no drop in glucose levels was registered after the first cognitive effort task (but only at the end of the whole experiment), the diminished self-control capacity does not directly relate to peripheral blood glucose levels and thus depletion of glucose per se in this setup was not the cause for self-regulation failure. The association of low BGL to arousal levels (related to decreased epinephrine levels) could potentially explain ego-depletion effects on self-control.

An alternative complementary interpretation relies on the involvement of the pre-genual anterior cingulate cortex as one of the main hubs of the default mode network (Smith et al. 2009; Uddin et al. 2009; Callard and Margulies 2014) and the default mode

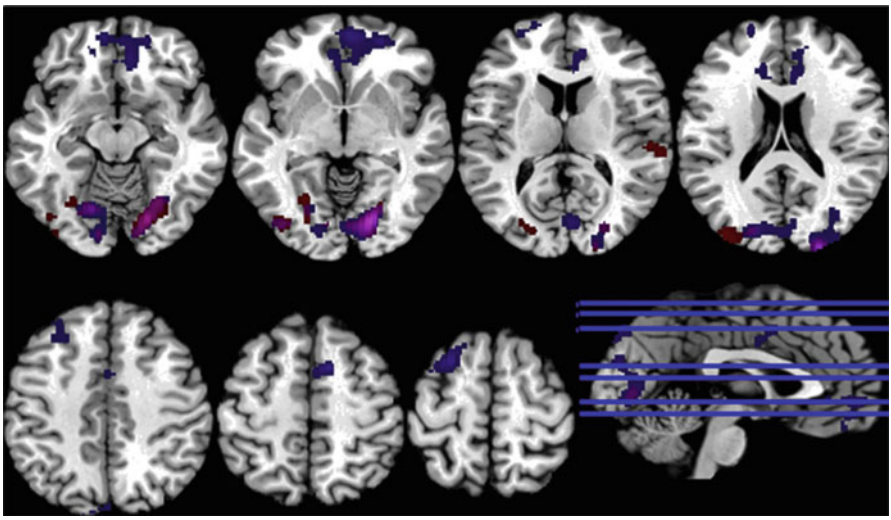

Fig. 5 Effects of glucose levels on brain activations during mood induction task after working memory task. Strong brain activity is found into the orbitofrontal and frontal cortex, temporal pole, and the dorsal anterior cingulate gyrus (in *blue*) in low blood glucose levels circumstances. Higher blood glucose levels are associated to stronger brain activation in the pregenual anterior cingulate cortex, the posterior cingulate cortex and the thalamus (in *red*). Brain activations during mood induction after the working memory task are showed in *violet* (From: Kohn et al. 2015. Neuroimage 113: 246–256. Copyright: Elsevier Inc.)

network has been shown to be strongly related to glucose metabolism (Passow et al. 2015). Therefore, a functional connectivity analysis using the template map of the default mode network (Smith et al. 2009) on the fMRI data of the task indeed revealed a stronger connectivity of the default mode network in anterior subsystems in the non-modulated fasting condition. This pattern of elevated connectivity to the anterior part of the default mode network in fasting, and also stronger connectivity in posterior subsystems in the elevated condition, could potentially be interpreted as a sign for a segregation of anterior and posterior parts of the default mode network in the low BGL group (Laird et al. 2011).

Policies and Protocols

To date, the negative consequences of behaviors of prolonged fasting for aesthetical reasons or simply because of time pressure are extremely underestimated and undervalued by the general population. Additionally, such effects are scarcely taken in account by educational programs as well as in psychopathological circumstances. The present manuscript reports and discusses how prolonged fasting can potentially affect higher cognitive processes like emotional and attentional processes, especially after cognitive efforts. One main position is that cognitive, attentional, and emotional processes are not per se affected by fasting behavior. Yet, empirical evidence points to the

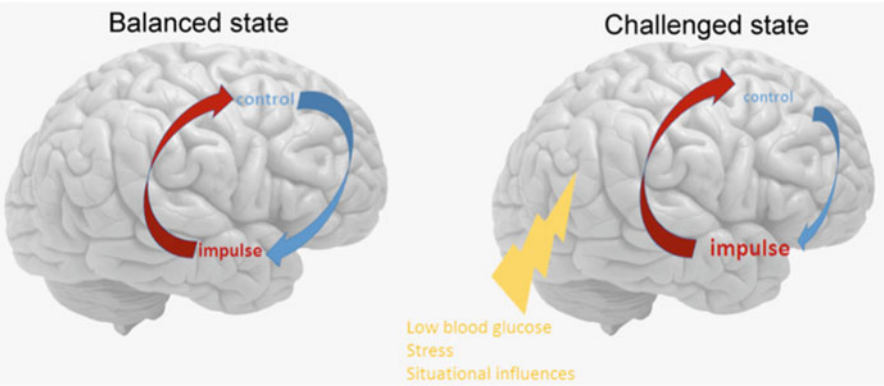

Fig. 6 The role of glucose in self-regulation and stressful circumstances. The model shows how the combination of several factors, such as low blood glucose levels, cognitive effort and stress occurrence might drive loss of control and cognitive-attentive decline (in *left panel*). This combination might potentially increase psychopathological symptoms. Stress, cognitive effort and low blood glucose can be seen as a challenge to the system, which in response may be more prone to self-regulation failure

possibility that fasting may render the individual in an "elevated risk state" in which certain cognitive, attentional, or emotional processes are performed and maintained under strong pressure to the whole system.

In prolonged fasting, cognitive effort can furthermore lead to a critical state in which the relative scarcity of glucose may pose a sufficient challenge to the organism to lead to self-regulation failure (Fig. 6). This may in consequence lead to emotional dysregulation, loss of cognitive control capacity, and attentional decline. A similar process can be observed in stressful contexts (Hermans et al. 2014). Stress, by activating catecholaminergic and endocrine systems, is thought to have negative effects on executive functions and attentional processes in general. These stress-induced hormonal changes have been proposed to give rise to characteristic large scale brain network modulations: The executive control network is decreased and its proposed counterpart, the salience network, is increased in activity under stress (Hermans et al. 2014). Similar processes as proposed in the large scale brain network dynamics of stress might be elicited by prolonged fasting and in consequence lie at the heart of fasting induced self-regulation failure. Consequences of challenged states and subsequent regulation failure may be far ranging and potentially life threatening. Also they may explain a part of what is in police reports or accounts of accidents usually referred to as "human error" (Hagger et al. 2009).

A more practical, specialized advice derived from the results presented here pertains to neuroimaging research. Based on the presented set of studies, we would advise to check participants for fasting state and ideally also include screening of blood glucose levels. A level of above 80 mg/dl seems to be sufficient to serve as a guideline. Yet, future research should aim at further elaborating on this suggestion.

Dictionary of Terms

- **Adenosine Triphosphate (ATP)** – is a molecule that consists of one adenin nucleotide, ribose, and three phosphate groups. Metabolic processes, for instance the oxidative phosphorylation, use the energy produced by losing of one or two phosphate groups from the ATP molecule, in order to keep each cell alive.
- **Rate of blood oxygenation** – is the fraction of hemoglobin binding to oxygen relative to the total amount of hemoglobin in the blood. Optimal human rates are between 95–100%. Hypoxemia state follows a blood oxygenation rate below 95%.
- **Cerebral metabolic rate of oxygen (CMRO2)** – refers to the quantity of oxygen consumed by the brain necessary to support the activity of neurons cells.
- **Cerebral blood flow (CBF)** – is the volume of blood (milliliters) passing through some brain regions (gray or white matter) per time unit (minutes).
- **Cerebral blood volume (CBV), or relative CBV (rCBV)** – is the volume of blood (milliliters) presents in a brain region (usually 100 g of brain tissue).
- **Continuous performance test CPT** – neuropsychological task to evaluate "executive function" such as working memory and attention (and the relative deficits). Repetitive stimuli (sounds, letters, numbers) are presented in series over the time. The task of the subject is to respond to specific target (for instance, to a specific letter) or inhibit responses to other stimuli.
- **Functional Magnetic Resonance Imaging (fMRI)** – measures brain functional activity associated to changes into the blood flow. Changes into the blood flow are coupled with change in neuronal activity while the subject is involved in a particular task or setting. Brain activities are shown as bright, by indicating functional association between those areas and the cognitive processes involved in the task used.
- **Blood-Oxigen-Level Dependent contrast (BOLD)** – reflects the changes in oxygen levels of particular brain regions involved in a specific task that can be detected with MRI technologies.
- **Salience Network (SN)** – network that trigger visceromotor responses to stimuli that attract our attention (salient stimuli). The fronto-insular and the anterior cingulate cortex play a major role in integrating the top down and ascending signals that are relate to salient stimuli.
- **Default Mode Network (DMN)** – network of brain regions activated during wakeful rest conditions and deactivated during task-related activities (for instance, goal-direct tasks).
- **Working memory** – is brief-term capacity. It retains and regulates information.
- **Mood induction task** – task used to manipulate mood. Movies, pictures, and unpleasant odors can be used in order to induce positive or negative emotional states.
- **Independent Component Analysis (ICA)** – method to delineate each single component of a multivariate complex signal. This computational method requires the components to be independent to each other and with a non-Gaussian distribution.

Summary Points

The work presented in this chapter attempts to give to the reader an overview on how prolonged fasting can negatively affect brain processes. Particularly,

- Certain eating habits, such as uncoordinated fasting, has been shown to have negative influences on visual attention, by decreasing BOLD-signal in high order occipital regions during visual stimulation.
- Low normal BGL decrease the activation of working memory-related regions. The weak response of the thalamus during working memory task after a cognitive effort is even more accentuated. Therefore, certain eating habits that result in low normal BGL can potentially and negatively interfere with our daily performances.
- Low normal BGL can negatively affect self-control-related brain regions. This effect is reflected in increased activations in brain areas involved in emotional processing. Clinically, low glucose levels might have additional deleterious effects on psychopathological conditions were compromised emotional processes already exist.
- Negative effects of low normal BGL on self-control-related regions are even more extended after a cognitively demanding task. However, no empirical link to the amount of glucose consumed has been found. Low normal BGL possibly via attentional and/or catecholamergic processes modulate self-regulation.
- Low BGL is potentially able to compromise the validity of neuroimaging studies that involve attentive, mnemonic, and emotional processes. Particularly, the control of the glycemic state (i.e., metabolic diseases) of the participants during the recruitment phase in fMRI studies is recommended.

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Intermittent Fasting and Caloric Restriction: Neuroplasticity and Neurodegeneration

65

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Contents

Abstract

The central nervous system plays a key and important role in regulating dietary energy consumption. Studies in the literature have shown that high calorie intake is deleterious to the physiological function of neurons. On the other hand, low-calorie intake has demonstrated to be beneficial, protecting neurons against harmful effects that could lead to the development of neurodegeneration. This chapter aimed to review the main aspects of dietary energy restriction protocols, such as intermittent fasting and calorie restriction, in relation to neuronal plasticity, cognition, and neurodegeneration.

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Dietary energy restriction · Caloric restriction · Intermittent fasting · Cognition · Cognitive function · Memory · Neurogenesis · Neuroplasticity · Brain · Hormesis · Hippocampus · Neurodegeneration · Alzheimer's disease · Parkinson's disease

List of Abbreviations

Aβ	Amyloid-β peptide
AD	Alzheimer's disease
ADAM10	A disintegrin and metalloproteinase 10
APP	Amyloid precursor protein
BDNF	Brain-derived neurotrophic factor
CaM	Ca^{2+}/calmodulin-sensitive
CNS	Central nervous system
CR	Caloric restriction
CREB	Cyclic AMP response element-binding protein
DAT	Dopamine active transporter
DER	Dietary energy restriction
DG	Dentate gyrus
FOXO	Forkhead box O
GDNF	Glial cell line-derived neurotrophic factor
Grp78	Glucose-regulated protein 78
GLUT3	Glucose transporter 3
HO1	Heme oxygenase 1
Hsp70	Heat-shock protein 70
IF	Intermittent fasting
LPS	Lipopolysaccharide
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MWM	Morris water maze
NF-κB	Nuclear factor κB
NMDAR	N-Methyl-D-aspartate receptor
PD	Parkinson's disease
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-α
PPARs	Peroxisome proliferator-activated receptors
ROS	Reactive oxygen species
SIRT	Sirtuin
SN	Substantia nigra
SOD2	Superoxide dismutase 2
TrkB	Tropomyosin receptor kinase B
VMAT	Vesicular monoamine transporter
VTA	Ventral tegmental area
WHO	World Health Organization

Introduction

The central nervous system (CNS) plays a central role in regulating dietary energy consumption. Those individuals that are capable to overcome others in the acquisition of food (calorie) are evolutionary selected (Mattson 2002). Nevertheless, studies consistently show that persistent excessive calorie intake can impair cognitive performance. On the other hand, long-term restriction in calorie consumption, at lower levels than the habitual *ad libitum* intake, could improve cognitive function (Fig. 1) (Fontan-Lozano et al. 2007; Mattson 2010).

Dietary energy restriction (DER) stimulates neurogenesis and cognitive function. These beneficial effects of energy deprivation on the CNS are due to the induction of adaptive response pathways in a process called hormesis, which involves many mechanisms, including mitochondrial biogenesis, reduction of oxidative stress and inflammation, and increase of brain-derived neurotrophic factor (BDNF) levels, among others (Fig. 2) (Abrous et al. 2005; Mattson 2008a, b; Vasconcelos et al.

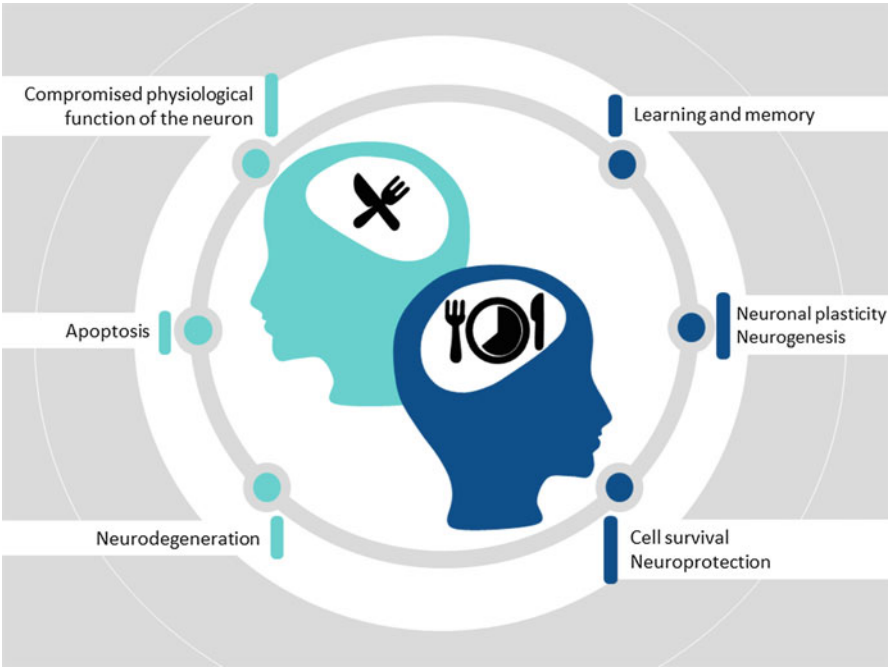

Fig. 1 A schematic view of DER effects in neuronal plasticity, cognition, and neurodegeneration. High calorie intake (light blue scheme) is deleterious to physiological function of neurons. On the other hand, DER (dark blue scheme) has demonstrated to be beneficial, protecting neurons against harmful effects, which could lead to neurodegeneration (DER, dietary energy restriction) (Fontan-Lozano et al. 2007; Mattson 2010)

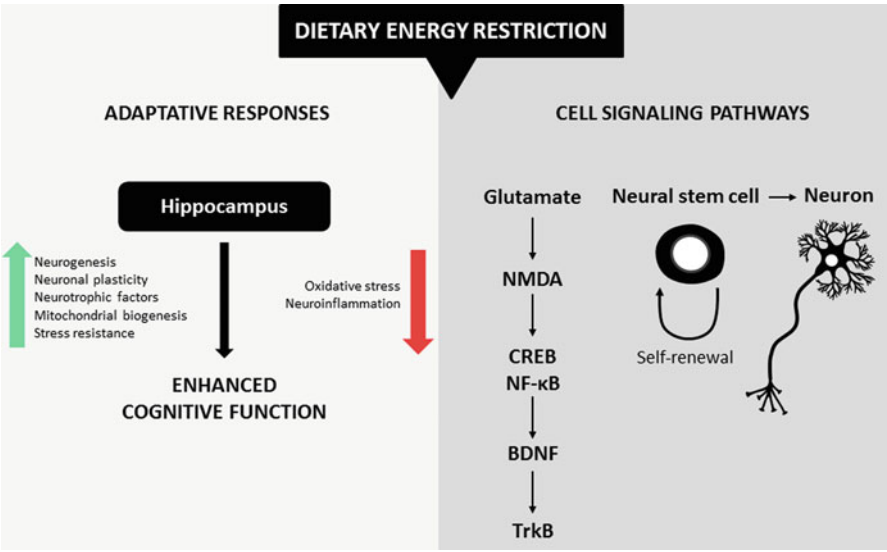

Fig. 2 DER-related adaptive responses and cellular signaling pathways on the CNS that contribute to enhancement of cognitive function. In the left, DER changes brain signaling and neuronal network activity resulting in improved cognition. The hippocampus, a brain region responsible for cognitive processing, is particularly important for the adaptive responses to DER. In the right, the hippocampus neurons play central roles in learning and memory. These neurons have an increased activity in response to DER, resulting in augmented glutamate neurotransmitter release. NMDA receptors, leading to Ca^{2+} influx followed by CaM kinases activation which, in turn, can modulate activity of transcription factors such as CREB and NF-κB which induce an increase of BDNF levels and enhanced association with its receptor, TrkB (CaM, Ca^{2+}/calmodulin-sensitive; CREB, cyclic AMP response element-binding protein; DER, dietary energy restriction; NMDA, N-methyl-D-aspartate; NF-κB, nuclear factor κB; TrkB, tropomyosin receptor kinase B) (Marosi and Mattson 2014; Longo and Mattson 2014)

2014). The remarkable DER effects are often viewed as a response of adaptation to food deprivation (Mattson 2008a, b).

There are two main protocols of DER that have been extensively studied due to promising effects on cognition and longevity: intermittent fasting (IF) and caloric restriction (CR) (Horne et al. 2015), the first associated with a restriction in frequency of feeding and the latter associated with restriction of calories ingested. While IF is a protocol that intercalates fasting periods with free access to food, CR refers to a chronic 20–40% decrease in calorie intake without malnutrition, both resulting in extended longevity, improved metabolic health and fitness, and improved physiological and molecular markers (Horne et al. 2015). Also, it has been demonstrated that DER could counteract many age-related diseases, such as reduction of the risk of metabolic deregulation, atherosclerosis, and neurological and cognitive diseases (Halagappa et al. 2007; Vasconcelos et al. 2014; Horne et al. 2015).

Although the term fasting is frequently interchanged by starvation, they do not have the same meaning. Starvation is a chronic nutritional insufficiency usually

meaning extreme fasting, causing deterioration and death. On the other hand, fasting was shown to optimize general health and stress resistance and prevent many age-related diseases through ketogenesis and changes in metabolic signaling pathways (Muller et al. 2001; Hartman et al. 2013).

Many studies have indicated that DER protocols also enhance neuroplasticity, cognition, and behavioral outcomes (Zainuddin and Thuret 2012), as reviewed below.

Effects of DER on Neurogenesis and Neuroplasticity

The hippocampus is an essential brain region for cognitive functions like learning and memory, in which neurogenesis occurs through life. During this process, proliferative cells form novel neurons in dentate gyrus (DG) region of the hippocampus, and these neurons integrate into the existing neuronal circuitry (Abrous et al. 2005). Another critical brain region involved in cognitive function is prefrontal cortex (Martinet et al. 2011), and it was shown a direct pathway communicating this brain area to the CA1 region of the hippocampus as a mechanism of memory and learning (Soares-Simi et al. 2013).

It has been shown a strong correlation between learning and memory abilities and the number of new neurons, pointing to a link between cognitive function and neurogenesis. For instance, Drapeau et al. (2003) evaluated in the same animals the amount of novel neurons and spatial navigation learning and found a positive correlation between these measurements.

Hormetic stimulus (mild stressors that induce hormesis), such as exercise, enriched environment, and DER were shown to increase new neuron formation (Lee et al. 2000; Abrous et al. 2005). Corroborating the association between cognition and neuroplasticity, Kaptan et al. (2015) showed that CR increased proliferation and the amount of neuronal cells in DG accompanied by an improvement in spatial learning and memory. Moreover, CR also mitigates the progressive neuronal loss, which is frequently associated with cognitive deficit (Graff et al. 2013).

BDNF is a neurotrophin that plays widespread roles in neuronal plasticity, survival, and differentiation, which is essential for learning and memory processes. It has been demonstrated that IF and CR can enhance BDNF expression in the hippocampus and other brain regions, which mediates adaptive responses. In turn, this neurotrophin seems to positively influence processes such as neurogenesis and cognition through stimulation of novel neuron survival and preventing cognitive dysfunction (Lee et al. 2000, 2002a; Marosi and Mattson 2014; Vasconcelos et al. 2014). Cyclic AMP response element-binding protein (CREB) transcription factor is the main regulator of BDNF expression (Lipsky and Marini 2007). Indeed, activation of CREB by phosphorylation was proven to be essential for memory formation. Furthermore, BDNF was shown to increase, in neurons, levels of N-methyl-D-aspartate receptor (NMDAR), which under physiological stimulation could contribute to induce plastic changes in synapses which are fundamental for memory and learning processes (Fig. 2) (Marosi and Mattson 2014).

Kaptan et al. (2015) showed that restriction of only 15% of calories during adolescence can increase cell proliferation in DG and BDNF levels in the prefrontal cortex and hippocampus, accompanied by an enhancement of learning and memory in female rats.

Effects of DER on Cognitive Function

Cognitive function can be defined as the ability to realize, memorize, and manage information from the environment (Shettleworth 2009). Cognitive abilities, such as learning and memory, play a central role in the success of a subject to continuously adapt to its changing surroundings (Morand-Ferron et al. 2016).

Severe DER results in a decrease of most organs size in mammals, except for the brain (Weindruch and Sohal 1997). This fact, from an evolutionary point of view, suggests that maintaining the cognitive function is of primordial importance under periods of limited food availability. Indeed, not only mammals are more active during periods of fasting, but also DER can improve cerebral functionality, as shown by a better execution of behavioral tasks of motor, sensory, learning, and memory abilities (Fontan-Lozano et al. 2007; Singh et al. 2012). These adaptive effects have been mostly associated with augmented generation of novel neurons from neuronal progenitor cells and improved synaptic plasticity (Lee et al. 2002b).

Although extremely important for adaptation, cognitive function and plasticity are very energetically costly (Kennedy et al. 1978; Leybaert et al. 2007). Brain consumes 20 to 25% of the glucose and oxygen, mostly for synapses and to restore resting membrane potential of axons (Attwell and Iadecola 2002), and it represents only 2% of the body weight (Clarke and Sokoloff 1999). Therefore, it is not surprising that changes in energy availability can impact cognition.

Numerous animal and human projects have attempted to study the effects of DER on cognition, as described below. Although the vast majority of DER research was performed with animals, there is also preliminary evidence in humans pointing to health enhancements (Horne et al. 2015).

Neurodegeneration

The neurodegenerative diseases consist of a group of neurological disorders that can affect different subsets of neurons in specific cerebral areas with no determined cause and slow progression. The neural degeneration can disrupt many levels of neuronal activity starting with molecular pathway impairment, disruption of synapses, and changes in local circuits and finally affecting higher-order neuronal networks (Fig. 3) (Palop et al. 2006). Regarding molecular level, some mechanisms seem to be more relevant to neural degeneration such as mitochondrial dysfunction, oxidative stress, and apoptosis (Przedborski et al. 2003). The most studied benefits of DER on neurodegenerative diseases include Alzheimer's disease (AD) and Parkinson's disease (PD), which will be the focus of this topic.

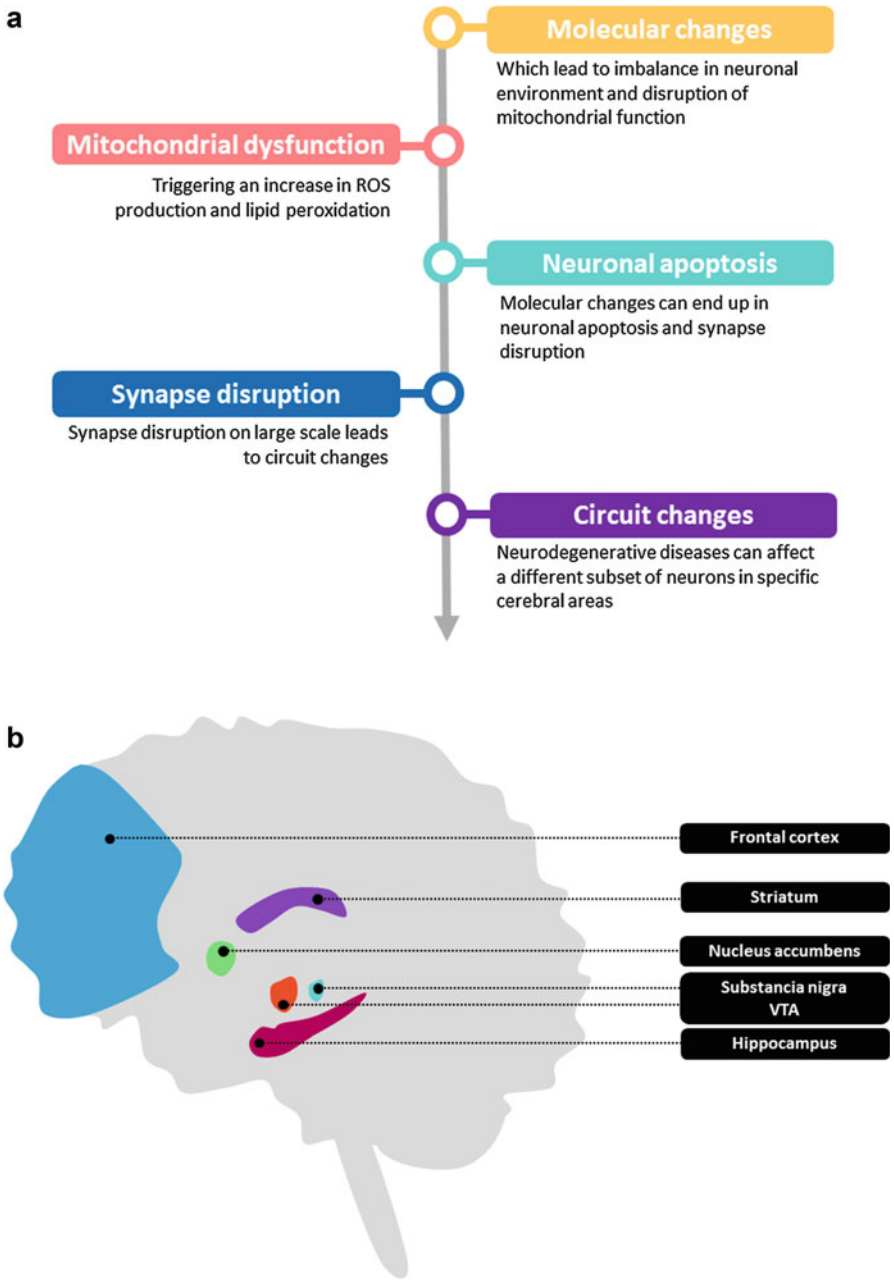

Fig. 3 General changes observed in neurodegeneration: from molecular to circuit disruption. (**a**) The neuronal degeneration can start with molecular pathway impairment that leads to an imbalance in neuronal environment and disruption of mitochondrial function triggering an increase in ROS and lipid peroxidation. All of these changes can result in neuronal apoptosis, synaptic

The beneficial effects of DER on neurodegenerative process are mainly focused in its ability (1) to modulate some of the molecular pathways that are impaired in these illnesses and (2) to enhance neurogenesis and synaptic plasticity as previously presented in this chapter.

It is well established that along normal CNS aging numerous modifications occurred to nucleic acids, proteins, and lipids together with a cumulative burden of excitotoxic, metabolic, and oxidative stress. All these changes are intensified in the neurodegenerative process. However, animal studies have proposed that dietary changes such as in alimentary frequency and calorie intake could alter the incidence and severity of these disorders (Mattson et al. 2003). The mechanism proposed to the observed benefits of reduced dietary intake is based on energy availability changes that are perceived by neurons as mild cellular stress stimulus leading neurons to react increasing the production of stress resistance response proteins. The insulin-like/forkhead box O (FOXO) signaling pathway, sirtuins (SIRTs), and peroxisome proliferator-activated receptors (PPARs) are among the principal switches that stimulate the synthesis of neurotrophic factors such as BDNF, chaperones, and antioxidant enzymes (Gillette-Guyonnet and Vellas 2008).

Some studies performed in animal model of either excitotoxicity or metabolic insults as well as AD have proposed that short-term CR or IF could be neuroprotective and alleviate amyloid-β (Aβ) peptide aggregates and microglia activation, respectively (Patel et al. 2005; Halagappa et al. 2007). AD is histologically characterized by two well-known pathological hallmarks: senile plaques composed of Aβ peptide and the neurofibrillary tangles of abnormally phosphorylated tau. Both protocols (IF or CR) increase the expression of some key proteins such as sirtuin-SIR2 (first identified in yeasts subjected to CR with expanded lifespan) or SIRT1 (in mammals) (Fig. 4). This protein can regulate DNA repair, gene silencing, aging, and programmed cell death. In vitro studies suggest that increased levels of SIRT1 protect cells from apoptosis triggered by reactive oxygen species (ROS) generated by Aβ aggregates (Gillette-Guyonnet and Vellas 2008). Higher levels of SIRT1 lead to increased generation of catalytically active A disintegrin and metalloproteinase 10 (ADAM10) proteins that can maintain an anti-amyloidogenic activity either in rodent brains (Wang et al. 2005) or in monkey brains (Qin et al. 2006). ADAM10 is a constitutive α-secretase protein that cleaves the amyloid precursor protein (APP) generating a secreted and non-amyloidogenic product. Some studies have demonstrated that active ADAM10 not only reduces Aβ but

Fig. 3 (continued) disruption, and dysfunction of neuronal circuits. (**b**) Neurodegenerative diseases can affect different subsets of neurons in specific cerebral areas, such as dopaminergic neurons from mesolimbocortical pathways, including neurons from VTA to nucleus accumbens, hippocampus and frontal cortex or mesostriatal pathway, from substantia nigra to striatum in Parkinson's disease and can affect cholinergic neurons and to a lesser extent non-cholinergic neurons in the hippocampus, in Alzheimer's disease (ROS, reactive oxygen species; VTA, ventral tegmental area) (Przedborski et al. 2003)

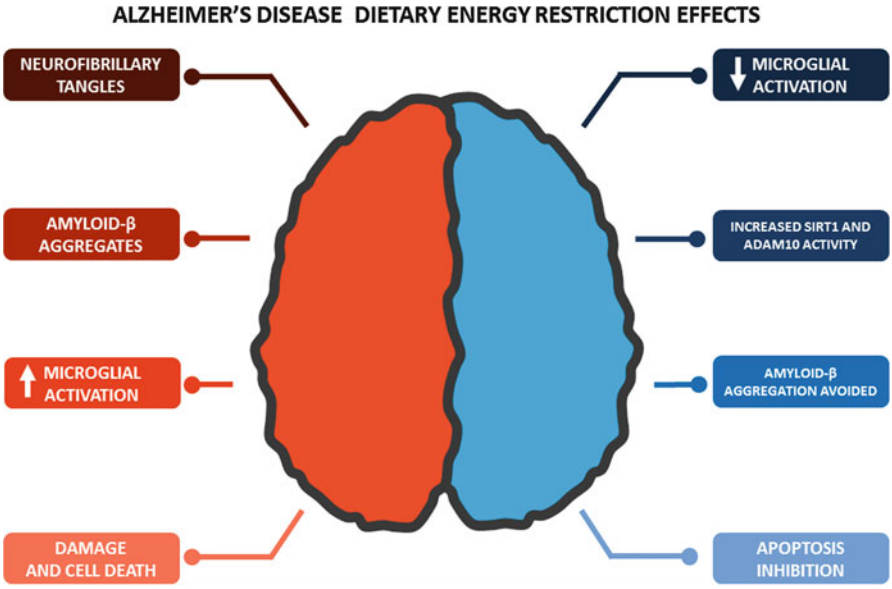

Fig. 4 DER effects in Alzheimer's disease animal models. In red, neurofibrillary tangles in cytosol and Aβ peptide aggregates that intensify microglia activation. In blue, DER can decrease microglia activation and due to an increase in SIRT1 can modulate ADAM10 activity avoiding Aβ peptide aggregation (Yuan et al. 2017). Furthermore, SIRT1 seems to inhibit apoptosis (Aβ, amyloid β; DER, dietary energy restriction; ADAM10, A disintegrin and metalloproteinase 10; SIRT1, sirtuin 1)

also can reduce tau pathology, increase hippocampal neurogenesis, and help to maintain normal physiological synaptic functions (Yuan et al. 2017).

Notwithstanding the PD can be characterized by pathologic accumulation of presynaptic protein α-synuclein or microtubule-associated protein tau within vulnerable dopaminergic neurons and often glial cells, as well as in striatum (Fig. 5). In experimental models of PD, DER has also been associated with a reducing death vulnerability of substantia nigra (SN) dopaminergic neurons (Duan and Mattson 1999). A study performed in nonhuman primate rhesus monkey that received 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin that selectively degenerates dopaminergic neurons leading to PD-like motor, neurochemical, and histopathological alterations in humans and primates, showed that monkeys that were at a reduced caloric diet for up to 6 months before receiving neurotoxin injection had an increase in neurotrophin levels such as glial cell line-derived neurotrophic factor (GDNF) and BDNF in striatum that protected dopaminergic neurons from degeneration measured by less motor deficits and higher levels of dopamine (Maswood et al. 2004). The same protection was also observed in mouse model (Duan and Mattson 1999).

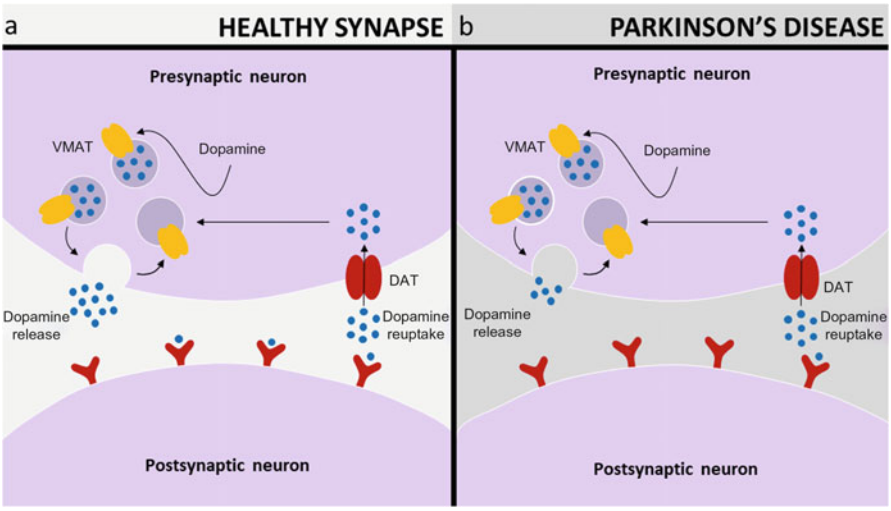

Fig. 5 Role of normal and mutated α-synuclein in synapse. (**a**) Presynaptic terminal of a dopaminergic neuron represents the well-known hallmark of Parkinson's disease: α-synuclein is an important protein for presynaptic dopaminergic vesicle release. (**b**) The loss of normal function of this protein promotes the accumulation of dopamine in the cytoplasm that together with α-synuclein oligomers is toxic to neuron (DAT, dopamine active transporter; VMAT, vesicular monoamine transporter) (Duan and Mattson 1999)

Similar to exercise effect, DER, especially IF, upregulates the antioxidant enzyme expression such as superoxide dismutase 2 (SOD2), glutathione peroxidase and heme oxygenase 1 (HO1), chaperones such as heat-shock protein 70 (Hsp70) and glucose-regulated protein 78 (Grp78), proteins involved in stress resistance and mitochondrial bioenergetics, antiapoptotic proteins, and neurotrophins (Camandola and Mattson 2017) (Fig. 6). Increased levels of BDNF can, in turn, raise the glucose transporters expression in neurons (GLUT3) stimulating neuronal energy metabolism. Furthermore, BDNF can also induce mitochondrial biogenesis through peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1α) (Camandola and Mattson 2017). It is well established that in PD there are an increase in membrane lipid peroxidation, protein nitration and oxidation, and a remarkable decrease in mitochondrial activity (Duan and Mattson 1999).

Although no safe protocol of DER had already been established for humans, it is becoming clearer that this challenge induces a complex array of molecular mechanisms that seems to optimize brain function and protect against neurodegenerative diseases and injuries (Camandola and Mattson 2017).

Animal Studies

Animal studies are very critical to evaluate the potential effects of DER at the cellular level on CNS, which could guide us to a better understanding of its impact in clinical

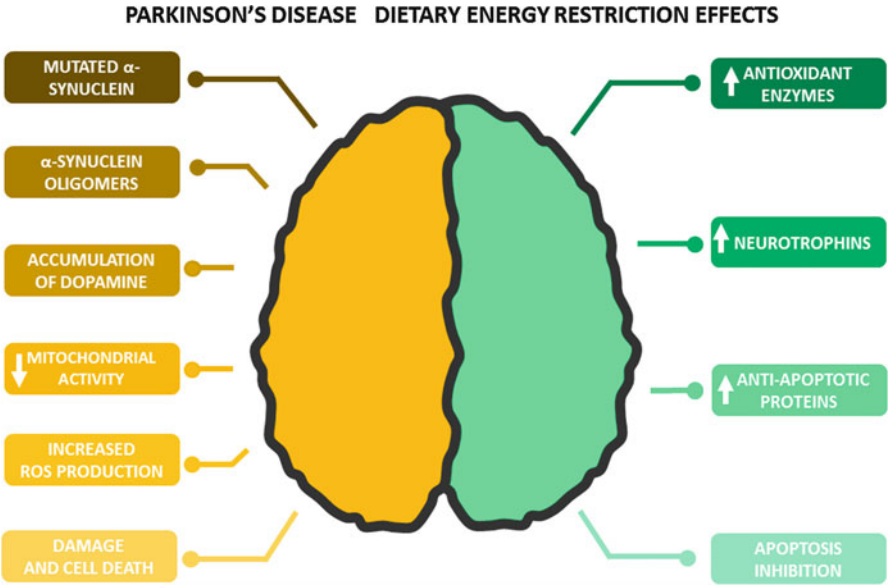

Fig. 6 DER effects in Parkinson's disease animal models. In yellow, mutated α-synuclein promotes the accumulation of dopamine in the cytoplasm that together with α-synuclein oligomers is toxic and can decrease mitochondrial activity as well as increase ROS production. All this cellular changes can lead to neuronal apoptosis. In green, DER can increase antioxidant enzymes, anti-apoptotic proteins, and neurotrophins protecting neurons from apoptosis (DER, dietary energy restriction; ROS, reactive oxygen species) (Duan and Mattson 1999)

trials (Mattson 2010). Several animal studies have consistently shown that DER enhances cognitive function, as some of them described ahead.

Literature data have provided evidence that a lifelong CR in mice is able to increase memory and prevent age-related decline in long-term potentiation (LTP) (Eckles-Smith et al. 2000; Kuhla et al. 2013). Likewise, CR started at midlife promoted an extension of life and of cognitive function in aged mice (25-month-old) (Means et al. 1993).

Ferreira et al. (2006) showed that 7-week-old rats subjected to mild (30%) or strong (60%) CR presented improved learning compared to rats in ad libitum diet, although memory was ameliorated in rats fed a 30% CR diet only. Moreover, 10% to 30% CR improved learning ability of mice in the Morris water maze (MWM) test (Ma et al. 2014).

Xu et al. (2015) showed that 30% CR in mice, unlike 30% caloric enhancement, improved learning and memory abilities, also evaluated in the MWM test. Accordingly, mice in high calorie diet (30% higher calorie intake) for 10 months had decreased memory capacity and neurodegeneration, while 30% CR resulted in improved memory in the MWM test (Dong et al. 2015). It was also investigated the effects of 20 and 35% CR on cognitive function in mice, and improved learning was observed in male but not in female, which suggests a sex-dependent DER effect on cognitive function (Wu et al. 2003).

It is well known that inflammation can induce cognitive dysfunction. Vasconcelos et al. (2014) demonstrated that IF is able to mitigate memory impairment associated with neuroinflammation, induced by lipopolysaccharide (LPS, a component of gram-negative bacteria membrane) stimulus. These results seem to be due to the anti-inflammatory effects of this DER protocol, which involves reduction of circulating inflammatory cytokines and pro-inflammatory gene expression.

Obesity is an inflammatory disease associated with increased systemic inflammation and cognitive deficits. Jeon et al. (2016) showed that CR in obese mice promoted weight loss and reversed learning, metabolic, and brain glucose dysfunctions. Accordingly, dietary-induced obesity rats subjected to CR for 28 days had improved cognitive function and BNDF signaling (Kishi et al. 2015).

Human Studies

Numerous religious groups have to fast during specific periods of the day or of the year, including Christians, Buddhists, Jews, Hindus, and Muslims (Longo and Mattson 2014). In many clinics, patients are being advised to adopt a very low-calorie diet (less than 200 calories per day) or even water-only diet under medical supervision in order to prevent diseases or for weight control (Longo and Mattson 2014). In fact, many studies have confirmed the beneficial effects of DER in improving general health in humans, including reduction of body weight in obese subjects (Harvie et al. 2011, 2013), hypertension (Goldhamer et al. 2001; Al-Shafei 2014), rheumatoid arthritis (Kjeldsen-Kragh et al. 1991; Muller et al. 2001), and asthma (Johnson et al. 2007).

Although DER was shown to increase cognitive function in different species, studies in humans are limited and with conflicting results (Rashotte et al. 1998; Qasrawi et al. 2017). In one study, nonobese volunteers subjected to a controlled experimental underfeeding during 1 week showed, during fasting, enhanced concentration, energy during the day, and emotional balance (Michalsen et al. 2003). Witte et al. (2009) showed that 30% CR in elderly humans resulted in memory improvement. Conversely, Green et al. (1994) showed that women students under DER had impaired cognitive performances in vigilance and short-term memory parameters. Moreover, the same group showed 1 year later that meal skipping had no effect on cognition of women (Green et al. 1995).

One month a year, during the holy month of Ramadan, around 1.6 billion Muslims practice intermittent fasting, in which they are refrained to eat and drink from sunrise to sunset. This reoccurring fasting diet is an interesting model for studying IF. Several researches have aimed to check Ramadan effects on cognitive function (Qasrawi et al. 2017). Results are inconsistent and frequently controversial.

One of the aspects of cognitive function is alertness. Three studies reported a shift of the alertness to later hours of the day during Ramadan (Roky et al. 2000, 2003; Bahammam 2003). However, another study compared eight Muslim and eight non-

Muslim volunteers before and on the first and second weeks of Ramadan and reported no change in sleepiness or reaction time during the day (Bahammam et al. 2013a). Accordingly, another study with controlled conditions (fixed caloric intake, sleep duration and schedule, and exposure to light and dark) also reported that there was no change in drowsiness or vigilance caused by Ramadan intermittent fasting, measured by the total blink duration and mean reaction time (Bahammam et al. 2013b).

It was also reported that there is no change in cognitive function in cyclists during Ramadan (Roky et al. 2000). However, Farooq et al. (2015) showed that 4 weeks of IF during Ramadan in teenager boys resulted in improved working memory and spatial planning ability. Another study demonstrated that in tasks which demand fast responses a better cognitive performance was observed in the morning, but no change in tasks non-dependent of speed (Tian et al. 2011). In the same study, it was also reported improved vigilance and psychomotor function in the morning (09:00 h) and poorer memory and visual and verbal learning in the afternoon (16:00 h) during fasting. However, the study did not assess sleep quality and duration (Tian et al. 2011).

Changes in sleep quality and deprived or disrupted sleep cannot be excluded as a possible reason for the findings cited above since most studies did not control sleep parameters (Devoto et al. 1999). Variations between results may also be attributed to different DER intensities, protocols, and duration before data collection. In hormesis theory, the stressful stimulus should not be so intense that the organism is not able to fully recover and should last enough to elicit an adaptive hormetic response (Mattson 2008a, b). Unfortunately, studies in humans are limited, and there is still no consensus on which DER protocol is the best one to achieve cognitive benefits.

Policies and Protocols

According to the World Health Organization (WHO), many chronic diseases are consequences of unhealthy diet. Opposite to malnutrition, high caloric intake that leads to obesity is the major risk factor to illnesses such as cardiovascular diseases, diabetes, and cancer and more recently has been associated with dementias. In 2014, around 39% of adults worldwide were overweight (Waqanivalu and Nederveen 2015).

In the face of such challenge, the University of Wisconsin–Madison and the National Institute on Aging have been studying the effects of DER on aging in rodents and nonhuman primates, and preliminary results suggest that a reduction in caloric intake can delay the onset of cardiovascular diseases and diabetes and control chronic inflammatory disorders and brain atrophy (Colman et al. 2009). Other groups have already established many protocols to deeply understand the changes promoted by caloric restriction and intermittent fasting on health.

Dictionary of Terms

- **Hormesis** – is a process in which a moderate (usually intermittent) stressor elicits an adaptive beneficial response of the cell or organism.
- **Neuroplasticity**, also known as neuronal or brain plasticity, is the ability of the brain to change, adapt, reorganize itself, and form new neural connections in response to various stimulus.
- **Neurogenesis** – is the formation of new neurons from neural stem cells in the brain.
- **Hippocampus** – is a brain region that plays a key role in memory consolidation and spatial navigation.
- **Cognitive function** – is the ability to interpret, memorize, and use information from the environment and is primordial for the adaptation of an individual.
- **Morris water maze** – is a classic and widely used test in behavioral neuroscience to access spatial learning and memory in rodents (hippocampus-related cognitive function).
- **Excitotoxicity** – is a neurotoxicity process that can lead neurons to damage or death mediated by high levels of excitatory neurotransmitters being most commonly related to glutamate.
- **Apoptosis** – is a type of programmed cell death.

Summary Points

- Dietary energy restriction is a hormetic stimulus that is associated with enhanced adult hippocampal neurogenesis and cognitive function.
- The two main dietary energy restriction protocols are caloric restriction and intermittent fasting.
- Caloric restriction involves the chronic restriction of 20 to 40% of the total calorie intake.
- Intermittent fasting involves the restriction of the frequency of food consumption (for instance, every other day fasting or Ramadan intermittent fasting).
- Many animal studies have proved the beneficial effects of dietary energy restriction on cognitive function and neural plasticity.
- The brain region of the hippocampus plays a central role in learning and memory.
- The neurodegenerative diseases consist of a group of different neurological disorders that affect different subsets of neurons in specific brain areas. They are usually characterized by slow progression with no determined cause.
- In neurodegenerative diseases, some common molecular processes such as mitochondrial dysfunction, oxidative stress, and apoptosis are among the more relevant causes of neuronal degeneration.
- Intermittent fasting and caloric restriction increase the production of stress resistance response proteins and neurotrophins.

- Caloric restriction or intermittent fasting could alleviate amyloid-β peptide aggregates and microglia activation, respectively, in Alzheimer's disease, and reduce neuronal death vulnerability in substantia nigra dopaminergic neurons in Parkinson's disease.

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Coordinating Evolutionarily Conserved Response of Muscle and Brain to Optimize Performance During Starvation

66

Donard S. Dwyer

Contents

Abstract

During evolution, a multitiered physiological response has emerged to deal with the repercussions of food deprivation and starvation that has been conserved across species. The main point of convergence of this system is on the Forkhead box O (FOXO) transcription factors. FOXO proteins control the expression of a wide array of genes involved in the use/disposal of oxygen, cell energetics, and proteasome/autophagy pathways. Upstream regulation is provided by insulin/IGF-1 signaling (IIS) mediated via Akt and modulated by additional kinases, including 5′-AMP-activated kinase (AMPK) and mammalian target of rapamycin (mTOR). This chapter describes the workings of this evolutionarily conserved starvation response as it relates to maintaining the performance of muscle and

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brain during chronic food deprivation. This system allows tight coordination of energy usage by muscle and brain to achieve optimum function in both tissues despite changing sources of energy – from glucose and amino acids to fatty acids and ketone bodies. The IIS pathway is critical for this coordination; however, either too little or too much IIS can be detrimental during starvation. The evolution of the starvation response has conferred both assets (support for long-range foraging during famine vs. efficient storage of energy supplies in times of plenty) and liabilities (anorexia nervosa vs. obesity and diabetes) to human populations. The IIS-FOXO-AMPK-mTOR pathway may offer new therapeutic targets for the treatment of anorexia and diabetes as well as muscle wasting syndromes, including sarcopenia and cachexia.

Keywords

Anorexia · FOXO · Insulin/IGF-1 signaling · Target of rapamycin · Sarcopenia

List of Abbreviations

ACC	acetyl-CoA carboxylase
AMPK	5′-AMP-activated kinase
DMSO	dimethyl sulfoxide
FOXO	Forkhead box O
IIS	insulin/IGF-1 signaling
InsR	insulin receptor
mTOR	mammalian target of rapamycin
PI3K	phosphatidylinositol 3-kinase
SNF1	sucrose non-fermeting-1
TCA	tricarboxylic acid cycle
TNF-α	tumor necrosis factor-α
TORC1	TOR complex 1
TORC2	TOR complex 2

Introduction

Throughout most of man's history, a major question faced on a daily basis was where would the next meal come from? With advances in toolmaking, hunting, and cultivation of crops, this became a less-pressing issue, although sporadic famines and food shortages continue to occur even today. The uncertainties about the next meal have always plagued lower animal species. Consequently, mechanisms have evolved for dealing with periods of acute and even prolonged starvation. This chapter will consider the problem from a molecular and systems-based perspective with some insights into the whole organism. Other reviews (e.g., Cahill 1970; this volume) deal better with starvation at the organism and population levels.

During periods of food deprivation and starvation, two major body systems compete for the limited energy supply: the musculoskeletal system, which produces movement aimed at finding food, preparing it, and eating, and the brain, which

analyzes environmental signals to locate food and directs foraging and feeding behavior. In view of these competing demands, survival depends on the tight coordination of physiological responses aimed at preserving energy resources and using limited fuel supplies in the most efficient manner. The insulin/IGF-1 signaling (IIS) pathway has evolved as the system best suited for coordinating energy metabolism in brain, muscle, and other tissues. In this chapter, I will describe how the IIS pathway integrates functional activity of muscle and brain to promote survival during starvation. The chapter will also highlight evolutionary factors that shaped the development of this highly integrated system. A theme that will emerge from this analysis is that optimum muscle performance during starvation requires the perfect blend of IIS – either too little or too much signaling via this pathway can be harmful. Finally, I will discuss how eating disorders, diabetes, and sarcopenia may have emerged as a consequence of the biological activities produced at either extreme of IIS.

Evolutionarily Conserved Response to Starvation

Depletion of nutrients and starvation represent a constant challenge to all biological organisms. As a result, a fundamental physiological response evolved that has been conserved from yeast to man. To understand how muscle and brain adapt to starvation, it will be helpful to first explore the origins and molecular mechanisms involved in this adaptation. At the core of the starvation response is the forkhead box O (FOXO) family of transcription factors (Fig. 1). Most species typically express multiple genes belonging to the general family; yeast have four members, Fkh1, Fkh2, Fhl1, and Hcm1 (Linke et al. 2013). Notable counterparts in *C. elegans* and humans include DAF-16 and FOXO1 and FOXO3, respectively. FOXO transcription factors regulate the expression of genes that affect cell cycle progression, development, energetics (especially glucose metabolism), resistance to oxidative stress, and mitochondrial biogenesis (Calnan and Brunet 2008). The overarching theme is that FOXO governs the use/disposal of oxygen to: (1) oxidize glucose, (2) facilitate oxidation reactions (e.g., mediated by tyrosine hydroxylase), (3) minimize oxidative stress, and (4) maximize mitochondrial capacity in support of growth and development. The FOXO pathway ultimately determines the metabolic state and cell fate.

Regulation of FOXO proteins is complex and is mediated by phosphorylation, changes in the rate of protein turnover, and compartmentalization in the cell (Calnan and Brunet 2008). In yeast, the major regulator of FOXO is the serine-threonine kinase, sucrose-nonfermenting-1 (SNF1), which is an ortholog of 5′-AMP-activated kinase (AMPK) in invertebrates and mammals (Hedbacker and Carlson 2008). Sch9 is a secondary kinase that also regulates FOXO in yeast; it is the yeast version of protein kinase B/Akt (Sobko 2006), which has assumed a far greater role in the regulation of FOXO in invertebrates and vertebrates. Akt is activated downstream of different growth factor receptors, but in *C. elegans* this is mainly achieved by activation of the IIS pathway (Fig. 1). This nearly exclusive relationship suggests

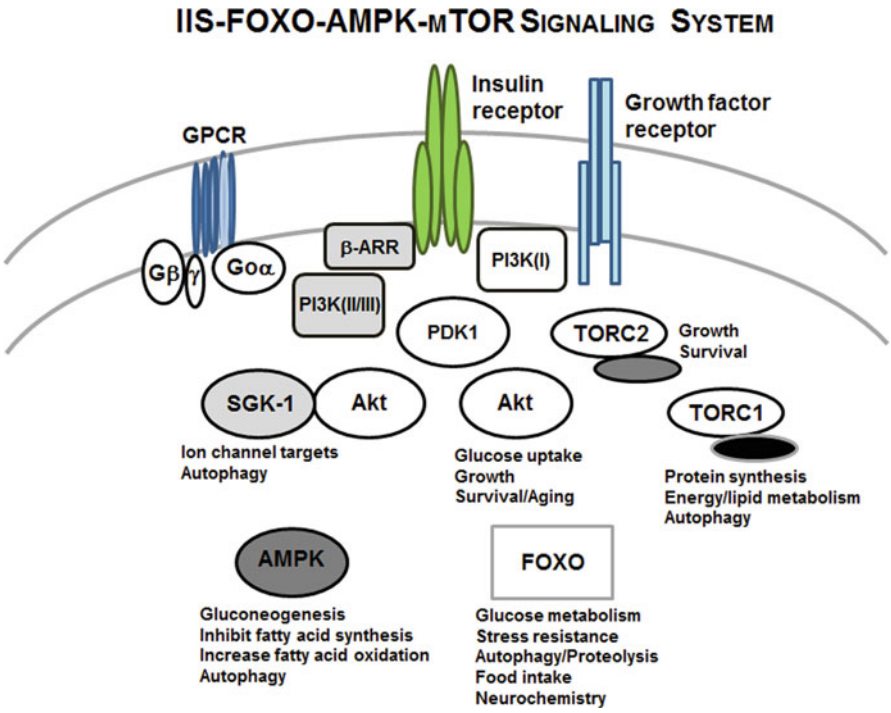

Fig. 1 The IIS-FOXO-AMPK-mTOR signaling system. The major components of these pathways are depicted here. *Arrows* showing connections were omitted for simplicity. The main responses mediated by these components are listed

that the insulin receptor may have been the original cell-surface signaling component to co-evolve with Akt.

In addition, to FOXO, AMPK, and Akt, the mammalian target of rapamycin (mTOR) pathway provides additional important feedback to the starvation response (Fig. 1). Again, this signaling mechanism has been shared from yeast to man. At the molecular level, regulation of IIS-FOXO-AMPK-mTOR signaling is very interactive and highly complex. Reviews on this topic provide the details and evidence for connections that will be discussed here in a more superficial way (Memmott and Dennis 2009; Goodman et al. 2011). There are two mTOR-containing kinase complexes: TORC1, which includes Raptor as a subunit and TORC2, which includes Rictor instead (Caron et al. 2015). TORC1 is activated in response to growth factors, amino acids, energy levels, oxygen, and stress and stimulates protein synthesis, lipid metabolism, mitochondrial biogenesis, and autophagy (Caron et al. 2015). It is broadly involved in cell growth and proliferation. TORC2 is activated by growth factors, especially insulin, and mediates changes in energy metabolism and cell survival. It targets Akt and serum and glucocorticoid-inducible kinases (SGKs) leading to their activation. There is complex interplay and feedback involving TORC1, TORC 2, Akt, and AMPK

(Memmott and Dennis 2009; Goodman et al. 2011). It is based on the type and magnitude of environmental signals, the sites on the target proteins that are phosphorylated, the momentary energy status of the cell (e.g., ATP:ADP:AMP ratios), and the cellular location where activation/deactivation takes place.

At the organismal level, there is conservation of adaptive responses to low-nutrient conditions. In *C. elegans*, the combination of low food supply and population crowding alters larval development causing young animals to enter a diapause stage also known as dauer formation (Riddle 1988). This alternate developmental trajectory is determined by IIS, including FOXO and mTOR. The dauer stage resembles seasonal hibernation observed in some mammalian species. Hibernation is also regulated by IIS, FOXO, and AMPK (Wu and Storey 2014; Lanaspa et al. 2015). The goal of dauer formation and hibernation is to diminish physical activity and therefore energy metabolism during periods of food limitation. A critical aspect of dauer/hibernation is preservation of muscle function despite a lengthy reduction in the supply of nutrients over several months. The evolution of this specialized response to fluctuations in food provides additional insights into the more general response to starvation observed on an intermittent basis.

Aside from its involvement in dauer formation and hibernation, the IIS-FOXO pathway regulates foraging and food-seeking behavior in an evolutionarily conserved fashion. Overexpression of FOXO in yeast during starvation significantly reduces a form of foraging known as pseudohyphal growth – a cell-based filament formation – aimed at dispersing yeast to nearby locations with higher nutrient value (Zhu et al. 2000). Similarly, enhanced expression of FOXO in *Drosophila* reduces food consumption (Kramer et al. 2003) and results in an anorexic phenotype (Dwyer et al. 2011). Interestingly, loss-of-function mutations in the insulin/IGF-1 receptor of *C. elegans*, along with brief food deprivation and exposure to low levels of dimethyl sulfoxide (DMSO), inhibit foraging and induce an immobile state reflecting diminished motivation (Dwyer and Aamodt 2013). Thus, insulin appears to play a role across higher species as a modulator of neural motivation circuits that govern the search for food and mates, and the avoidance of harm. Finally, the IIS-FOXO pathway has been related to the eating disorder anorexia nervosa (Dwyer et al. 2011), in further support of the evolutionary significance of this pathway.

As a logical extension of the role of IIS-FOXO in dauer/hibernation and foraging, this pathway, including mTOR and AMPK, modulates lifespan across species – from yeast to *C. elegans*, *Drosophila*, and mice (Kenyon et al. 1993; Tatar et al. 2001; Taguchi and White 2008). However, the effects on lifespan are complex and paradoxical. When IIS is partially reduced in *C. elegans* strains with temperature-sensitive *daf-2* (insulin receptor) mutations by raising the cultivation temperature from 15 °C to 20 °C, lifespan is significantly increased across the population By contrast, when the temperature is further elevated to 25 °C to produce a more complete loss of *daf-2* function, about half of the animals lives longer than normal, as expected, whereas the other half surprisingly dies prematurely (Kenyon et al. 1993). Similar observations have been made in aging studies with *Drosophila* (Tatar et al. 2001). Therefore, both too much and too little signaling via the IIS pathway can produce deleterious effects on organismal growth and development (Fig. 2). The

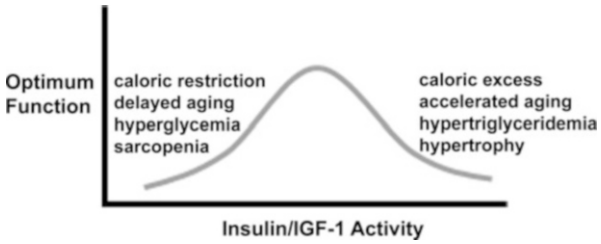

Fig. 2 The "sweet spot" between IIS and optimum physiological function. When insulin/IGF-1 signals are either too low or too high, muscle function and organism health may be adversely affected. The adverse effects and characteristics of these conditions are listed at the *left* and *right* sides of the figure

same appears to be true of IIS-FOXO effects on muscle function during starvation (Dwyer and Aamodt 2013). For example, loss of function in IIS-FOXO signaling components adversely affects recovery of pharyngeal pumping in *C. elegans* upon acute food deprivation, whereas gain-of-function mutations are associated with poorer muscle performance after 24 h, despite an initial boost in the recovery of pumping over the first 6 h of starvation. Thus, the IIS-FOXO pathway is a dual-edged sword in muscle.

Part of the reason for this duality of function can be attributed to the nature of the signals that emanate from the insulin/IGF-1 receptor upon activation. Classically, IIS is mediated via phosphatidylinositol 3-kinase (PI3K), PDK-1, and TORC2 all converging on Akt and leading to downstream phosphorylation of its targets (Manning and Cantley 2007). Evidence is emerging for a second pathway mediated via β-arrestin, class II/III PI3Ks, and Akt/SGK complexes (Fig. 1) acting at different intracellular sites and perhaps on different substrates (Dwyer and Aamodt 2013). Which of these two options is exercised will depend upon existing set points of the insulin/IGF-1 receptors (e.g., complexation with G proteins vs. β-arrestin) and coincident signals determined by nutritional state, glucose availability, etc. With this bifurcating signal, a very ancient system has been adapted and modified to cope with the increased challenges faced by complex mammals such as man during starvation.

Caloric restriction in man is accompanied by changes in appetite and behavior that may confer evolutionary advantages in certain settings. Keys et al. (1950) found that males subjected to chronic (24 week) reduction in food intake experienced loss of appetite, obsessions toward food, increased anxiety, restlessness, and early psychotic features. These psychological outcomes of caloric restriction were recognized as being the same as putative driving forces behind the development of anorexia nervosa (Guisinger 2003; Dwyer et al. 2011). A vicious cycle of self-starvation, blunted appetite, and muscle wasting ensues (Fig. 3). In addition, studies of hunger strikers revealed that this population developed denial of the adverse effects of starvation and a reduced sense of harm during a hunger strike (Fessler 2003). Guisinger (2003) proposed that anorexia nervosa may be an evolutionary adaptation to flee famine. It is informative to directly quote his reasoning for the advantages of the anorexic phenotype.

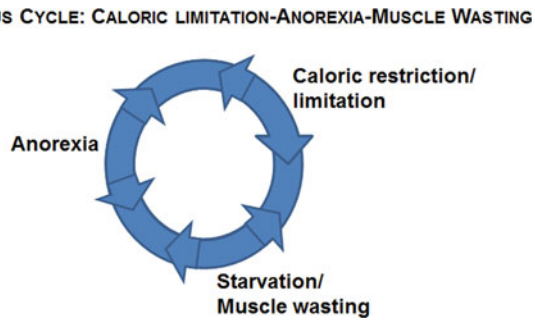

Fig. 3 Vicious cycle of caloric limitation, anorexia and starvation/muscle wasting. Any of these responses can produce the others and generally there is feed forward in the system

To migrate efficiently (during famine), individuals' bodies would have to turn off the usual adaptations to starvation. The ability to stop foraging locally, to feel restless and energetic, and optimistically to deny that one is dangerously thin could facilitate a last-ditch effort. Here it is proposed that core anorectic symptomatology includes three distinct adaptations specifically relevant for surviving past famine conditions: ignoring food, hyperactivity, and denial of starvation, including distorted body image. (Guisinger 2003, 748).

We attribute this anorexic phenotype, in part, to genetic variation in the components of the IIS-FOXO-AMPK-mTOR system (Dwyer et al. 2011). The underlying evolutionary changes that mediate this type of protective behavior must also have affected the function of tissues responsible for promoting foraging and surviving famine, namely muscle and brain.

Effects of Starvation on Muscle

Muscle has evolved efficient anabolic mechanisms to promote growth in times of plentiful food and exercise, and catabolic mechanisms to promote survival during famine and disuse. Growth is mainly regulated by growth hormone/IIS, follistatin, androgens, and β-adrenergic agonists (Schiaffino et al. 2013). Catabolic signals include TNF-α, myostatin, reduction of IIS, and starvation. Autophagy plays a paradoxical role in muscle – it is activated during atrophy and starvation (Mammucari et al. 2007), which makes sense, but it is also necessary to build and maintain muscle mass (Masiero et al. 2009). Clearly, there is a delicate balance between protein synthesis and degradation to maintain optimum muscle function that fluctuates depending on environmental conditions and endogenous signaling pathways.

The effects of starvation on muscle function need to be considered from both acute and chronic perspectives. With short-term food deprivation, glycogen stores are used first to meet the energy needs of muscle, which progressively shift to the oxidation of fatty acids, including intramyocellular triglycerides (IMTGs) (Stannard and Johnson 2003). As insulin levels fall with prolonged food deprivation, insulin-mediated suppression of proteolysis is reversed, and FOXO upregulates ubiquitin-proteasome and autophagy-lysosome protein degradation. Part of the purpose of

proteolysis is to provide glucogenic amino acids, in particular alanine and glutamine, for gluconeogenesis. Felig (1973) described the glucose-alanine cycle wherein hepatic conversion of alanine to glucose dominates the usage of glucogenic amino acid substrates from peripheral tissues. Importantly, a rise in IMTGs not only provides a substitute for glucose oxidation in muscle, but also decreases insulin sensitivity, thereby leaving more glucose in circulation for use by the brain (Stannard and Johnson 2003). If starvation continues, fatty acids are converted to ketone bodies, which become the main fuel source for the brain. Proteolysis in muscle begins to slow, but is still necessary to provide amino acids as anapleurotic substrates for the tricarboxylic acid (TCA) cycle in other tissues.

Widespread reductions in protein synthesis and other adaptations kick in at this stage to preserve muscle mass in support of last-ditch foraging efforts described by Guisinger (2003). These physical efforts and other forms of exercise feedback to inhibit autophagy and protein degradation (Zheng et al. 2015). To explain contradictory effects of exercise on autophagy (Jamart et al. 2013; Zheng et al. 2015) as well as reversal of the positive effects of gain-of-function mutations in Akt-FOXO on long-term recovery of pharyngeal pumping during starvation (Dwyer and Aamodt 2013), I propose the following sequence of events. At 6–8 h after food deprivation, autophagy is initiated at a low level. This can be enhanced by aerobic exercise, which adds to the negative energetic balance. After 24 h of food deprivation, proteolysis is in full swing. If this FOXO-mediated adaptation is blocked by gain-of-function mutations in Akt, it is detrimental for muscle function. Moreover, after 24 h of food deprivation, exercise has the opposite effect on autophagy – it is inhibited. This inhibition appears to be mediated via reactivation of the mTOR pathway and S6 kinase via PDK1 (Zheng et al. 2015). Thus, for preservation of muscle function, autophagy must be initiated 6–8 h after food restriction, but it must also be limited after prolonged starvation by exercise or adaptive signals to prevent muscle damage. This is yet another example of physiological performance being impaired by either too little or too much activity in the IIS-FOXO-AMPK-mTOR system.

The release of amino acids following protein degradation activates TORC1 in the mTOR pathway, which can directly phosphorylate FOXO (Greer et al. 2007). This leads to transcriptional regulation of atrogin-1 and enhancement of autophagy. As the ATP:AMP ratio decreases over time with starvation, AMPK activation promotes the utilization of fatty acids for energy via inhibition of acetyl-CoA carboxylase (ACC) by phosphorylation (Hopkins et al. 2003). Consequently, starving muscle undergoes a series of shifts in the main source of energy being used – from glucose derived from glycogen to glucose derived from amino acids and then to fatty acids and IMTGs derived from adipose tissue – a process known as metabolic flexibility.

Because the IIS pathway normally downregulates proteolysis and a decrease in protein degradation is needed to spare muscle during chronic starvation, how does this pathway operate when circulating levels of insulin are very low? IIS can be elicited by several mechanisms that are independent of plasma insulin concentrations: (1) transactivation via G proteins, (2) direct phosphorylation by AMPK, (3) PDK1-S6 kinase activity, (4) local IGF-1 synthesized in muscle, and (5) alternate complexes of the InsR, for example with β-arrestin. The InsR exhibits intrinsic activation that is modulated by

G proteins (O'Hare and Pilch 1990). Furthermore, drugs affecting G protein-coupled receptors can produce transactivation of the InsR (Weeks et al. 2010). The InsR is directly phosphorylated and activated by AMPK in response to low glucose (Chopra et al. 2012), so this is yet another possibility for residual IIS activity. Sustained activation of PDK1 is an additional mechanism for preserving muscle function over 24 h of starvation (Dwyer and Aamodt 2013). This kinase appears to be permissive of autophagy at early stages of food deprivation, but is protective of muscle at later stages. Low levels of PDK1 activation may occur as the result of stimulation via other growth factor receptors or by alternative kinase signaling pathways. In terms of the fourth possibility, muscle synthesizes IGF-1, and while levels normally decrease with starvation, there are reports to the contrary (Troncoso et al. 2012). Local autocrine actions of IGF-1 could limit the loss of muscle mass during starvation.

As discussed above, the InsR is activated as part of a complex with β-arrestin (Povsic et al. 2003). This pathway requires class II/III PI3Ks and the serum and glucocorticoid-inducible kinase-1, SGK-1 (Dwyer and Aamodt 2013). Glucocorticoid levels will rise as a result of the stress of starvation and act as a cosignal. This alternative IIS pathway regulates autophagy in muscle and the phosphorylation of downstream targets that may include voltage-gated and other types of calcium channels and the Na + −leak current channel. Phosphorylation of various channel substrates may maximize the function of these proteins, thereby sustaining muscle performance even while protein degradation is taking place and energy metabolism is partially compromised. Exercise achieves some of the same outcomes, although the signaling mechanisms have not been fully established. Of course, non-IIS components are likely to be involved in the maintenance of muscle performance during chronic starvation. The net effect is the preservation of muscle function for essential tasks and coordination of metabolic pathways to spare glucose for use by the brain.

Effects of Starvation on the Brain

To fully understand the effects of starvation on muscle function, it is necessary to consider the interplay between the peripheral response to food deprivation and the central response of the brain. The brain depends almost entirely on glucose or glucose-derived substrates for energy under normal circumstances. With prolonged starvation, there is a switch to the use of ketone bodies that can penetrate the blood-brain barrier (Cahill 1970). Consequently, the brain too displays a kind of metabolic flexibility. Glucose, lactate, pyruvate, and glutamine are interchangeably used in the brain for fuel in the fed state. In the early stages of starvation, muscle supplies alanine for the glucose-alanine cycle and hepatic gluconeogenesis as discussed earlier and glutamine, which can directly be used in the brain after conversion to the TCA cycle intermediate, α-ketoglutarate (see Fig. 4). In addition, a gradual reduction in glucose uptake by muscle resulting from decreased surface expression of glucose transporters means that more of the circulating glucose produced by gluconeogenesis is available for the brain.

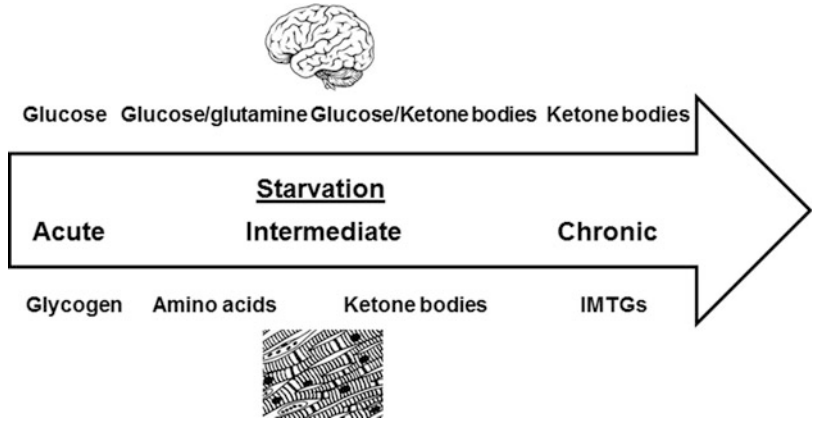

Fig. 4 Shift in energy resources as a function of starvation. Muscle (*lower diagram*) and brain (*upper diagram*) coordinate production and usage of energy substrates (*listed*) over time during food deprivation (See the text for a detailed description of this interplay)

In the next stage of starvation, ketone bodies are produced from fatty acids stored in adipose tissue. Initially, these ketone bodies provide the energy needs of both brain and muscle (Fig. 4). At this stage, the need for amino acids from muscle is reduced, which diminishes protein degradation to preserve muscle function. With prolonged starvation, muscle uses fewer ketone bodies for energy and ramps up its use of free fatty acids and IMTGs (Kelley 2005). Much like the spared use of glucose by muscle described earlier, this reduction in the use of circulating ketone bodies allows greater flux to the brain. Clearly, there is tight coordination between muscle and brain to minimize competition for the use of crucial, but limiting, energy sources such as glucose and ketone bodies.

Starvation causes neurochemical changes in the brain along with altered behavioral patterns. Here, I will focus on those changes mediated via the evolutionarily conserved IIS-FOXO pathway, which is the main focus of this chapter. As IIS is reduced in the periphery and brain as a function of the duration of food deprivation, FOXO is activated and promotes transcriptional activity in the nucleus. Among the genes whose expression is regulated are tryptophan hydroxylase and tyrosine hydroxylase (Estevez et al. 2006; Ferri et al. 2007), enzymes that catalyze rate-limiting steps in the synthesis of serotonin and dopamine, respectively. Serotonin and dopamine both regulate appetite and feeding at various levels. They stimulate feeding, control meal size and food preference, modulate reward pathways, and signal satiety. These neurotransmitters also control locomotion and foraging across species. Collectively, these findings attest to the importance of the IIS-FOXO-AMPK-mTOR system for fully integrating the behavioral response to food availability.

Insulin's direct effects in the nervous system appear to have been conserved through evolution as well. Thus, insulin regulates motivation and effort to find food (Dwyer and Aamodt 2013) and is a satiety factor in brain. Decreased insulin signaling in mice with experimentally induced diabetes is associated with increased

immobility in the forced swimming test – a measure of despair and diminished motivation (Gupta et al. 2014). Mobility is restored in these mice with exogenous insulin. Watve and Yajnik (2007) have proposed that acquired insulin resistance mediates a behavioral switch that determines whether organisms faced with environmental stress adopt a "stronger to smarter" strategy that favors brain over brawn (muscle). This fits with the picture painted above; moreover, the hyperinsulinemia that typically accompanies insulin resistance may have positive cognitive effects.

Adaptation to Starvation

Starvation causes significant adaptations at the level of both muscle and brain involving the IIS-FOXO pathway. Expression of the InsR is significantly increased along with its affinity for insulin (Kolterman et al. 1979). Therefore, the low levels of insulin that remain can more easily trigger receptor activation. In addition, insulin enhances muscle performance and efficiency by stimulating the phosphorylation of various ion channels and transporters. Therefore, excitable cells such as myocytes and neurons can maintain their capacity for generating ion fluxes with fewer channels and transporters. This adaptation preserves excitable cell function despite increased protein turnover or removal by proteolysis/autophagy. In *C. elegans*, starvation for 48 h reveals that FOXO returns from the nucleus to the cytoplasm (Weinkove et al. 2006), which is another mechanism to potentially limit excessive protein degradation. This adaptive response requires normal IIS components, in particular PI3K.

With chronic food deprivation, there is a progressive increase in the metabolism of ketone bodies and fatty acids in muscle because fat is the by far the largest storage depot for energy substrates. The IMTGs are a source of energy, but also produce insulin resistance in muscle, which shuts down protein synthesis and glycogen storage (Stannard and Johnson 2003). When fat tissue is depleted, there is an increase in protein degradation that eventually leads to atrophy and wasting. However, pushback against the loss of muscle mass occurs via several independent mechanisms. Activation of the autonomic nervous system can be directly anabolic for muscle via release of epinephrine/norepinephrine and their actions at β2-adrenergic receptors (Schiaffino et al. 2013). In addition, adrenergic receptors are reported to enhance the hepatic expression of follistatin, which counteracts induction of proteolysis by myostatin (Zhang et al. 1997). S6 kinase can be activated by amino acids (via TORC1), proteolysis, exercise, and residual PDK1 activity (Zheng et al. 2015). This would inhibit latter stages of protein degradation and reduce muscle loss. Behaviorally, feelings of anxiousness and restlessness induced by long-term food deprivation are known to cause hyperactivity (Guisinger 2003). The increase in muscle activity, like endurance exercise, would block or limit proteolysis and produce other positive effects in muscle.

The complex regulation of IIS-FOXO-AMPK-mTOR leads to a dynamic response that shifts as a function of the length of starvation. In addition, it is influenced by coincidence detection. For instance, amino acids activate TORC1 to support protein synthesis and growth (Avruch et al. 2009). However, if there is an

increase in amino acids at the same time as a decrease in the ATP:AMP ratio, AMPK is activated and TORC1 is downregulated. This dynamic regulation is also needed to promote metabolic flexibility. Specifically, there must be changes in the expression of metabolic enzymes geared for usage of the different energy substrates (e.g., glucose, lactate, glutamine, and ketone bodies) that fluctuate over the course of food deprivation. Some of this is accomplished by metabolite-mediated feedback regulation of catalytic enzymes, but multiple layers of regulation are necessary. In another twist on adaptive changes that take place, some of the molecules that inhibit the mTOR pathway are themselves degraded during ongoing proteolysis (Ghosh et al. 2008), which leads to mTOR reactivation and prevention of runaway protein degradation that would compromise muscle function.

Epigenetic changes in muscle and brain represent an important very long-term adaptation to starvation. Although this mechanism will only be briefly mentioned here, the reader is referred to informative reviews of this subject (Bocock and Aagaard-Tillery 2009; Gonzalez-Aquilera et al. 2014). Epigenetic modifications have been documented to occur as a consequence of starvation. Some of these are transgenerational and pass along to the F_2 and even F_3 offspring (Gonzalez-Aquilera et al. 2014). Epigenetic changes can be mediated by histone deacetylases that confer stress resistance during chronic food deprivation by upregulating ribosomal RNA synthesis and protein degradation pathways (Nakajima et al. 2016). In other cases, AMPK-FOXO regulation of histone arginine methyltransferases affects the expression of autophagy genes (Shin et al. 2016). A third potential regulatory strategy involves changes in ubiquitination of histone proteins that control transcription of autophagy components (Chen et al. 2017). The physiological effects of epigenetic-mediated modifications in muscle as a result of starvation have not been characterized in any detail. Nevertheless, the fact that some of these changes are transmitted to the next generation attests to the survival value of the regulatory pathways responsible and suggests additional evolutionary advantages (and disadvantages) may exist.

Unintended Outcomes of the Starvation Response: Anorexia Nervosa and Diabetes

Evolution of the starvation response has led to the emergence of both assets and liabilities for overall health. What is protective in one setting may be harmful in another. Guisinger (2003) and our group (Dwyer et al. 2011) suggested that adaptations that enhanced survival during famine may today predispose toward the eating disorder, anorexia nervosa. As local food sources were depleted, early humans faced the choice to stay and wait for a return of sustenance or to go and seek food elsewhere. Both strategies are fraught with inherent challenges: harsh weather conditions may last longer than anticipated by the "stayers" and delay the return of food, and conversely, leaving to find food elsewhere will mean exploration by the "goers" of unfamiliar territory, which may harbor new dangers and little food. Success following

the second approach required that our distant ancestors ignore feelings of hunger, get by on fewer calories, disregard harm so as to take the risks needed to find food or take on more dangerous prey, and hyperactivity to support long-range foraging. These survival advantages perfectly describe the symptoms/features of anorexia nervosa (Guisinger 2003). Depending on the gene complement specifying components of the starvation response (e.g., mutations altering expression/function of FOXO, AMPK, mTOR, etc.), certain individuals in the population (goers) may have been better suited for this approach leading to dispersal of the tribe and settlement of new areas during famine. According to this idea, behavior that is today considered pathological may be a remnant of a successful strategy that actually enabled man's survival.

Likewise, success with staying put may have depended on a set of different mutations (e.g., in IIS, Akt, and mTOR) that enhanced storage of energy substrates as fat and glycogen and the efficiency of energy metabolism to maximize accumulation of energy stores during times of plenty and limit expenditure during famines. This is a restatement of the thrifty gene hypothesis of Neel (1962), who initially proposed the idea to explain current-day prevalence of diabetes and obesity as relics of this survival strategy. In summary, goers were built to forage over great distances, but were prone to anorexia nervosa as a result. On the other hand, stayers were well suited to make the most of available food resources but tended to develop obesity, insulin resistance, and diabetes when food is plentiful. It is worth noting that the local population densities would have been very different for individuals adopting these two survival strategies. The goers would have spread out over greater areas, thus reducing local population density and competition for limiting resources, whereas the stayers would generally have higher population densities because their approach depended on the cooperative cultivation and/or acquisition of food. Interestingly, population density also affects the developmental decision of *C. elegans* to undergo dauer formation in the context of diminished IIS (Riddle 1988). Dauer larvae show distinct changes in muscle and have bouts of hyperactivity that presage the dispersal behavior of the goer contingent of human populations. Thus, adaptation to starvation during evolution has influenced the makeup of molecular signaling pathways, cellular and tissue responses aimed at preserving locomotion/foraging, and population behavior geared toward survival of the species.

Muscle Wasting in Hemodialysis, Cachexia, and Sarcopenia

In addition to starvation, various medical conditions plus aging have adverse effects on muscle mass and function. Here, I will focus on muscle wasting as a result of hemodialysis, cancer, and aging. The general unifying theme is that these conditions are associated with the induction of catabolic reactions in response to altered nutritional states and insulin resistance. Hemodialysis for kidney disease often produces muscle wasting caused by multiple factors, including uremia, negative nitrogen balance possibly secondary to metabolic acidosis, and increased proteolysis and insulin

resistance (Franch 2009). Anorexia is very common in patients undergoing hemodialysis and contributes to overall weight loss (Bossola et al. 2011). It is also a feature of cachexia and fairly common in elderly patients who develop sarcopenia (Morley 2001; Muscaritoli et al. 2010). In cachexia due to cancer, various inflammatory cytokines are released, such as TNF-α, IL-1, and IL-6, that promote catabolism in different tissues and loss of muscle mass (Keller 1993). TNF-α also counteracts IIS leading to insulin resistance. Sarcopenia associated with aging is thought to result from a combination of poorer nutritional status, oxidative stress in muscle and age-related decline in IGF-1 plasma levels and production in muscle (Sandri et al. 2013). In addition, insulin resistance frequently emerges with aging. Whereas early on IIS is needed to drive development and reproductive maturation, late in life this influence is greatly diminished to reduce the possibility that genetic liabilities of aged individuals are passed on through reproduction and to decrease competition with the young and/or fertile for food resources. Consequently, sarcopenia with aging can be considered a normal, if unfortunate, outcome of the programmed IIS trajectory. Along with muscle wasting, there is impairment in muscle function in these various conditions. Together, these maladaptive changes in muscle compromise health outcomes and quality of life. Treatment is generally focused on enhancing nutrition, including protein feeding; however, nutritional intervention alone has limited success. Exercise is more beneficial for maintaining muscle mass and performance as seen earlier in the case of starvation. In the future, the IIS-FOXO-AMPK-mTOR pathway may offer additional therapeutic targets for disorders featuring muscle wasting.

Policies and Protocols

Policies

- In starvation and muscle wasting, the goal should be to maintain muscle performance through better nutrition and exercise, as appropriate, especially in the aged population and hemodialysis patients.
  - Useful websites for nutritional information and exercise guidelines:
    https://www.nutrition.gov/life-stages/seniors
    http://www.nhs.uk/Livewell/fitness/Pages/physical-activity-guidelines-for-adults.aspx
    https://www.cdc.gov/physicalactivity/basics/older_adults/
- Because starvation for any reason is associated with emergence of anorexia, interventions should include strategies to increase appetite.
  - Helpful information for managing decreased appetite:
    https://my.pearlpoint.org/resources/loss-of-appetite-during-cancer-treatment
    https://www.cancer.org/treatment/treatments-and-side-effects/physical-side-effects/eating-problems/poor-appetite.html

Protocols

- **Detecting Akt activation**. As a measure of IIS function, the phosphorylation state of Akt and other kinases can be determined by western blotting with specific monoclonal antibodies.
- **Monitoring FOXO activation**. Activated FOXO transits from the cytoplasm to the nucleus and this can be detected by immunofluorescence staining with appropriate antibody reagents.

Dictionary of Terms

- **Anorexia nervosa** – An eating disorder characterized by failure to maintain normal body weight, excessive caloric restriction, and disturbances in the perception of body image.
- **Autophagy** – Proteolytic degradation of organelles and proteins via the lysosomal pathway in cells.
- **Epigenetics** – Nongenomic changes in DNA (e.g., cytosine methylation) that regulate expression of marked genes.
- **Phosphorylation** – A modification of proteins by addition of phosphate to serine or threonine residues that alters the function of the phosphorylated protein.
- **Sarcopenia** – Prominent loss of muscle mass and function often associated with aging.

Summary Points

- The major components and function of the IIS-FOXO-AMPK-mTOR pathway have been conserved from yeast to man.
- This system has been adapted in higher organisms to coordinate energy production and usage by muscle and brain, to reduce competition between these tissues for limited energy substrates and maintain optimum function.
- Chronic starvation causes sequential metabolism of available energy stores – from glycogen (glucose), and amino acids in muscle to adipose tissue (fatty acids and ketone bodies).
- Muscle proteolysis during starvation is both a positive factor, through contribution of glucogenic amino acids, and a negative factor, through excessive autophagy, which must be tightly regulated to prevent muscle wasting.
- Either too little or too much activity of the IIS pathway can be detrimental for muscle performance during chronic food deprivation.
- Evolution of the starvation response has entailed a downside as well, namely emergence of eating disorders (anorexia nervosa) and obesity/diabetes.

- The IIS-FOXO-AMPK-mTOR pathway offers promising targets for therapeutic intervention aimed at preserving muscle performance in syndromes associated with muscle wasting.

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Defining and Assessing Skin Changes in Severe Acute Malnutrition (SAM)

67

Sofine Heilskov, Christian Vestergaard, and Mette Soendergaard Deleuran

Contents

Abstract

Specific skin changes in severe acute malnutrition have been known since the earliest publication on the subject in 1933. They vary from mild dryness or pigmentary changes to severe and widespread erosions. A common standardized way to document the skin changes observed in severe acute malnutrition is still under development. Currently five specific skin changes, characteristic to severe acute malnutrition, have been identified in African children. There is no knowledge of the global prevalence, but most reports on severe skin affections are from sub-Saharan countries.

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The etiology is still unknown and the recommendations on treatment are mostly based on expert opinion. Skin changes in severe acute malnutrition have proven to be a prognostic marker for the risk of death. The mortality rate for patients treated for severe acute malnutrition is persistently high and thus the skin is a target for new treatment approaches. The skin is easily accessible for administration of treatment and assessment and is potentially a good additional target to the existing treatment protocols.

Keywords

Severe acute malnutrition (SAM) · Kwashiorkor · Marasmus · Edematous malnutrition · Dermatosis of Kwashiorkor · Skin changes · Dermatitis · Lichenification · Hair changes · Pigmentation disturbances · Skin scoring · SCORDoK · Prognosis

List of Abbreviations

BSA	Body surface area
SCORDoK	Clinical score for SAM specific skin changes
EFAD	Essential Fatty Acid Deficiency
HR	Hazard ratio
MUAC	Mid Upper Arm Circumference
F75 and F100	Milk based therapeutic diets
SAM	Severe acute malnutrition

Introduction

The skin is the largest organ of the body. It acts as our first-line defense against pathogens, it contributes to the regulation of body temperature and water balance, and it is an important part of the sensory system. The skin is an important clinical tool in accessing the health status of a patient and in the diagnosis of many illnesses that are reflected by dermal changes.

Patients suffering from malnutrition may develop skin changes as a sign of single nutrient deficiency. These skin changes will be referred to as *single nutrient-specific skin changes*. In the case of severe acute malnutrition (SAM) a patient may develop characteristic skin changes which have not yet been attributed to one specific dietary deficiency and these are considered unique to SAM. These characteristic skin changes will be referred to as *SAM specific skin changes*. SAM-specific skin changes can be severe and widespread, and new research has found that they have prognostic value for the patient (Heilskov et al. 2015).

Skin changes in patients suffering from malnutrition have been described in the scientific literature since the early publications on malnutrition (Williams 1933). Despite this there is still no verified explanation to the etiological background for SAM-specific skin changes, and their impact on prognosis still needs to be further elucidated. Additionally, suitable treatment directed specifically against the skin changes has yet to be explored. As skin in SAM is so poorly investigated, this is a

potential focus area for improvement of the treatment of SAM and lowering of the persistently high mortality rates. The clinical assessment of the skin is easy and there is no need to involve expensive analytic methods. It is therefore a relevant clinical tool to be used in low resource clinics.

The geographical distribution is not yet fully mapped, but reports on skin changes tend to come from the African continent. An approach to develop a common language using dermatological terminology has only recently been published (Heilskov et al. 2015). Five different skin signs were identified as specific to SAM. These results are based on Ugandan children and therefore further investigation is needed to generalize these findings on a global level.

Focus areas of this chapter are:

- Etiology and pathogenesis
- Histopathology
- Diagnosis and clinical manifestations of SAM specific skin changes
- Review clinical management options and prognosis
- Present two clinical protocols supporting proper assessment and monitoring of the skin changes

The sparse research literature on SAM and skin affection mainly focuses on preschool children. Therefore, most of the research cited in the current chapter is based on study subjects of the age of 6–59 months.

Etiology and Pathogenesis

The patho-physiological background for the skin changes in SAM is poorly understood. The clinical manifestation of the skin changes seems to be unique to SAM. Still, descriptions of skin changes in SAM tend to highlight similarities with known single nutrient deficiencies. The sparse research on the subject has focused on mechanisms in single nutrient deficiencies, but studies have not yet proven the skin changes to be connected to one nutrient alone. In patients who survive stabilization and who recover on therapeutic foods, rich in vitamins, minerals, and fatty acids, the result is healing of the skin changes without serious sequelae. A clinical example of this is shown in Figs. 1 and 2.

Etiological Focus Areas

Giving an overview, of what is known and what has yet to be explored in the search for an etiological explanation for the skin changes in SAM, is challenging. A standardized characteristic, using dermatological terminology, has only recently been published (Heilskov et al. 2015). This common language is only based on African children and thus needs to be generalized to other skin types and cultural

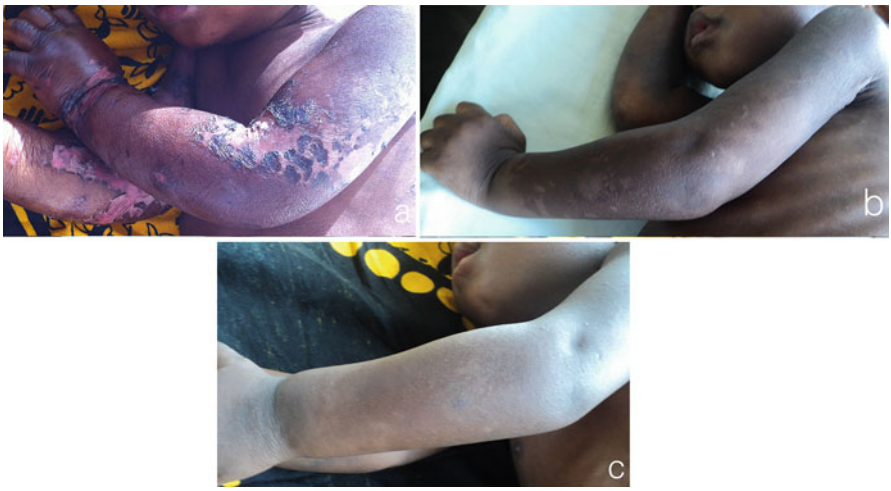


Fig. 1 Severe skin changes in a Ugandan patient admitted to hospital, with severe acute malnutrition and generalized edema (**a**). (**b**) The same patient after 13 days of treatment with milk-based diets (F75 and F100). (**c**) And the patient before leaving hospital, 28 days after admission to hospital. Pictures are provided by the authors

settings. Therefore, we must settle for the term "skin changes" vs. no skin changes, when considering results on the etiological research.

Edema-Specific Skin Changes

Skin changes are commonly mentioned as a clinical sign of kwashiorkor (of which edema is a diagnostic criteria), and it has been suggested that some skin changes are only seen in edematous SAM. This has not yet been systematically investigated. One study found the three stages of bullae-erosion-desquamation to be edema specific (Heilskov et al. 2015). In etiological research, some studies have concluded that measured nutrients were lower in edematous patients (Giovanni et al. 2016) and even lower in those with skin affection (Golden and Golden 1979; Vasantha 1969; Vasantha et al. 1970; Wolff et al. 1984), suggesting that the state with edema could proceed to skin changes.

Amino Acid Deficiency

It has been suggested that the skin could play a role as a protein reserve, like muscles upholding the function of other tissues, during starvation (Waterlow et al. 2006). To answer this question more research on epidermal protein turnover, compared to other tissues during starvation, must be made.

A theory on impaired maturation of collagens and cross-linking of fibers in the skin of edematous SAM patients has been suggested. This is based on the findings of an increased proportion of labile collagen in edematous patients. Supporting this theory, the same authors found lowered levels of both collagen and noncollagen

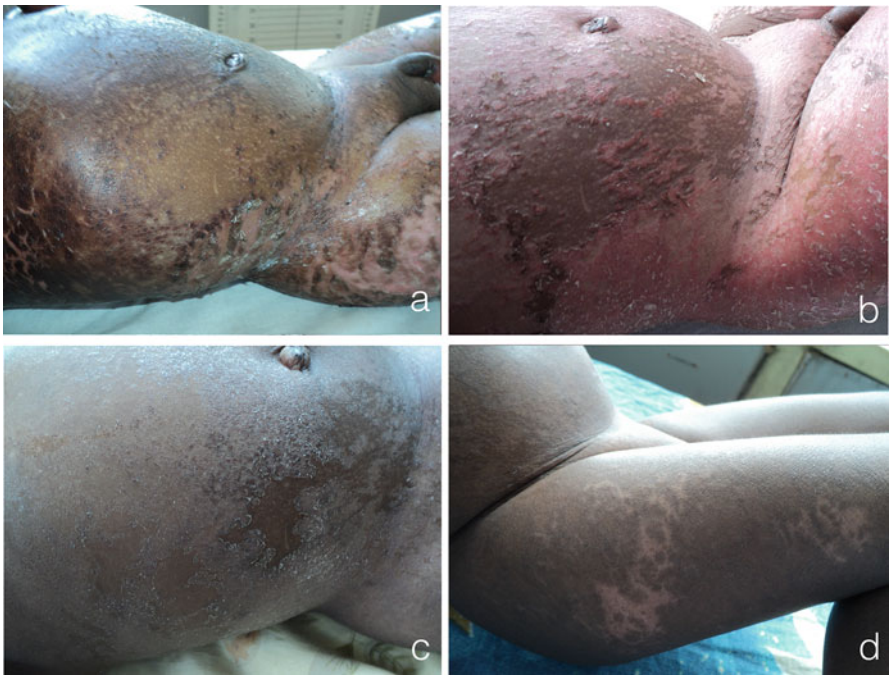


Fig. 2 Severe skin changes in a Ugandan patient admitted to hospital, with severe acute malnutrition (**a**). (**b**) The same patient, stabilized after 6 days of treatment with milk-based therapeutic diet (F75) and antibiotics. (**c**) After 23 days, the patient is shifted to F100. (**d**) Before leaving hospital, 31 days after admission to hospital. Pictures are provided by the authors

nitrogen in the skin of children with edematous SAM, compared to healthy controls, and that levels decreased with the severity of skin lesions. Furthermore, analysis of skin from SAM patients showed decreased levels of several amino acids (proline, tyrosine, and glycine) in those with skin changes compared to those without. The levels of these amino acids also decreased with the severity of the skin changes. Glycine and proline being a consistent part of the triple helical structure of collagen connect these findings to the hypothesis (Vasantha 1969; Vasantha et al. 1970).

Low availability of methionine, a sulfur-containing amino acid, has been suggested to cause the changes in skin and hair that is seen in SAM by impairing the creation of sulfur bonds in keratin (Roediger 1995). The connection to the sulfur amino acid has been mentioned in other studies (Jahoor et al. 2008; Jahoor 2012; Amadi et al. 2009), but the theory has not yet been properly investigated. Plasma levels of methionine have been found to be lower in SAM patients compared to stunted controls and the lowest levels were found among those with edema (Giovanni et al. 2016).

As methionine is essential in the initiation of protein synthesis, it would be surprising if the effect of methionine depletion was restricted only to affect keratin synthesis. Inadequate amounts of methionine should theoretically affect stability of

secondary and tertiary protein structures in general. Furthermore, as part of the coenzyme s-adenosyl methionine, methionine depletion would have effect on the regulation of several metabolic processes.

Skin changes did play a central role in a study on dogs fed a diet devoid of methionine. Apart from weight loss and anorexia, the study subjects showed evidence of a marked dermatitis (Milner 1979).

Fatty Acids

Essential fatty acid deficiency (EFAD) is accompanied by dry and scaly skin (Collins et al. 1971). Severe cases show weeping erosions in the flexural folds (Braun-Falco et al. 2009). A study of whole blood samples from children with SAM showed a different fatty acid balance compared to healthy controls. This included a lower n-6: n-3 ratio in the SAM patients. Arachidonic acid levels have been found to be lower in edematous SAM compared to nonedematous patients (Leichsenring et al. 1995; Wolff et al. 1984) and to be highly correlated with the presence of skin changes (Wolff et al. 1984). Animal models studying EFAD have shown that an impaired cutaneous permeability barrier due to EFAD causes a 50% increase in DNA synthesis of the skin, reflecting epidermal hyper proliferation (Proksch et al. 1991). This is in contrast to findings of lowered total protein and DNA content of the hair, in malnourished patients (Bradfield 1972).

Niacin Deficiency (B3)

Skin changes in SAM have been suggested to be a variant of pellagra caused by niacin deficiency (Stannus 1935). The skin changes in pellagra forms a symmetrical, sharply bordered erythema developing into exudative eruptions restricted to sun exposed areas such as hands and neck (Casal's neckless) (Hegyi et al. 2004). Other clinical features of pellagra such as diarrhea are common clinical features in fulminant SAM. The mood changes seen in children with SAM could also be a clinical expression of the dementia seen in pellagra. This thesis has not yet been confirmed in a clinical setting where the skin changes are successfully treated with nicotinic acid (Ground 1957; Trowell 1954). One study found decreased urinary excretion of N1-methylnicotinamide, compatible with pellagra, in seven out of nine adults with various degrees of malnutrition and additional skin changes of lower extremities (Maltos et al. 2015).

In a recent publication serum, tryptophan levels were found to be decreased in patients with edematous SAM (kwashiorkor) compared to those with nonedematous SAM (marasmus). This difference was one of the ten most distinguishing features when comparing the two patient groups (Giovanni et al. 2016).

Deficiency of other vitamins in the B group has not yet been investigated.

Zinc Deficiency

In acrodermatitis enteropathica, where the intestinal absorption of zinc is impaired, the isolated consequences of zinc deficiency can be seen. Dermatological features are dermatitis, xerosis, and alopecia evolving into crusty and sharply demarcated erosions. Other symptoms are stunting, diarrhea, anorexia, and impaired cognitive

function. All of which are features observed in SAM. Low plasma levels of zinc have been described in edematous SAM. In this study, an insignificant tendency for lower levels among patients with skin changes was noted (Golden and Golden 1979).

Histopathology

There are only few studies on the histo-pathological changes of SAM, but they consistently describe an epidermis with changes of parakeratosis and acanthosis indicating a rapid cell turn-over. The dermis show changes characteristic of inflammation with papillary edema and a tendency to lymphocytic inflammation. Table 1 gives an overview of the histological cohort studies in SAM (Rangam et al. 1962; Sims 1968; Thavaraj and Sesikeran 1989).

Diagnosis and Clinical Manifestation of SAM Specific Skin Changes

Skin assessment is performed on admission to hospital. When a patient with SAM presents with skin changes, it is to be considered as SAM with medical complications (see treatment).

The patient must be naked to estimate the body surface area (BSA) affected. A photo protocol can be used to monitor the skin status during treatment and as a tool for registration of data in research setups (Protocol, Fig. 10).

Setting:

- Ensure warm room temperature to avoid hypothermia
- Ensure privacy
- Ensure involvement of the caretaker when assessing a child
- Involve a local healthcare staff as assistant, if you cannot speak the local language

SAM Specific Skin Changes

Five specific skin changes in SAM have been identified, in a recent dermatological publication on skin changes in Ugandan preschool children (Heilskov et al. 2015):

- Telogenic effluvium
- Pigmentary changes (hyper- and hypopigmentation)
- Ichthyosiform skin changes (grade 1–3)
- Lichenoid skin changes (grade 1–3)
- Various stages of bullae, erosions, and desquamation (grade 1–3)

Table 1 Overview of histological findings in biopsy material from skin in severe acute malnutrition. First published in JEADV 2014 (Heilskov et al. 2014) (With permission from publisher. Licence number 3933660523976)

Study	Epidermal findings	Dermal findings	Study subjects and material
(Rangam et al. 1962**) cohort study**	In areas of athrophy, the entire thickness exist of *stratum granulosum* and -*lucida* and there are no kerato-hyaline granula	*Papillary layer* shows oedematous thickening, separation of fibrils and fragmentation	31 African children
			Oedematous malnutrition
	Increase in alkaline phosphatase. Placed in a band-like appearence in *stratum basale* and -*spinosum*	Generalized infiltration of histocytes, especially in the *papillary layer*	Biopsy from crus and lateral abdomen
	Displacement of cells and loss of cohesion in the basal layer	Elastic fibres show fragmentation and clumping	Light microscopy
		Few sweat and sebaceous glands	
(Sims 1968**) cohort study**	Reduced overall thickness	No observations	15 Zulu children, Durban
	Reduced thickness of *stratum corneum*		Oedematous malnutrition
	Reduced thickness of *rete Malpighi* (*basale, spinosum, granulosum*)		Biopsy from medial side of the axilla. Area with no visible skin affection
	Reduced length of desmosomes		Electron- and light microscopy
(Thavaraj and Sesikeran 1989**) cohort study**	Exaggeration of *stratum corneum*	Atrophy of hair bulbs	20 African children
	Atrophy of *stratum granulosum* and -*spinosum*		Mixed oedematous and non-oedematous malnutrition
	In subjects with dermal oedema collagen was reduced and there was crowding of elastic fibres		Biopsy from less affected areas
			Light microscopy

The clinical manifestation can be a mixture of the five skin changes (Fig. 3). Therefore, the systematic clinical score for SAM specific skin changes, SCORDoK (Protocol, Fig. 9), can be used to standardize the registration of the skin changes.

The presence of lichenoid skin changes has shown to be an independent predictor of death and the hazard ratio (HR) increases with severity of the lesions (Heilskov et al. 2015).

Fig. 3 Features in skin changes, related to severe acute malnutrition, on admission to hospital. Severe acute malnourished Ugandan child, complicated by oedema and severe skin involvement with mixed skin changes: Lichenoid skin changes grade 3 and bullae, erosions and desquamation grade 2. Hyperpigmentation is seen in relation to lichen and erosions. Scalp hair has been razed of. Pictures are provided by the authors

Telogenic Effluvium

Telogenic effluvium is loss of telogen hair caused by a disturbed hair cycle (Fig. 4). This can vary from mild thinning to total hair loss. Telogen hairs are in a preserved state of rest opposite to growing hairs in the anagen phase. During research on hair, it was noted that the hair was easier pulled out in malnourished patients. The hair bulbs had a smaller diameter and this was directly related to a lower weight for age. A higher proportion of hair bulbs were in the resting state (telogen phase) in the case of

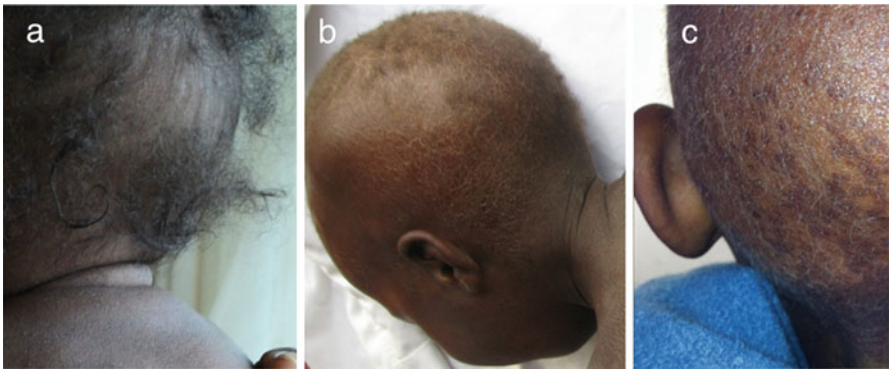


Fig. 4 Telogenic effluvium. Various manifestations of increased telogenic effluvium, also showing depigmentation and straightening of the hair (**a**, **b**, **c**). First published in JEADV 2015 (Heilskov et al. 2015) (With permission from publisher. Licence number: 3,933,580,522,015)

malnutrition (Bradfield et al. 1968). Telogenic effluvium is known to be secondary to changes in several physiological mechanisms that are expected to be compromised in SAM and related to co-morbidities such as infections (Braun-Falco et al. 2009).

SAM-related telogenic effluvium is often combined with depigmentation and/or straightening of curly hair. The *flag sign*, where light bands in the hair reflect the nutritional status of a child over time, can be observed. Changes in hair status are connected to hair cycle and thus are slow. It takes 2–5 months before a telogen hair is shed, after the shift from the anagen phase. Telogenic effluvium is therefore expected to be a sign of more chronic malnutrition. It has also been noticed that changes in the status of the hair are not seen during the short time of recovery from SAM in a hospital (Heilskov et al. 2015).

Pigmentary Changes

Pigmentary changes (Fig. 5) are hyper- and hypopigmentation. The change in pigmentation can be general, as seen in systemic disease, or well-defined areas. In SAM, pigmentary changes often follow the other SAM-specific skin changes but can be the only skin change in a SAM patient.

Ichthyosiform Skin Changes

Ichthyosis is generalized scaling of the skin and is histologically characterized by hyperkeratosis. Ichthyosis can be coupled to the protein synthesis of the keratinocytes, as in ichthyosis vulgaris, or the lipid components of the extra-cellular matrix, as in X-linked recessive ichthyosis, both resulting in retention hyperkeratosis, which is abnormal shedding of the corneal layer (Braun-Falco et al. 2009; Williams 1992). Grades of affection in ichthyosiform skin changes in SAM are shown in Fig. 6.

Grade 1 is characterized by dry skin, hyperpigmentation, and accentuation of the lines of the skin. Mild shedding of fine grey scales is observed in few areas. In **grade 2**, the dark skin turns greyish and the dusty scales loosen easily. Areas with

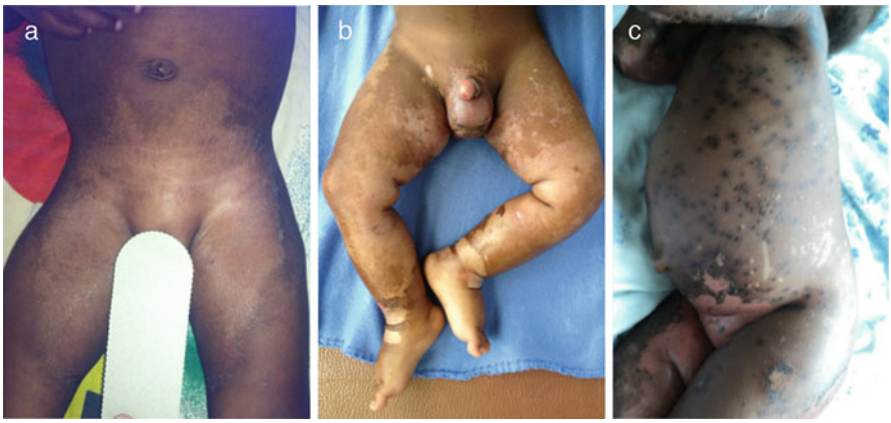


Fig. 5 Pigmentary changes. Pigmentary changes in SAM. Hypopigmentation caused by desquamation (**a**, **b**) and erosion (**b**, **c**) and hyperpigmentation in lichenoid skin changes (**c**). First published in JEADV 2015 (Heilskov et al. 2015) (With permission from publisher. Licence number: 3,933,580,522,015)

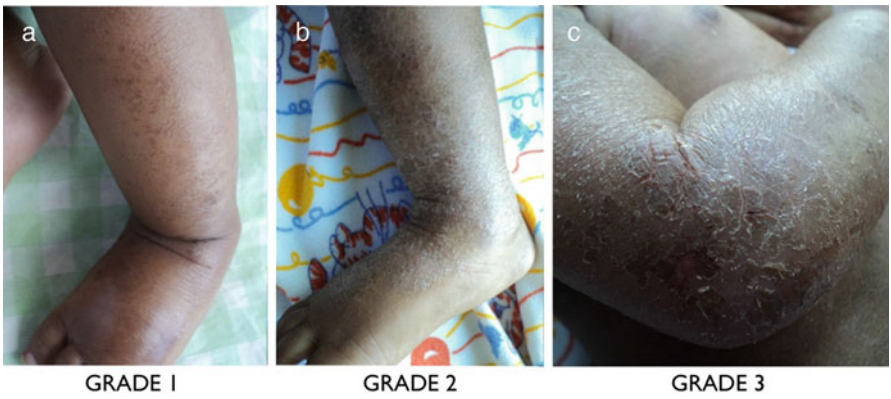


Fig. 6 Ichthyosiform skin changes. Grades of affection in ichthyosiform skin changes on extremities. Grade 1 (**a**), grade 2 (**b**), grade 3 (**c**). First published in JEADV 2015 (Heilskov et al. 2015) (With permission from publisher. Licence number: 3,933,580,522,015)

hyperkeratosis appear. In these areas, the scales are more infiltrated and bigger. In **grade 3** scales are hard, shiny, and slightly hyperpigmented. Erosions appear where the scales are shed.

Lichenoid Skin Changes

Lichenoid skin change is a term for skin changes that clinically resemble the prototype lichen planus. Lichen is noninfectious and is histologically characterized by lymphocytic infiltrate at the epidermal function forming a band-like pattern.

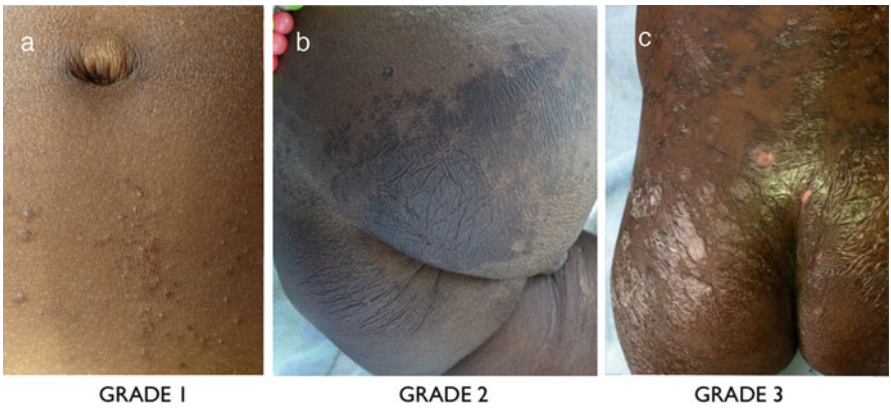


Fig. 7 Lichenoid skin changes. Grades of affection in lichenoid skin changes. Grade 1 (**a**), grade 2 (**b**), grade 3 (**c**). First published in JEADV 2015 (Heilskov et al. 2015) (With permission from publisher. Licence number: 3,933,580,522,015)

Papules tend to fuse into flat-topped patches bordered by the skin lines. There are many clinically variants of lichen.

In research on SAM, no authors have commented on pruritus, but a distribution of the skin changes to areas of mechanical stress rather than sun-exposed areas has been noted (Heilskov et al. 2014). Grades of affection in lichenoid skin changes in SAM are shown in Fig. 7.

Grade 1 is characterized by small ($\leq$0.5 cm) papules. These are hyperpigmented and irregular on the surface but not hyperkeratotic. The smallest (1–2 mm) are purple-brown. In **grade 2**, the areas affected are bigger and coalesce to highly hyperpigmented plaques of various sizes. These can be hyperkeratotic and the lines of the skin are accentuated. The border is irregular, but well defined and in these areas, the original papules can be identified. Plaques can loosen and peel off. In **grade 3,** the plaques are more infiltrated and rigid and the surface can be shiny. Plaques peel off leaving thin epidermis or erosions. The whole body can be affected, but palms and soles are spared.

Bullae, Erosions, and Desquamation

A bulla is a raised circumscribed lesion containing serous fluid and measuring $>$5 mm. The involved layers of the epidermis can vary and no histological research has yet been made concerning the bullae in SAM. A bulla tends to rupture and the outer layer desquamates from the skin, leaving the epidermis exposed as an erosion. An example of bullae, erosions, and desquamation in SAM is shown in Fig. 8. As the erosions affect primarily the epidermis, it tends to heal without scarring.

Severity grade of this SAM-specific skin change is defined by the affected BSA. Bullae, erosions, and/or desquamation is observed on the body, affecting $<$5% of the body in **grade 1**, from 5 to 30% in **grade 2** and more than 30% in a **grade 3**.

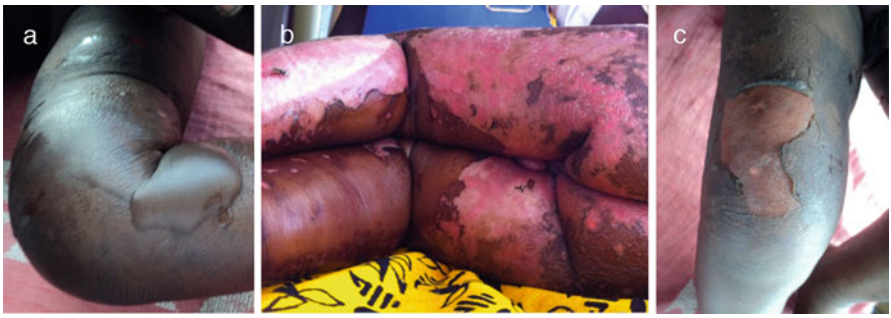


Fig. 8 Bulla, erosion and desquamation. Bullae, erosions and desquamation on lower extremities (**a**, **b**, **c**). First published in JEADV 2015 (Heilskov et al. 2015) (With permission from publisher. Licence number: 3,933,580,522,015)

The cut-off values for grade of affection are not based on experience on outcome or investigation on prognosis.

Differential Diagnosis

Burns, diaper dermatitis, atopic eczema, staphylococcal scalded skin syndrome (SSSS), and skin changes due to a single nutrient deficiency.

Skin Changes in Single Nutrient Deficiencies

A malnourished patient is most likely to be lacking several vitamins that are important for maintenance of healthy skin. Still the clinical skin manifestation can be dominated by the signs of a single nutrient deficiency of which niacin (B_3), zinc, some amino acids, and essential fatty acids have been described. The fat-soluble vitamin A, water-soluble riboflavin (B_2), pyridoxine (B_6), and vitamin C and biotin are also connected to skin and mucosal changes. The diagnosis of the single nutrient deficiencies, based on clinical findings, is difficult as the clinical appearance is often less characteristic.

Clinical Management Options

Skin changes in SAM account for one criterion of complicated SAM and these patients are preferably referred to hospital care. There are currently few well-documented recommendations on treatment of skin changes in SAM.

Table 2 Focus areas in the hospital treatment of skin changes, complicating severe acute malnutrition

Milk based therapeutic food (Ashworth et al. 2003; WHO and UNICEF 2009).	Admission and stabilisation: F75, containing 75 kcal/100 ml and 0.9 g protein/100 ml.
	After stabilisation: F100, containing100 kcal and 2.9 g protein/100 ml.
	Give the food orally ore use nasogastric tube. Never parenteral substitutes.
	Encourage continued breastfeeding.
Possible regimes for topical treatment (Ashworth 2005; Ashworth et al. 2003).	Zinc (Golden et al. 1980) and castor oil ointment, or petroleum jelly or paraffin gauze) to raw areas.
	Soak the affected areas for 10 min/day in 0.01% potassium permanganate solution. Disadvantages of this regime has been discussed (Golden and Grellety 2012).
	Application of oils rich in essential fatty acids, like sunflower seed oil (linoleic acids) has proven to approve skin barrier function, enhance weight gain and lower the risk of skin infection in preterm infants (Salam et al. 2013). This regime is for ulcer-free skin affections and is, for now, to be considered as a low-cost alternative to the above mentioned.
Antibiotics (Ashworth 2005; WHO 2013; WHO and UNICEF 2009).	Gentamicin IV or IM (7.5 mg/kg), once daily for 7 days, plus ampicillin IV or IM (50 mg/kg), every 6 h for 2 days.
	Followed by amoxicillin oral (15 mg/kg), every 8 h for 5 days.

Recommendations on treatment of SAM complicated by skin changes are summarized in Table 2 (Ashworth 2005; Ashworth et al. 2003; Golden and Grellety 2012; Golden et al. 1980; Salam et al. 2013; WHO 2013; WHO and UNICEF 2009).

Prognosis and Complications

Complicated SAM has a high mortality rate even when being managed in hospital. Known complications in SAM that theoretically can be related to a broken skin barrier are hypothermia, dehydration, electrolyte imbalance, and infection/sepsis.

Three studies have confirmed skin changes to be a predictor of death (Becker et al. 2005; Heilskov et al. 2015; McLaren et al. 1969). In a characteristic of specific skin changes in SAM, it was found that lichenoid skin changes significantly lowered the chance of survival and that the HR increased with the grade of severity (Table 3) (Heilskov et al. 2015). It was also revealed that lichenoid skin changes could be used in a logistic regression model, to forecast death when admitting SAM patients to hospital. The misclassification error of the model, constructed of known risk factors, was improved when adding lichenoid skin changes as a variable.

Table 3 P-values from Cox-regression for correlation between six skin predictors and risk of death (hazard). From the table, it is seen that lichenoid skin changes significantly lower the chance of survival and that the risk of death relative to healthy controls, increase with the grade of severity (hazard rate, HR) Data are from JEADV 2015 (Heilskov et al. 2015) (With permission from publisher. Licence number: 3,933,580,522,015)

Predictor	Hazard ratio	Unadjusted model (0/1)	Adjusted model (0/1)[a]	Unadjusted model (grade 0–3)	Adjusted model (grade 0–3)[a]
	[HR]	[P]	[P]	[P]	[P]
Telogenic effluvium[b]	0,65	0,64	0,58	–	–
Pigmentary changes[b]	1,26	0,42	0,73	–	–
Ichthyosiform skin changes^		–	–	–	–
Lichenoid skin changes		0,34	0,23	**0,03**	**0,02**
Grade 1	^				
Grade 2	6,00				
Grade 3	13,79				
Bullae – erosions – desquamation		0,30	0,38	0,09	0,35
Grade 1	^				
Grade 2	2,65				
Grade 3	3,22				
Body surface area[c]	–	–	–	0,30	0,42

[a]Adjusted for age, sex and oedema
[b]Telogenic effluvium and pigmentary changes are both dichotomous (1/0) variables and there are therefore no p-value for these as graded predictors
[c]Body surface area is a continuous predictor (0–100%) and therefore there is no p-value for a stratified analysis.
^ no events observed

Policies and Protocols

Protocol-Assessing Skin Status

Standardized Clinical Score of Skin manifestations in SAM-SCORDoK

Comparability of results and objectivity in registration are important factors when reporting research results. This protocol is a tool for registration of the characteristic skin changes in SAM, with an estimate of severity and affected BSA.

Setting:

- It is important to have sufficient light in order to evaluate the skin changes.
- Ensure warm room temperature to avoid hypothermia.

- Ensure privacy.
- Ensure involvement of the caretaker when assessing a child.
- Involve a local healthcare staff as assistant, if you cannot speak the local language.

Figure 9 below is a guide to register the SAM-specific skin changes and to estimate affected BSA in preschool children. Be aware that the BSA distribution is different in adults, children, and babies.

Protocol-Standardized Photo Protocol

Photography is traditionally used in the dermatological field for reporting and registering skin changes.

This photo protocol is a practical tool to monitor the development of the skin changes during treatment and when monitoring intervention.

A good picture protocol is also an advisable supplementation to a study setup, to ensure objectivity and when later assessing intra- and interobserver variability. Dermatologists are often not available in developing countries and the use of photos in telemedicine requires standardized picture of good quality.

Setting:

- Assure informed consent before taking pictures
- Use a camera of good quality
- Establish good light and a matt colored background
- Ensure warm room temperature to avoid hypothermia
- Ensure privacy
- Ensure involvement of the caretaker when assessing a child
- Use the assistance of a local healthcare staff, if you cannot speak the local language

A guide to a systematic photo protocol, using the preschool child as an example, is shown below (Fig. 10).

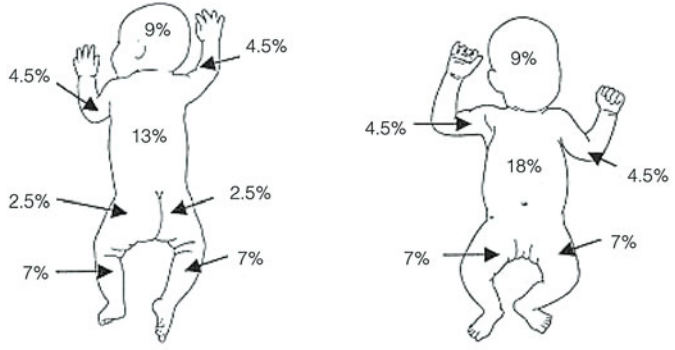
Dictionary of Terms

- **Severe acute malnutrition** – It is the acute form of malnutrition usually affecting children and is characterized by low weight for height (wasting) or bilateral pitting edema. The diagnosis is supported by anthropometric measures and a history of insufficient food intake.
- **Body surface area** – It is widely used in the dermatology to reflect the grade of affection in skin disease. Best known is the “Rule of Nines” chart, used in the assessment of skin damage from burns.

SCORDoK

Standardised clinical score of skin manifestations in SAM

ID: _____ Phase: Admission ☐ Transition ☐ Discharge ☐



Body Surface Area		
Skin manifestation	**No**	**Yes**
Telogenic effluvium		
Pigmentary changes		

			Grade I	**Grade II**	**Grade III**
Ichthyosiform skin changes					
Lichenoid skin changes					
Bullae - Erosions - Desquamations					

Fig. 9 Standardised score for skin changes in severe acute malnutrition, SCORDoK. © Copyrighted all rights reserved. Copyright number: TXu 1–948-432. First published in JEADV 2015 (Heilskov et al. 2015) (With permission from publisher. Licence number: 3,933,580,522,015)

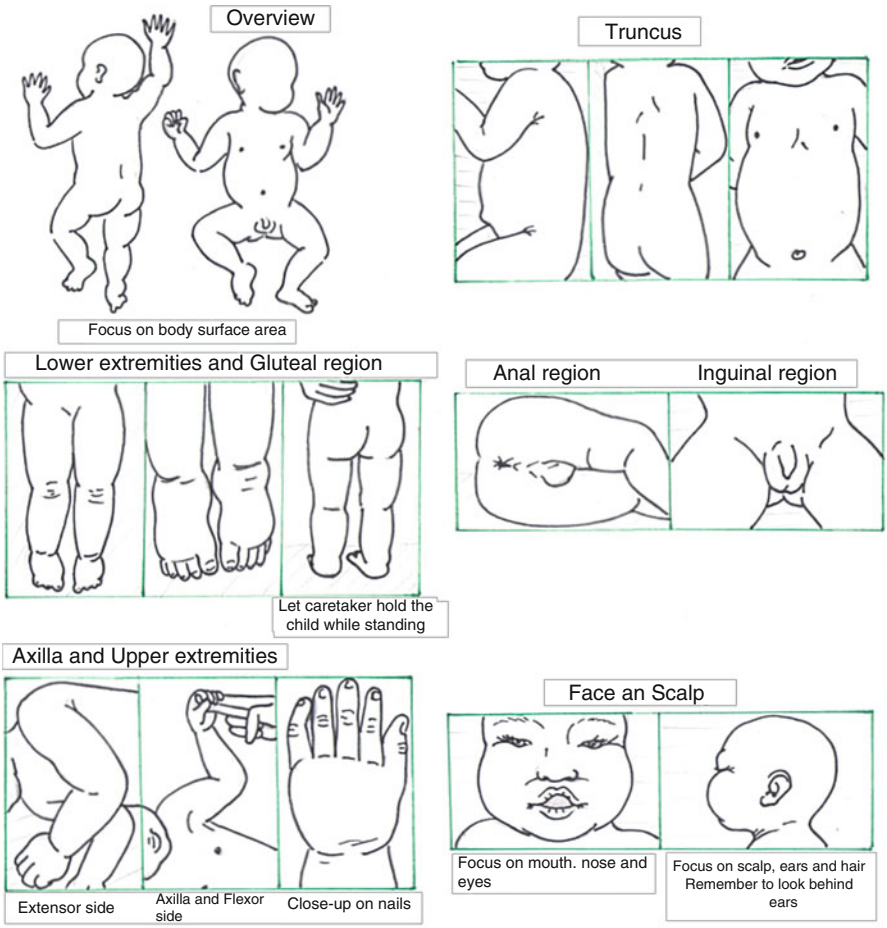


Fig. 10 Guide to standardised photo documentation when monitoring the skin changes in severe acute malnutrition. © Copyrighted all rights reserved. Copyright number: TXu 1–948-432. Edited by Asbjoern Axelsen. First published in JEADV 2015 (Heilskov et al. 2015) (With permission from publisher.Licence number: 3,933,580,522,015)

- **Mortality rate** – It is a measure of deaths per unit time, in a given population.
- **Hazard ratio** – It is used in survival analysis to reflect the risk of death in a study cohort relative to a control group. If the treated cohort has a mortality rate twice as high than the control group, the hazard ratio equals 2. A hazard ratio > 1 indicates increased risk of death in the cohort.
- **Logistic regression model** – It estimates a probability of an outcome, for example death. It is based on binary variables and gives the opportunity to conclude whether the presence of a variable increases the risk of the current outcome.

Summary Points

- This chapter focuses on characteristic skin changes in severe acute malnutrition.
- The sparse research on the subject has focused on mechanisms in single nutrient deficiencies, but studies have not yet proven the skin changes to be connected to one nutrient alone.
- Focus areas in the etiological research have been amino acid deficiency, essential fatty acid deficiency, niacin deficiency (B_3), and zinc deficiency.
- Five skin changes have been identified as specific to severe acute malnutrition (telogenic effluvium, pigmentary changes, ichthyosiform skin changes, lichenoid skin changes and stages of bullae, erosions, and desquamation).
- Skin changes in severe acute malnutrition can be severe and comprehensive and research has found that they increased risk of death if present.
- Lichenoid skin changes significantly lower the chance of survival and the hazard ratio increases with the grade of severity.
- Skin changes in severe acute malnutrition are one criterion of complicated severe acute malnutrition, and these patients are preferably referred to hospital care for intravenous therapy with antibiotics, in addition to therapeutic feeding.
- The standardized clinical score of skin manifestations in severe acute malnutrition, SCORDoK, ensures a standardized system to monitor the skin during treatment and secure comparability of results when reporting research observations.
- A photo protocol is a practical tool to monitor the development of the skin changes during treatment, when monitoring intervention, and is essential in tele-medical communication.
- The etiology of the skin changes is unknown and suitable treatment, based on research results, has yet to be developed.
- The epidermis has changes of parakeratosis and acanthosis indicating a rapid cell turn-over.
- The dermis show changes characteristic of inflammation with papillary edema and a tendency to lymphocytic inflammation.
- The geographical distribution is not yet mapped out but reports on skin changes tend to come from the African continent.

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Effects of Energy Deficiency: A Focus on Hospitalized and Critically Ill Patients

68

Lisa Santoriello and Rafael Barrera

Contents

Abstract

Malnutrition is a broad term used to describe any imbalance in nutrition that can develop as a result of dietary intake, increase in basal requirements, poor absorption, excessive nutrient losses due to an underlying disease state, or any combination of such factors. Upon admission, 15–60% of hospitalized patients are at

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risk or already qualify as being malnourished, with 30–40% of hospitalized patients experiencing further nutritional decline during their hospital course. When left unnoticed, malnourishment, especially in the hospitalized patient, is associated with several negative sequelae, including immunosuppression, higher infection rates, increased muscle loss with delayed functional recovery, impaired wound healing, increased risk of treatment complications, fewer ventilator-free days, longer hospital stays, higher overall treatment costs, and increased mortality rates. Several nutritional risk scoring systems exist to help clinicians risk-stratify these patients upon admission, with the goal of starting early nutritional therapy when deemed necessary.

Keywords

Malnutrition · Energy deficiency · Critical illness · Nutritional assessment · Nutritional therapy · Caloric deficiency · NUTRIC · NRS-2002 · Enteral · Parenteral

List of Abbreviations

APACHE II	Acute Physiology and Chronic Health Evaluation II
ASPEN	American Society for Parenteral and Enteral Nutrition
BMI	Body mass index
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
GALT	Gut-Associated Lymphoid Tissue
IC	Indirect calorimetry
NICE SUGAR	Normoglycemia in Intensive Care Evaluation and Survival Using Glucose Algorithm Regulation
SCCM	Society of Critical Care Medicine
SOFA	Sequential organ failure assessment

Objectives

- Overview of malnutrition and its effects on hospitalized and critically ill patients
- Review of nutritional assessment
- Recognize the importance of early nutritional support in patients
- How and when to initiate nutritional therapy
- Review nutritional goals in various clinical conditions
- Postdischarge nutritional maintenance

Malnutrition is a broad term used to describe any imbalance in nutrition that can develop as a result of dietary intake, increase in basal requirements, poor absorption, excessive nutrient losses due to an underlying disease state, or any combination of such factors. In 2012, the Academy of Nutrition and Dietetics and the American Society for Parenteral and Enteral Nutrition released a consensus statement recommending an etiology-based approach for the detection of malnutrition in adults. Three etiology-based definitions fall

under this guideline: starvation-related malnutrition without inflammation, chronic disease-related malnutrition with mild to moderate inflammation, and acute disease- or injury-related malnutrition with marked inflammation. These definitions reflect that inflammation, whether chronic or acute, acts as an underlying factor in the pathogenesis of malnutrition associated metabolic alterations, further stressing the importance of early nutritional therapy in critically ill.

While traditionally thought of as adjuvant care, nutritional goals have become more focused to prevent cellular injury from oxidative stress and improve overall immune response, abating the metabolic response to bodily stresses endured in critical illness. In this chapter, we will discuss several methods clinicians can implement to achieve these goals.

Effects of Energy Deficiency

Upon admission, 15–60% of hospitalized patients are at risk or already qualify as being malnourished, with 30–40% of hospitalized patients experiencing further nutritional decline during their hospital course (Mauldin and O'Leary-Kelly 2015). More often than not, patients had poor nutritional intake in the days leading up to admission and often continue to receive poor nutritional support while admitted. This cycle leads to weight loss and further nutritional decline, making it difficult for patients to ultimately recover from illness Naber et al. 1997; Norman et al. 2008).

Over recent years, it has become increasingly apparent that proper nutritional balance plays a vital role in aiding the recovery of hospitalized patients and the critically ill. Several prospective observational studies have shown that malnutrition is frequently associated with several negative outcomes in hospitalized patients, including immunosuppression, higher infection rates, increased muscle loss with delayed functional recovery, impaired wound healing, increased risk of treatment complications, fewer ventilator-free days, longer hospital stays, higher overall treatment costs, and increased mortality rates (Norman et al. 2008; Hiesmayr et al. 2009).

Critical illness is a catabolic stress state characterized by a systemic inflammatory response that increases the body's nutritional needs from baseline requirements. When present, critical illness can lead to multi-organ dysfunction, exacerbating many of the negative outcomes already associated with underlying malnutrition, thus resulting in a higher likelihood of adverse outcomes. Nutritional therapy is vital to abate the inflammatory catabolism of lean body mass and the associated poor prognostic and economic outcomes.

Assessment of Nutritional Risk

Integral to the care of the critically ill patient is an initial assessment of malnutrition or risk thereof. A simple evaluation can be done with history and physical, however, several nutritional risk scores can be used for a more robust assessment. While

(i) Score of Disease Severity	
Score 1	General malignancy, hip fracture, long-term hemodialysis, diabetes, chronic disease (COPD, cirrhosis, etc)
Score 2	Hematologic malignancy, severe pneumonia, major abdominal surgery, stroke
Score 3	Head/brain injury, bone marrow transplant, intensive care patients with an APACHE score higher than 10
(ii) Score of the Impaired Nutrition Status	
Score 1	Weight loss > 5% over 3 months Food intake below 50-75% of normal requirement in preceding week
Score 2	Weight loss > 5% over 2 months Food intake below 25-50% of normal requirement in preceding week, BMI < 20.5 with poor general condition
Score 3	Weight loss > 5% over preceding month Food intake below 25% of normal requirement in preceding week BMI < 18.5 with poor general condition
Score of the Age	
Score 1	> 70 years old

Fig. 1 Nutritional Risk Score (NRS-2002); Risk screening score = Score of severity of disease + Score of impaired nutrition status + Score of age

nutritional assessment scales such as the Mini Nutritional Assessment, Malnutrition Universal Screening Tool, Short Nutritional Assessment Questionnaire, Malnutrition Screening Tool, and the Subjective Global Assessment may suffice for initial admission screening for hospitalized patients, they do not take disease severity into account. Due to this, the Society of Critical Care Medicine (SCCM) and American Society for Parenteral and Enteral Nutrition (ASPEN) recommend the use of the Nutritional Risk Score (NRS-2002) or NUTRIC score for all critically ill patients thought to be high risk for or whom are admitted with malnutrition (Taylor et al. 2016).

The NRS-2002 (Fig. 1) is a validated scoring system supported by several randomized controlled studies in predicting postoperative complications. Patients are considered "at risk" with a score > 3 based on components such as severity of disease, recent weight loss, baseline body mass index, general food intake, and age (Taylor et al. 2016).

The NUTRIC score (Fig. 2) is a nutritional risk assessment tool designed to quantify critically ill patients' risk of malnutrition related morbidity and mortality that may be preventable with aggressive, early nutritional therapy. Notably, it was developed and validated specifically for ICU patients, taking six main factors into account: age, APACHE II score, baseline sequential organ failure assessment score (SOFA), existing comorbidities, length of hospital stay prior to ICU admission, and

Variable	Range	Points
Age	< 50 years old 50-75 years old >75 years old	0 1 2
APACHE II	< 15 15-20 20-28 >28	0 1 2 3
SOFA	< 6 6-10 > 10	0 1 2
Number of Co-Morbidities	0-1 ≥ 2	0 1
Days from hospital to ICU admission	0-1 > 1	0 1
IL-6	0-400 ≥ 400	0 1

Fig. 2 NUTRIC Score; Scores associated with worse clinical outcome vary depending on availability of IL-6 level. When IL-6 level is available, a score of 6–10 is considered high; a score of 5–9 is considered high when IL-6 is not available. These patients are most likely to benefit from early, aggressive nutritional therapy

interleukin-6 level (if available). Patients are considered high risk if scored ≥6 (≥ 5 if interleukin-6 not available). In a prospective, observational study of 597 patients, Heyland et al. showed an association between high NUTRIC scores and prolonged time on mechanical ventilation as well as mortality rate. While there may be some limitations to this scale, it does serve as a good initial assessment to classify ICU patients that may benefit from early nutritional therapy (Heyland et al. 2011; Taylor et al. 2016).

While outpatient nutritional evaluations may benefit from the use of traditional serum protein markers such as albumin, prealbumin, transferrin, and retinol-binding protein, these markers are not validated in the critically ill patient. Because albumin has a long half life, 2–3 weeks, acute protein deficiency due to critical illness will only gradually affect serum levels. Prealbumin and retinol-binding protein, with half lives of 2–3 days and 11 hours respectively, may show variations related to malnutrition sooner; however, several disease states and the acute inflammatory response can alter these levels irrespective of nutritional status. These proteins may be falsely decreased due to increased vascular permeability in the setting of an acute inflammatory response, thus they may not accurately reflect nutritional status. Similarly, anthroprometrics such as body mass index (BMI), adjusted body weight (ABW), and arm muscle circumference (AMC) are not reliable in this population either, as they do not account for the edema that typically accumulates in the critically ill (Raguso et al. 2003; Whitson and Barrera 2014; Martindale et al. 2009).

Nutritional Therapy

When to Start Nutritional Therapy

As previously discussed, hospitalized and critically ill patients are at nutritional risk, and as such, nutritional therapy should be considered once the patient is deemed hemodynamically stable. For patients with baseline malnutrition or those in a high catabolic state such as that seen in critical illness, nutritional therapy should be initiated within the first 24–48 hours of admission when it is expected that the patient will be unable to voluntarily maintain caloric intake or meet their nutritional needs (Martindale et al. 2009). Several observational studies have shown improved clinical outcomes when nutritional therapy is given during this time period. These studies found that early nutritional therapy was associated with improved maintenance of functional and structural intestinal integrity, thus decreasing the risk of translocation of gut flora, modulation of systemic immunity via GALT within intestinal walls, minimized effects of oxidative stresses leading to decreased activation and release of inflammatory cytokines, and an overall decrease in length of stay, morbidity, and mortality (Kudsk 2002; Jabbar et al. 2003; Kang and Kudsk 2007).

Enteral Versus Parental Nutrition

When nutritional therapy is required, the preferred route is enteral over parenteral infusion. Enteral nutrition presents a significantly decreased rate of infectious complications, and it does not require central line access, further reducing complication risk. Parenteral nutrition may, however, be required in certain circumstances. Patients at high nutritional risk that are unable to tolerate oral diet or enteral nutrition within 5 days of admission and are not expected to be able to do so within the next 7 days may require parental nutrition. However, unless already at high risk of malnourishment, parenteral nutrition has not been shown to provide much benefit in well-nourished patients within the first 5–7 days of hospitalization. Parenteral dependent patients, such as those with short-gut syndrome, should continue with parenteral nutrition upon admission, with the exception of when bacteremia is suspected (Martindale et al. 2009; Taylor et al. 2016).

Supplemental parenteral nutrition may be required in patients of both low and high nutritional risk that are receiving enteral nutrition, yet are unable to meet at least 60% of their energy and protein requirements. However, there is no evidence in the literature that has clearly shown the optimal time to start supplemental parenteral nutrition in such patients. Once initiated, tolerance of enteral nutrition should continually be monitored, with down-titration of supplemental parenteral nutrition until discontinuation as enteral tolerance allows (Martindale et al. 2009; Taylor et al. 2016).

Meeting Energy Needs

The primary goal of any nutritional regimen is to provide sufficient caloric support without overfeeding. When available, indirect calorimetry (IC) is recommended to

determine energy needs. However, elements of ICU care, like mechanical ventilation, renal replacement therapy, and sedation with certain anesthetic agents, may affect it's accuracy. IC also requires invasive monitoring, resulting in a point estimate that may not give an accurate depiction of the patient's overall nutritional requirements (Bartlett et al. 1982; Whitson and Barrera 2014). Thus, when not available or feasible, published, simplistic weight-based equations (25–30 kcal/kg/day) should be used to determine energy needs, as other anthropometric equations have not been validated in the critically ill patient. Regardless of initial method used, energy requirements should be reassessed, at the minimum, on a weekly basis to ensure energy provision is fully optimized. Providers must also be cognizant of any additional caloric intake that may be provided through other treatments, such as dextrose-containing solutions and lipid-based medications like propofol which are commonly used in the ICU setting and contribute to the calculation of any nutritional regimen used to meet nutritional goals (Villet et al. 2005; Alberta et al. 2009; Whitson and Barrera 2014).

Equally as important as adequate overall energy provision is adequate protein delivery. While no evidence exists for the accurate determination of protein needs in the critically ill, many clinicians tend toward the use of simplistic weight-based equations. One could also use nitrogen balance as an estimate of protein turnover, however, this has limited value in the critically ill patient, as several conditions common to this population, including renal failure, burns, and fluid shifts, could alter these levels outside of the expected values one may see in an otherwise healthy patient. For non-critically ill patients, the protein goal is typically to provide 0.8 g/kg actual body weight/day. However, several studies found that despite meeting overall energy requirements, critically ill patients that were given this protein range did not exhibit the same improvement in mortality as those that had higher protein goals, typically in the range of 1.2–2 g/kg/day (Weijs et al. 2012; Plank 2013).

Monitoring Tolerance

Once nutritional therapy has been initiated, the next step is to ensure that patients are tolerating what is being provided. When continuous infusion is initiated, it is typically started at a rate below goal to allow for assessment of tolerance, usually starting at 20–30 ml/hr and increased by 10–20 ml/hr every 4–8hr as tolerated until the goal rate is reached. Multiple studies show that the greatest benefit of nutritional therapy is seen when started within 48 hours of presentation. Treatments common to the critically ill, including mechanical ventilation and use of vasoactive agents, should not be thought to be contraindications to the initiation or continuation of nutritional therapy. Clinical evidence of gastrointestinal function (bowel sounds, flatus, passage of stool, etc) are also not required prior to initiation; on the contrary, the initiation of enteral feeds is likely to promote gut motility (Martindale et al. 2009; Taylor et al. 2016).

Enteral feeds may be held for several reasons, including pausing for planned diagnostic tests and procedures or concern for patient tolerance. Thus, patients will commonly only receive approximately 80% of their prescribed nutritional therapy. Presumed patient intolerance accounts for about 30% of cessations for an average of

8–20% of prescribed infusion time, yet only half of these episodes are true intolerance. Several physical exam findings can be suggestive of patient intolerance of enteral nutrition, including vomiting, diarrhea, abdominal distention, decreased flatus/stool output, high naso/oropharyngeal output, and high gastric residual volumes (Martindale et al. 2009; Taylor et al. 2016).

While several feeding protocols exist, a broad distinction can be made based on methods of achieving goal rate and dealing with intolerance. In the volume-based approach, a target 24 hour rate is determined, and the hourly rate of feeds can then be adjusted, taking into account periods during which feeds were held, in order to meet the established 24 hour goal. The top-down approach, while similar to the volume based approached, also includes methods to enhance tolerance, such as the addition of prokinetic agents, adjustments in feed formulation, and feeding tube placement. Both of these approaches allow providers to correct for nutrition-free periods when feeds had been held, optimizing enteral nutritional delivery and ultimately aiding in meeting daily nutritional goals (Taylor et al. 2016).

A common method used to monitor patient tolerance is the periodic evaluation of gastric residuals, typically performed every 4 hours. The goal should be to minimize pauses in nutritional delivery whenever possible. As such, enteral nutrition should only be held if residuals are greater than 500ml or when lesser volumes are associated with clinical evidence of a change in tolerance. When these criteria are met, feeds should be discontinued until symptoms or residual volumes improve. Studies suggest no correlation between residual volumes less than 500cc and adverse outcomes, however, these patients may benefit from pro-kinetic agents, such as metoclopramide or erythromycin (Taylor et al. 2016).

In additional to high residuals, patients on enteral feeds also commonly develop diarrhea. If this occurs, the first step should be to assess the formulation provided. The common causes include the amount of fiber in the formulas, formula osmolality, mode of delivery (continuous vs bolus), other medications received, and infectious causes (e.g., C.dif). In the absence of infectious processes, an initial step to address persistent diarrhea could be the addition of a mixed soluble and insoluble fiber solution, but this should be avoided if there is thought to be a high risk of bowel ischemia or severe dysmotility. If diarrhea still persists, a small peptide formula can be substituted in the setting of a possible malabsorption process not responsive to the addition of fiber. It is also imperative to remember to monitor the sequelae of persistent diarrhea, such as electrolyte disturbances, dehydration, perianal skin breakdown, and wound contamination, while attempting to correct the underlying cause (Taylor et al. 2016).

Effect of Different Disease States on Caloric Requirements

For the majority of hospitalized and critically ill patients, basic polymeric formulations should be sufficient when initiating enteral nutrition, as the routine use of specialty formulas has not been found to provide any clear benefit in morbidity or mortality. Below we will discuss specific nuances to nutritional therapy for several commonly encountered medical and surgical conditions.

1. Medical
   (a) Sepsis

   Specific studies addressing nutrition in severe sepsis/septic shock are lacking, thus much data has been extrapolated from general data pertaining to all critically ill patients. However, compared to other critically ill patients, this subset is at a higher nutritional risk due to the hypermetabolic state that results from the exaggerated inflammatory response that occurs in sepsis. As such, nutritional therapy should be initiated once fluid resuscitation is complete and the patient is hemodynamically stable. The Surviving Sepsis Campaign published in 2012 recommended giving trophic feeds (10–20 kcal/kg/day) in the initial phase of sepsis, advancing as tolerated within 24–48 hours, with the aim to achieve >80% of determined energy goal within the first 7 days of admission. Protein goals are difficult to determine in septic patients, but current recommendations are to provide 1.2–2 g/kg/day as extrapolated from other critical illness criteria. Energy expenditure varies greatly in states of severe sepsis, thus follow-up nutritional assessments should be performed every 4–5 days to assure adequate nutrition is being provided. Immune modulating formulas supplemented with arginine, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), glutamine, nucleic acid, and the like are not recommended in sepsis, as no improvement in overall outcomes has been documented. The results of several observational studies would also suggest avoidance of exclusive or supplemental parenteral nutrition, as it seems to be associated with worsening clinical outcomes, including increased infectious rate, longer ICU and hospital length of stay, and longer duration of organ support (renal replacement therapy, mechanical ventilation, etc) (Taylor et al. 2016).

   (b) Diabetes Mellitis

   Given recent multicenter trials comparing tight and moderate glucose control, it is recommended that goals for glycemic control should aim for a range of 140–180 mg/dL in critically ill patients, as discussed in both SCCM and ASPEN guidelines. The Normoglycemia in Intensive Care Evaluation and Survival Using Glucose Algorithm Regulation (NICE SUGAR) study found that tighter glycemic control was found to be associated with increased hypoglycemic episodes and increased overall mortality. Glycemic control can be achieved with a short-acting insulin intermittent sliding scale given in 6 hour intervals, or by regular insulin given as a continuous infusion and titrated hourly based on point of care glucose testing. If hyperglycemia remains difficult to control despite these means, formulas with a lower proportion of calories from carbohydrate may be substituted until glycemic control is achieved (Whitson and Barrera 2014; Taylor et al. 2016).

(c) Respiratory Failure

Patients with existing chronic lung disease may tend toward baseline carbon dioxide retention depending on the degree of alveolar ventilation limitation. It was thus postulated that high carbohydrate diets, associated with a higher respiratory quotient, could raise the V_{co2} which varies in proportion to the fat:carbohydrate caloric load. While providing a higher proportion of calories from fat should theoretically lead to a lower CO_2, it is now likely of little consequence given current recommendations for caloric requirement goals. High-fat/low-carbohydrate enteral formulations had previously been thought to be associated with decreased duration of mechanical ventilation, however, these findings could not be reproduced and were only found clinically significant in instances of overfeeding. Providers should, however, carefully monitor phosphate levels, as phosphate is essential for synthesis of ATP and 2,3 DPG which are vital for normal diaphragmatic contractility and thus optimal respiratory functioning (Whitson and Barrera 2014; Taylor et al. 2016).

(d) Renal Insufficiency/Failure

Patients with poor renal function and subsequent decreased glomerular filtration rate are susceptible to multiple electrolyte abnormalities – hyperkalemia, hyperphosphatemia, hypermagnesemia, and, when oliguric or anuric – are also at risk of fluid overload. Thus, close monitoring and careful consideration is needed when selecting the appropriate nutritional therapy for these patients. Patients with acute kidney injury (AKI) or acute renal failure (ARF) should receive standard formulation, energy requirements, and protein requirements. If significant electrolyte abnormalities do develop, specialty formulations with lower potassium and phosphate may be used. For patients requiring renal replacement therapy, there is a baseline tendency toward lean body mass metabolism, as well as significant amino acid loss during hemodialysis. As such, protein should not be restricted in these patients; on the contrary they may have higher protein requirements, upwards of 2.5 g/kg/day. Nitrogen intake above this has not been shown to give any added benefit and may only serve to increase the rate of urea production in such patients. Similarly, hemodialysis patients also require higher doses of water-soluble vitamins, as these too are removed during hemodialysis (Whitson and Barrera 2014; Taylor et al. 2016).

(e) Hepatic Failure

Nutritional risk in these patients is directly correlated to the severity of liver disease, and they usually tolerate general nutritional recommendations. Calculating energy and protein requirements can, however, be difficult in this population, as they commonly have edema and ascites that can render weight-based formulations inaccurate. While dry weight may be difficult to obtain, it is recommended that ideal or dry weight be used in any predictive equations to determine nutritional needs, though IC should be used whenever available. Although patients with portal hypertension are prone to the

development of hepatic encephalopathy, protein should not be restricted. With appropriate lactulose therapy, patients can usually tolerate typical protein recommendations. There is also no current evidence to suggest that branched-chain amino acids lead to improved outcomes compared to standard protein formulations (Whitson and Barrera 2014; Taylor et al. 2016).

(f) Acute Pancreatitis

The delivery of nutritional therapy in acute pancreatitis is directly based on disease severity. No specialized nutrition is required for patients with mild pancreatitis. Instead, these patients should be advanced to an oral diet as tolerated. However, if any complications occur or the patient remains unable to tolerate oral intake for >7 days, enteral nutrition should be initiated. In moderate to severe disease, enteral nutrition should be initiated at a trophic rate and advanced to goal in conjunction with volume resuscitation, with the goal to reach target energy delivery within 24–48 hours of admission. Enteric nutrition is preferred over parenteral because of the better risk/benefit ratio, and there have been no differences in outcome based on gastric or jejunal enteral tube placement. Standard polymeric formula may be used, as current data pertaining to use of immune-modulating formulas is insufficient. If not tolerated, the formula may be changed to a polymeric formula with small peptides or a nearly fat free elemental formulation. If still not tolerating well, the infusion level may also be diverted more distally within the GI tract, and feeds should be given via continuous infusion rather than bolus. Patients that have inadequate nutrition for ≥7 days or have developed complications (fistula, abscess, pseudocyst, ascites, pain exacerbated by feeds) may require parenteral nutrition to meet their energy requirements (Windsor et al. 1998; Whitson and Barrera 2014; Taylor et al. 2016).

2. Surgical

(a) Severe Burn

Patients that have suffered severe burn injury have notably higher caloric requirements due to the hypermetabolic state that ensues post-injury. These patients should have enteral nutrition started early in their hospital course, within 4–6 hours of injury if feasible. Predictive equations are notoriously inaccurate in burn patients, thus IC should be used whenever available. Despite these high requirements, many burn patients do meet their energy needs when provided 25–30 kcal/kg/day. Both the 2001 American Burn Association and the 2013 SPEN guidelines recommend protein in the range of 1.5–2 g/kg/day, as patients that had received daily protein amounts upward of these were found to have increased rates of protein catabolism. Fluid status should also be closely monitored, as these patients have extensive evaporative loses dependent on the percentage of body surface area affected (Rousseau et al. 2013; Whitson and Barrera 2014; Taylor et al. 2016).

Although these patients will likely have frequent trips to the operating room interrupting delivery of enteral feeds, enteral nutrition has still been shown to be more beneficial, as patients that received parenteral nutrition alone or in combination with enteral nutrition were found to have higher infectious rates. Use of a volume-based approach would be advantageous in this patient population, so as to better deliver daily goal energy requirements. These patients may also benefit from immune-modulating formulas containing glutamine, arginine, and omega-3 fatty acids, with studies showing such formulations had a positive effect on indices of morbidity and may reduce the degree and duration of the associated systemic inflammatory response (Martindale et al. 2009; Drover 2011; Taylor et al. 2016).

(b) Trauma

The 2008 Trauma Nutrition Guidelines recommend that a high protein polymeric enteral formula similar to that recommended for other critically ill patients should be initiated within 24–48 hours of injury. Gastric enteral feeding has been found to be safe in this patient population once hemodynamically stable. Because trauma patients typically have frequent pauses in feeds for return trips to the operating room, these patients would likely benefit from a volume-based protocol (Taylor et al. 2016).

Unlike the basic starvation response, the metabolic response to trauma drastically changes metabolism, promoting skeletal muscle breakdown in order to generate the substrates needed to support the immune response and recovery. This, combined with muscle breakdown from prolonged bedrest and subsequent decreased muscle protein synthesis, lead to a rapid deterioration of lean body mass if adequate nutrition is not provided early on in the clinical course. The body's response to traumatic injury is also a dynamic process, with resting energy expenditure peaking 4–5 days post-injury, but remaining elevated for the up to 2–3 weeks. Energy goals should aim for the 20–35 kcal/kg/day range, starting toward the lower end of this range early on with liberalization as the patient clinically improves. Protein goals are similar to that of other critically ill patients, but trauma patients would likely benefit from the higher end of the 1.2 to 2 g/kg/day range due to the increased skeletal muscle breakdown previously discussed. Little data is available suggesting benefit from the use of immune-modulating formulas in this population (Taylor et al. 2016).

(c) Traumatic Brain Injury

Recommendations for traumatic brain injury (TBI) patients regarding energy goals are similar to those of other critically ill patients with a trend toward better outcomes when nutritional therapy is initiated within 24–72 hours of presentation. Protein requirements are, however, slightly higher (1.5–2.5 g/kg/day). While further studies are required, immune-modulating formulations containing arginine or EPA/DHA supplements have recently gained popularity in this population due to the potential for hastened recovery (Taylor et al. 2016).

(d) Open Abdomen

In the absence of bowel injury, patients with an open abdomen are still recommended to receive early enteral nutrition with standard energy and protein requirements. Retrospective studies have found these patients to be safely fed within 24–48 hours of injury, and the use of early nutritional therapy was associated with earlier abdominal fascial closure, less fistula formulation and intra-abdominal complications, and decreased mortality compared to delayed nutritional provision. An additional 15–30g of protein per liter lost is also required in excess of baseline protein requirements to correct for the significant protein loss that occurs as a consequence of the high protein exudate produced from the exposed peritoneum (Taylor et al. 2016).

(e) Post-operative Critically Ill (Surgical Intensive Care Unit – SICU)

As previously discussed, nutritional risk scores, such as the NRS-2002 and NUTRIC score should be used upon admission to SICU in order to determine nutritional risk. When feasible, nutritional therapy should begin within 24 hours of surgery, and enteral nutrition remains the preferred route over parenteral nutrition. While bowel discontinuity or high concern for bowel ischemia would preclude a patient from receiving enteral nutrition, instances such as high output fistulas, bowel anastomoses, open abdomen, use of vasopressors, and mechanical ventilation should not be considered contraindications to its initiation. Meta-analyses have shown that early initiation of enteral feeding has decreased post-operative infectious rates, noninfectious complications, and hospital length of stay. While energy requirements are calculated similarly to medical patients, surgical patients may benefit from high protein formulas, upward of 1.5–2 g/kg/day of protein, to aid in wound healing and recovery. Immune-modulating formulas are recommended over standard polymeric formulas for the post-operative SICU patient. Major surgery can lead to a depletion of arginine levels, which can adversely affect T-cell function, leading to immunosuppression, and EPA and DHA help to reduce systemic inflammation (Drover 2011; Marimuthu et al. 2012; Taylor et al. 2016).

While enteral nutrition is preferred, some procedures may preclude patients from receiving enteral feeds (i.e., upper GI procedure). For such patients, parenteral nutrition should be initiated 7–10 days before the scheduled procedure, as it has not been found to provide the same beneficial effects if only given in the post-operatively period. For patients at high nutritional risk that underwent an emergent procedure and thus were not able to receive pre-operative parenteral nutrition, the post-operative period prior to initiation should be shortened from the usual 5–7 days (Taylor et al. 2016).

For patients that will be receiving an oral diet post-operatively, literature does not suggest any physiologic advantage to advancing from a clear to solid diet. While clears may transit more quickly through the GI tract,

they are also more easily aspirated. Studies showed no increased morbidity or mortality by initiating a solid diet, with some data suggesting quicker return of bowel function in patients that were started with a regular diet rather than slowly advanced from clears (Pearl et al. 2002; Lassen et al. 2008).

3. Obese

Obese patients pose a challenge to clinicians in providing proper nutritional therapy, as clinicians frequently misinterpret high BMI as evidence for adequate nutritional reserves. As such, nutritional assessments may be overlooked in these patients. These patients are at greater risk of insulin resistance and are prone to having difficulty with fuel utilization compared to their lean counterparts. A baseline nutritional assessment should be performed like that of any other hospitalized patient, with special attention paid to determining actual, usual, and ideal body weight, as well as waist circumference. Biomarkers (glucose, lipid panel, etc.) can be used to assess for metabolic syndrome and emerging comorbidities that may make nutritional management more difficult. The presence of central adiposity, metabolic syndrome, BMI $>$ 40, and SIRS places these patients at higher risk of nutritional associated morbidity and mortality, and recommendations are thus based on nutritional optimization (Mauldin and O'Leary-Kelly 2015; Taylor et al. 2016).

Target energy goals should not exceed 65–70% of that predicted by IC. This permissive underfeeding promotes steady weight loss while still providing adequate nutrition. If IC is not available, weight-based equations can be used based on body weight. For a BMI 30–50, 11–14 kcal/kg *actual* body weight/day should be given. For a BMI $>$ 50, 22–25 kcal/kg *ideal* body weight should be provided. Protein requirements are calculated using ideal body weight, with recommendations of 2 g/kg/day for patients with BMI 30–40 and 2.5 g/kg/day for patients whose BMI is $>$40. Subsequent protein requirements can be adjusted throughout the hospital course using nitrogen balance studies to achieve equilibrium whenever possible. A high protein, hypocaloric formula with a low nonprotein calorie to nitrogen ratio is recommended to achieve these goals, as hypocaloric formulations have been associated with shorter length of stay, lower infectious rate, and more vent-free days compared to eucaloric formulas (Dickerson et al. 2002; Taylor et al. 2016).

Complications

A variety of complications can be associated with nutritional therapy, whether enteral or parenteral. The nutritional therapy provided by clinicians is now responsible for meeting the patient's needs for macronutrients, water, vitamins, and minerals, and as such, several metabolic complications can ensue. These complications can range from simple electrolyte imbalances requiring repletion, poor glycemic control and fluid disturbances, or life-threatening conditions like refeeding syndrome.

Energy intake in the form of glucose in excess of the patient's needs can cause various complications. Excessive glucose intake can lead to poor glucose control and hyperglycemia that can, in turn, lead to increased infectious risks. Breakdown of this glucose for utilization via glycolysis generates carbon dioxide, which when present in excess can lead to hypercapnia. Similarly, overfeeding can lead to other metabolic derangements, including hyper- or hypo-osmolarity, acid-base derangements, hypertriglyceridemia, and weight gain. Thus, close monitoring of electrolytes and blood glucose is required to enable detection of electrolyte deficiencies, disturbances in water balance, and glycemic instability. Careful consideration of both formulation and rate must occur in order to ensure patients are getting optimal nutrition while prudently avoiding these complications (Whitson and Barrera 2014; Taylor et al. 2016).

Refeeding syndrome occurs when nutritional support provides a carbohydrate source to a patient that was previously dependent on fatty acid metabolism for energy in starvation state. Patients with prolonged malnutrition are at highest risk, however, anyone with little or no nutritional intake for >5 days can develop this condition, typically within the first 3–4 days of nutritional supplementation. This occurs because the starvation state depletes the body's stores of intracellular cations (magnesium, potassium, phosphorous), as well as ATP and thiamine which serve as substrates for bodily processes that serve in the breakdown of macronutrients to their usable forms. Rapid reintroduction of carbohydrates increases insulin secretion while decreasing glucagon production and stimulates the synthesis of protein, fat, and glycogen, further depleting the body's already limited stores. This in turn leads to cardiac, neurologic, pulmonary, renal, and hepatic dysfunction and failure. Refeeding syndrome can be avoided with early recognition, notably aggressive electrolyte repletion and avoidance of rapid provision of high carbohydrate feeds (Whitson and Barrera 2014).

In addition to complications caused by the formulation of the selected nutritional therapy, there are also mechanical complications that may occur. Aspiration of enteral feeds is a common complication that occurs in patients receiving enteral nutrition. Careful attention should be paid to identifiable risk factors, such as nasogastric feeding tube, mechanical ventilation, poor oral hygiene/care, age $\geq$ 70 years old, frequent transport, bolus feeds, neurological deficits, and existing acid reflux disease. Once recognized, efforts can be made aimed at prevention. These patients should have the head of the bed elevated to 30–45 degrees unless there is a valid reason to do otherwise. Other protective methods include continuous infusion in place of intermittent bolus feeds, use of prokinetic agents and avoidance of unnecessary opioid use and neuromuscular blockade, correction of electrolyte abnormalities, and the use of small bore feeding tubes placed within the small bowel instead of the stomach. Such measures can help to reduce the risk of aspiration in patients known to be prone to this occurrence (Whitson and Barrera 2014; Taylor et al. 2016).

An uncommon but serious complication seen in less than 1% critically ill patients receiving enteral nutrition is bowel necrosis, with patients receiving vasopressor drugs at highest risk. An imbalance between the metabolic oxygen demand required

for digestion and the availability of splanchnic oxygen supply is thought to be the mechanism. While enteral nutrition has generally been proven to be safe, a high index of suspicion is required for patients showing clinical signs concerning for ischemic bowel (Whitson and Barrera 2014).

Post-Discharge Nutritional Maintenance

Recovery from critical illness does not end at discharge, and appropriate nutritional care should be continued after hospitalization in order to improve patient outcomes and prevent hospital readmission. It is imperative that patients have outpatient follow-up arranged to ensure that their nutritional needs continue to be met after leaving the hospital.

Policies and Protocols

In this chapter, we describe at risk populations for malnutrition and its adverse effects on hospitalized patients. Though difficult to fully define malnutrition, we provide two validating scoring systems, NUTRIC and NRS-2002, to help identify at risk patients. Once such patients are identified, we also provide methods to assist in decisions involving mode of nutrient delivery, monitoring tolerance, and disease-specific caveats to nutritional provision.

Dictionary of Terms

- **Anthroprometric** – Any means of measuring body dimensions
- $\mathbf{V_{co2}}$ – Rate of elimination carbon dioxide
- **Enteral Feeds** – Nutritionally complete food (carbohydrates, fat, and protein) delivered to the gastrum or jejunum
- **Parenteral Feeds** – Nutrition delivered through a vein, bypassing the gastrointestinal system
- **Trophic Feeds** – Use of low-volume enteral feeds to stimulate gastrointestinal function
- **Respiratory Quotient** – Ratio of the volume of carbon dioxide delivered to oxygen consumed

Summary Points

- Malnourishment is predominant in the outpatient community, and further nutritional decline is typically seen during patients' hospital course.
- Malnourishment in hospitalized patients is associated with immunosuppression, higher infection rates, increased muscle loss with delayed functional recovery,

impaired wound healing, increased risk of treatment complications, fewer ventilator-free days, longer hospital stays, higher overall treatment costs, and increased mortality rates.

- A nutritional assessment should be performed on all patients presumed to be at risk for malnutrition, utilizing various existing nutritional scores. NRS-2002 and NUTRIC are recommended for use in critically ill patients, as both scores address disease severity.
- Adequate and appropriate nutrition should be initiated within 24–48 hours (earlier in burn and some surgical patients) and is associated with decreased morbidity and mortality.
- Enteral nutrition is the preferred route over parenteral whenever possible.
- Nutritional recommendations may vary slightly based on the underlying medical/surgical condition.
- Outpatient follow-up should be arranged to ensure patients' nutritional needs continue to be met in the community after discharge.

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Effects of Dietary Restriction on Cancer Development and Progression

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Contents

Abstract

The effects of caloric restriction on tumor growth and progression are known for over a century. Indeed, fasting has been practiced for millennia, but just recently

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has emerged the protective role that it may exert toward cells. Fasting cycles are able to reprogram the cellular metabolism, by inducing protection against oxidative stress and prolonging cellular longevity. The reduction of calorie intake as well as short- or long-term fasting has been shown to protect against chronic and degenerative diseases, such as diabetes, cardiovascular pathologies, and cancer. In vitro and in vivo preclinical models showed that different restriction dietary regimens may be effective against cancer onset and progression, by enhancing therapy response and reducing its toxic side effects. Fasting-mediated beneficial effects seem to be due to the reduction of inflammatory response and down-regulation of nutrient-related signaling pathways able to modulate cell proliferation and apoptosis. In this chapter, we will discuss the most significant studies present in literature regarding the molecular mechanisms by which dietary restriction may contribute to prevent cancer onset, reduce its progression, and positively affect the response to the treatments.

Keywords

Caloric restriction · Cancer · Cell proliferation · Diet · Dietary restriction · Fasting · Feeding · IGF-1 · Inflammatory response · Long-term starvation · Molecular pathways · Oxidative stress · Short-term starvation · Therapy response

List of Abbreviations

AKT	Serine/threonine kinase
AMPK	AMP-activated protein kinase
APN	Aminopeptidase N
ATM	Ataxia telangiectasia mutated
BER	Base excision repair
Bm1	B Lymphoma Mo-MLV insertion region 1 homolog
C/EBPβ	CCAAT/enhancer-binding protein β
CAAs	Cancer-associated adipocytes
ChK2	Checkpoint kinase 2
CR	Caloric restriction
DEN	Diethylnitrosamine
DR	Dietary restriction
DSBs	Double-strand breaks
DSR	Differential stress resistance
EGFR	Epidermal growth factor receptor
ERK	Extracellular signal-regulated kinase
FGF21	Fibroblast growth factor 21
FOS	FBJ murine osteosarcoma viral oncogene homolog
FOXO	Forkhead box subgroup O
GCN2	General control nonderepressible 2
H2AX	H2A histone family member X
HCC	Hepatocellular carcinoma
HDAC1	Histone deacetylase 1
HER2	Human epidermal growth factor receptor 2
HopX	HOP homeobox

HSL	Hormone-sensitive lipase
IGF-1	Insulin-like growth factor 1
IGFBP-1	IGF-binding protein 1
IL-6	Interleukin 6
KD	Ketogenic diet
Lgr5	Leucine-rich repeat containing G-protein-coupled receptor 5
LTS	Long-term starvation
MAPK	Mitogen-activated protein kinase.
Msn2/4	Moesin 2/4
mTOR	Mammalian target of rapamycin
mTORC1	mTOR complex 1
NF-KB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NSCLC	Nonsmall cell lung cancer
OGG1	8-Oxoguanine DNA glycosylase 1
PARP-1	Poly (ADP-ribose) polymerase 1
PI3K	Phosphoinositide 3-kinase
PKA	Protein kinase A
PSA	Prostate-specific antigen
RAF	V-Raf-1 murine leukemia viral oncogene homolog
Ras	Rat sarcoma viral oncogene homolog
REV1	DNA-directed polymerase
ROS	Reactive oxygen species
SIRT1	Sirtuin 1
SOD2	Superoxide dismutase 2
SSBs	Single-strand breaks
STS	Short-term starvation
TKIs	Tyrosine-kinase inhibitors
VEGF	Vascular endothelial growth factor

Introduction

In recent years, increasing evidences showed that several types of intermittent, chronic, or periodic dietary approaches, including short-term starvation (STS), long-term starvation (LTS or fasting), caloric restriction (CR), may exert a protective role against aging and other age-related pathologies as well as cancer in humans and numerous animal models (Lee and Longo 2016; Brandhorst and Longo 2016; Longo et al. 2015; Trepanowski et al. 2011). Interestingly, these dietary restriction (DR) regimens showed significant anticancer effects mostly in preclinical models, suggesting the possibility of using these methods to increase lifespan and improve therapy response in cancer patients. However, prolonged fasting periods could impair the patient health conditions already unfavorable due to physiological weight loss (Cleary and Grossmann 2011; Lluch et al. 2014). For this reason, STS (or intermittent fasting), consisting of the lack of food intake for a short time, appears to be the most suitable approach for cancer patients, although there are conflicting

opinions about it. STS aims to slow down growth of tumor, by restricting temporarily its exposure to different nutrients, including glucose, and generating protective effects against cancer (Robertson and Mitchell 2013; Anton and Leeuwenburgh 2013). Conversely, LTS consists of a prolonged food deprivation, resulting in adaptive cellular responses able to decrease inflammatory processes and oxidative stress, enhance energy metabolism, and strengthen cell protection (Longo and Mattson 2014). For example, a serum starvation able to bring down basal cellular activity was applied to several in vitro models, in order to study molecular mechanisms underlying apoptosis, cellular stress response, and autophagy (Pirkmajer and Chibalin 2011). Finally, CR is defined as the reduction in calorie intake aimed to inhibit tumorigenesis and prevent other diseases, including diabetes and cardiovascular pathologies, by inducing an improved insulin sensitivity and reducing the oxidative damage and metabolic rate (Lv et al. 2014; Lefevre et al. 2009).

This chapter aims to provide an overview of the most recent studies present in literature concerning the molecular mechanisms by which dietary restriction may contribute to prevent cancer onset, slow down its progression, and positively affect the response to anticancer therapies, also suggesting a close correlation between diet and reduction of treatment-induced side effects.

Molecular Pathways Involved in Dietary Restriction and Cancer-Related Events

Molecular Changes Induced by Dietary Restriction

Nowadays, the link between cancer and metabolism is becoming increasingly evident (Longo and Mattson 2014; Brandhorst et al. 2017). It is clear that beneficial effects mediated by fasting, in particular by CR, do not involve a single gene, a pathway or a unique molecular mechanism. The benefits are due to the negative regulation of nutrient-signaling pathways, including insulin-like growth factor 1 (IGF-1) pathway and its effector extracellular signal-regulated kinase (ERK), mitogen-activated protein kinase (MAPK), and phosphoinositide 3-kinase (PI3K), which are known to modulate important proliferation pathways (Cangemi et al. 2016). Furthermore, it is well known that genomic instability is a distinctive feature of cancer, and CR tumor response seems to play a key role for the maintenance of genomic integrity (Robertson and Mitchell 2013; Duan et al. 2017). Due to these evidences, new metabolic approaches are being sought today for anticancer treatment. CR, also used in combination with the conventional chemotherapies, has allowed to obtain good results in animal models (Klement and Fink 2016).

CR-Induced Changes in Tumor Microenvironment

The most recent data in literature showed a correlation between aging and neoplastic diseases. It has been observed that aging promotes neoplastic cell growth and

proliferation through surrounding microenvironment alterations. This process, named "adaptive oncogenesis," is determined by tissue decline caused by age and oncogenic cell alterations (Cadoni et al. 2017). Changes in age-associated tissue microenvironment seem to play an important role in cancer and cancer-related diseases. Although the mechanisms responsible for delays in aging and carcinogenesis have not been fully identified, CR is today the only known nongenetic approach able to extend organism life. Nutrient-sensing pathways play a pivotal role in cellular response to CR probably because these regulatory processes are responsible for maintaining a microenvironment that promotes aging and carcinogenicity (Cadoni et al. 2017).

The deacetylase SIRT1 is a protein implicated in regulation effects downstream of CR, in both human and murine models (Cohen et al. 2004). The SIRT1 levels are low in senescent cells probably due to the formation of the C/EBPβ complex and HDAC1, which bind and inhibit SIRT1 promoter. Several studies showed that long-term CR is able to block the formation of the C/EBPβ and HDAC1 inhibitory complex, restoring the functionality of SIRT1 promoter in murine liver cells (Jin et al. 2011).

The SIRT1-activated pathway is also involved in the regulation of forkhead box subgroup O (FOXO) protein, which is deacetylated by SIRT1 in response to oxidative stress. The FOXO1 levels seem to be increased in rat liver cells during long-term CR. This suggests that both SIRT1 and FOXO1 have a modulating role in long-term CR and are responsible for creating a microenvironment that delays aging and prevent cancer (Yamaza et al. 2010).

DR and Inflammatory Response

Several studies showed that DR also plays a role in modulating inflammatory response. Liver cells of diethylnitrosamine (DEN)-induced HCC mice models submitted to DR showed a reduction in levels of NF-kB, a mediator of inflammation associated with cell proliferation and cancer (Duan et al. 2017). A decrease in levels of cytokines and inflammatory chemokines was observed in murine liver, kidney, and spleen tissues (Chiba and Ezaki 2010). Also, mice under 4 weeks DR condition display a reduction of proinflammatory gene expression and an increase in anti-inflammatory gene expression (Robertson and Mitchell 2013; Fig. 1).

DR and Chemotherapy Protection

Proliferation pathways regulated by Ras and AKT are almost always constitutively activated in cancer cells. Cells dramatically reduce the cell division number and become more resistant to stress in response to poor nutrition conditions, such as fasting or DR. This occurs because DR inactivates nutrient-sensing signaling pathways (Brandhorst et al. 2017). The link between cell proliferation, which depends on the nutrient-sensing pathways, and stress resistance is the basis of the protective effect that DR exerts on normal cells compared to tumor cells. This resistance is

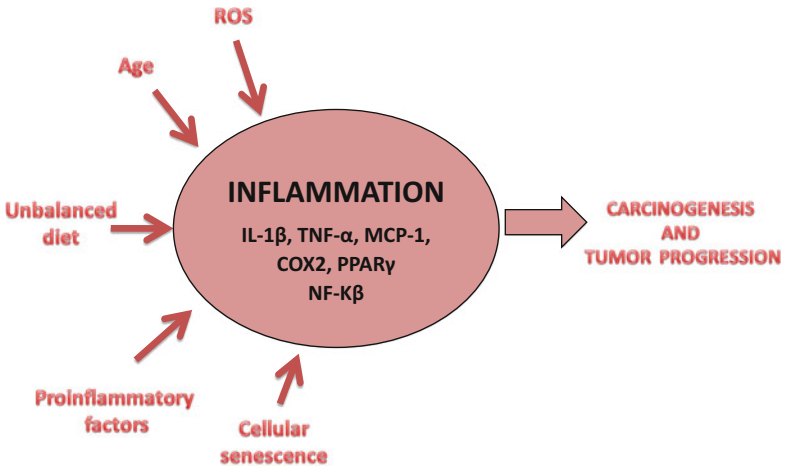

Fig. 1 Association between inflammation and cancer. Unbalance diet, age, cellular senescence, and accumulation of proinflammatory factors and ROS cause cell inflammation

called differential stress resistance (DSR). In fact, tumor cells are unable to protect themselves from stress, because oncogenes negatively regulate resistance genes (Brandhorst et al. 2017; Raffaghello et al. 2008). Moreover, several mutations accumulated in cancer cells make them less able to adapt to extreme environmental conditions created by fasting (Longo and Fontana 2010). Several studies showed that DR-induced DSR may be used to protect healthy cells from the toxic effects of chemotherapy (Brandhorst et al. 2017).

The fasting-induced protection has been shown also in in vivo experiments. CR protects mice from high dose etopoxide toxicity 4, nausea and vomiting induced by doxorubicin, and irinotecan-induced weight loss (Raffaghello et al. 2008; Tinkum et al. 2015). It has also been found that IGF-1 gene deletion protects against chemotherapeutic toxicity of doxorubicin and cyclophosphamide (Brandhorst et al. 2017). Probably, the protective effect induced by DR and starvation is due to a change in microenvironment of the intestinal cryptic stem cells. Indeed, fasting before chemotherapy preserves the correct architecture and functioning of intestinal cells by maintaining the expression of genes such as Lgr5, Bm1, and HopX (Tinkum et al. 2015).

In other studies, it was observed that DR makes cancer cells susceptible to cisplatin-based chemotherapy effects through activation of the ATM/ChK2/p53 signaling pathway, which causes temporary loss of coordination between cell proliferation and growth stimulated by nutrients (Shi et al. 2012).

Correlations Between DR and IGF-1, Insulin, and Cancer

One of the nutrition-related pathways involved in carcinogenesis is the IGF-1 signaling, which affects both sensitivity to oxidative stress and DR. Insulin and

IGF-1 play a pivotal role in controlling metabolism and growth in response to nutritional signals and nutritional state of cells (Shi et al. 2012). IGF-1 pathway regulates cell proliferation and differentiation, showing a tumorigenic effect through apoptosis inhibition (Ramsey et al. 2002; Prisco et al. 1999). Epidemiological studies highlighted the role that IGF-1 pathway plays in cancer pathology. Indeed, high serum IGF-1 concentration is associated with an increased risk of prostate, breast, and colon cancers (Renehan et al. 2004).

A study performed on murine xenograft models showed that deregulation of IGF-1 and PI3K/AKT pathways results in DR resistance. IGF-1 recruits PI3K on cell membrane via binding to tyrosine kinase receptor, resulting in AKT activation. AKT, in turn, phosphorylates and activates downstream effectors that induce cell proliferation (Kalaany and Sabatini 2009).

FOXO1 protein is an effector downstream of IGF-1/AKT pathway, negatively regulated by AKT. This protein is able to modulate the expression of genes involved in oxidative metabolism, stress resistance, and longevity (Cangemi et al. 2016). It has been observed that DR-sensitive cells show a decrease in AKT cytoplasmic levels. This results in FOXO1 nuclear relocation and induction of the proapoptotic and antiproliferative gene transcription. In addition, in vivo studies on xenograft models demonstrated that DR-induced apoptosis increases in tumor cells that overexpress FOXO1. These results are consistent with the antitumorigenic effect of FOXO1 in DR conditions (Kalaany and Sabatini 2009).

A significant role is played by downstream effectors of the PI3K pathway, such as mTOR, AMPK, and SIRT1, which are probably related to cellular sensitivity to DR. Moreover, it has been observed that mutations constitutively activating PI3K protein are important for tumor sensitivity to DR. In fact, an increased sensitivity to DR is observed when PI3K levels decrease. This suggests that molecular analysis of PI3K mutational state could represent an interesting tool to identify DR resistance markers (Lee et al. 2012b; Fig. 2).

Several epidemiological studies showed that there is a strong correlation between increased adiposity and tumor risk (Lee et al. 2012b; Fanale et al. 2017; Wang et al. 2012; Toren et al. 2013; La Paglia et al. 2017). Adiposity is associated with an increase in insulin serum levels. Insulin, an anabolic hormone produced by pancreatic β-cells, exhibits mitogenic effects on many cell types, especially on preneoplastic cells. Furthermore, insulin increases IGF-1 activity, reducing synthesis and secretion of IGF-binding protein 1 (IGFBP-1) (Esposito et al. 2003). Hyperinsulinemia increases concentration of circulating sex hormones, in particular stimulating the production of androgens involved in the growth of different tumors. Several studies showed that DR counteracts metabolic anomalies associated with excessive adiposity, by reducing insulin levels, sex hormones, IGF-1, inflammatory cytokines, prostaglandins, and other various markers of oxidative stress and DNA damage (Esposito et al. 2003, Heilbronn et al. 2006; Fig. 3). According to the data of epidemiological studies carried out on dietary style of Western countries, DR associated with low protein intake has been shown to decrease serum IGF-1 levels in humans (Fontana et al. 2008; Giovannucci et al. 2003).

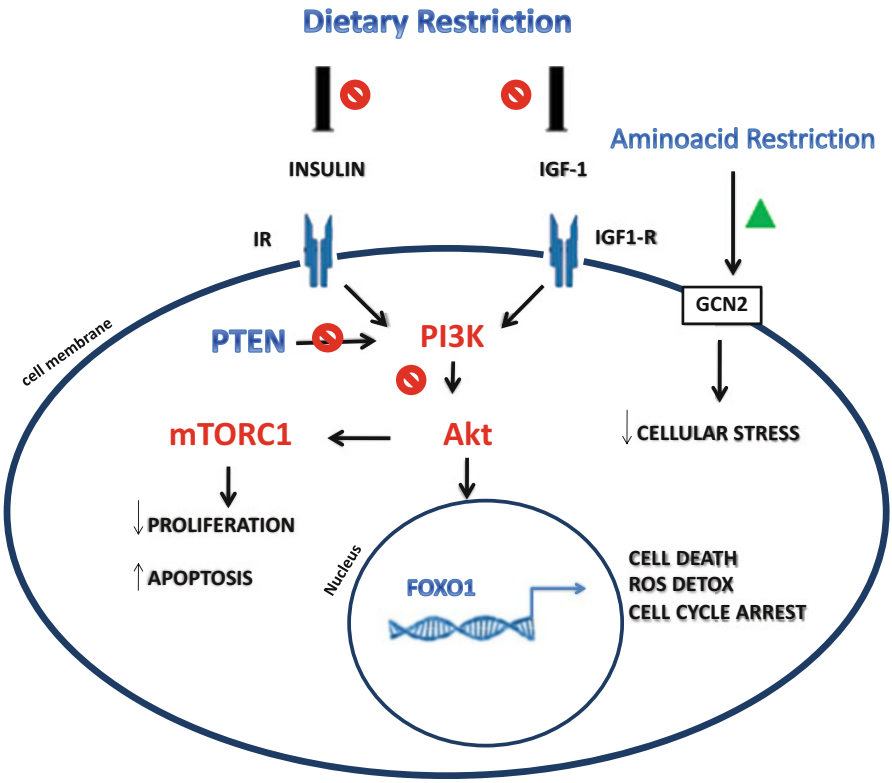

Fig. 2 Molecular pathways modulated by dietary restriction. Dietary restriction decreases the circulating levels of insulin and IGF-1, resulting in inhibition of PI3K/AKT pathway, and leading to increased apoptosis and decreased proliferation

DR in combination with deprivation of essential amino acids triggers protective events for cells, by inducing a decrease in mTORC1 cellular levels and a concomitant increase in amino acid deprivation sensor (GCN2) (Brandhorst et al. 2017).

Although there is still no data regarding the effect of fasting in preventing cancer in humans, the most likely hypothesis is that the effect of DR on IGF-1 levels could generate a protective environment for healthy cells and an adverse environment for tumor cell growth (Longo and Mattson 2014). Another protein restriction marker is FGF21, the fibroblast growth factor, regulated by PPARα whose plasma levels increase during DR associated with protein restriction. In summary, DR has effects on FGF21, IGF-1, and mTOR activity, which are probably linked to carcinogenesis (Klement and Fink 2016).

DR and Oxidative Stress Response

Many studies indicated that the increase of antioxidant factors in tumor cells is mediated by threonine tyrosine kinase Rim15 and transcription factors Msn2/4 and

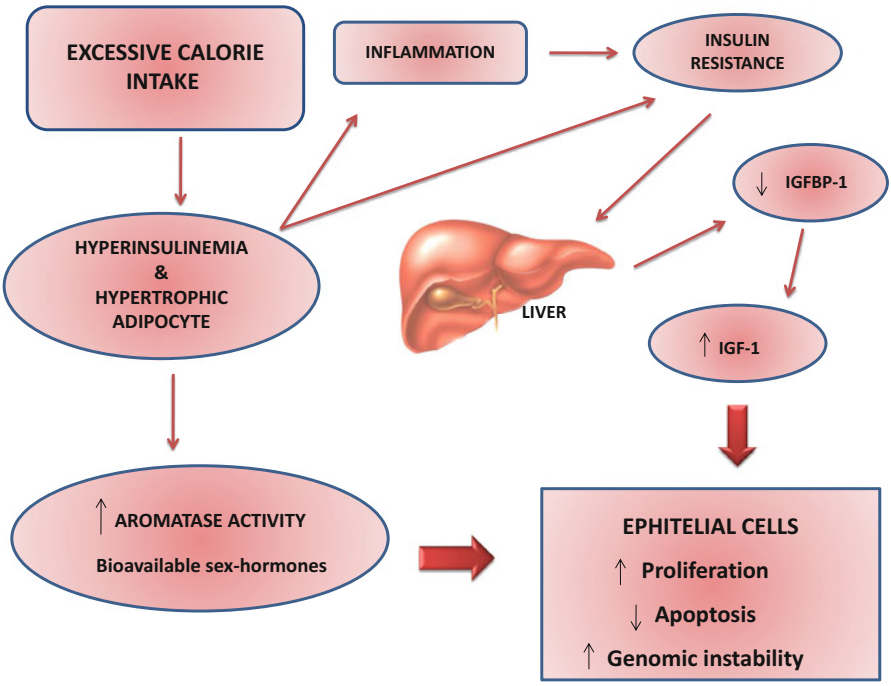

Fig. 3 Correlation between calorie intake and adiposity. Excessive calorie intake causes hyperinsulinemia, hypertrophy of adipose tissue, and increased inflammation

Gis1, which regulate several genes, including mitochondrial SOD2, implicated in oxidative stress resistance (Madia et al. 2009; Hlavata et al. 2003). Additionally, Tor/Sch9 and Ras/AC/PKA pathways regulate the expression of several DNA repair genes, including REV1 gene (Madia et al. 2009).

According to this evidence, Mn-superoxide dismutase (MnSOD) heterozygous knockout mice showed an increased DNA oxidative damage and tumor incidence. This suggests a complex interaction between oxidative stress and cancer (Van Remmen et al. 2003).

Another mechanism induced by DR is the autophagy response to oxidative stress. Autophagy is a process by which cells under DR conditions convey nutrients to essential metabolic processes. DR-induced autophagy is activated by poly (ADP-ribose) polymerase 1 (PARP-1), a nuclear enzyme induced by DNA damage. ROS (reactive oxygen species) production under DR conditions causes DNA damage, which determines PARP-1 activation and fasting-induced autophagy (Cangemi et al. 2016). ROS induce different types of DNA damage, including single-strand breaks (SSBs), double-strand breaks (DSBs), and ionized DNA nucleotides. The repair of latter damage requires the intervention of the base excision repair (BER) system, in particular the OGG1 (8-oxoguanine DNA glycosylase) enzyme 1. Both in vitro and in vivo experiments showed that BER activity is influenced by the availability of nutrients. Indeed, autophagy has no effects on OGG1 expression in the absence of fasting (Siggens et al. 2012).

DEN-induced mice HCC cells exhibit high levels of Caspasi 3, PARP, and Citocromo C, which are proteins involved in mitochondria-mediated autophagy, suggesting that DR suppresses proliferation and promotes apoptosis (Lu et al. 2008; Duan et al. 2017).

According to previous studies (Xie et al. 2007; Standard et al. 2014), gene expression analysis of DEN-induced mice HCC cells revealed that DR restores functioning of many MAPK genes. MAPK pathway regulated by RAS promotes tumor growth and is one of the most important molecular targets for treatment of several types of cancers (Duan et al. 2017).

The Implication of Dietary Restriction in Cancer

The different molecular signature that distinguishes a normal cell from a tumor cell is the main reason that could explain the different susceptibility to growth stimuli. In fact, tumor cells undergo a series of genetic and epigenetic modifications that make their growth independent of the presence of growth factors (Hanahan and Weinberg 2011; Fig. 4). The accumulation of these genetic alterations constitutively activates key components of intracellular pathways. Among these, the most common deregulated signal pathways are Ras/Raf/MAPK and PTEN/PI3K/AKT, responsible for an uncontrolled cell proliferation (Massihnia et al. 2016). The deprivation of nutrients both in vitro and in vivo results in a decrease of growth factor levels in normal cells, forcing thus the cell to enter a proliferative quiescent status (Flemstrom

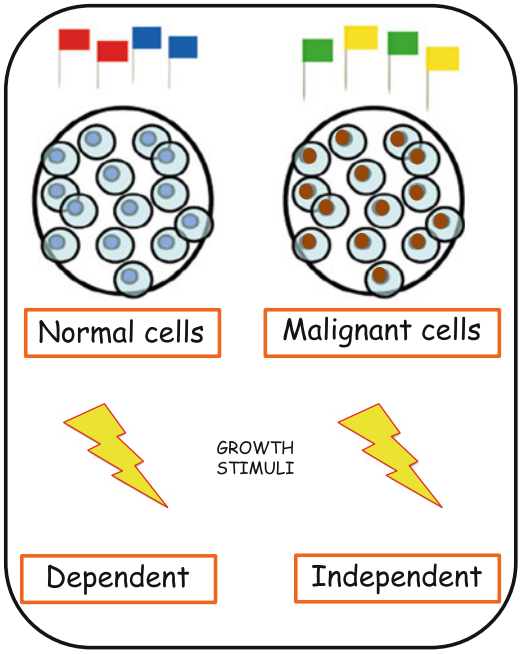

Fig. 4 Growth stimuli of normal and malignant cells. The different molecular profile between normal and malignant cells is responsible for the differences in behavior toward growth stimuli

et al. 2010; Pirkmajer and Chibalin 2011). Unlike normal cells, tumor cells overcome this block by reprogramming their metabolic state and thus maintaining high proliferative abilities (Hanahan and Weinberg 2011). The discovery that different types of dietary restrictions can protect normal cells from the most common side effects of chemotherapy has recently raised interest in its possible clinical application. Moreover, STS seems to protect not only healthy cells but also increase the sensitivity of various types of cancer to the therapy (Lee and Longo 2011). Indeed, fasting in combination with chemotherapy determines increased cytotoxic effects in malignant cells from different types of cancers (Russo and Rizzo 2008). The validity of STS has been evaluated in immunosuppressed nude mice xenograft models in which human neuroblastoma cells were subcutaneously injected. Surprisingly, after 34 days of fasting combined with cyclophosphamide treatment the tumor mass was reduced (Lee et al. 2012a). The usefulness of STS has also been demonstrated for its attenuating properties on chemotherapy side effects. Indeed, its cardioprotective properties have been recently demonstrated during doxorubicin-based treatments (Dirks-Naylor et al. 2014). In addition, a recent work on murine models revealed that following a prolonged fasting of 48–60 h prior to the administration of a high dose of etoposide, the side effects, generally resulting from the treatment, were attenuated (Raffaghello et al. 2008). The synergy between refeeding and DNA damage caused by pharmacological treatment may favor the growth of new foci in various organs including liver, colon, and rectum (Laconi et al. 1995; Premoselli et al. 1998). Interestingly, in a tumor mass, malignant cells are strictly connected with the so-called "cancer-associated adipocytes" (CAAs) and interact with them (Calle and Kaaks 2004). In particular, CAAs show the reduction of peculiar markers including HSL, APN, and resistin, and increased proinflammatory cytokine expression such as IL-6 and IL-1β and TNF-α (Berstein et al. 2007; Ribeiro et al. 2012; Dirat et al. 2011). This altered expression, associated with the production of adipokines, results in a tumor microenvironment variation that favors uncontrolled growth. Therefore, fasting, having a massive effect on the size of adipocytes, can consequently decrease the secretion of tumor-favorable factors (Hermsdorff et al. 2009). Recently, CR efficacy has also been demonstrated in relation to radiotherapy, leading to an increase in the sensitivity to radiation-induced cytotoxicity (Champ et al. 2013). As alternative to standard chemo−/radiotherapy, another type of metabolic therapy has been proposed (ketogenic diet) whose beneficial effects have been demonstrated in the multiform glioblastoma and brain cancer for its antiangiogenic, anti-inflammatory, and antiapoptotic abilities (Seyfried et al. 2015). Below we will discuss deeper the association of chemotherapy and dietary restriction in some of the most spread cancers worldwide looking at the benefits deriving from their combination (Fig. 5).

Breast Cancer

Breast cancer is one of the main causes of cancer deaths in the female population (Fanale et al. 2013). Various clinical studies have shown the efficacy of fasting in the

Fig. 5 Association between dietary restriction and chemotherapy. The figure shows the relevant benefits arising from the association between different types of dietary restriction and drug administration

favorable outcome of the chemotherapy treatment to which some patients affected by breast cancer have undergone. In particular, it seems that a short period of fasting pre- and posttreatment will have a better outcome in terms of patient's tolerability by reducing the side effects. Indeed, the case report of three different patients treated with different therapies and subjected to different times of fasting is below described. A first woman of 51 years with a breast cancer at stage 2A did not show any side effect once subjected to fasting 140 h before and 40 h after treatment with docetaxel and cyclophosphamide. The validity of the association was confirmed in a second 53-year-old patient, also suffering of a tumor in stage 2A and HER2+. In particular, chemotherapy cycles associated with fasting 64 h before and 24 h after were not accompanied by high toxicity effects or in any case with negligible and/or reversible transient effects. The third case saw a 78-year-old patient with a HER2+ tumor, after mastectomy and subjected to variable fasting periods in the course of carboplatin-based, docetaxel and trastuzumab-based chemotherapy cycles. Significant levels of pharmacological toxicity have not been reported (Safdie et al. 2009; Table 1).

Ovarian Cancer

Among gynecological tumors, ovarian cancer is one of the most common and the fifth cause of death in the female population (Reid et al. 2017). Al-Wahab et al. (2014) published a study showing the effects of energy balance in mouse models

Table 1 Case reports of different tumors. The table summarizes the cases described in the text and related fasting schedules adopted in pre- and posttreatment

Breast cancer	Case I (51yo)	140 h before 40 h after treatment
	Case II (53yo)	64 h before 24 h after Treatment
	Case III (78yo)	Not shown
Ovarian cancer	Case I (44yo)	62 h before 24 h after treatment
Lung cancer	Case I (61yo)	48 h before 24 h after treatment
Prostate cancer	Case I (74yo)	60 h before 24 h after treatment

subjected to high energy diet or CR conditions. Mice group under high-energy diet showed the most extensive tumor formation accompanied by the highest tumor score at multiple sites. Moreover, they showed increased levels of insulin, leptin, IGF-1, VEGF, and proinflammatory factors (IL-6). Instead, the mice group under CR showed a lower tumor burden as well as a great reduction in insulin, IGF-1, leptin, MCP-1, VEGF, and IL-6 levels (Al-Wahab et al. 2014). Also, clinical trials demonstrated the effectiveness of the association chemotherapy/DR. The case of a 44-year-old woman suffering from ovarian cancer has been emblematic, because she has benefited from the antineoplastic treatment in combination with STS carried out 62 h before and extended 24 h after drug treatment (Safdie et al. 2009; Table 1).

Lung Cancer

Lung cancer is one of the major causes of cancer-related morbidity and death in men and women population worldwide. Depending on EGFR mutational status, therapy may vary in favor of tyrosine-kinase inhibitors (TKIs). Among them, erlotinib is one of the most commonly used TKIs in I and II line of treatment (Passiglia et al. 2017). Currently, the recommended dose is 150 mg under complete fasting conditions or 2 h after the meal consumption. Two modalities of administration are resulted able to determine a different drug absorption and consequent increase in therapeutic efficacy. In particular, drug seems to have greater effect when the administration takes place 2 h after the meal (Katsuya et al. 2015). An interesting clinical case is that of a 61-year-old NSCLC patient who has seen mitigating the side effects of drug therapy after STS 48 h before and 24 h after therapy (Safdie et al. 2009; Table 1).

Prostate Cancer

Prostate cancer is considered as the second cause of cancer-related death in male population (Vanacore et al. 2017). Interesting is the case of a 74-year-old man

diagnosed with stage 2 prostate adenocarcinoma. The patient has undergone several cycles of chemotherapy during which he faced many side effects such as fatigue, weakness, short-term memory impairment, and peripheral neuropathy. Moreover, PSA levels raised with an unstoppable trend. After many failed attempts, patients were enrolled in a rigorous fasting schedule consisting of restrictions 60 h prior to and 24 h post drug administration. After treatment, PSA levels dropped dramatically and a marked reduction of side effects was reported (Safdie et al. 2009; Table 1).

Conclusion

Recently, accumulating evidence showed that starvation condition seems to play a pivotal role in preventing cancer development and progression as well as improving the response to different therapeutic treatments. The above presented studies and considerations seem to confirm the protective role exerted by fasting against cancer. However, a potential limitation is represented by time required for each cancer patient to obtain an optimal fasting condition, since a severe nutrient deprivation for several months may be needed, as showed by in vivo preclinical analyses. Furthermore, not all patients are fit to undergo such dietary regimens, because many of them are subject to weight loss due to the chemotherapy toxicity and tumor itself. For this reason, since CR and ketogenic diet (KD) have been shown to be approaches particularly effective unlike intermittent fasting whose role is still controversial, further clinical studies are yet necessary in order to assess the safety and efficacy of these methods.

Policies and Protocols

Protocol for Maintaining Cancer Cells Under Short-Term Starvation Conditions

In this chapter, we have discussed the most significant studies present in literature concerning the molecular mechanisms by which dietary restriction may contribute to prevent cancer development, slow down its progression, and positively influence therapy response. Since most of the current experimental evidences concerning the correlation between dietary restriction and cancer arise from studies mainly performed on in vitro preclinical models, here we discuss a rapid and convenient method for maintaining the healthy and malignant breast cells under short-term starvation conditions restricting the supply of glucose. Glucose metabolism represents a primary source of energy able to support cell proliferation and regulate cell death-related signaling pathways. The impaired balance between excessively high glucose consumption and its poor supply determines glucose deprivation in the tumor microenvironment, activating a positive feedback mechanism that involves ROS production by NADPH oxidase and mitochondria, inhibition of tyrosine

phosphatases by oxidation, and amplification of tyrosine kinase signaling in cells dependent on glucose for their survival.

Both cell lines are grown at 37 °C, 5% CO2, and 80% confluence, in a culture medium DMEM (Dulbecco's Modified Eagle Medium) containing high glucose concentration (4.5 g/l D-glucose, 110 mM pyruvate) and enriched with fetal bovine serum (10% FBS), nonessential amino acids (NEAA-1%), and streptomycin-penicillin (1% Strepto/Pen). For short-term starvation experiments aimed at establishing a glucose deprivation, cells are washed twice with PBS (phosphate-buffered saline) and then incubated in glucose-free DMEM without pyruvate and supplemented with 10% FBS, 1% NEAA, 1% Strepto/Pen.

Dictionary of Terms

- **Caloric restriction** – Reduction of calorie intake that implicates feeding once daily or thrice weekly. It can be distinguished in two forms: intermittent caloric restriction and chronic caloric restriction.
- **Dietary restriction** – Condition of short- or long-term fasting which can involve the lack of food consumption for short or prolonged periods. It includes the caloric restriction, short- and long-term starvation, and ketogenic diet.
- **Hyperinsulinemia** – The increase in circulating insulin levels that lead to a greater IGF-1 activity, by reducing synthesis and secretion of IGF binding protein 1, and elevated concentration of circulating sex hormones.
- **Inflammatory response** – Set of actions exerted by the immune system to fight an inflammation through release of several factors that mediate this response, including chemokines and cytokines.
- **Therapy response** – Assessment of the extent of sensitivity or resistance of a tumor to a specific anticancer treatment.

Summary Points

- This chapter focuses on molecular mechanisms by which dietary restriction may contribute to prevent cancer onset and improve therapy response.
- Dietary restriction involves food deprivation for short or prolonged periods.
- Dietary restriction includes short-term starvation, long-term starvation, caloric restriction, and ketogenic diet.
- Numerous experimental evidences showed that fasting may exert a protective role against aging and other age-related pathologies as well as cancer.
- Preclinical models suggested the potential use of fasting to induce anticancer effects and improve patient life's quality.
- However, prolonged fasting periods could impair the patient health conditions already unfavorable due to physiological weight loss induced by tumor itself.

- Fasting-mediated benefits seem to be mediated by the reduction of inflammatory response and down-regulation of nutrient-related signaling pathways able to modulate cell proliferation and apoptosis.
- Good results were obtained in animal models by associating caloric restriction with conventional chemotherapies.
- Deregulation of IGF-1 and PI3K/AKT pathways causes dietary restriction resistance.
- This chapter describes some case reports for different types of cancer.

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Part IX

Life Stages, Pregnancy, the Young, and Elderly

Maternal Undernutrition and Developmental Programming: Implications for Offspring Reproductive Potential

70

Stella Chadio and Basiliki Kotsampasi

Contents

Abstract

A growing body of evidence suggests that the concept of developmental programming can also be applied to reproductive system development and function. Variation in the nutrient supply during fetal life and particularly maternal undernutrition has been proposed as a dominant cause of programming. This chapter reviews the existed data from animal and human studies on the impact of maternal undernutrition on later reproductive health and competence. Specific outcomes depend on the severity, duration, and stage of development when nutritional perturbations are imposed, while sex-specific effects are also manifested.

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Human cohort studies linking maternal undernutrition, reflected by weight at birth, to a number of reproductive health outcomes and fertility measures are also presented and critically evaluated. Maternal undernutrtion affects offspring gamete quality and reproductive parameters, while effects on fertility are still questionable. Evidence exists for an effect of maternal undernutrition on ovarian reserve, indicating an impact on the time of menopause and reproductive ageing. Mechanisms underlying reproductive programming are still poorly understood. They might include altered cell proliferation/apoptosis, changes in hormone levels or receptor abundance. The periconceptional period, during which major epigenetic changes take place, is of critical importance. Maternal malnutrition can disturb the apposition of epigenetic marks throughout this period, leading to detrimental reproductive outcomes in later life. Evidence also exists that adverse outcomes extend beyond first generation to induce transgenerational effects, through epigenetic mechanisms.

Delineating the mechanisms responsible for long-lasting effects of early nutritional programming will help in developing useful interventions during periconceptional and fetal life to ensure reproductive health in later life.

Adopting policy strategies to prevent and improve maternal nutritional status is a long-term investment that will benefit the present as well as the future generation.

Keywords

Maternal undernutrition · Fetus · Programming · Periconceptional · Offspring · Reproduction · Fertility · Ovarian reserve · Epigenetics · Transgenerational

List of Abbreviations

AGA	Appropriate for gestational age
AMH	Anti-Müllerian hormone
BW	Body weight
DOHaD	Developmental origin of health and disease hypothesis
HPG	Hypothalamic–pituitary–gonadal
IGF-1	Insulin-like growth factor-1
IVF	In vitro fertilization
SGA	Small for gestational age
TTP	Time to pregnancy: The time it takes to become pregnant when planning pregnancy

Introduction

Maternal nutrition is of critical importance for fetal growth and development. A compromised nutrient supply will result in low-birth-weight neonates which suffer an increased risk of perinatal mortality. Apart from short-term outcomes, accumulating evidence from animal and human studies indicate that maternal nutritional status can also affect offspring future health and well-being. This has given rise to the "developmental origins of health and disease"(DOHaD) or developmental

programming hypothesis, which implies that adverse environmental factors acting in utero program the development of fetal tissues, resulting in permanent changes to the structure, physiology, and metabolism in offspring, leading to detrimental consequences and diseases in later life (Wadhwa et al. 2009).

Variation in the nutrient supply during fetal life in terms of both quantity and quality and especially maternal undernutrition has been highlighted as a dominant cause of programming. To date such nutritional programming effects have been largely characterized in terms of susceptibility to cardiovascular and metabolic diseases (McMillen and Robinson 2005). However, in the last years, the DOHaD approach has been extended to encompass programming of reproductive function and health, as reproductive axis and its hormonal control systems are largely established in fetal life, thus being particularly vulnerable to nutritional programming (Chadio and Kotsampasi 2014).

The way through which early nutrition contributes to the onset of later detrimental outcomes likely involves a complex interaction between the maternal environment, placental changes, and epigenetic programming of the embryo. The mechanisms underlying programming effects may include tissue remodeling by alterations in cell proliferation or differentiation, resulting to altered organ structure and function (Hoppe et al. 2007). Endocrine programming is also considered a possible mechanism, since hormones influenced by maternal undernutrition can directly or through changes in placenta phenotype act on the fetal tissues to alter cell growth and differentiation, consequently affecting their function later in life (Harding et al. 2010). Particularly, glucocorticoid exposure has been implicated as a mediator of developmental programming effects (Seckl 2004) and in this respect maternal undernutrition has been shown to alter HPA axis function in young and adult offspring (Chadio et al. 2007).

Finally, epigenetic modulation of gene transcription provides the most plausible mechanism through which fetal nutrient supply can alter gene expression in the developing fetus, leading to later permanent effects. Epigenetic regulation of gene expression include DNA methylation, chromatin and histone modifications, and noncoding RNAs, which can initiate and regulate epigenetic changes in both DNA and histones (Sabin et al. 2013). Effects on reproductive potential and fertility could possibly be mediated by altered expression of genes involved in gonadal development and function and/or in the regulation of the HPG axis. Moreover, alteration in gene expression, if present in germline, may also result in transgenerational effects (Fig. 1).

Nutritional Programming and Gonadal Development

Animal models most widely used in developmental programming studies have been rodents and sheep. Rodents offer significant advantages due to their short gestation period, while studies in sheep provide power for translation to human pregnancy, as sheep have a long gestation period enabling targeting of specific developmental windows during pregnancy and produces a fetus comparable in size to humans.

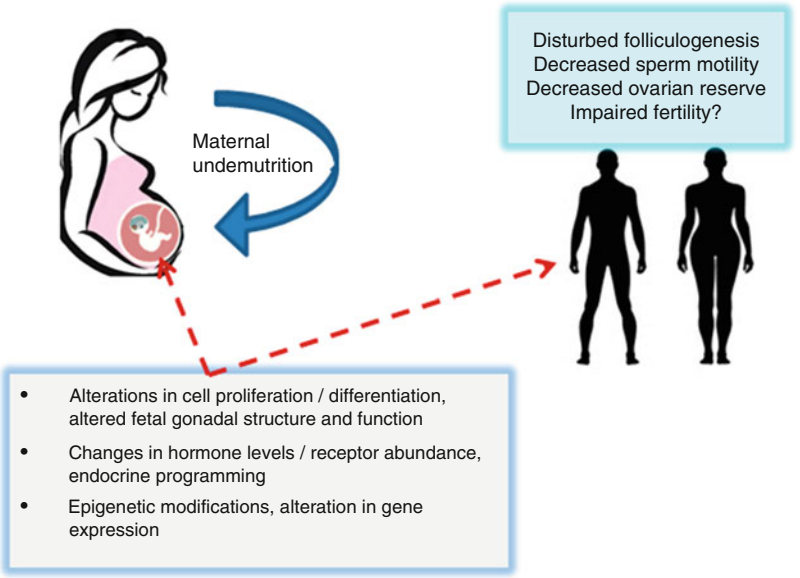

Fig. 1 Nutritional programming of reproductive function

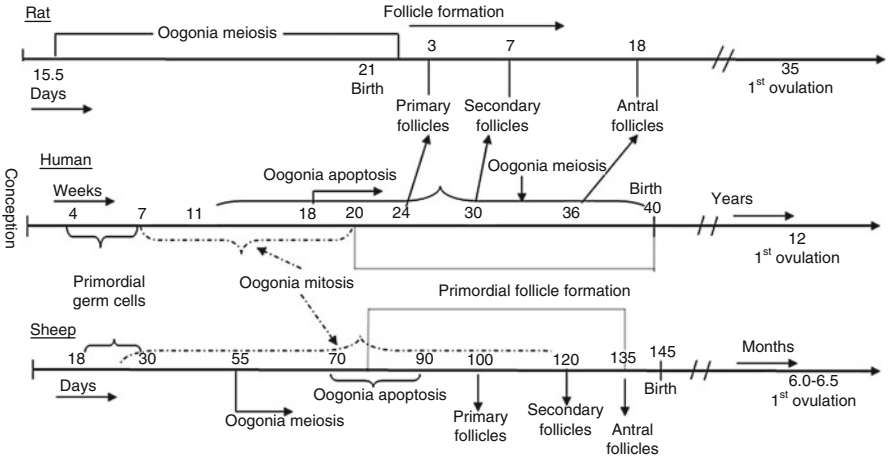

Fig. 2 Milestones of ovarian development in rat, sheep, and human

In females, normal ovarian development during embryogenesis determines the fertility and reproductive capacity later in life (Sarraj and Drummond 2012), while in males, spermatogenesis relies on the establishment of a normal adult Sertoli cells number which are the primary determinant of sperm production and testes size in adulthood and proliferate during fetal, neonatal, and peripubertal period (Sharpe et al. 2003) (Figs. 2 and 3).

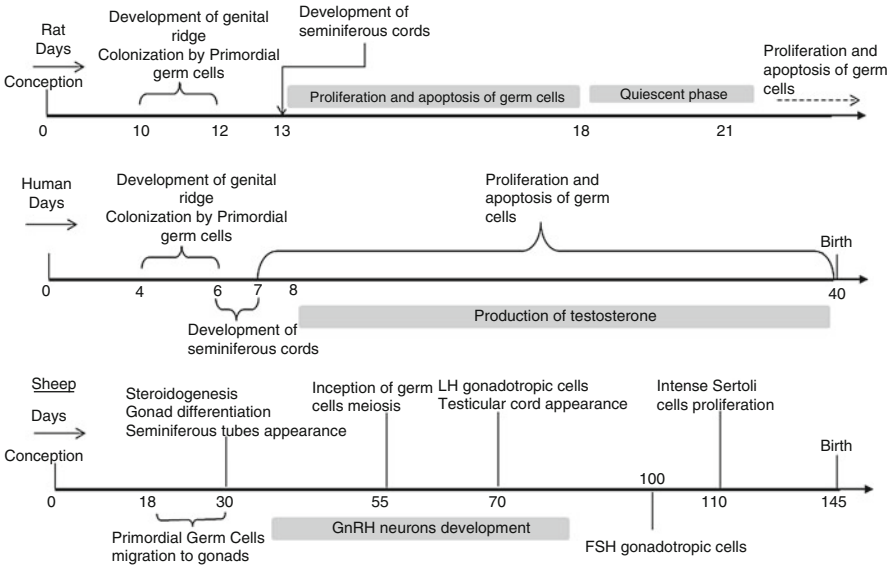

Fig. 3 Milestones of testis development in rat, sheep, and human

A number of animal studies examining the effects of in utero undernutrition on fetal gonadal development indicate a detrimental effect on follicular number and development. In female rats, maternal food restriction imposed during late gestation and/or lactation resulted in a decrease in uterine and ovarian weight, along with disturbed folliculogenesis, reflected by a greater number of antral follicles of small size and a reduced number of graafian follicles of large size (Leonhardt et al. 2003).

In sheep fetuses undernourished during the first month of gestation, a delay in oogonial meiosis and follicular development has been reported (Rae et al. 2001), probably due to alterations in the apoptosis-proliferation balance, leading to reduced follicle number postnatal (Lea et al. 2006). In the same animal model, maternal undernutrition increased the incidence of DNA damage in fetal oogonia (Murdoch et al. 2003), while from early to mid-gestation, it decreased fetal ovarian vascular development, possibly affecting follicular quality (Grazul-Bilska et al. 2009).

On the other hand, it should be emphasized that early perturbations could be of significance only if long-lasting effects are considered. In this regard, rat adult offspring of nutritionally restricted dams showed reduced numbers of primordial and antral follicles, along with changes in key ovarian gene expression and elevated levels of ovarian oxidative stress, which has been associated with ovarian ageing (Bernal et al. 2010). Furthermore, data from sheep showed a sex and window of exposure effect of undernutrition in utero on subsequent gonadal development and function in adult animals. In particular, increased accumulation of antral follicles of small size or reduced number of corpora lutea, consistent with anovulation, were found in 10-months-old female offspring born to mothers undernourished from day 1–30 or day 30–90 of gestation, respectively (Kotsampasi et al. 2009a). In males,

Table 1 Impact of maternal undernutrition on gonadal development and function in offspring

Species	Duration of gestational undernutrition	End points	Effects	Reference
Rats	Late gestation/ lactation	At birth	Disturbed follicullogenesis	Leonhardt et al. 2003
Rats	Whole gestation and/ or lactation	Adult offspring	Reduced number of primordial follicles, increase in oxidative stress, accelerated ovarian ageing	Bernal et al. 2010
Rats	Low protein diet during gestation	12/ 24 week of age	Reduced number of primordial follicles	Aiken et al. 2013
Sheep	0–30 day	Fetal day 50	Delayed ovarian follicular development	Rae et al. 2001
Sheep	65–110 day; 0–110 day	Fetal day 110	Increased rate of apoptosis	Lea et al. 2006
Sheep	28–78 day	Fetal day 78	Increased oxidative damage in oogonia	Murdoch et al. 2003
Sheep	50–135 day	Fetal day 135	Decrease in ovarian vascular development, follicular quality	Grazul-Bilska et al. 2009
Sheep	0–30/31–100	10 months of age	Reduced Sertoli cell number	Kotsampasi et al. 2007a
Sheep	0–30/31–100	10 months of age	Increased number of small antral follicles, decreased number of corpora lutea	Kotsampasi et al. 2007b
Cows	−11 to 110 day	Adult	Diminished ovarian reserve, lower AMH levels, reduction of antral follicle count	Mossa et al. 2013

maternal undernutrition during early to mid-gestation resulted in a reduction in the number of Sertoli cells, accompanied by an increased apoptotic rate, indicating a direct gonadal effect (Kotsampasi et al. 2009b).

These findings in sheep were more recently confirmed in cows, in which maternal nutrient restriction during the first third of pregnancy resulted in diminished ovarian reserve, as measured by AMH and FSH levels and antral counts up to 86 weeks of age (Mossa et al. 2013) (Table 1).

In human studies, given the difficulty to retrospectively assess nutrition in utero, birth weight is usually used as a proxy for fetal nutritional status. In particular, small weight at birth may be the result of either maternal undernutrition or reduced nutrient delivery to the fetus due to different placental insufficiency.

Prospective studies with SGA girls, followed by catch-up growth, showed reduced uterine and ovarian volume and a marked low ovulation rate during their

adolescence (Ibáñez et al. 2002a), suggesting poor ovarian reserve. Furthermore, prenatal growth restraint was found to be followed by elevated serum FSH concentrations in infant girls and boys (Ibáñez et al. 2002b), probably reflecting reduced fractions of granulosa and Sertoli cells within the gonads.

In SGA males, a number of studies have shown reduced gonadal function, as compared with those born of AGA weight (Vanbillemont et al. 2010), while others failed to detect any significant relationship between BW and gonadal function (Olsen et al. 2000; Jensen et al. 2007).

Taking together, the above data point out to sex-specific effects of developmental programming and further emphasize the significant influence of timing and duration of maternal undernutrition on programming gonadal development and function. However, to date there is no direct link between maternal undernutrition and epigenetic modifications that relate to a fertility phenotype, mainly because these mechanisms have not been widely investigated so far.

Does Nutritional Programming Compromise Future Fertility?

A common finding among studies in rodents, sheep, and humans is the disruption of follicular development that follows undernutrition in utero. Therefore, it is of significant importance to determine if this prenatal compromise translates into any significant functional deficit in subsequent fertility, the main outcome of reproductive function.

Data from human studies regarding nutritional programming of reproductive function are very limited. Apart from the well-recognized general limitations of epidemiological studies due to various methodologies, definitions, follow-up periods, and inclusion criteria involved, there are some added parameters related to the study of reproductive function in human populations, such as social or cultural differences which could potentially complicate the interpretation of the observed outcomes. The Dutch famine paradigm (the consequence of a German-imposed food embargo in the western part of the Netherlands toward the end of World War II in the winter of 1944–1945) provided a unique opportunity to address the impact of maternal undernutrition during different gestational windows on later health parameters, including reproductive competence. Follow-up studies of this historical women cohort, examining a number of fertility markers (age at first pregnancy, completed family size, and interpregnancy interval) do not support a detrimental effect on fertility of those exposed to famine as embryos (Lumey and Stein 1997; Lumey 1998). In contrast, a substantial increase in the risk for sterility among rural, but not urban, Chinese women was detected for those conceived and born during the 1959–1961 Chinese famine, indicating that exposure to acute malnutrition in utero may lead to a long-term negative impact and impaired fecundity (Song 2013). No consistent results have also been reported when birth weight was taken as a surrogate for intrauterine nutritional status. A French cohort study reported no evidence of any

relation between BW and fertility of both men and women (Meas et al. 2010), in agreement with recently reported findings from a large longitudinal study based on the Aberdeen Maternity and Neonatal Databank (AMND) records (Shayeb et al. 2014). On the contrary, reports from a case control study in Swedish women seeking infertility treatment revealed that those with low birth weight or SGA had an increased risk for infertility due to female factor (Vikstrom et al. 2014). In support of this, reports from the Danish National North Cohort study revealed an association between low maternal birth weight and a TPP of more than 1 year, indicating effects on fecundity (Nohr et al. 2009).

However, given the well-recognized impact of the catch-up growth that follows in utero growth restriction in mediating many of the adverse metabolic outcomes in adulthood (Gluckman et al. 2005), it is likely that a mismatch between the predicted and actual environment may also underlies some of the effects on reproductive function, a hypothesis that needs further elucidation. Furthermore, it should be not ignored that birth weight cannot be considered an accurate measure, since the critical windows during which the fetus is vulnerable to maternal nutrient disruptions occurs before measurable effects on fetal weight are expressed, and more importantly programming effects may well be expressed in the absence of any changes in birth weight (Chadio et al. 2007; Kotsampasi et al. 2009a, b). Evidence for epigenetic differences among individuals who were exposed to famine early in gestation, but exhibiting a normal birth weight, strengthens this aspect further (Heijmans et al. 2008).

In males, data on the relationship between birth weight and gonadal function are still conflicting, ranging from no effect of birth weight on semen quality (Ramlau-Hansen et al. 2010; Olsen et al. 2000; Ozturk et al. 2001) to a significant association between birth weight and testosterone levels, independent from adult weight (Vanbillemont et al. 2010). Previous studies reported lower BW in subfertile men of unknown etiology (Francois et al. 1997), while birth weight was found to be associated with sperm DNA fragmentation and inversely correlated with total sperm count in primary infertile men (Faure et al. 2015).

Interestingly, very recent data support an association between birth weight and male reproductive function, as men with low birth weight were presented with reduced sperm motility, but normal sperm morphology compared to normal or high birth weight (Boeri et al. 2016).

Overall, despite the methodological inadequacies of individual study results, accumulating evidence from animal and human studies points toward an impairment of gonadal function caused by perinatal growth restriction, probably associated with increased risk of reproductive health.

Nutritional Programming and Reproductive Life Span

Accumulating evidence support a relationship between early life events and increased risk of premature adrenarche, early puberty and associated fertility problems. In this respect birth weight, taken as a surrogate for in utero nutrition has been

reported to affect sexual maturation (Hernández and Mericq 2008), although data on the relationship between birth weight and age at menarche are controversial, mainly due to heterogeneity in the study designs. An association between low birth weight and earlier onset of puberty or menarche has been reported in a number of Cohort studies (Cooper et al. 1996; Ibáñez et al. 2000), suggesting an effect of birth weight per se. On the other hand, different lines of evidence clearly indicate that SGA children gaining body weight rapidly develop central adiposity, accompanied by elevated IGF-1 and leptin concentrations and insulin resistance, factors that contribute to precocious pubarche and earlier menarche (Ong et al. 2000). The normalizing effect of treatment with an insulin sensitizer confirms that insulin resistance is the key mechanism linking a post-SGA state to early menarche (Ibáñez et al. 2006). These findings clearly support a central role for an interplay between low birth weight and accelerated weight gain in determining age at menarche and further support the suggestion the timing and intensity of the catch-up growth that follows prenatal growth restraint may have consequences on the endocrine, metabolic, and reproductive systems that persist over time (Ibáñez et al. 2011).

On the other hand, evidence for an association between maternal nutrition and age at menopause is more straightforward. It is well accepted that in females the follicular reserve is set up during the fetal life and serves as the source of developing follicles and oocytes for life time supply (McNatty et al. 1995). Therefore, a key end point to evaluate the possible effects of nutrition in utero on ovarian reserve is offered by examining the age at natural menopause. Data on the relation between maternal nutrition and age at menopause are very limited, while more frequently reported are studies on the association between birth weight, taken as a proxy for intrauterine nutritional status and age at menopause. In this regard, although earlier reports from the Dutch Hunger paradigm found no such an association, more recent data from the same cohort reported an increased likelihood of menopause at any given age for those women exposed to Dutch famine in utero (Yarde et al. 2013). Furthermore, data from a British birth cohort study using birth weight standardized for gestational age revealed that both extremes of birth weight (<2.5 kg or >4 kg) were indeed associated with an earlier age at menopause (Tom et al. 2010), suggesting that prenatal nutrition can probably influence reproductive lifespan in adult life. Evidence for such an association has also been shown in SGA girls that are thought to be at an increased risk of experiencing premature infertility (Vikstrom et al. 2014), although these results are still contentious (Meas et al. 2010).

It is interesting to note that a reduction in the age at menopause was also detected for women exposed to Dutch winter famine during their childhood (Elias et al. 2003). These observations along with data from poorer, rural communities (Kapur et al. 2009) points out to an effect of nutrition on age at menopause. However, there is still need to clarify the relative contributions of early versus later life nutrition, especially in the light of the well-known effects of catch up growth in developmental programming paradigm (Gluckman et al. 2005).

It is well accepted that ageing of the reproductive system occurs more rapidly than somatic ageing (Li et al. 2012), making the reproductive tract particularly sensitive to the ageing aspects of developmental programming exposures, such as maternal

Table 2 Impact of low birth weight or undernutrition in utero on fertility parameters in humans

Females/ males	Birth weight/ nutrition in utero	End point	Effect	Reference
Females	SGA	Adolescence	Reduced ovulation rate	Ibáñez et al.
Females	Low BW	Adult	Reduced fecundity	Nohr et al. 2009
Females	Low birth weight/ SGA	Adult	Increased risk for infertility	Vikstrom et al. 2014
Females	BW <2.5 kg or >4 kg	Adult	Early age at menopause	Tom et al. 2010
Females	Undernutrition in utero	Adult	No effects on fertility	Lumey 1998
Females	Undernutrition in utero	Adult	Earlier menopause	Yarde et al. 2013
Males	SGA	Adult	Reduced gonadal function	Vanbillemont et al. 2010
Males	Low birth weight	Adult	No effects on semen quality	Ramlau-Hansen et al. 2010; Olsen et al. 2000; Ozturk et al. 2001
Males	Low birth weight	Adult	Reduced sperm motility	Boeri et al. 2016
Males-females	Low birth weight	Adult	No impact on fertility	Meas et al. 2010; Shayeb et al. 2014

undernutrition. A number of elegant studies in the rat model of low-protein maternal nutrition showed an increased oxidative stress in offspring ovaries and oviducts along with increased mitochondrial DNA copy number and telomere shortening, all indicative of accelerated ageing. Most importantly, this accelerated cellular ageing was accompanied by a decline in the ovarian reserve in programmed animals (Aiken et al. 2013). This kind of insights from animal studies shed more light on the molecular mechanisms that underlay reproductive senescence and might have significant implications in light of the current trend toward later childbearing in many populations. However, there is need to be further explored in longitudinal human studies, before any safe conclusion can be drawn (Table 2).

The Importance of Periconceptional Nutrition and the Role of Micronutrients

Maternal nutrition can influence the development of the fetal reproductive system at all stages of development. However, recently the periconceptional period has attracted more attention in the context of DOHaD concept, as being the potentially more critical period in which developmental plasticity is vulnerable to environmental challenge, including maternal malnutrition. This period

encompasses gametogenesis, fertilization, conceptus formation, implantation, and placentation, which represent particular time windows during which epigenetic changes can have long-lasting consequences on the development and function of the tissues and therefore long-term effects on phenotype and diseases (Attig et al. 2010). The gametes and the embryo represent particularly sensitive stages, during which the epigenome can be altered. Changes to the immediate environment surrounding oocytes, probably resulting from nutritionally induced changes in hormone and metabolite levels, can alter the pattern of genes expressed by ovarian follicles, impacting immediate and longer-term development (Ashworth et al. 2009). Moreover, preimplantation embryo has been shown to be sensitive to environmental perturbations with long-term consequences (Fleming et al. 2004).

The vital role of periconceptional nutrition has been highlighted in studies involved in diet manipulation of donor animals. In particular, a reduced number of cleaved oocytes following IVF has been reported for ewes undernourished from 8 weeks prior to conception (Grazul-Bilska et al. 2012), while maternal global undernutrition has been shown to reduce blastocyst and trophectoderm cell number in the same species (Borowczyk et al. 2006).

Accumulating evidence also suggest that apart from macronutrients, micronutrient imbalance can adversely affect fertility, embryogenesis, and placentation (Cetin et al. 2010). In particular, gametogenesis and early embryogenesis are critical developmental windows for erasure, acquisition, and maintenance of genomic imprints (Ashworth et al. 2009). Malnutrition is well known to disturb a number of metabolic pathways and among these the 1-carbon metabolism, which determines the flux of methyl groups via the linked folate–methionine cycle toward synthesis or methylation of DNA (Thuesen et al. 2010). The excellent review by Steegers-Theunissen et al. (2013) considering different lines of evidence, including human and animal studies, support a link between 1-C derangements and reproductive outcomes. Elevated homocysteine levels, a sensitive biomarker of deranged maternal 1-C metabolism, have been negatively associated with the number of oocytes recovered, embryo quality, and pregnancy outcome (Boxmeer et al. 2008).

In humans, in which periconceptional period is thought to start around 14 weeks prior up to 10 weeks after fertilization in women and about 10 weeks for the spermatogenic cycle in men, data on the effects of periconceptional nutrition on later reproductive outcomes are very scarce. However, it is worth noting that a Dutch study demonstrated that women adhered to a Mediterranean (rich in 1-C metabolites, such as folate and B12 derived from vegetables, fruits, etc.) diet in the weeks before IVF were 65% more likely to achieve an ongoing pregnancy (Twigt et al. 2012).

Apart for the well-established role of micronutrients in short-term pregnancy outcomes, accumulating evidence support also a role in developmental programming leading to later onset risk of noncommunicable diseases (Christian and Stewart 2010). Further studies are required to establish links with fertility as well.

Policies and Protocols

Despite the well-recognized improvement in maternal nutrition during the last decades, disparities still exist among populations not only in developing, but in developed countries as well, particularly in adolescent girls (Mouratidou et al. 2006; Black et al. 2008). In the context of long-term consequences of poor maternal nutrition for the fertility and reproductive performance of next generations, a life time approach with respect to intervention strategies should be considered. The periconceptional period, because of its paramount significance, is crucial for the implementation of measures to provide within an integrated preconceptional care approach nutritional advice to women planning pregnancy. Central to the preventive strategies will also be efforts to improve the nutrition of adolescent girls and women of reproductive age, especially those in lower socioeconomic groups.

The fact that maternal undernutrition may impact on the ovarian reserve should be taken into account considering fertility and assisted reproduction counseling. Moreover, the consequences of diminished ovarian reserve may impact not only fertility, but also general health due to a number of early menopause-related commodities. These latter effects are of particular interest nowadays, in light of current trend toward late motherhood.

Evidence that maternal undernutrition may lead to transgenerational effects creates new policy challenges about protecting and promoting reproductive competence and health through maternal nutrition, to ensure reproductive health across the generations that follow.

Regarding future research, although historical cohort studies provided useful results, there is a need for more randomized controlled trials to assess the impact of maternal nutrition on later reproductive health. Apart from macronutrients, specific attention should also be paid to ascertain which micronutrients are important and therefore should be supplemented before conception and during pregnancy. The results from contemporary cohort studies, which are more descriptive and provide more material for biological analysis, particularly for epigenetic studies will help in elucidating the underlying mechanisms.

There is an emerging need for a fruitful collaboration between the scientific community and policymakers. The participation of scientist in the decision-making process is of crucial significance to provide an evidence-based perspective and effective implementation.

A recent report from the WHO Regional Office for Europe, in the context of HEALTH 2020, highlights the need for development and updating of national guidelines to protect and promote public health through the improved nutrition of women of reproductive age, especially during the preconception and pregnancy periods (WHO 2016).

The prevention of maternal undernutrition is a long-term investment that will benefit the present as well as the future generation.

Dictionary of Terms

- **DOHaD** – The developmental origin of health and disease hypothesis implies that a stimulus or insult acting during critical periods of growth and development may result in developmental adaptations that permanently affect structure and metabolism, leading to increased risk of diseases in later life.
- **Nutritional programming** – The process through which variations in the nutrient supply during fetal life, in terms of both quantity and quality, exerts permanent effects upon the developing fetus.
- **Epigenetics** – The study of heritable changes in gene expression that occur without alterations in genomic sequence.
- **Periconceptional period** – The period before and immediately after the time of conception. It encompasses gametogenesis, fertilization, implantation, and early embryogenesis stage.
- **Transgenerational effects** – The ability of environmental insults during development to promote a phenotype state not only in the individual exposed but also in successive generations.

Summary Points

- Evidence from human epidemiological and animal studies clearly indicate that maternal undernutrition affects offspring gamete quality and reproductive parameters, but effects on fertility are not yet well documented.
- Data from human famine paradigms are not consistent, reporting either no detrimental effects of maternal undernutrition on fertility or a decrease in the ovulation rate in women undernourished as fetuses.
- Outcomes depend on the timing and duration of maternal undernutrition, while sex-specific effects are also evident.
- A substantial body of evidence, both from animal and human studies, indicates that maternal undernutrition may impact on ovarian reserve, probably affecting the time of menopause and thus reproductive longevity. These findings represent an issue of translational interest, especially in light of global population trends toward late childbearing.
- The periconceptional period, during which major epigenetic changes take place, is of critical importance. Maternal malnutrition can disturb the apposition of epigenetic marks throughout gametogenesis, fertilization, and the first steps of embryonic development, leading to detrimental outcomes in later life.
- Recent evidence indicates that parental nutrition during the periconceptional period can affect reproductive performance via 1-C metabolic pathways.
- Evidence also exists that adverse outcomes extend beyond first generation to induce transgenerational effects through epigenetic mechanisms.

- Delineating the mechanisms responsible for long-lasting effects of early nutritional programming may lead to develop useful interventions during periconceptional and fetal life to ensure reproductive health in later life.

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Fetal Undernutrition and Oxidative Stress: Influence of Sex and Gender

71

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Contents

Abstract

Low birth weight is increasing worldwide. In poor societies, this is linked to maternal undernutrition and in high-income countries it is mainly due to

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gestational complications associated with the delay in first pregnancy. In both situations, the consequence is fetal undernutrition and deficient growth. In addition to the high risk of perinatal mortality and morbidity, low birth weight has long-term health consequences, increasing the risk of cardiometabolic disease development. The process that associates a deficient fetal growth with a higher risk of adult disease is known as fetal programming. In addition to undernutrition, other stress factors during intrauterine life – such as exposure to toxic substances – also contribute to programming. All of them are known to disrupt the physiological systems responsible for cardiovascular and metabolic control, being oxidative stress a common denominator. Fetal responses to stress factors seem to be modulated by sex. Under adverse intrauterine conditions, females exhibit a better placental adaptation and fetal growth. Later on, during fertile life, estrogens also represent a biological advantage for females, due to their cardiovascular protective actions. However, gender-related factors, such as social or cultural inequities, are likely to counteract the better biological adaptation of females. Sex and gender are often difficult to separate and their relative influence in fetal programming is still far from being understood. Additional research is needed to design-specific interventions and policies in order to reduce the impact of fetal programing on cardiometabolic health in future generations.

Keywords

Antioxidants · Cardiometabolic diseases · Fetal programming · Gender · Malnutrition · Oxidative stress · Placenta · Sex

List of Abbreviations

BMI	Body Mass Index
CMD	Cardiometabolic disease
CVD	Cardiovascular disease
GPX	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced Glutathione
HPA	Hypothalamic-Pituitary-Adrenal axis
IUGR	Intrauterine growth restriction
LBW	Low birth weight
MDA	Malondialdehyde
NO	Nitric oxide
RAAS	Renin-Angiotensin-Aldosterone system
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SNS	Sympathetic nervous system
SOD	Superoxide dismutase
T2DM	Type 2 Diabetes Mellitus

Introduction

Over 20 million low birth weight (LBW) babies are born every year, related to intrauterine growth retardation (IUGR), prematurity, and other causes, and the rate is growing worldwide (World Health Organization 2014). The incidence of LBW is much higher in poor societies, but is also increasing in high-income countries. LBW not only contributes to neonatal morbidity and mortality, but also predisposes to long-term noncommunicable diseases, a process known as fetal programming. In particular, LBW associates with cardiovascular diseases and their risk factors – hypertension, obesity, and type II Diabetes Mellitus (T2DM) – referred in this chapter as cardiometabolic diseases (CMD).

LBW is mainly the result of fetal undernutrition related either to a poor maternal diet or to placental complications. However, factors such as exposure to toxic substances or psychological stress may also contribute. All these fetal stressors seem to converge on the same organic alterations – oxidative stress being a common underlying mechanism. In addition, the sex of the individual seems to modulate the responses to fetal stressor and may exert an influence on CMD development. Furthermore, gender aspects, including social and educational factors, might also modulated fetal programming.

The aim of this chapter is to summarize current knowledge on the role of oxidative stress in fetal programming with a focus on the influence of sex and gender.

Fetal Programming: Stress Factors and Mechanisms Implicated

Intrauterine life is a period of high plasticity, the individual being highly vulnerable to stress factors – particularly nutritional limitations. Over four decades of study have provided sufficient evidence of the relationship between fetal stress, LBW, and CMD. This association was postulated by Barker, based on epidemiological studies in populations that suffered starvation in early twentieth century (Forsdahl 1977; Barker and Osmond 1986).

Barker's hypothesis has been extensively confirmed in populations of different ethnic origin and geographical location exposed to malnutrition in fetal life, which exhibited an increased risk of hypertension, T2DM, and obesity in adult life (Alexander et al. 2015; Liu et al. 2016). Postnatal growth acceleration following inadequate fetal growth has an additional negative impact, further contributing to CMD programming (Singhal and Lucas 2004).

The main stress factors interfering with fetal growth are maternal malnutrition, obstetric complications, exposure to toxic substances, and psychological stress.

Although the term "malnutrition" is often used as synonym of undernutrition, it can also be used to indicate a dietary imbalance or excess. Maternal undernutrition was the first recognized stress factor responsible for LBW and CMD programming, and continues to be the most important contributor. More than 4.5% of pregnant women worldwide suffer undernutrition, mainly in low- and middle-income countries

(Yasmin 2016). Furthermore, micronutrient-deficient diets are also common and also contribute to LBW and prematurity (Black et al. 2013). Pregnant women from high-income countries might also have micronutrient deficiency due to unbalanced diets related to lifestyle factors. Another form of malnutrition is obesity, which is a worldwide growing problem. It has been estimated that around 57% of pregnant women are overweight, which is also a risk factor for LBW (Tarrade et al. 2015).

Fetal undernutrition might also be a consequence of obstetric complications, such as gestational diabetes mellitus (GDM) and preeclampsia, unrelated to the maternal nutritional status. These complications contribute to LBW by restricting nutrient and oxygen availability to the fetus and are associated with CMD programming (Boney et al. 2005).

Some toxic substances related to lifestyle habits, like tobacco and alcohol intake, interfere with fetal growth, increase the risk of LBW (Ko et al. 2014; Nykjaer et al. 2014), and program hypertension (Simonetti et al. 2011). It is remarkable that alcohol intake has negative effects on fetal growth not only when consumed during pregnancy, but also during the period prior to conception and women should be advised when planning to conceive (Nykjaer et al. 2014). Similarly, some environmental contaminants, which are difficult to evade due to their ubiquitous presence, interfere with fetal development and might program CMD (Al-Gubory 2016).

Other substances known to interfere with fetal growth are glucocorticoids and, in particular, cortisol, which might be elevated in the mother as a consequence of pharmacological treatment or psychological stress (Alexander et al. 2015). The passage of cortisol from the mother to the fetus is limited by the placental enzyme 11-β-hydroxysteroid dehydrogenase-2 (11-β-HSD-2). In situations of undernutrition, placental insufficiency, or maternal exposure to toxic substances, this enzyme is inhibited, allowing cortisol to reach the fetus in excess and, therefore, contributing to LBW. Epidemiological studies provide evidence that cortisol elevation might contribute to LBW and cardiovascular disease in adult life (Van Montfoort et al. 2005). A schematic diagram summarizing the main fetal stress factors is shown in Fig. 1.

Studies in animals mimicking gestational complications, exposure to malnutrition, toxic substances, or excess cortisol during intrauterine life, confirm that these stressors lead to LBW and subsequent CMD. These studies have also investigated the mechanisms through which an adverse fetal environment translates into physiological alterations, demonstrating that different fetal stressors converge onto similar alterations in the systems responsible for cardiometabolic control (Nuyt 2008; Alexander et al. 2015). Thus, studies in animal models of fetal programming reveal a deficient kidney, heart, and vascular development, evidenced as low organ weight and cell number at birth. These initial alterations are associated with later insufficient renal function, cardiac hypertrophy, and arterial stiffening – factors contributing to hypertension development in adult life (Alexander et al. 2015; Rodríguez-Rodríguez et al. 2017). Fetal programming of hypertension has also been associated with alterations in the renin-angiotensin-aldosterone and the sympathetic nervous systems (RAAS; SNS), which are key for blood pressure regulation (Alexander et al. 2015).

Regarding the organs responsible for metabolic control, alterations in fetal pancreatic β-cells, adipocytes, skeletal muscle, and liver have been found in animals

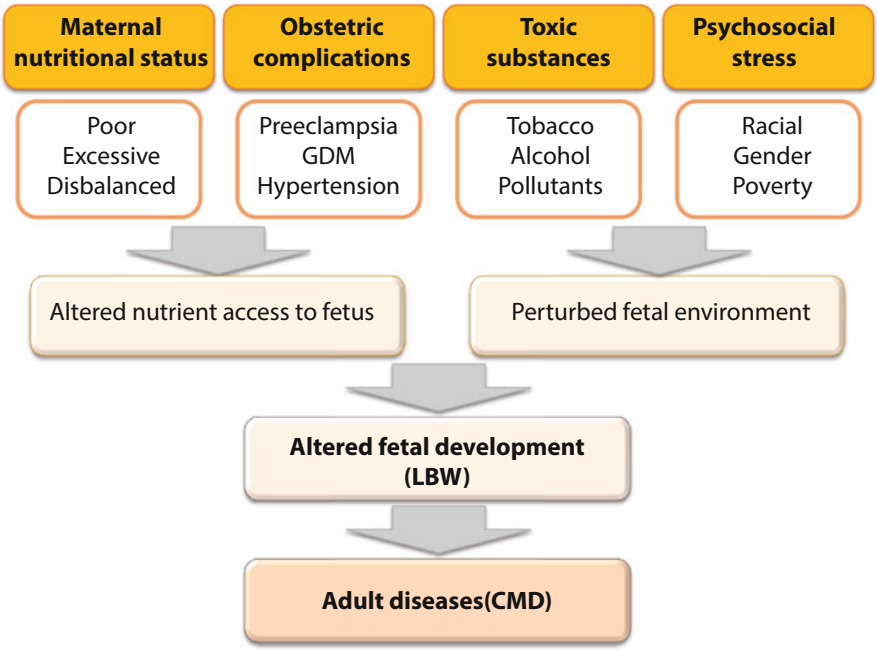

Fig. 1 Main stress factors implicated in fetal programming of cardiometabolic disease. Adverse factors known to interfere with fetal growth contributing to low birth (*LBW*), and subsequent development of cardiometabolic diseases (*CMD*) in adult life. *GDM* Gestational Diabetes Mellitus

exposed to fetal stress factors. These initial alterations have been associated with abnormal control of glucose and fat metabolism and the subsequent development of T2DM and obesity in adult life.

Some of the aforementioned alterations might be mediated by epigenetic processes, i.e., the modification in gene function and expression without changes in the DNA sequence. Several fetal stress factors seem to alter the expression of important genes for cardiometabolic control, including 11β-HSD2, several RAAS components, and some glucose transporters – through epigenetic modulation (Ingelfinger and Nuyt 2012; Alexander et al. 2015). Epigenetic changes are inheritable, and thus, CMD programming can be transmitted from one generation to the other. Figure 2 summarizes the main alterations induced by fetal stressors in organ growth and physiological systems responsible for cardiometabolic control.

Oxidative Stress as Mechanism Implicated in Fetal Programming

Oxidative stress can be defined as an imbalance between pro-oxidative substances (reactive species) and antioxidants, favoring the former.

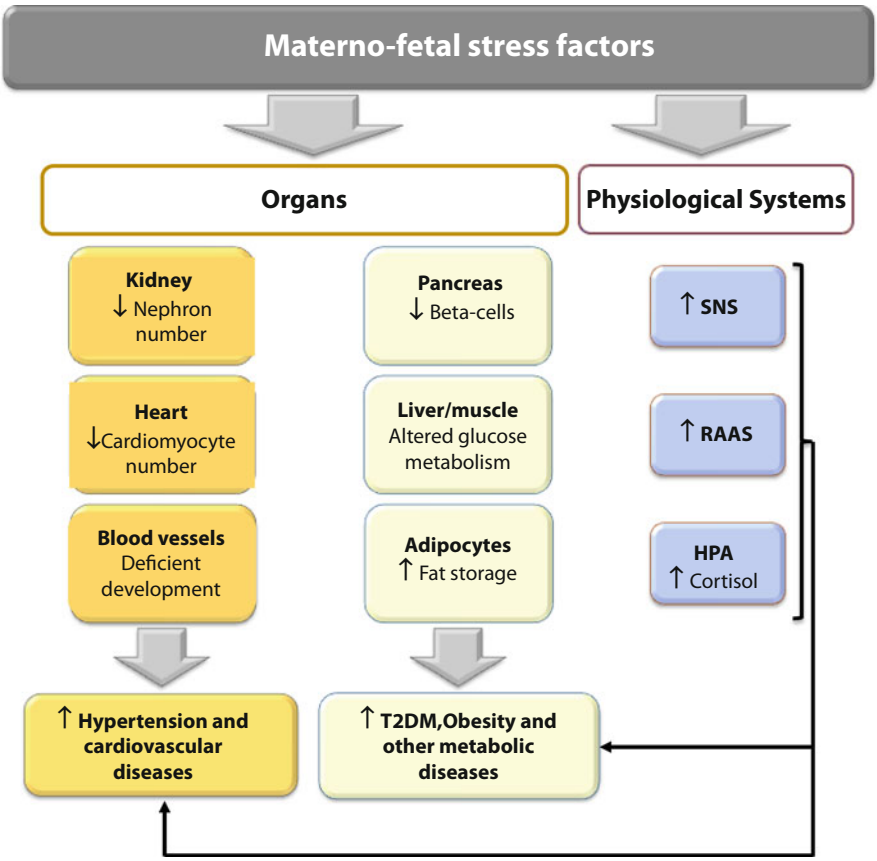

Fig. 2 Alterations implicated in fetal programming of cardiometabolic diseases. Stress factors during fetal life lead to cardiometabolic diseases, such as hypertension, type 2 diabetes mellitus (*T2DM*), and obesity, through modifications in several organs and physiological systems such as the sympathetic nervous system (*SNS*), the renin-angiotensin-aldosterone system (*RAAS*), and the hypothalamic-pituitary-adrenal axis (*HPA*)

Oxidative stress is well known to contribute to kidney, heart, and vascular pathophysiological alterations associated with CMD diseases through oxidative damage to macromolecules. Therefore, it is plausible that oxidative stress may be the underlying common link between adverse fetal environment and increased risk of CMD (Thompson and Al-Hasan 2012; Alexander et al. 2015).

Reactive Species and Antioxidants

Biologically relevant reactive species includes reactive oxygen species (ROS) – generated as a consequence of aerobic metabolism – and reactive nitrogen species (RNS) which can be either free radicals or nonradical species. Their common

characteristic is their high instability. Thus, reactive species have a tendency to react with surrounding molecules, thereby oxidizing them. ROS and RNS have important physiological functions as cell signaling molecules and in the immune defense, among others. However, in excess, they produce oxidative damage to lipids, proteins, and DNA, resulting in functional loss (Halliwell and Gutteridge 2015; Nikolaidis et al. 2015).

The main cellular ROS are superoxide anion ($O_2\bullet^-$) and derived molecules, such as hydrogen peroxide (H_2O_2) and hydroxyl radical (OH•). Biologically relevant RNS include nitric oxide (NO) and peroxynitrite ($ONOO^-$).

To counteract the high oxidative capacity of reactive species, the organisms display a well-organized endogenous antioxidant system. From a wide point of view, an antioxidant can be defined as a substance that delays, prevents, or removes oxidative damage to target molecules. Endogenous antioxidants can be enzymatic systems, low molecular weight molecules, and the hormone melatonin (Reiter et al. 2016).

Figure 3 shows a schematic diagram of biologically relevant reactive species and antioxidants contributing to redox balance.

Biomarkers to Assess Oxidative Stress in Biological Samples

Oxidative stress is considered an important mechanism in diseases and its assessment, through biomarkers present in accessible biological samples, provides important information for disease diagnosis and progression. However, this is not an easy target and the question on which biomarker is most suitable remains under debate. To evaluate oxidative stress, it would desirable to have information on the levels of both reactive species and antioxidants, which represent the two sides of the balance. While the majority of endogenous antioxidants can be easily measured, reactive species are difficult to assess due to their short half-life.

Oxidative stress is frequently determined by the products of the oxidative damage to lipids, proteins and DNA. Lipids are the first target of oxidation by reactive species, altering cell membrane function, and lipid peroxidation products are frequently used to assess their damage. Protein oxidation by reactive species alters the function of key physiological molecules such as antibodies, enzymes, or receptors. Carbonyl groups in proteins are highly susceptible for oxidation and they are frequently used to assess the degree of protein damage. DNA oxidation occurs continuously in cells, but it is usually followed by repair. However, if the repair process is overwhelmed, cell mutation or death might occur. DNA oxidative damage can be assessed in plasma and urine by several biomarkers (Nikolaidis et al. 2015).

An elevation in these biomarkers over normal levels is indicative of oxidative stress. However, it does not identify the origin, i.e., an increase in reactive species, a decrease in antioxidants, or both. Further information can be obtained by evaluating antioxidants, which can be assessed in biological fluids. There are many endogenous antioxidants with different chemical characteristics and the assessment of a particular one does not provide sufficient information about the global antioxidant status.

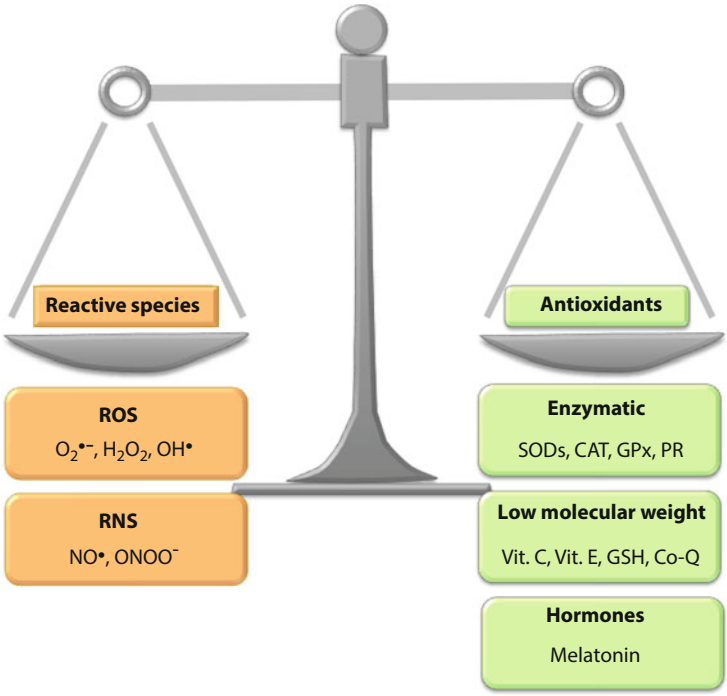

Fig. 3 Pro-oxidant/antioxidant balance in biological systems. Main biological pro-oxidant (reactive species) and antioxidant substances that are balanced in physiological situations. A pro-oxidant excess or an antioxidant deficiency induces a disbalance known as oxidative stress. Reactive species include Reactive Oxygen Species (*ROS*) and Reactive Nitrogen Species (*RNS*). The main biological ROS are superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$). The main RNS are nitric oxide ($NO^{\bullet}$) and peroxynitrite ($ONOO^-$). Endogenous antioxidants include the enzymatic systems superoxide dismutases (SODs), catalase (CAT), glutathione peroxidase (*GPx*) and peroxiredoxins (*PR*), several vitamins (*Vit.*), reduced glutathione (*GSH*) and coenzyme Q (*Co-Q*), and the hormone Melatonin

Therefore, it would be desirable to quantify as many antioxidants as possible. A better approach is to combine all antioxidants measured into an index, a parameter representative of the global antioxidant status of the individual. The use of an antioxidant index might be useful to predict obstetric and fetal complications (Ramiro-Cortijo et al. 2016).

Oxidative Stress and Fetal Programming

There is wide evidence supporting the implication of oxidative stress in fetal programming. Different stress factors, including malnutrition (Gupta et al. 2004), nicotine (Stone et al. 2014), environmental pollutants (Al-Gubory 2016), or glucocorticoids (Stark et al. 2011), have been shown to associate with increased oxidative

damage, measured both in pregnant women and in the offspring. The implication of oxidative stress in fetal programming is reinforced by the demonstration in animals that maternal exposure to fetal stress factors during gestation together with an antioxidant treatment avoids hypertension development in the offspring (Alexander et al. 2015).

Oxidative damage may be related to poor antioxidant levels during gestation, as found in neonates with growth deficiency and their mothers (Saker et al. 2008). Antioxidant deficiency can be a consequence of deficient passage of antioxidants through the placenta, particularly important during the last weeks of pregnancy (Davis and Auten 2010). However, oxidative disbalance may also occur in the first trimester of pregnancy and has been associated with higher risk of obstetric complications and deficient fetal growth (Potdar et al. 2009; Ramiro-Cortijo et al. 2016). The finding that low antioxidant levels in maternal plasma during gestation associate with diets poor in fruits and vegetables suggests the importance of maternal nutrition and micronutrients in particular for oxidative balance (Ramiro-Cortijo et al. 2016).

Influence of Sex and Gender in Fetal Programming

Sex and gender are terms which are often exchanged, but have specific meanings. Sex refers to the classification according to biological genetic differences and can be considered as fixed. Gender is used to distinguish the socio-cultural differences assigned to women and men, which vary with time and among societies. Sex influences can be easily determined from studies in experimental animals using males and females. However, in humans, sex and gender are closely interrelated and their relative influence is difficult to separate.

Despite growing evidence that human diseases present a sexual dimorphism in the prevalence, symptoms, and severity, sex and gender-based considerations are still not sufficiently taken into account in biomedical research or in clinical practice. In particular, their role in fetal programming is still not completely understood.

Sex Influences

There is evidence that fetal programming of CMD exhibits a sexual dimorphism. Among the biological factors which contribute, the best characterized are the placenta and sex hormones. The majority of experimental animal studies conclude that males exposed to fetal stress exhibit a higher susceptibility to hypertension and cardiovascular diseases development than females (Dasinger and Alexander 2016). However, in humans, sexual dimorphism is not evident and women and men who suffered starvation in utero exhibit similar CMD risk (Smith et al. 2016; Liu et al. 2016). The apparent discrepancy might be related to age influences, menopause being a possible confounding factor. This is supported by longitudinal studies using animals exposed to undernutrition during intrauterine life, which show that young females exhibit a better cardiovascular response compared to males. However, at old

age females and males exhibit similar cardiovascular alterations (Rodríguez-Rodríguez et al. 2017). Interestingly, females seem to exhibit similar or even higher vulnerability than males in the development of CMD when the fetal stressor is overnutrition or nutritional disbalance (Alexander et al. 2015). Despite these findings, there is insufficient information on the long-term responses of females to fetal stress factors and an effort should be made to increase the number of studies to clarify the role of sex and age in fetal programming.

Role of the Placenta

The placenta has endocrine, metabolic, and barrier functions and also participates in the suppression of maternal immunity against the fetus. When exposed to an insult, failure or inadequate placental response may contribute to alterations in fetal growth and subsequent disease programming.

The placenta is generated by the fetus and, therefore, has the same biological sex. Male and female placentas exhibit structural and molecular differences and respond differently to adverse stimuli. Female placenta seems to have a better buffering capacity against suboptimal nutrition (Eriksson et al. 2010; Tarrade et al. 2015; Rosenfeld 2015). However, in a situation of overnutrition, females seem to be at disadvantage and more prone to programming. This may explain why there is a larger prevalence of T2DM and strokes in women from obese mothers (Eriksson et al. 2014). Differences in genetic regulation between male and female placenta might also account for the better adaptation of females to fetal stress factors. Thus, female placenta are able to upregulate the enzymatic barrier 11β-HSD2 in response to excess glucocorticoid exposure, leading to a better adaptation than males against alterations in HPA axis (Rosenfeld 2015).

Sex differences in placental function may be related to oxidative stress. During gestation, there is an elevation of ROS, which play important physiological roles. Incapacity of the placenta to counteract this ROS elevation through a concomitant increase in antioxidants is a predisposing factor for maternal and fetal complications (Wu et al. 2015). Male and female placentas also respond differently to stressors which modify oxidative status. In male placentas glucocorticoids induce a pro-oxidant state, with a detrimental impact on placental function and fetal growth (Rosenfeld 2015). Among endogenous antioxidant systems, melatonin – which may be synthesized by the placenta – has been suggested to play a protective role in obstetric and fetal complications, reducing oxidative stress (Marseglia et al. 2016). Whether melatonin levels in the placenta are influenced by sex remains to be determined.

Role of Sex Hormones

The abovementioned sexual dimorphism in the placenta may be related to the effect of sex hormones. In males, testicular development and synthesis of the sex hormone testosterone starts around week 6–8 of gestation. This period marks the difference with females, which will be mainly exposed to maternal female sex hormones, estrogen, and progesterone, during the gestational period. Male and female sex hormones have opposing influences on placental vasculature. While estrogens stimulate production of NO (a well-known vasodilator) and stimulate vascular

development, testosterone has the opposite effects (Chinnathambi et al. 2013; Gopalakrishnan et al. 2016; Maliqueo et al. 2016). A deficient placental vasculature and inadequate vasodilatation would compromise feto-placental blood flow leading to fetal undernutrition. This might explain why excess in maternal testosterone levels (which occur in some pathological situations) are associated with inadequate fetal growth (Palomba et al. 2013; Gopalakrishnan et al. 2016).

In adult life, estrogens and testosterone also exert opposing influences and it is well recognized that female sex hormones exhibit cardiovascular protective effects (Maric-Bilkan et al. 2014). Therefore, sex hormones could modulate the effects of fetal programming. The favorable effects of estrogens are supported by the fact that female animals exposed to a fetal stress usually do not exhibit high blood pressure, while males develop hypertension. Furthermore, ovariectomy induces blood pressure elevation, together with alterations in cardiovascular, RAAS, and renal systems, which are reversed by estrogen replacement. On the other hand, androgens seem to be implicated in fetal programming of hypertension, as suggested by the higher circulating testosterone levels in animals exposed to fetal stress and by the inhibition of hypertension development upon castration (Dasinger and Alexander 2016).

The effects of sex hormones may be related to oxidative stress. Estrogens stimulate antioxidant systems and inhibit ROS-producing enzymes, while testosterone activates ROS synthesis (Maric-Bilkan et al. 2014). Furthermore, in animal models of fetal stress, ovariectomy increases vascular ROS production (Dasinger and Alexander 2016).

Sex influences on fetal programming are summarized in Fig. 4.

Gender Influences

Cardiovascular diseases have been perceived for many years as man's diseases (Lerner and Kannel 1986). This perception has been proved wrong and explained by a gender bias due to a combination of factors, such as the delay in women seeking medical care or the difficulties in the recognition of the clinical symptoms in women (Mosca et al. 2011). Moreover, epidemiological studies have focused on male-related cardiovascular risk factors, such as tobacco consumption excluding others (such as plasma tryglicerides) which are more relevant in the prediction of cardiovascular events in women (Ridker et al. 2002). The importance of heart disease in women has been gradually recognized and substantial efforts have been made to reduce gender disparities in research and clinical care.

Gender factors may also modulate some aspects of fetal development. Since social, cultural, and educational factors varies among different societies according to their economic status, their influence on fetal programming is separately analyzed in low- and high-income countries (Fig. 5).

Gender Aspects in Low- and Middle-Income Countries

One of the gender-related factors, which may influence fetal programming in poor societies, is the lack of reproductive health and family planning control. Around 180

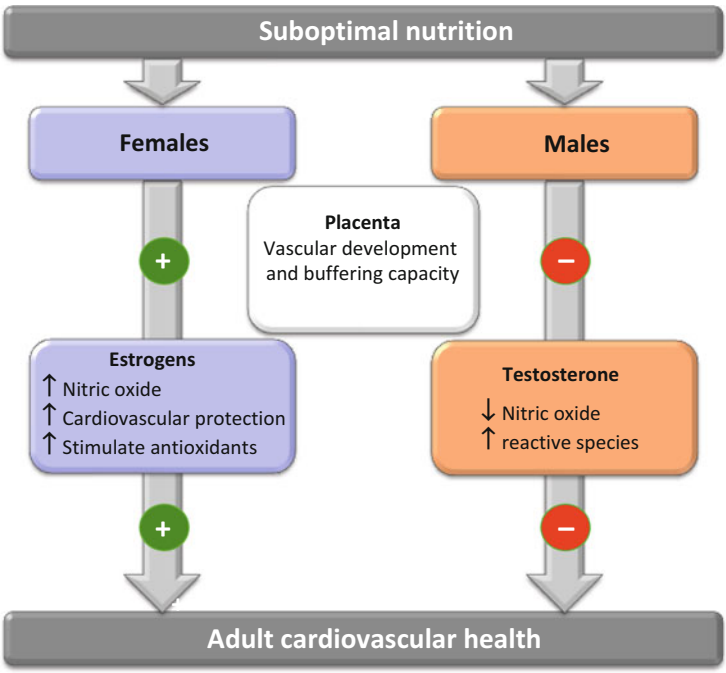

Fig. 4 Sex influences on fetal programming of cardiovascular diseases. Females are better adapted than males to suboptimal nutrition, both during fetal life, through placental adaptations, and during adult life through the cardiovascular protective actions of estrogens

million pregnancies occur in low-income and only around 2 million in high-income countries. Moreover, one-third of the world pregnancies have not been planned in poor societies, in situations of chronic poverty, starvation, and deficient hygiene conditions. The immediate consequence is LBW. In fact, LBW has been estimated in approximately 20 million per year, the majority born in poor countries (World Health Organization 2014).

In situations of poverty, pregnant women are at higher risk of malnutrition, since gestation requires an increased intake of macro- and micronutrients to cover maternal and fetal needs. Gender aspects might contribute to maternal undernutrition in societies where women have a low social status and little decision-making power, particularly in rural settings. Furthermore, the poor educational level and taboos about food lead to inadequate dietary practices, further contributing to malnutrition and poor fetal development (Shannon et al. 2008). Even if maternal diet has sufficient macronutrients, micronutrient deficiencies are very common in developing countries, particularly iron (Black et al. 2013). Many micronutrients are cofactors of antioxidant enzymatic systems or are antioxidants themselves. Therefore, as stated in previous sections, deficiencies in vitamins and minerals might also contribute to fetal programming through oxidative stress.

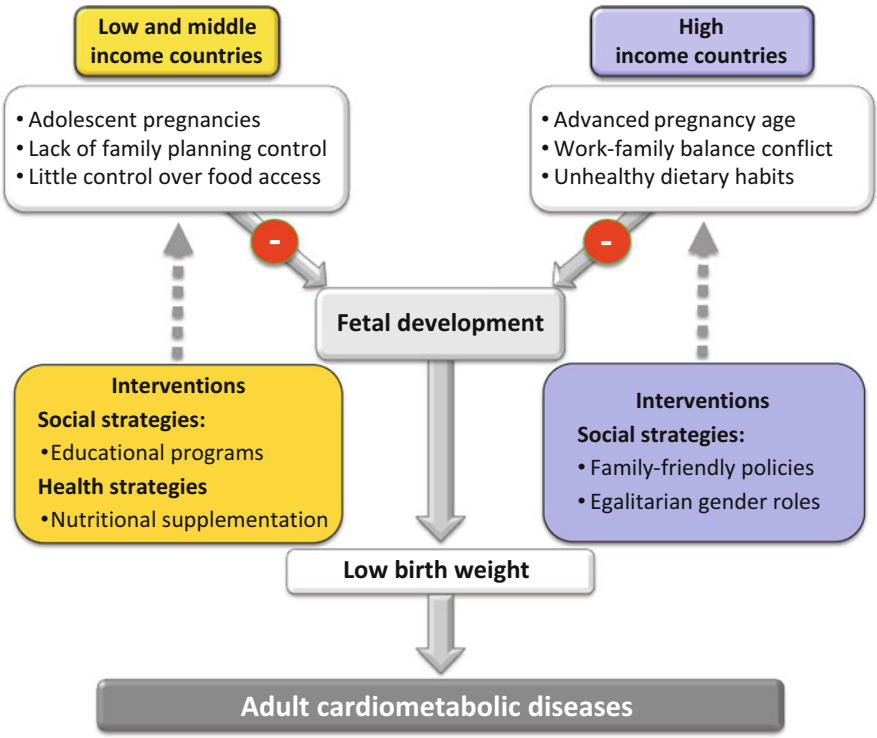

Fig. 5 Gender influences on fetal programming of cardiometabolic disease. Contribution of gender factors to low birth weight and possible intervention strategies, according to socioeconomic development level

A number of international initiatives have been taken to address the nutritional needs of women during periconceptional and gestational periods. A Cochrane database systematic review provides evidence that educational programs aimed at increasing energy intake in undernourished pregnant women reduces the risk of prematurity and LBW (Ota et al. 2015). Intervention strategies with micronutrient supplementation also demonstrate a reduction in LBW (Haider and Bhutta 2015) and an improvement of metabolic health of the offspring (Ekstrom et al. 2016). It has been put forward that educational programs should not only include pregnant women, but also engage other family members, particularly those with a high social role (Shannon et al. 2008).

In addition to maternal undernutrition, a second problem is pregnancy age. In low- and middle-income societies, it is not uncommon that the first pregnancy occurs in adolescence, which is an important developmental period. In this situation, nutrients have to cover the energy requirements for maternal and fetal growth and, in a situation of dietary deficiency, both would compete for nutrients, increasing the risk of LBW (Wemaux-Denis et al. 2017). This problem has been perceived by some

authorities who have targeted adolescent girls as a priority group for nutritional intervention (Vogt et al. 2016).

Gender Aspects in High-Income Countries

Developed societies are witnessing a progressive increase in the age of first pregnancy (Bréart et al. 2003; Ventura et al. 2009). The increase in maternity age is directly linked with a decline in fertility, which has increased the use of assisted reproduction techniques and multiple pregnancies, frequently associated with LBW and prematurity. Advanced pregnancy age also associates with obstetric complications, such as preeclampsia, gestational hypertension, or gestational diabetes, all linked to LBW. In high-income countries, these problems are not associated with perinatal mortality due to a better health care system. However, it is foreseen that, in future generations, the increase in the age of pregnancy will increase the incidence of CMD, through fetal programming (Tarrade et al. 2015).

The increase in maternity age in high-income countries is associated with the change in the social role of women and their gradual access to higher education and employment, as well as an increase in family planning control. Despite these social changes, in many developed societies egalitarian gender roles are not sufficiently developed and it is frequent that women experience a conflict between work and family, particularly in professionals of high educational background. This conflict has a direct influence on the decision to postpone maternity (Ramiro-Cortijo et al. 2016).

Another gender aspect is psychological stress derived from strenuous work, particularly in women with education and health-related jobs, where gender-specific difficulties arise and higher levels of burnout have been reported (Cassidy-Vu et al. 2017). Psychosocial stress by itself has adverse consequences for the fetus, through the effects of excess cortisol, increasing the risk of LBW and prematurity (Guardino et al. 2016). Furthermore, psychological stress might favor addictive behaviors such as tobacco or alcohol, as well as fast-food-based diets, poorer in micronutrients.

In summary, micronutrient deficiencies, obstetric complications, and psychological stress are likely to have a negative impact on oxidative balance, contributing to LBW and fetal programming of CMD. Regarding sex and gender influences, we conclude that although female sex seems to have a better biological adaptation to fetal stressors, gender inequities might counterbalance this advantage, leading to a similar risk of CMD development compared to men, or even higher after menopause. Therefore, specific nutritional interventions as well educational programs should be applied. In addition, it is desirable to increase social awareness to promote egalitarian gender roles.

Policies and Protocols

Governmental Policies This chapter points out the importance of LBW as key factor in fetal programming of CMD. In low- and middle-income countries LBW is a direct consequence of maternal malnutrition, while obstetric complications due to increase in

gestational age are the main determinants in high-income countries. Therefore, specific governmental policies aimed at counteracting these problems are required.

Low- and Middle-Income Countries

- Educational programs involving not only pregnant women, but also key family figures, would help to spread good dietary practices during pregnancy.
- Adolescent girls should be targeted as a priority group for education on nutritional aspects during gestation.
- Supplementation programs with macro- and micronutrients would help to reduce the incidence of LBW and prematurity in vulnerable populations.

High-Income Countries

- Health educational programs to raise awareness on the negative effects of alcohol and tobacco consumption on the gestational health, not only during pregnancy but also prior to conception.
- Specific governmental programs designed to extend maternity leave periods could have a positive impact on obstetric problems associated with maternity age delay.
- Specific guidelines could be designed to promote family-friendly practices at work.

In addition, environmental health policies should be applied worldwide in order to reduce toxic pollutants and their deleterious consequences on maternal and offspring health.

Dictionary of Terms

- **Antioxidants** – Substance that delay, prevent, or remove oxidative damage to molecules.
- **Epigenetic alterations** – Heritable genetic modifications without changes in DNA sequence.
- **Intrauterine growth restriction (IUGR)** – Refers to poor growth in utero evidenced by at least two ultrasound measurements.
- **Free Radicals** – Molecules or fragments of molecules containing one or more unpaired electrons. Free radicals are highly unstable and tend to react with other molecules inducing oxidative processes.
- **Gender** – Gender is used to distinguish the socio-cultural differences assigned to women and men, which vary with time and among societies.
- **Oxidative stress** – Disbalance between pro-oxidant reactive species and antioxidants.
- **Prematurity** – Broad term defining neonates born prior to 37 weeks' gestation.

- **Reactive species** – Molecules or fragments of molecules which induce oxidation to macromolecules. Biologically relevant are those derived from oxygen (Reactive Oxygen Species, ROS) and nitrogen (Reactive Nitrogen Species, RNS)
- **Sex** – Sex refers to the classification according to biological genetic differences between women and men.

Summary Points

- LBW is increasing worldwide; in low-income countries due to maternal undernutrition and in high-income countries due to obstetric complications.
- In high-income countries, the increase in obstetric complications is associated with maternity age delay.
- Epidemiological and experimental studies support the negative impact of LBW due to undernutrition and other stress factors on CMD development in adult life.
- Fetal undernutrition and programming is likely to contribute to the burden of CMD in the future generations worldwide.
- Oxidative stress is implicated in the alterations in physiological systems associated with fetal programming of CMD.
- Female placenta has a better adaptation to suboptimal conditions in utero compared to male placenta.
- Female hormones might counteract fetal programming improving placental vascular development and protecting the cardiovascular system during adult life.
- Gender aspects may counteract the better biological adaptation of female sex to fetal stress factors.
- It is necessary to expand the knowledge on the mechanisms responsible for the sexual dimorphism in fetal programming, including females in experimental animal studies.
- The influence of sex and gender in fetal programming of CMD should be further investigated in epidemiological studies.

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Effects of Protein Deficiency on Perinatal and Postnatal Health Outcomes

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Abstract

There are a variety of environmental insults that can occur during pregnancy which cause low birth weight and lead to poor neonatal outcomes. One such insult is maternal malnutrition, which can be further narrowed down to a low protein diet during gestation. Studies demonstrate that perinatal protein deficiencies can impair proper organ growth and development, leading to long-term metabolic dysfunction. Understanding the molecular mechanisms that underlie how this deficiency results in adverse developmental outcomes is essential for establishing better therapeutic strategies that may alleviate or prevent diseases in later life. This chapter reviews how perinatal protein restriction in humans and animals leads to metabolic disease, and it identifies, to date, some of the underlying molecular mechanisms that have been elucidated. These include alterations in transcriptional and epigenetic mechanisms, as well as as endoplasmic reticulum (ER) stress and oxidative stress. Furthermore, nutritional and pharmaceutical interventions are highlighted to illustrate that the plasticity of the underdeveloped organs during perinatal life can be exploited to prevent the onset of long-term metabolic disease.

Keywords

DOHaD · Amino acids · Liver · Adipose · Pancreas · Maternal LP diet · Diabetes · Dyslipidemia · Longevity · Epigenetics · Posttranslational histone modifications · DNA methylation · Endoplasmic reticulum stress · MicroRNAs · Taurine · Oxidative stress

List of Abbreviations

ADP	Adenosine diphosphate
Akt1	Protein kinase B
ALS	Amyotrophic lateral sclerosis
Cyp1A2	Cytochrome P450 1A2
Cyp2c11	Cytochrome P450 2c11
Cyp3a1	Cytochrome P450 3a1
Cyp7a1	Cholesterol 7 alpha-hydroxylase
DOHaD	Developmental origins of health and disease
ER stress	Endoplasmic reticulum stress
G6Pase	Glucose-6-phosphatase
GLUT4	Glucose transporter type 4
GR	Glucocorticoid receptor
GRP78	Glucose-regulated protein 78
IGF-1	Insulin-like growth factor 1
IRS-1	Insulin receptor substrate 1
IUGR	Intrauterine growth restriction
LDL	Low-density lipoprotein
LP	Low protein
LPL	Lipoprotein lipase
LXR	Liver X receptor

LXRE	LXR response element
MEF2	Myocyte enhancer factor-2
miRs	MicroRNAs
MPR	Maternal protein restriction
p-eIF2α	Phosphorylated eukaryotic translation initiation factor 2
PND	Postnatal day
PPARα	Peroxisome proliferator-activated receptor alpha
PPAR-γ	Peroxisome proliferator-activated receptor gamma
ROS	Reactive oxygen species
SAM	Severe acute malnutrition
SGA	Small for gestational age
SIRT1	Sirtuin 1
UPR	Unfolded protein response
XBP1	X-box binding protein 1

Introduction

There are a variety of insults that can occur during pregnancy leading to intrauterine growth restriction (IUGR). IUGR is characterized by a delay in fetal growth rate; therefore, IUGR infants are often categorized as being small for gestational age (SGA) due to low birth weight. One of the most common insults that can underlies IUGR is maternal malnutrition, a global problem across all classes of socioeconomic status. Over the past half century, a sizable amount of evidence has revealed the important relationship between birth weight and postpartum development (Barker 1994; Ong et al. 2000). One of the leading contributors to this finding was Dr. David Barker, an English epidemiologist who is well known for establishing the "Predictive Adaptive Response" hypothesis (Hales and Barker 2001). This hypothesis is highly supportive of the developmental origins of health and disease (DOHaD), as it suggests that unfavorable in utero events can permanently alter physiological processes that manifest in the metabolic syndrome. The hypothesis states that fetal programming is altered in preparation of a nutritionally scarce postnatal environment, thereby producing a "thrifty" phenotype that is characterized by fetal energy conservation (Hales and Barker 2001). Unfortunately, these metabolic adaptations become harmful when the fetus is born into a nutritionally rich environment because the neonate is programmed to store energy rather than spend it. Individuals who are affected by this thrifty phenotype therefore tend to become obese early in life and have an increased risk for early-onset type II diabetes mellitus, cardiovascular disease, and stroke among other chronic conditions (Ravelli et al. 1998; Eriksson 2006; Barker et al. 2002).

The composition of maternal diet during pregnancy plays a large part in fetal development, as an absence or excess of nutrients can impact organ growth and development. Maternal malnutrition can exist in a variety of forms, including global nutrient abnormalities (i.e., high or low caloric intake) or atypical supplementation of specific macromolecules and nutrients. Regardless of the source, human and animal

studies have demonstrated that maternal malnutrition in pregnancy also leads to placental insufficiency, an idiopathic condition by which reduced maternofetal nutrient transfer leads to IUGR (Ogata et al. 1986; Simmons et al. 1992). One such model is the maternal protein restriction (MPR) model of undernutrition, which investigates the impact of perinatal protein deficiency in IUGR offspring. Amino acids have been shown to be critical for fetal growth and development, as they are the structural building blocks for all proteins (Crosby 1991; Petry et al. 2001). Inadequate supplementation of amino acids during pregnancy has been shown to cause asymmetrical IUGR, as LP animal offspring have reduced growth of organs such as the liver, muscle, and pancreas at the expense of more essential organs like the brain (Desai and Hales 1997). These offspring consequently have impaired metabolic programming that persists into adulthood, and thus exhibit a phenotype that is characteristic of the metabolic syndrome. Moreover, as Barker's hypothesis would suggest, MPR offspring that are fed a normal protein diet after birth undergo rapid growth during early periods of life (Ozanne and Hales 2004). Moreover, postnatal catch-up growth exacerbates the symptoms and incidence of metabolic deficits (Sohi et al. 2011; Bol et al. 2009; Bieswal et al. 2006), and this dietary mismatch also appears to have significant effects on lifespan (Ozanne and Hales 2004). Considering these changes to metabolism and longevity, this review aims to demonstrate the importance of maternal protein during pregnancy on long-term outcomes of the offspring, with an emphasis on how postnatal catch-up growth can modify the mechanisms responsible for regulation of glucose, lipids, hormones, and lifespan.

Protein Restriction and Long-Term Outcomes: Clinical Evidence

In 1986, Barker and his colleagues discovered birth records for over 15,000 English persons born prior to 1931. These records were collected by Miss Ethel Burnside, Lady Inspector of Midwives for Herfordshire, England, who documented birth weight and body weight at 1 year of age (Barker 2003). These follow-up records allowed Barker to assess the growth trajectory of individuals within the first year of life, and he was able to further inquire about adult health for those still living at the time. The data revealed that those who were born of low birth weight had disproportionately higher rates of coronary heart disease (Barker 2003; Ravelli et al. 1976), and these individuals also had impaired liver size and/or function at birth (Barker et al. 1993). This is not surprising, as IUGR often results in asymmetric organ development (Desai and Hales 1997). Furthermore, studies of individuals born around the time of the Dutch Hunger Winter reveal that prenatal exposure to famine confers increased risk for glucose intolerance in adulthood (Ravelli et al. 1998). This population also had high rates of obesity after exposure to famine during the first half of gestation (Ravelli et al. 1976), suggesting that timing of maternal malnutrition during pregnancy can influence long-term metabolic outcomes of offspring.

While the previously mentioned epidemiological studies are focused on caloric restriction, there is also evidence to support that protein deficiency during critical periods of development gives rise to poor metabolic outcomes. Populations of children with severe acute malnutrition (SAM) are often used to study the repercussions of malnutrition, as these individuals see the effects of a low calorie diet (marasmus) or a low protein, high carbohydrate diet (kwashiorkor; Forrester et al. 2012; Spoelstra et al. 2012). In 1967, a study of Ugandan children revealed that individuals with kwashiorkor had low serum protein levels in comparison to those with marasmus (Hadden 1967). These individuals also exhibited glucose intolerance and elevated plasma free fatty acids (Hadden 1967); however, children with kwashiorkor displayed normal glucose tolerance after a 2 week dietary intervention (Hadden 1967). It was proposed that the original impairment in glucose tolerance may be due to an inability to utilize free fatty acids as a substrate in the citric acid cycle, so adequate dietary protein may be essential for normal aerobic metabolism (Hadden 1967). More recent studies also show that children with kwashiorkor exhibit reduced lipolysis and fatty acid oxidation relative to children with marasmus (Badaloo et al. 2006), while children with kwashiorkor or marasmus have pancreatic beta cell dysfunction that contributes to glucose intolerance (Spoelstra et al. 2012). Studies have also established that SAM has early life origins, as low birth weight infants have high risk for exhibiting either marasmus or kwashiorkor when exposed to a nutrient-poor postnatal environment (Francis-Emmanuel et al. 2014). Interestingly, individuals who exhibit marasmus tend to be of lower birth weight than those who develop kwashiorkor; however, individuals from both groups of SAM tend to have poor metabolic outcomes as adults (Francis-Emmanuel et al. 2014). As mentioned previously, nutrition-induced accelerated growth influences the onset of metabolic disease in low birth weight offspring (Eriksson 2006). Unfortunately, none of the discussed human SAM studies contained data on childhood growth rate, so it remains unknown as to whether catch-up growth is involved in metabolic outcomes of individuals who experienced SAM in early life. Furthermore, because a typical kwashiorkor diet has low protein and high carbohydrate content, it is not clear whether long-term metabolic dysfunction occurs in adulthood due to low dietary protein, high carbohydrates, or both for these individuals.

Is Veganism Safe in Pregnancy?

Veganism and vegetarianism is also of interest when studying the effects of protein restriction, as a vegan/vegetarian diet relies solely on plant-sourced nutrients. Individuals who practice veganism or vegetarianism must be careful to ensure that they ingest an adequate amount of protein, often in the form of legumes, lentils, grains, etc. There are mixed opinions on whether consumption of a vegan/vegetarian diet is safe during pregnancy, as observational human studies report conflicting data on both maternal and fetal outcomes. A literature review by Piccoli et al. (2015) revealed that multiple studies found infants of vegetarian mothers to be of lower

birth weight than nonvegetarian mothers, while two different studies reported that infants of vegans/vegetarians actually have higher birth weight and length. Gestational age was not disclosed for either of these studies; therefore, the association between a vegetarian/vegan diet and high birth weight is not necessarily meaningful (Piccoli et al. 2015). It was also noted that most studies did not report maternal protein intake levels, so it is hard to conclude whether there is a relationship between veganism/vegetarianism and fetal outcomes. Moreover, a case report by Mariani et al. (2009) revealed poor short-term outcomes of an infant born to a vegan mother. The infant had been breast-fed exclusively up until 10 months of age and showed developmental delay, failure to thrive, and megaloblastic anemia among other conditions (Mariani et al. 2009). Furthermore, the infant exhibited major improvement with vitamin supplementation, so it may be that the health impairments were due to vitamin deficiencies rather than low protein (Mariani et al. 2009). Regardless of these reports, organizations such as the American Dietetic Association maintain that a vegan or vegetarian diet is safe during pregnancy (Craig and Mangels 2009). That said, physicians must assess protein intake of pregnant women who consume these diets, and future studies are warranted to determine any long-term detrimental effects on offspring.

Maternal Protein Restriction (MPR) Rodent Model: Relevance to Human IUGR

Protein and amino acids are an essential part of the human diet, and many studies have determined that amino acids have a key role in fetal growth and development (Crosby 1991; Petrik et al. 1999). An absence of amino acids is known to occur in cases of both maternal malnutrition and placental insufficiency, thereby leading to low birth weight and asymmetrical IUGR. It is for this reason that the MPR model can be used to study fetal undernutrition in response to maternal malnutrition or placental insufficiency. With the MPR model, pregnant rat dams are fed a diet of 20% (control) or 8% protein. Offspring born to control diet-fed dams continue to have a 20% "normal" protein throughout life, while offspring born to LP dams are assigned to one of three groups: low protein 1 (LP1), low protein 2 (LP2), or low protein 3 (LP3). LP1 offspring are fed an 8% protein diet throughout life, while LP2 offspring are fed an 8% protein diet until weaning (i.e., PND 21). Alternatively, LP3 offspring are exposed to a LP environment exclusively during gestation – these pups are fed a 20% protein diet from birth through adulthood. It is also important to note that the reduction in calories in the 8% protein diet is compensated for by the addition of carbohydrates (Fig. 1). This makes each diet isocaloric with each other, thereby eliciting no maternal stress and no changes in maternal food intake or weight gain (Fig. 2). Furthermore, while there are no differences in postnatal food intake across all dietary groups of offspring, LP offspring were lower in body weight in postnatal life compared to control offspring (Fig. 2). It is also important to note that the MPR diet is not

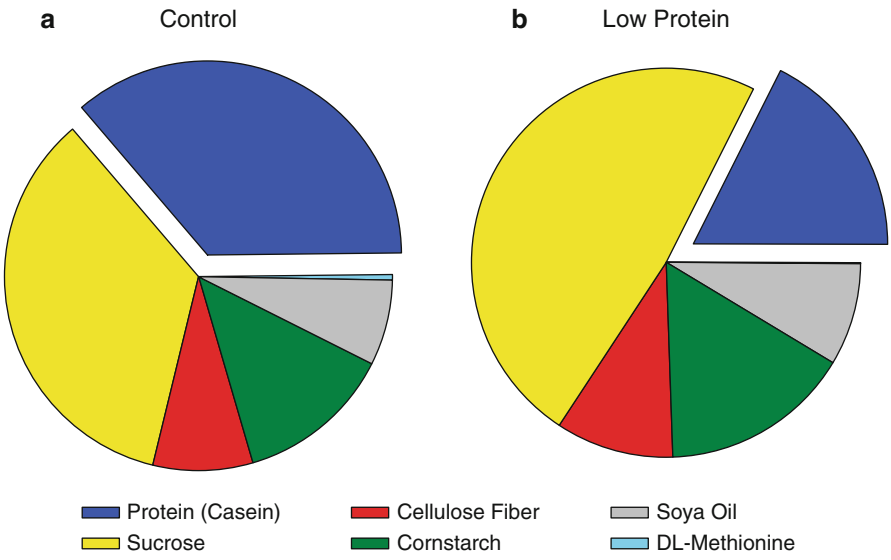

Fig. 1 Overview of control and low protein rodent diets. Composition of (**a**) Control (20% protein) and (**b**) Low protein diet (8% protein) are described. The low protein diet is attributed to decreased casein content but is made isocaloric by a slight increase (13%) in carbohydrates (i.e., sucrose)

considered to be a "high carbohydrate" diet, as the slight percent increase in carbohydrates (13%) is negligible relative to the substantial decrease in protein content (greater than 50%).

Outcomes I: Liver

Studies involving the MPR model have demonstrated that mammalian fetal liver development is impaired due to the low protein insult. While there is an overall reduction in birth weight of LP offspring (Fig. 2), there is also a significant decrease in fetal liver to body weight ratio (i.e., the liver is proportionally small; Sohi et al. 2011). This finding suggests that fetal liver growth is compromised at the expense of more "vital" organs such as the heart and brain (Williams et al. 2005). Furthermore, the timing of protein restoration appears to be significant during the neonatal period as LP2 and LP3 offspring display liver and whole body postnatal catch-up growth despite no differences in food intake (Fig. 2) (Sohi et al. 2011). Offspring having undergone asymmetrical IUGR are believed to be prone to symptoms of the metabolic syndrome, and previous studies confirm that LP2 rat offspring exhibit glucose intolerance at PND 130 due to altered hepatic gluconeogenesis (Vo et al. 2013). In addition, adult male recuperated offspring (e.g. LP2) have dyslipidemia and impaired drug metabolism due to altered expression of various hepatic cytochrome P450 enzymes (Fig. 3; Sohi et al. 2011, 2014).

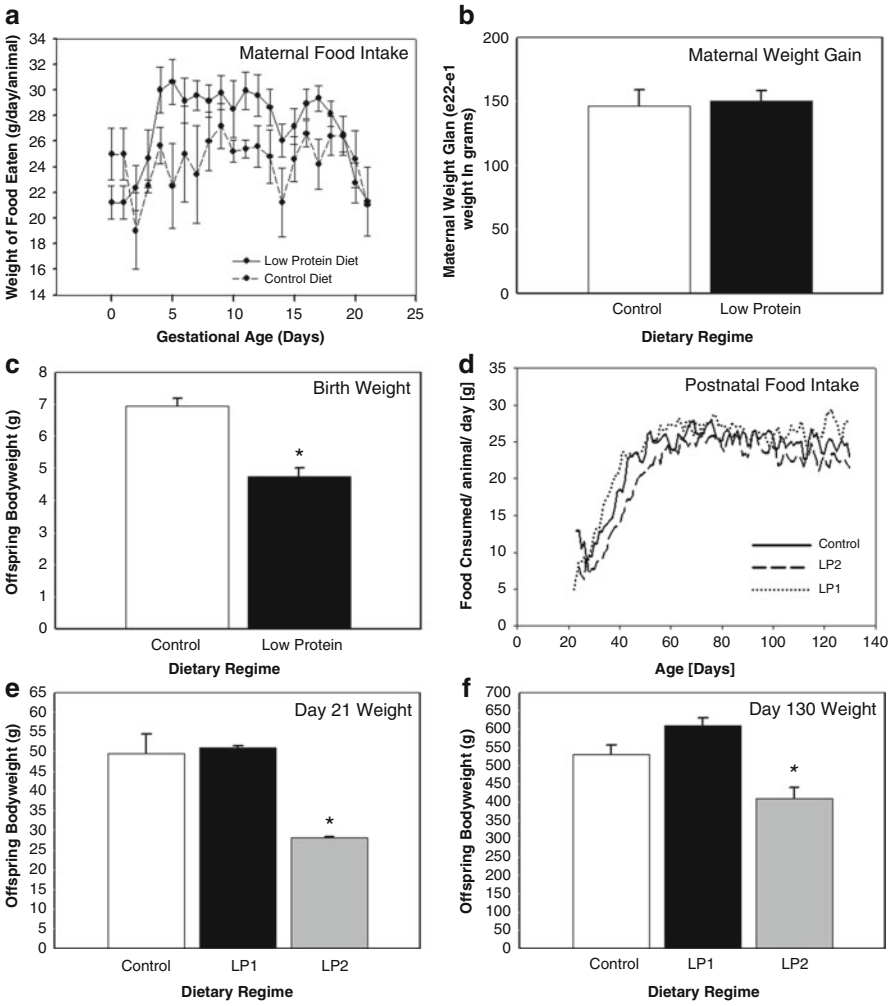

Fig. 2 Effect of maternal low protein diet on (a) Maternal food intake, (b) maternal weight, (c) birth weight, (d) food intake of offspring, (e) weight of offspring at day 21, and (f) weight of offspring at day 130. Pregnant rats were given either a control diet (20% protein) or a low protein diet (8% protein) during gestation only (LP1) and lactation (LP2). Weight of food eaten in g/day/animal and maternal weight gain from gestation day 1 to gestation day 22 in grams were measured, respectively. Total maternal food intake, maternal weight gain, and birth weight results are expressed as the mean ± SEM and significance was assessed using Student's unpaired t-test. For postnatal day 21 and 130 weight analysis, the dietary groups were compared by ANOVA and significant difference was determined by a Tukey HSD post hoc test for individual pairwise comparisons (*$P < 0.05$, indicates significance between both the control and LP1 group). $n = 5$–8/group, where each n represents an offspring derived from a different mother (Reprinted from "Higher Hepatic MiR-29 Expression in Undernourished Male Rats During the Postnatal Period Targets the Long-term Repression of Insulin-like Growth Factor 1", G Sohi et al., Endocrinology (2015) 156(9): 3069–3076, with permission from Oxford University Press)

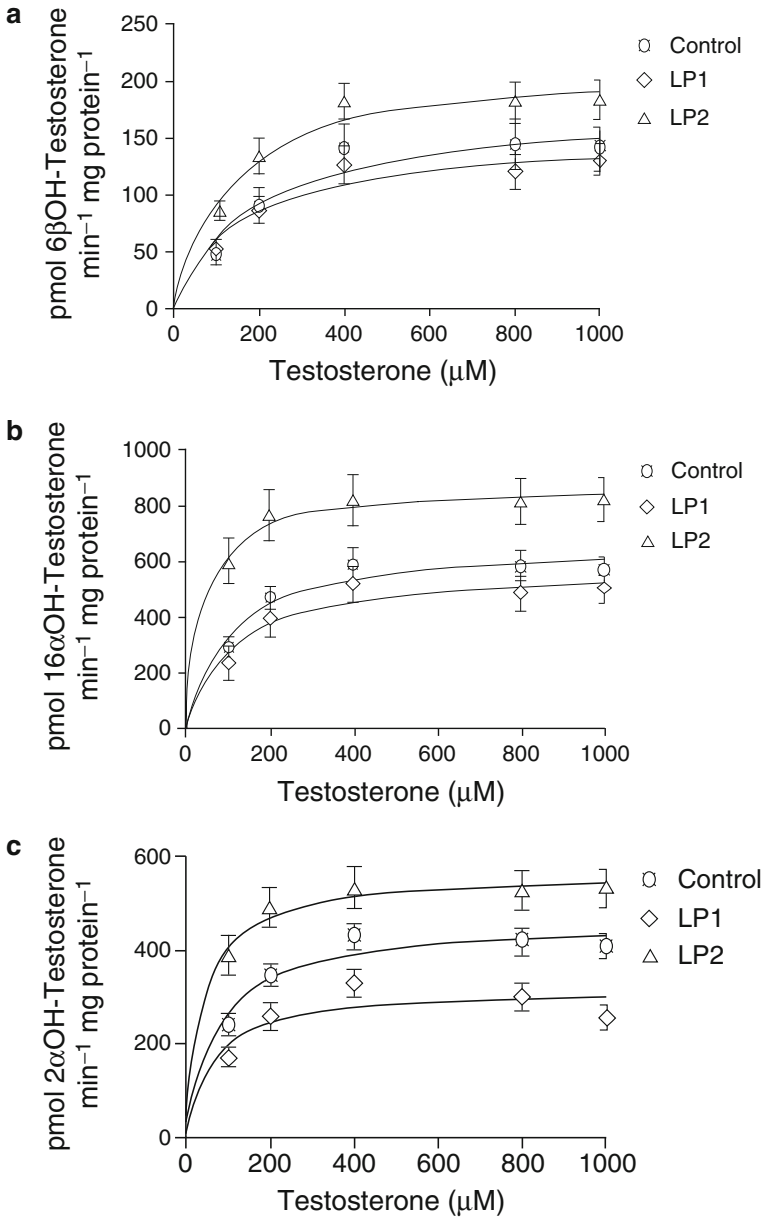

Fig. 3 Michaelis-Menten plots of (a) 6β-OH testosterone, (b) 16α-OH testosterone, and (c) 2α-OH testosterone after incubation of day 130 rat liver microsomes (Control, LP1, and LP2) with 1 mM NADPH and various concentrations of testosterone. Liver microsomes were extracted from control, LP1 (low protein all life), and LP2 (low protein diet during pregnancy and weaning) dietary regimes in postnatal day 130 offspring. Timed enzyme reactions were performed for testosterone metabolite analysis via solid-phase extraction followed by UPLC-

Outcomes II: Other Organs

The effects of MPR are not exclusive to the liver. Epidemiological studies indicate that there is an association between visceral obesity and poor fetal growth and this has been further confirmed via the MPR rat model (Guan et al. 2005). The increase in visceral adiposity occurs due to increased rates of preadipocyte proliferation, as indicated by increased incorporation of [3H]-thymidine into the DNA of primary rat preadipocytes (Zhang et al. 2007). It is also interesting that these studies showed no apparent alteration in preadipocyte differentiation, as there were no significant differences in the expression of peroxisome proliferator-activated receptor gamma (PPAR-γ) or lipoprotein lipase (LPL; Zhang et al. 2007). Early studies by Ozanne et al. (1996a) also demonstrate that MPR leads to increased insulin sensitivity of muscle at 3 months of age, as LP offspring have increased glucose uptake into skeletal muscle upon stimulation with low doses of insulin. This increased sensitivity is brought about by increased expression of GLUT4 and insulin receptors in myocyte plasma membranes (Ozanne et al. 1996a). While the mechanisms behind this are not well understood, it is also known that this enhanced glucose tolerance is lost later in adult life due to insulin resistance (Hales et al. 1996).

Fetal brain development also appears to be compromised by protein restriction, as LP-born rat offspring exhibit changes in kynurenine metabolism in the brain. Kynurenine metabolites are involved in neuronal development (Honório de Melo Martimiano et al. 2017), so an imbalance of these compounds within fetal brain tissue is believed to contribute to an increased risk for mental health disorders. Additionally, there is an increase in reactive oxygen species (ROS) within the brainstem of LP male offspring at weaning, so neuronal mitochondrial function may be diminished (Ferreira et al. 2016). Based on the extensive amount of studies concerned with this particular diet, it is clear that LP-born offspring have gross organ impairment contributing not only to metabolic dysfunction, but to the onset of other adult diseases as well.

Outcomes III: Diabetes

Long-term effects to glucose homeostasis are highly promoted by maternal protein restriction, as demonstrated by glucose intolerance and insulin resistance in adult humans and adult rat offspring (Sohi et al. 2013; Chamson-Reig et al. 2009; Phipps et al. 1993). In the liver, MPR leads to hyperglycemia in 4 month offspring due augmented expression of gluconeogenic enzymes such as glucose-6-phosphatase (G6Pase) and 11β-hydroxysteroid dehydrogenase type I (11β-HSD1; Vo et al. 2013). Moreover, Burns

Fig. 3 (continued) PDA detection. Each data point on the curves were expressed as the mean ± SEM. $n = 5–6$/group, where each n represents an offspring derived from a different mother (Reprinted from "Protein Restoration in Low Birth Weight Rat Offspring Derived from Maternal Low Protein Diet Leads to Elevated Hepatic Cyp3a and Cyp2c Activity in Adulthood," G Sohi et al., Drug Metabolism and Disposition (2014) 42: 221–228, with permission from The American Society for Pharmacology and Experimental Therapeutics (ASPET))

et al. (1997) demonstrated that MPR adult rats have significantly reduced hepatic glucokinase expression, thus contributing to increased glucose output. Impaired liver function leading to insulin insensitivity is further evident in MPR offspring when examining both phosphorylated eukaryotic initiation factor 2 α (p-eIF2α) and phosphorylation of Akt1 (Sohi et al. 2013). Adult MPR offspring with postnatal catch-up growth have increased p-eIF2α [Ser51], a marker of protein translation attenuation and ER stress, and this is associated with a decrease in the phosphorylation of protein kinase B (Akt1) [Ser473], a marker of insulin resistance (Sohi et al. 2013). Interestingly, MPR offspring have unchanged levels of p-eIF2α at embryonic day 19; therefore, the relationship between p-eIF2α and insulin sensitivity appears to be affected by postnatal catch-up growth rather than LP insult directly. This is in support of the predictive adaptive response hypothesis, as this molecular change occurs only in cases of a mismatched nutritional environment. Finally, expression of hepatic glucagon receptors was reduced fivefold in studies of MPR offspring by Ozanne et al. (1996b), along with a threefold increase in hepatic insulin receptors. These changes were reflected by reduced hepatic glucose output (relative to control animals) upon stimulation with glucagon, as well as increased glucose output with administration of insulin (Ozanne et al. 1996b). These studies clearly verify the importance of perinatal protein supplementation in fetal liver development, as the augmentation of many hepatic targets can negatively impact plasma glucose and insulin sensitivity.

In addition to poor outcomes seen in the developing liver, MPR appears to impact growth and function of other organs involved in glucose homeostasis, such as the pancreas. Epidemiological studies of adults who suffered from SAM during childhood have demonstrated that these individuals have glucose intolerance and poor insulin sensitivity later in life as a result of compromised beta cell development (Francis-Emmanuel et al. 2014). Similarly, the Preston and Hertfordshire studies by Barker and his colleagues revealed that there is an inverse relationship between birth weight, plasma glucose, and insulin concentrations of individuals exposed to famine during pregnancy (Hales et al. 1991; Phipps et al. 1993). Animal studies have since confirmed that this occurs due to reduced beta cell mass, increased islet cell apoptosis, altered beta cell cycle length and lower pancreatic islet vascularization (Petrik et al. 1999; Boujendar et al. 2003). In cases of perinatal protein restriction, this phenotype can be rescued with administration of meat-sourced amino acids (e.g., taurine) during gestation and the first weeks of neonatal life (Boujendar et al. 2002, 2003). Supplementation of a LP diet with 2.5% taurine leads to restoration of beta cell mass by PND 130 in vivo, while in vitro studies demonstrate that this is due to normalization of DNA synthesis, apoptosis, and fetal islet vasculogenesis (Boujendar et al. 2002, 2003). A study by Chamson-Reig et al. (2006) also determined that deficient beta cell development occurs in response to MPR during early, mid, and late gestation; however, males are more susceptible to this insult during late gestation and females during mid-gestation. Not only does this emphasize that there are sex-specific differences in organ development in response to MPR, but also that timing of perinatal protein deficiency plays a role in the severity of offspring outcomes.

Studies in humans and animals also support the idea that postnatal catch-up growth confers increased risk for diabetes later in life. A study of men and women

in Helsinki demonstrated that individuals who developed type II diabetes mellitus in adulthood were of low birth weight but had also caught up to average weight and height by 7 years of age. Likewise, Blesson et al. (2017) identified that female rat MPR offspring have rapid catch-up growth in the first 4 weeks of life and exhibit elevated glucose at 3 months of age. Assessment of gastrocnemius muscle from these female offspring revealed that they express altered phosphorylation of molecules involved in insulin signaling, including insulin receptor substrate-1 (IRS-1), Akt-1, and glycogen synthase. This is again in support of the idea that postnatal catch-up growth is detrimental to metabolic organ function, as in utero adaptations are not conducive in a mismatched postnatal environment. In contrast with this, Zheng et al. (2012) demonstrated that female LP offspring have increased expression of Glucose Transporter Type 4 (GLUT4) mRNA and protein in skeletal muscle at PND 38. These offspring also have increased expression of myocyte enhancer factor 2A (MEF2A), a coactivator of *GLUT4* transcription, and increased glycogen synthase (Zheng et al. 2012). The authors suggest that this may be an adaptive response to MPR during gestation, and it is possible that estrogen may be involved due to the apparent sex-specific differences (Zheng et al. 2012).

Outcomes IV: Dyslipidemia

With respect to lipids, perinatal protein appears to play a role in the maintenance of healthy cholesterol levels in adult offspring. Male rats exposed to severe MPR (4% protein) during the last third of gestation exhibit elevated LDL and reduced high-density lipoprotein (HDL; de Oliveira et al. 2016). In addition, male rat MPR offspring with catch-up growth show increases in cholesterol due to decreased expression of Cyp7a1, the critical enzyme in cholesterol metabolism (Sohi et al. 2011). While hepatic and circulating cholesterol was increased for both males and females at PND 21, there was an increase exclusively in males at PND 130 (Sohi et al. 2011). It is noteworthy that these adult offspring exclusively with catch-up growth (e.g., LP2 offspring) also have increased expression and activity of hepatic Cyp3a1 and Cyp2c11, which are involved in the catabolism of many drugs, including statins (Fig. 3, Sohi et al. 2014). Therefore, it is very conceivable that these animals that exhibit hypercholesterolemia also do not respond as well to cholesterol-controlling drugs. In addition, considering that testosterone is a major substrate for these particular Cyp enzymes, this may explain why MPR male offspring have lower circulating testosterone levels, and consequentially, the long-term sexual dimorphism that exists in this model (Chamson-Reig et al. 2009).

Besides the changes seen in expression and function of hepatic Cyp enzymes, MPR also influences cholesterol levels by way of altered insulin-like growth factor-1 (IGF-1). IGF-1 is a hormone that is known to play a large role in fetal and placental growth (Koutsaki et al. 2011), and its decreased expression has been proposed to induce dyslipidemia and hyperinsulinemia (García-Fernández et al. 2008). Administration of exogenous IGF-1 leads to significantly reduced cholesterol levels in old mice relative to untreated old mice; however, cholesterol levels in treated mice still

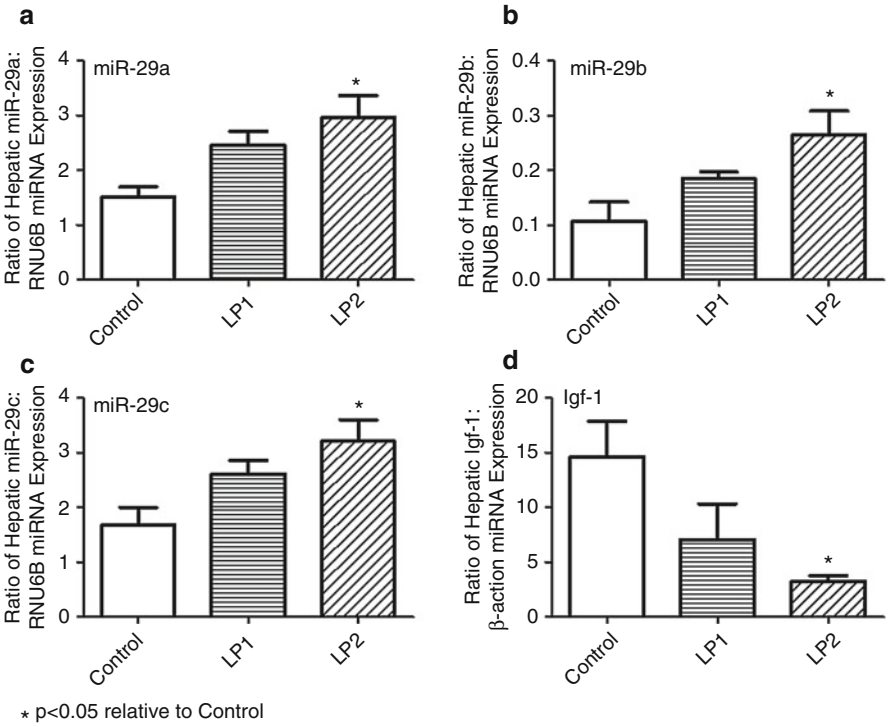

Fig. 4 **Quantitative RT-PCR microRNA analysis of** (**a**) miR-29a, (**b**) miR-29b, (**c**) miR-29c, and (**d**) Igf1 mRNA in the livers of rat offspring (Control, LP1, and LP2) derived at postnatal d130. Pregnant rats were given either a control diet (20% protein) or a low protein diet (8% protein) during gestation only (LP1) and lactation (LP2). The relative amounts of miR-29a, 29b, and 29c mRNA were normalized to that the expression of RNU6B. The relative expression of each Igf1 mRNA transcript was normalized to that of the each b-actin mRNA transcript. Results were expressed as the mean ± SEM. The groups were compared by ANOVA and significant difference was determined by a Tukey HSD post hoc test for individual pairwise comparisons (*$P < 0.05$, indicates significance between control and LP2 cohort). For Fig. 2d, given the variances were not equal, the Tukey HSD post hoc test was performed on log-transformed data. $n = 5$–8/group, where each n represents an offspring derived from a different mother (Reprinted from "Higher Hepatic MiR-29 Expression in Undernourished Male Rats During the Postnatal Period Targets the Long-term Repression of Insulin-like Growth Factor 1," G Sohi et al., Endocrinology (2015) 156(9): 3069–3076, with permission from Oxford University Press)

do not reach levels as low as those found in young, untreated mice (García-Fernández et al. 2008). Similar to uterine-ligated offspring, MPR offspring exhibit significantly reduced levels of *Igf-1* at PND 21 and 130 (Fig. 4; Sohi et al. 2015). These offspring consequentially have reduced growth rate in comparison to control offspring, as indicated by a significantly lower body weight at PND 21 and PND 130 (Fig. 2). Given that this group of offspring also exhibits dyslipidemia in adult life (Sohi et al. 2011), it seems feasible that low levels of *Igf-1* contribute to abnormally high levels of cholesterol. It is noteworthy that offspring exposed to a LP diet

exclusively during lactation exhibit an even greater reduction in expression of hepatic *Igf-1* (Sohi et al. 2015), which suggests that the neonatal window of development plays a significant role in the regulation of *Igf-1* expression.

Outcomes V: Premature Aging

Many studies have determined that there is an existing relationship between birth weight and longevity, and this is again due to alterations in fetal programming that underlie impeded fetal development. Lifespan becomes reduced when impaired fetal development is followed by postnatal catch-up growth, as demonstrated by studies of the MPR diet by Ozanne and Hales (2004). Specifically, they demonstrated that MPR offspring have reduced fetal growth and these offspring tend to have increased lifespan when maintained on a LP diet (Hales et al. 1996). Conversely, MPR offspring that undergo postnatal catch-up growth after birth have a significantly reduced lifespan relative to their LP counterparts (16.3 vs. 13.1 months; Hales et al. 1996). Additionally, expression of sirtuin 1 (SIRT1) protein, a deacetylase enzyme believed to play a role in regulation of lifespan and glucose homeostasis (Michan and Sinclair 2007), was significantly decreased in skeletal muscle of MPR animals with postnatal catch-up growth (Chen et al. 2009). These offspring also have decreased levels of insulin signaling molecules such as IGF-1 and phosphorylated IRS-1, so the authors predicted that impairments to insulin sensitivity may contribute to regulation of lifespan (Chen et al. 2009). A similar model of MPR also demonstrated that markers of cell senescence (e.g., p21 and p16) are upregulated in pancreatic islets of recuperated rat offspring, as well as significantly shorter telomere length (Tarry-Adkins et al. 2009). This further consolidates the relationship between glucose homeostasis and longevity, given the role of pancreatic islets in insulin and glucagon production. It also is noteworthy that offspring born to normal protein mothers and cross-fostered to LP-fed dams during lactation (i.e., MPR lactation only) have significantly increased lifespan in comparison to control offspring (17.0 vs. 15.1 months; Hales et al. 1996). The authors speculated that offspring might therefore benefit from slow postnatal growth; however, adequate dietary protein still remains essential during pregnancy (Hales et al. 1996).

Epigenetic Mechanisms Linking Protein Restriction and Adverse Metabolic Outcomes

It is well understood that transcriptional changes directly compromise fetal development in utero; however the role of epigenetic alterations in fetal metabolic programming has not been investigated to great extent. Epigenetic mechanisms act to influence long-term gene expression without altering the primary genetic sequence, often by modifying interactions between transcriptional and/or translational machinery with regulatory sequences. Mechanisms such as direct DNA methylation, posttranslational histone modifications, and microRNAs (miRs) have been implicated in cases of fetal

undernutrition, and LP-born offspring are no exception. In 2005, a study by Lillycrop et al. demonstrated that CpG island methylation status of hepatic glucocorticoid receptor (*GR*) and *PPARα* promoters are significantly reduced in MPR offspring, and this hypomethylated state is associated with increased expression of these genes. Interestingly, feeding of a LP diet in combination with folic acid supplementation prevented these epigenetic changes, indicating that one-carbon metabolism is essential in preventing the effects of this maternal insult (Lillycrop et al. 2005). Further studies also confirmed that this alteration exemplifies transgenerational effects, as methylation status is decreased in the F2 generation at PND 80 (Burdge et al. 2007). This is characteristic of many epigenetic mechanisms, thereby illustrating relevance of perinatal insult to health outcomes of future generations.

Chromatic structure is also greatly affected by posttranslational histone modifications, including histone acetylation, methylation, ubiquitination, ADP-ribosylation, and phosphorylation. In MPR, the long-term expression of gluconeogenic enzymes (e.g., G6Pase and 11β-HSD1) is increased due to the histone-mediated silencing of hepatic liver X receptor alpha (LXRα) at 4 months (Vo et al. 2013). LXRα is a transcription factor involved in the silencing of genes associated with glucose production. Vo et al. (2013) demonstrated that there is a significant decrease in histone H3 acetylation [K9, 14] at the transcriptional start site of *Lxrα* in 4-month protein recuperated MPR offspring. This is concomitant with decreased association of LXRα at the LXR response element (LXRE) of *G6Pase* and *11β-HSD1*, culminating in glucose intolerance (Vo et al. 2013). As mentioned previously, MPR offspring also exhibit decreased expression of hepatic Cyp7a1 leading to hypercholesterolemia in male offspring at PND 21 and 130 (Sohi et al. 2011). This reduction in enzyme expression is due to epigenetic silencing at the *Cyp7a1* promoter region, as there is increased tri-methylation and decreased acetylation of histone H3 [K9, 14], markers of chromatin condensation. It is interesting that female MPR offspring from the same cohort are protected from these histone modifications in adulthood, as they show complete opposite trends in methylation and acetylation.

In addition to DNA methylation and histone modifications, miRs have also been demonstrated to influence long-term gene expression via epigenetic mechanisms. MiRs are short, noncoding RNA molecules that act to silence target genes via target mRNA degradation or translational repression. In 2016, Su et al. investigated the role of miR-15b in pancreatic beta cell proliferation of MPR-born mouse offspring. It was discovered that miR-15b is significantly increased in the pancreatic islets of MPR offspring, accompanied by reduced expression of cyclin D1 and D2 (Su et al. 2016). Given the role of cyclins in progression through the cell cycle, it is believed that the downregulation of these molecules contributes to impaired beta cell function and thus glucose intolerance. As discussed earlier, administration of the MPR diet during pregnancy and lactation has been demonstrated to cause the upregulation of hepatic miR-29 expression in LP offspring with postnatal catch-up growth (Sohi et al. 2015). MiR-29a, miR-29b, and miR-29c were all significantly increased in livers of 3 week and 4 month old offspring, and this further caused a reduction in expression of *Igf-1* (Fig. 4; Sohi et al. 2015). With that in mind, it is possible that timing of nutritional restoration for IUGR offspring may play a role in long-term disease via modulation

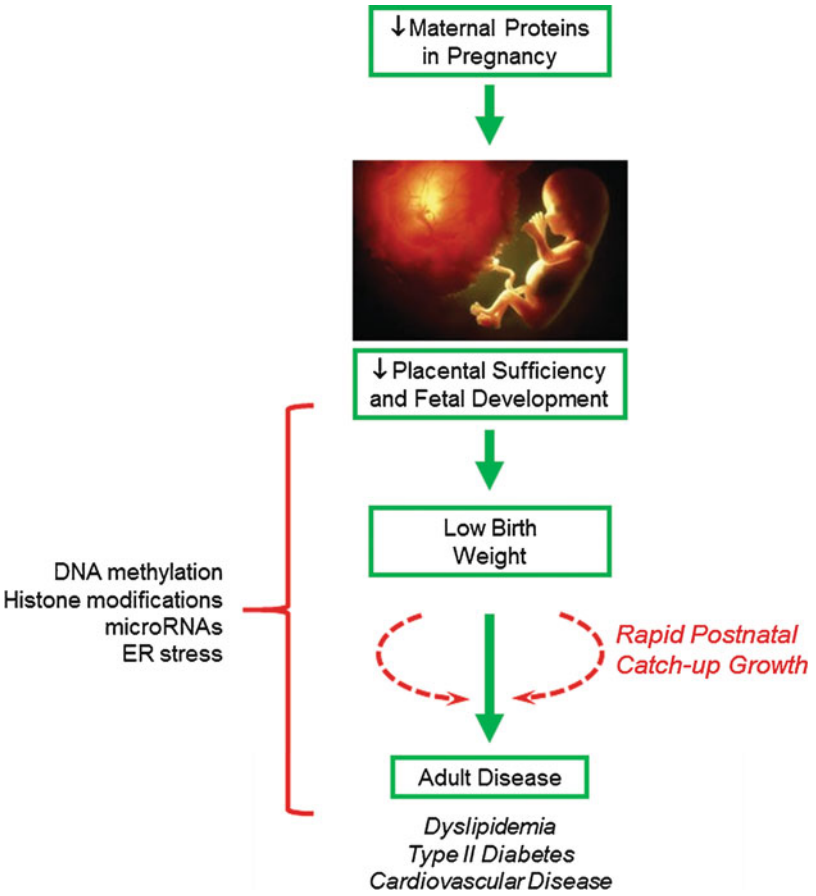

Fig. 5 Overview of the molecular mechanisms underlying how maternal protein restriction (MPR) during perinatal leads to long-term metabolic dysfunction in adulthood Direct pathways altered by maternal protein restriction are indicated by *green solid arrows*, while direct and indirect molecular mechanisms are indicated by *red arrows*

of miRs. Given that miRs also circulate in the blood, these animal studies could lead to novel therapeutic interventions with the use of miR inhibitors in neonatal treatment of the metabolic syndrome. An overview of the molecular mechanisms underlying MPR-induced metabolic dysfunction is illustrated in Fig. 5.

Other Mechanisms Linking Protein Restriction and Adverse Metabolic Outcomes

As previously mentioned, the fetal liver is proportionally small in MPR offspring at birth and undergoes rapid postnatal catch-up growth with introduction of a normal protein diet (Sohi et al. 2011; Hales et al. 1996). During this period of growth,

hepatocytes undergo rapid replication such that the neonatal liver becomes larger. It is therefore possible that ER stress may contribute to poor metabolic health outcomes in the recuperated adult MPR offspring. ER stress is a cellular event which ensues due to environmental insults leading to an increase in the presence of misfolded or unfolded proteins present within the ER (Sohi et al. 2013). In response to ER stress, the unfolded protein response (UPR) becomes activated in attempt to reverse this stress by refolding those misfolded proteins and/or attenuating protein translation through three signaling pathways (Sohi et al. 2013). In the case that the UPR cannot alleviate ER stress, apoptosis may further occur. The MPR offspring with catch-up growth exhibit hepatic ER stress at 4 months of age as indicated by increased hepatic p-eIF2α [Ser51], Grp78, and spliced Xbp1 (Sohi et al. 2013). P-eIF2α [Ser51] is known to negatively regulate the initiation stage of protein translation (Proud 2005). As with many other mechanisms discussed in this review, the LP diet itself does not a play a direct role given embryonic day 19 low protein fetuses do not exhibit ER stress (Sohi et al. 2013). In addition to affecting hepatic function directly, ER stress may also be involved in the regulation of epigenetic mechanisms such as miRs. ER stress has been shown to directly cause an increase in miR-29a expression in vitro (Nolan et al. 2016), and studies by Sohi et al. (2015) have implicated that MPR offspring with postnatal catch-up growth exhibit increased hepatic miR-29a and ER stress at 4 months of age. Considering that miR-29 targets *Igf-1*, this further suggests that ER stress may play an important role in the etiology of the metabolic syndrome in these IUGR offspring.

Conclusion

While maternal malnutrition exists in many forms, MPR has been shown to have major consequences for the long-term metabolic health of LP-exposed offspring. Epidemiological studies in humans have deduced that perinatal protein deficiency gives rise to low birth weight, and these individuals are at greater risk for development of the metabolic syndrome in adult life. Studies of individuals with SAM reveal that poor dietary protein can lead to glucose intolerance and abnormal plasma fatty acid levels. Moreover, animal studies of the MPR model have further established that LP-exposed offspring have low birth weight and asymmetrical IUGR, with liver growth and development taking a major hit relative to other organs. Additionally, the function of other organs such as the pancreas, muscle, and adipose becomes impaired, which further contributes to metabolic dysfunction. In adult life, these animals tend to have glucose intolerance, dyslipidemia, and increased visceral obesity. The onset of these deficits are further exacerbated by postnatal catch-up growth, as a nutrient-poor prenatal environment gives rise to altered fetal programming that is not beneficial in a nutrient-rich postnatal environment. Furthermore, these offspring exhibit reduced lifespan relative to animals that are fed either a control or LP-exclusive diet. While the mechanisms behind these defects are not fully understood, it is widely accepted that epigenetic alterations such as DNA methylation, posttranslational histone modifications, and miRs can influence fetal gene expression. Animal models of MPR have provided insight into what might be occurring in humans, but further investigation is warranted to better comprehend

the molecular basis of the metabolic syndrome in response to perinatal protein restriction. Until then, nutritional intervention during pregnancy is necessary to ensure that mothers consume appropriate amounts of dietary protein such that there are no negative effects to fetal growth and development.

Policies and Protocols

In this review, we have discussed the metabolic implications of perinatal protein restriction and postnatal catch-up growth in LP-born offspring. Models of protein restriction have confirmed that insufficient amino acids during pregnancy contribute to low birth weight, and this leads to the metabolic syndrome in adult life. Due to fetal adaptations that occur in utero, low birth weight offspring have rapid weight gain when presented with a mismatched postnatal environment (i.e., a "normal" protein diet), exacerbating the risk for adult metabolic disease. It is critical that primary health-care workers are informed regarding this information related to postnatal catch-up growth. Physicians, nurses, and midwives should emphasize to patients that a balance between prenatal and postnatal diet with respect to protein intake is essential. In addition, it is recommended that pregnant women ingest protein in the form of animal-sourced amino acids rather than plant-based amino acids. As previously mentioned, studies demonstrate that poor fetal pancreatic development (due to perinatal protein restriction) can be rescued with administration of taurine, a meat-sourced amino acid (Boujendar et al. 2003). While the role of animal-based amino acids has been only investigated in pancreatic development, it is conceivable that this may be the case for other metabolic organs as well. As always, prevention is a more successful strategy than treatment; therefore, it is highly encouraged that health-care workers and pregnant mothers work together to prevent maternal malnutrition for the sake of the developing fetus.

Dictionary of Terms

- **Asymmetrical intrauterine growth restriction (IUGR)** – A category of IUGR in which infants are not only small for gestational age but also exhibit disproportionately small organ size.
- **Dyslipidemia** – An increase in plasma cholesterol, triglycerides, or both, leading to the development of cardiovascular disease.
- **Epigenetics** – The study of heritable changes in gene expression without modification of the primary gene sequence.
- **Endoplasmic reticulum stress** – A cellular stress response characterized by increased accumulation of misfolded and/or unfolded polypeptides in the lumen of the endoplasmic reticulum.
- **Gluconeogenesis** – Production of de novo glucose molecules from non-carbohydrate sources.
- **Glucose intolerance** – A prediabetic condition in which affected individuals exhibit elevated blood glucose (i.e., hyperglycemia) in the fasted and/or fed

state. Glucose intolerance often precedes type II diabetes, which occurs when individuals also exhibit insulin resistance.

- **Heterochromatin** – Repressed region of DNA leading to a decrease in gene expression.
- **Malnutrition** – Either an excess or deficiency in one or more nutrients.
- **Metabolic syndrome** – A group of adverse metabolic symptoms that together confer increased risk for type II diabetes mellitus and cardiovascular disease.
- **MicroRNAs** – Endogenous, short, noncoding RNA molecules that post-transcriptionally regulate expression of target mRNA sequences.
- **Placental insufficiency** – An idiopathic condition occurring in 8% of pregnancies that leads to reduced maternofetal nutrient exchange due to inadequate placental blood flow.
- **Postnatal catch-up growth** – A period of growth after birth whereby low birth weight offspring exhibit rapid growth rate such that they "catch-up" to average body weight. Offspring that undergo postnatal catch-up growth are often referred to as "recuperated" offspring.
- **Senescence** – The process of biological aging due to loss of cellular division and function.
- **Severe acute malnutrition (SAM)** – An extreme form of undernutrition characterized by muscle atrophy and low body weight can be further categorized into cases of marasmus (extreme caloric restriction) or kwashiorkor (extreme protein deficiency).
- **Telomere** – A protective region of repetitive sequences at the end of a chromosome.

Summary Points

- In mammals, many organs are vulnerable to perinatal protein deficiency, which causes altered gene expression and leads to long-term metabolic effects in the offspring.
- Rat offspring exposed to maternal protein restriction have low birth weight and asymmetrical intrauterine growth restriction (i.e., many organs are proportionally small relative to the rest of the body).
- Given the role of the liver in glucose homeostasis, as well as the metabolism of cholesterol and a variety of drugs, impaired liver growth and development by maternal protein restriction leads to abnormal regulation of plasma glucose levels and hepatic enzymes.
- MPR indiced alterations to hepatic, pancreatic, or adipose function leads to dyslipidemia, obesity, glucose intolerance, and coronary artery disease.
- Transcriptional and epigenetic mechanisms (e.g., DNA methylation, post-translational histone modifications, microRNAs) facilitate adaptation of developing organs to amino acid deficiencies in utero; however, this can have dire consequences long-term and may have transgenerational effects.

- Endoplasmic reticulum stress is present in offspring with postnatal catch-up growth due to rapid growth of metabolic organs, and activation of the UPR further increases risk for the metabolic syndrome in adult life.

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Complementary Food Supplements After Disasters

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Caixia Dong and Shi-an Yin

Contents

Abstract

International experience has proved that even though in previously healthy population living in those areas attacked by natural disaster, elder infants and young children's morbidity and mortality resulted from acute malnutrition often dramatically increased in very short period since they are the most nutritionally vulnerable group due to poor or limited food choices. Present paper reviews the application of complementary food supplements for elder infants and young children after natural disaster. After a serious destructive event, quickly assessing

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and monitoring nutritional and health status of infants and young children will be important for providing basic information to the local government to make and implement nutrition intervention to the target vulnerable groups. First, taking methods dependent on the field situation evaluate the risk of nutritional deficiency, establish an effective transportation mechanism in distributing salvage food and guide the usage of complementary food supplements in affected areas. Second, the practical experience of complementary food supplements (CFSs) in application for elder infants and young children includes multi-nutrient powder with food matrix, lipid-based nutrient supplements (LNS), and corn-soy blend (CSB), and micronutrients Sprinkles. Third, it should be particularly paid attention to the issues of safety use and quality control procedures on CFSs for elder infants and young children, implementing CFSs intervention in affected-areas after natural disaster will cover two aspects which involve in hygiene and safety to meet the nutritional requirements. Hygiene is referring to the contamination of traditional microbiology and safety of nutritional needs is related to prevent nutritional deficiencies due to poor compliance and avoid excessive intake due to overuse.

Keywords

Complementary food supplements · Elder infants · Young children · Natural disaster · Nutrition · Nutrition deficiencies · Lipid-based nutrient supplements · Corn-soy blend · Sprinkles · Micronutrient

List of Abbreviations

ARI	Acute respiratory infection
CF	Complementary food
CFS	Complementary food supplement
CFs	Complementary foods
CFSs	Complementary food supplements
CRP	C-reactive protein
CSB	Corn-soy blend
DD	Diarrheal disease
FEP	Free erythrocyte proporphyrin
Hb	Hemoglobin
IDA	Iron-deficient anemia
IO	Intelligence quotient
LAZ	Length for age Z score
LNS	Lipid-based nutrient supplements
MAM	Moderate acute malnutrition
NGOs	Non-Governmental Organizations
RUSF	Ready-to-use supplementary food
RUTF	Ready-to-use therapeutic food
SAM	Severe acute malnutrition
SF	Serum ferritin
STR	Serum transferring receptor

TfR	Transferring receptor
WAZ	Weight for age Z score
WHO	World Health Organization
WHZ	Weight for height Z score
YYB	Yingyangbao, one kind of complementary food supplements in China

Introduction

Natural disasters such as earthquake, debris flow, floods, typhoon, severe drought, and volcanic eruptions are widespread and obvious characteristics are unpredictable and sudden, destructive, danger, and whole social emergency which make millions of people to be involved globally every year. In most of cases, a severe natural disaster might double damage to the human being, the substance and spirit disasters. Because food supply systems were severely damaged or even completely stopped, major food shortages became a primary post-disaster feature and the common characteristics were high prevalence of acute malnutrition and micronutrient deficiencies in infants and young children (Sun et al. 2013; Wang et al. 2010; Zhao et al. 2010a; Dong et al. 2014; Singh et al. 2006).

International experience has proved that even though in previously healthy population living in disaster area, elder infants and young children's morbidity and mortality resulted from acute malnutrition often dramatically increased in very short period (Singh et al. 2006) since they are the most nutritionally vulnerable group due to the supply of poor or limited food (Zhao et al. 2010a, b; Nishikiori et al. 2006; Wang et al. 2010). Malnutrition will increase the susceptibility to infectious diseases such as diarrhea and pneumonia and has an indirect association with the leading causes of death in infants and young children (Walker et al. 2013). However, malnutrition is preventable through effective nutritional intervention, for example, guiding advice on complementary feeding practices and/or giving complementary food supplements (CFSs) or multiple micronutrient supplements such as Sprinkles (Dong et al. 2013; Wang et al. 2007, 2009; Yu et al. 2007; Yin and Dong 2015). Frequently, a food supply and nutrition improving response combined with other public health interventions to reduce the risk of malnutrition will be required in those areas affected by a natural disaster (Dewey and Adu-Afarwuah 2008; Young et al. 2004; Bhutta et al. 2013). This chapter will review assessing and monitoring the nutritional and health status of infants and young children, the practical experience of CFSs in practical application, and the issues of safety use on CFSs for elder infants and young children after a natural disaster.

Quickly Assessing and Monitoring the Nutritional and Health Status of Infants and Young Children during or after Disaster

After a natural disaster, quickly assessing and monitoring nutritional and health status of infants and young children will be important for providing basic

information to the local government to implement nutrition intervention to the target vulnerable groups.

1. **Taking different evaluating methods depending on the field situation**: Infants and young children are a vulnerable group, but in the disaster-affected areas, relief and aid to such group are provided generally not to meet their needs in diversify of nutrition and quick growth development (Zhao et al. 2010; Sun et al. 2013). Therefore, we need to evaluate nutritional status of children young than 5 years through the nutrition survey, food consumption monitoring, sentinel monitoring, or the other simple and operable epidemiologic methods. Intervention measures and implementation for "assistance to such group" were based on the results obtained through evaluating or monitoring data which also can be used to evaluate the effectiveness of nutritional intervention implemented after disaster.
2. **Evaluate the risk of nutritional deficiency**: Nutritional status of populations as an early warning indicator would be very limited, because malnutrition generally is considered as a later indicator reflecting the deterioration or adverse effects of the nutritional and health status caused by natural disaster. However, we can analyze the changing trend of nutritional status of infants and young children through nutrition survey or monitoring, search the difference between present and past conditions, timely find priority nutritional problems resulted from natural disaster for implementing target intervention which include food resource, food security situation involved in availability and accessibility, food supplies and distribution, etc. (Programme 2005). Implementing evaluation on nutritional deficiency risk in infant and young children is very important after a severe natural disaster which includes malnutrition, morbidity, mortality caused by the disaster. All of these would have more practical and operable significance for effectively implementing assistance to the targeted populations.
3. **Establish an effective transportation mechanism in distributing salvage food**: Based on the results obtained from nutritional deficiency risk analysis and evaluation, we need to establish multiple-parallel support and protection ways to improve the nutritional status of infants and young children through providing food aid such as CFSs and with or without multiple micronutrient supplements progressively supplant single micronutrient. We can distribute special relief foods or CFSs to these populations through village clinics, schools, hospitals, orphanages, and other institutions or NGOs (Sun et al. 2011; Dong et al. 2013). Some organizations such as the Red Cross can provide more foods for vulnerable groups by increasing the general food ration to meet their needs.
4. **The usage of CFSs in the affected areas**: The period from 6 to 24 months of age is the most critical period because of their rapid growth and an increasing reliance on complementary food (CF). In order to improve nutritional status and reduce the mortality, adequate food-based nutrition interventions should be very important, since such interventions can play a key role in saving lives. Several strategies can be used to improve complementary feeding practices. These include education on child's caring knowledge to mothers or caretakers designed to promote healthy feeding practices; provision of CFSs offering extra energy with or without

micronutrient fortification; and increasing energy density of CFs. However, the usage of CFSs has been interested in recent years (Yang et al. 2013; Sun et al. 2011; Lopez Boo et al. 2014).

Before the intervention using CFSs, it can be best rapidly to monitor and evaluate the nutritional and health status, establish the nutritional risk and needs for infants and young children, and solve the transportation mechanisms how to effectively ship and distribute the CFSs to the affected areas.

The Practical Experience of CFSs on Application

Malnutrition children have a higher risk suffered from death and infectious diseases because of traditional CFs with a low nutrient and energy density in developing countries. Treating severe acute malnutrition (SAM) children in hospitals is not always desirable or practical, but home-based treatment will be better choice in most cases and complementary food supplement (CFS) usage can treat and prevent malnutrition in affected areas attacked by natural disaster. In those affected areas, no single of intervention measure can solve all complex nutritional problems that may exist in vulnerable groups. Malnutrition and other nutrition-related problems are generally closely related to insufficient intake of nutrients (especially micronutrients) caused by lack of animal foods, low dairy consumption, food insecurity, lack of maternal care, and poor public health environment. Therefore, implementing adequate nutrition intervention in early life is rather an opportunity for a better later life. At the present time, quickly providing CFSs at home level may play an important role in improving the nutritional status and is still considered as the overriding approach to control malnutrition of infants and young children. Home intervention has been recommended to use CFSs prepared by the career for young children. These include CFSs with food matrix such as soybean powder fortified with multiple micronutrients (Sun et al. 2011), CFSs without food matrix such as Sprinkles (provide micronutrients) (Lopez Boo et al. 2014), and commercially manufactured food such as lipid-based nutrient supplements (LNS) (Phuka et al. 2009).

Multi-Nutrient Powder with Food Matrix

Multi-nutrient powder is a CFS with food matrix for elder, infants, and young children. Through supplementing multiple-micronutrient, high-quality protein from soybean or its isolated proteins and/or cow milk, fat, and total energy could be provided which would be very useful for improving general nutritional status because these macronutrients play important role in the utilization and bioavailability and adequate reserve of micronutrients in the body. Such products are generally not water-based and relatively cheap, easy to transport from manufactured place to the mountain areas or inconvenient places, easy storing at room temperature and dry place without refrigeration for about 2 years, well compliance for long-term taking, and suitable for the mild malnutrition groups in these areas where hygiene is suboptimal after natural disaster. However, for SAM, we recommend taking ready-

to-use therapeutic food (RUTF) or increasing the dose as well as improving the quality of the complementary foods (CFs) (Wang et al. 2009; Phuka et al. 2009).

Such product can be added to the CFs or prepared and eaten directly for the elder infants and young children. For example, one kind of such product has been thoroughly evaluated and approved in China. This product (referred to as "Ying Yang Bao" in Chinese) was specially designed for the elder infants and young children based on a China's National Food Safety Standard for "CFSs" (GB 22570–2014). Essential composition of formulated supplementary foods are as follows (Nutrients/10 g/pack/d): vitamin A, vitamin D_3, vitamin B_1, vitamin B_2, iron, and zinc; the other optional ingredients include protein, calcium, magnesium, selenium, copper, vitamin E, vitamin K, niacin, vitamin B_6, vitamin B_{12}, folic acid, pantothenic acid, choline, biotin, and vitamin C. The composition in a commercial formulated CFSs and comparison with LNS and Sprinkles used in natural disaster areas or low-income rural areas for several years was shown in Table 1 and a program to market of such CFSs (Ying Yang Bao) has been implemented through the intervention efficacy in improving anemia and feeding practices in China (Sun et al. 2011).

The brewing and using methods for such product are very simply. One pack of this product was put into a small bowl with about 30 ml boiled cool water and then stir into a mud paste with a spoon, make it thick enough to stay in the spoon that would be best status for baby use. The preformed mud paste can be directly put into porridge, noodles or soup, and egg yolk or bread (or Chinese steam bread), well stir paste for baby use. For the best usage, it should take one pack daily alone or with a meal or taking half pack twice times per day for infants aged 6–12 months. Table 2 listed the effectiveness of CFSs (Yingyangbao, YYB) on improving status of elder infants and young children in low-income rural areas or affected-areas involved in natural disaster of China.

In China, a CFS (Yingyangbao, YYB) was first performed in 2001 by the International Life Science Institute and Chinese Center for Disease Control and Prevention. Such CFS has been used for the affected areas attacked by natural disasters or the intervention for nutritional improvement in poor areas since 2008, and national standard for this CFS has been established later (Huo 2017). Two prospective studies on the effectiveness of CFSs were carried out in the areas affected by Wenchuan Earthquake (Dong et al. 2013) and poor areas (Wang et al. 2015, 2017); these studies evaluated the improving role in growth rate and anemia prevalence of elder infants and young children. All children (6~18 months) in these affected areas were daily provided with formulated CFSs (compositions of this product were shown in Table 1) for up to 24 months of age. The intervention period lasted for one and half year. After the intervention, the growth and anemia status of children were significantly improved. Since YYB efficacy and effectiveness in reducing iron deficiency, underweight, and stunting have also been well documented (Wang et al. 2004; Dong et al. 2013), such kinds of products have been used in several national intervention studies for more than 10 years (Huo 2017), and Chinese government has launched a national project improving children's nutrition in poor areas since 2012. However, compliance to CFSs contributes to the improving effectiveness of nutritional and anthropometric status (Wang et al. 2017).

Table 1 Nutrient composition in different CFSs (Ying Yang Bao, YYB), LNS, and micronutrient Sprinkles

Nutrients	Unit	YYB[a](10~20g/pack/d)			LNS			Sprinkles[b]
		6~12m	13~36 m	37~60m	25 g/d[c]	50 g/d[c]	100g[d]	1 g/pack/d
Energy	kcal	–	–	–	127	256	520–550	–
Protein	g	>2.5	>2.5	>2.5	3.5	7.0	10–12	–
Fat	g	–	–	–	8.5	16.9	45–60%[e]	–
Carbohydrates	g	–	–	–	6.6	13.8	–	–
Vitamin A	μgRE	120~360	150~450	150~450	400	400	8,00–1,100	300
Vitamin D_3	μg	3.0~9.0	3.0~9.0	3.0~9.0	5	5	15–20	7.5
Vitamin K_1	μg	3.0~9.0*	4.5~13.5*	4.5~13.5*	–	–	–	–
Vitamin B_1	mg	≥0.12	≥0.24	≥0.24	0.5	0.5	≥0.5	–
Vitamin B_2	mg	≥0.2	≥0.24	≥0.24	0.5	0.5	≥1.6	–
Vitamin B_6	mg	≥0.12*	≥0.20*	≥0.20*	0.5	0.5	≥0.6	–
Vitamin B_{12}	μg	≥0.2*	≥0.36*	≥0.36*	0.9	0.9	≥1.6	–
Niacin	mg	1.2~6.0*	2.4~6.0*	2.4~6.0*	6	6	≥5	–
Folic acid	μg	18.8~150*	35.3~150*	35.3~150*	160	160	≥200	150
Pantothenic acid	mg	≥0.72*	≥0.8*	≥0.8*	2	2	≥3	–
Choline	mg	≥60*	≥80*	≥80*	–	–	–	–
Biotin	μg	≥2.4*	≥3.2*	≥3.2*	–	–	–	–
Vitamin C	mg	≥20*	≥24*	≥24*	30	30	≥50	50
Iron		3.0~9.0	3.6~10.8	3.6~10.8	8	8	10–14	30

(*continued*)

Table 1 (continued)

Nutrients	Unit	YYB[a](10~20g/pack/d)			LNS			Sprinkles[b]
Zinc	mg	2.0~6.0	2.0~7.0	2.0~7.0	8.4	8.4	11–14	5
Copper	mg	–	–	–	0.4	0.4	1.4–1.8	–
Iodine	μg	–	–	–	135	135	70–140	–
Magnesium	mg	–	–	–	60	60	80–140	–
Selenium	μg	–	–	–	17	17	20–40	–
Calcium	mg	120~240	180~360	180–360	283	366	300–600	–
DHA	mg	30~90*	30~90*	30~90*	–	–	–	–

[a]*YYB* Yingyangbao, a complementary food supplement, cited from China's National Food Safety Standard for "CFSs" (GB 22570 – 2014)
[b]Cited from Yang et al. (2004)
[c]Cited from Phuka et al. (2009)
[d]Cited from http://WWW.who.int/nutrition/publications/severemalnutrition/9789280641479/, 2010
[e]Lipids were expressed as percentage of total energy, n-3 and n-6 were 0.3~2.5% and 3~10% of total energy, respectively, and moisture content is less than 2.5%; the other compositions per 100 g can be added, which include sodium (≤290mg), potassium (1110~1400mg), phosphorus (300~600mg) excluding phytate, vitamin E (≥20mg), vitamin K (15~30μg), and biotin (≥60μg)
"–", without supplementation

Table 2 The effectiveness of complementary food supplements (Yingyangbao) on improving status of elder infants and young children in disaster-affected areas or low-income rural areas of China

Date	Site	Sample size	Children age (m)	Duration (m)	Observation index	Results	Reference
2009	Huguan & Changzhi counties	250	6–24	Daily use for 8 m	Hb assay	Reduce anemia & improving feeding practices	(Sun et al. 2011)
2009	Li county	510	6–23	Daily use for 15 m	Anthropometry and Hb	Effectively improve growth and decrease anemia	(Wang et al. 2011)
2010	MDG[a]	693	6–23	Daily sue for 18 m	Anthropometry, Hb & micronutrients assay.	Good compliance contributes to low prevalence of malnutrition, vit B_{12} def, & low risk for anemia	(Wang et al. 2015)
2010	Kang county	1019	6–23	Daily use for 18 m	Anthropometry, Hb	Improve nutritional status, elevate Hb & decrease anemia	(Dong et al. 2013)
2012	Huzhu county	2186	6–23	Daily use for 24 m	Anthropometry, Hb	Improve feeding practices & reduce anemia prevalence	(Zhang et al. 2016)
2014	Fengjie county	500	6–23	Daily use for 12 m	Anthropometry, Hb	Reduce prevalence of malnutrition & anemia	(Li et al. 2017)
2015	Ganzhou city	483	6–18	Daily use for 6 m	Anthropometry, Hb	Improve nutritional status & decrease anemia prevalence	(Ding et al. 2016)

[a]*MDG* program of improving nutrition and food safety for China's most vulnerable women and children funded by the Millennium Development Goals Spanish Achievement Fund involving Wuding county, Zhengan county, and Zhenan county
YYB Yingyangbao, *Hb* hemoglobin

Lipid-Based Nutrient Supplements (LNS) and Corn-Soy Blend (CSB)

Recently, different approaches for improving nutrition status have been used for treating moderate acute malnutrition (MAM) using fortified blended flours such as enhanced versions of corn-soy blend (CSB) or lipid-based nutrient supplements (LNS) in low-income countries or emergency settings (Lazzerini et al. 2013; Campbell et al. 2016; Muslihah et al. 2016; Flax et al. 2015; Abbeddou et al. 2017; Hemsworth et al. 2016; Adu-Afarwuah et al. 2016; Chaparro and Dewey 2010). The study in Burkina Faso showed that CSB was not as readily consumed as LNS (Iuel-Brockdorf et al. 2015) or the study in Malawi indicated that LNS might be superior to CSB for underweight children (Thakwalakwa et al. 2014). Most of studies used LNS and carried out in developing countries, and the international lipid-based nutrient supplements project have been began to prevent undernutrition in vulnerable populations in 2009 (Arimond et al. 2015).

LNS are soft or crushable foods, conveniently packaged, and the low percentage of free water and energy-dense fortified pastes that typically contain high proteins, carbohydrates, and micronutrients embedded in a lipid base (Briend et al. 1999; Phuka et al. 2009). The general nutrient compositions in such product were shown in Table 1. However, manufacture for such products is flexible, the exact recipe, energy, and protein contents can be tailored to specific needs, such as those for a fast-growing child or those recovering from mild or moderate undernutrition. LNS do not require cooking or preparation except adding water before use and can be easily consumed by children from the age of 6 months; hence feeding process is not easy to be contaminated by external bacteria which are particularly useful in those areas attacked by natural disaster.

LNS are simple to store and deliver, easy to use, fast acting, affordable, culturally acceptable, have appropriate shelf-life and stability without needing refrigeration under varied climate and temperature. Table 3 listed the effectiveness of LNS on improving status of elder infants and young children in low-income rural areas or affected-areas involved in natural disaster, and most of these studies showed that interventions using LNS could significantly reduce the incidence of stunting, improve linear growth and dietary diversity, macro- and micronutrient intakes. Adu-Afarwuah et al. (Adu-Afarwuah et al. 2016) have shown that provision of LNS would have more advantages in length, length for age Z score (LAZ), weight, and weight for age Z score (WAZ) compared with those in the standard iron and folic acid or multiple micronutrient group by 18 months of age. However, some factors that may affect the effectiveness include the duration and compliance of the intervention, composition and dosage of LNS given, and baseline demographics and nutritional status of children (Wang et al. 2017; Matsungo et al. 2017).

The term of LNS refers to a range of fortified, lipid-based products, including the products like RUTF and ready-to-use supplementary food (RUSF). RUTFs have revolutionized and proven to be effectively in the community-based treatment of SAM with weight for height Z score (WHZ) <-3SD attributable to the transient effects of disease or food shortages in emergencies (Briend et al. 1999; Yang et al. 2013) and now RUTF can be made according to a standard, energy-rich composition defined by the World Health Organization (WHO) for severely malnourished infants

Table 3 The effectiveness of LNS on improving status of elder infants and young children

Date	Site	Sample size	Age (m)	Design	Observation index	Results	Reference
2009–2014	Rural Northwest Bangladesh	5499	6 m	Control group, periodic child feeding counseling; treated group, counseling plus 1 of 4 CFSs, a supplement provided 125 & 250 kcal/d for 6–11 m & 12–18 m of age for 1 year[a]	Questionnaire & anthropometry	Daily CTSs improved linear growth and dietary diversity	(Campbell et al. 2016)
2014–2015	West Madura Island, Indonesia	269	>6 m	Non-randomized, controlled trial. SQ-LNS[b] group provided 118 kcal/d; biscuits group provided 3 pieces or 30 g/d containing 135 kcal/d for 6 m.	Questionnaire & anthropometry	SQ-LN group had lower stunting incidence than in biscuit & control groups	(Muslihah et al. 2016)
2009–2010	Three municipalities in Hunduras	298	6–18 m & WHZ≥-2SD	Intervention group received LNS with 46.3 g/d and 70 g/d for 6–11 m and 12–30 m lasting for 12 m	Questionnaire & 24 h dietary recalls	LNS improved macro- and micronutrient intakes.	(Flax et al. 2015)
2010–2012	Dande Health District in southwestern Burkina Faso	2435	9–18 m	Children daily received 20 g SQ-LNS, group one, placebo tablet without Zn; group two, placebo table with 5 mg Zn; group three, placebo tablet with 10 mg Zn; & group four, 5 mg Zn tablet, lasting for 9 m	Hb in whole blood, Zn, Fe and vitamin A, ferritin, sTfR and RBP in plasma[c,d].	SQ-LNS improved Fe and vitamin A status regardless of Zn amount and source.	(Abbeddou et al. 2017)

(*continued*)

Table 3 (continued)

Date	Site	Sample size	Age (m)	Design	Observation index	Results	Reference
2009–2012	Rural communities in Malawi	748	<6 m	Control without supplement, and 3 groups received 10, 20, or 40 g LNSs[e]/d for 12 m.	24 h dietary recalls & anthropometry	10–40 g LNSs/d increased energy and macronutrient intakes.	(Hemsworth et al. 2016)
2009–2014	Semi-urban Ghana	1228	6–18 m	Randomized controlled trial. From 6 m to 18 m of age, SQ-LNS was provided with118 kcal per 20 g/d.	Anthropometry	SQ-LNSs increased child's attained length by age 18 m of age.	(Adu-Afarwuah et al. 2016)

[a]*CFSs* complementary food supplements. Four CFSs included the first product was made of chickpeas and the second product was made of rice and lentils both developed and produced in-country, the third was fortified wheat-soy blend (WSB++) fed to children as a porridge supplied by the World Food Programme, and the forth was a commercially produced, read-to-eat, peanut-based product
[b]*SQ-LNS* small quantity of liquid-based nutrient supplements
[c]*sTfR*, soluble transferrin receptor
[d]*RBP* retinol-binding protein
[e]*LNSs* lipid-based nutrient supplements

and young children in low-income countries (Yang et al. 2013; Thakwalakwa et al. 2010; Thakwalakwa et al. 2014; Siega-Riz et al. 2014; Dewey and Arimond 2012; 'Community Based Management of Severe Acute Malnutrition', 2010) because of their specialized nutrient composition, the reduced risk of contamination that it requires no preparation, and the fact that children can consume RUFT at their homes and ensures rapid weight gain provided that they are free from clinical complications. RUFTs were generally higher in energy density (>700 kcal/d) than RUFT (≤500 kcal/d).

However, RUSF provides less energy at a relatively lower cost than RUTF and enriches a child's existing diet with the goal of treating mild or MAM with WHZ between -2SD and -3SD or prevent the progression from MAM to SAM, and thus would have the potential efficacy to reduce child mortality and morbidity (Yang et al. 2013; Phuka et al. 2009; Thakwalakwa et al. 2010; Chaparro and Dewey 2010).

Although such product has a relatively higher cost, it does have well compliance and very easy for transportation, suitable for malnourished populations, particularly for those children with SAM. RUTF and RUSF must be targeted appropriately to ensure cost-effectiveness. More than two third (73%) of children aged 6~60 months were recovered from MAM after 13 weeks treatment with RUSF (Karakochuk et al. 2012) and the overall recovery from SAM with use of RUTF is 88.3% with a mortality of less than 1% and with a mean weight gain of 3.2 g/kg/d after 8 weeks treatment (Gera 2010).

Micronutrients Sprinkles without Food Matrix

Micronutrients Sprinkles is a CFSs without food matrix and single-dose sachets containing iron and other multi-micronutrients, which are easily sprinkled onto any traditional CF in a powdered form for elder infants and young children prepared in the household. Ingredients of micronutrients Sprinkles were shown in Table 1 and the other micronutrients such as iodine, vitamin B_1, vitamin B_2, calcium, and the others can be added to micronutrients Sprinkles sachets depending on local iodine or the micronutrient status. This single-dose sachets can be put in the infant's daily CF such as porridge, soup, or steamed rice to prevent iron-deficient anemia (IDA) and deficient prevalence of iron and other micronutrients (Schauer and Zlotkin 2003; Yang et al. 2004; Jack et al. 2012; Suchdev et al. 2016). The provision of multiple micronutrient supplements would be better than single iron supplement. Table 4 showed the effectiveness of home fortification with micronutrient Sprinkles on improving status of elder infants, young, and preschool children in low-income areas or affected-areas involved in natural disasters which showed that such CFS can significantly improve iron status, decrease the prevalence of IDA, and shorten hospitalization for diarrhea and fever.

These products can be added directly to infant daily foods, cannot change the color and texture of the food itself and cannot affect child's appetite. The smell produced by iron is not obvious, and it does not cause irritation to the gastrointestinal tract of infants due to fortified microencapsulated iron, but the effect of adding micronutrients Sprinkles to home-prepared foods should consider the local food culture and acceptance. Because this product uses a single-dose sachets, it is easy to

Table 4 The effectiveness of home fortification with micronutrient Sprinkles on improving status of elder infants, young, and preschool children

Date	Site	Sample size	Children age (m)	Duration (m)	Observation index	Results	Reference
2001	Baotou city, China	353	36–72	Daily use for 2.5 mo	Anthropometry, Hb, SF[a] & FEP	Improve growth & reduce Fe deficiency	(Yang et al. 2004)
2001	Central Vietnam	377	5	Daily use for 6 mo	Hb, plasma ferritin, TfR, Zn, retinol	Improve Fe status & IDA prevalence	(Phu et al. 2010)
2002	Bijie county, China	273	36–60	Daily use for 2.5 mo	Anthropometry, Hb, FEP & IQ[b]	Improve iron status and IO	(Yang et al. 2005)
2003	Rural Bangladesh	79	12–24	Daily use for 2.5 mo	Anthropometry, Hb, SF & STR[c]	Improve iron status and decrease IDA	(Hyder et al. 2007)
2004	Koforidua Ghana	98	6–12	Daily use for 12 mo	Morbidity, Hb, plasma ferritin, TfR, CRP & Zn[d]	Reduce iron deficiency & IDA	(Adu-Afarwuah et al. 2008)
2005	Rural Haiti	254	9–24	Daily use for 2 mo	Hb	Reduce IDA	(Menon et al. 2007)
2007	Kyrgyz Republic	172	6–36	Daily use for 2 m	Hb	Improve Hb levels & reduce IDA	(Lundeen et al. 2010)
2007	Western Kenya	1062	6–36	Daily use and 12 mo follow	Diarrhea, fever, cough, malaria morbidity episodes	Decrease hospitalization for diarrhea & fever	(Suchdev et al. 2013)
2007	An urban district of Iran	120	6–18	Daily use for 4 m	Anthropometry, Hb, serum ferritin, retinol, Zn, 25(OH)D	Reduce proportion of zinc deficiency	(Samadpour et al. 2011)
2009	Rural area LPDR[e]	111	6–52	Daily use for 24 m	Anthropometry & Hb	Improve Hb level & reduce IDA	(Kounnavong et al. 2011)
2009	Bahia Brazil	143	6–48	Daily use for 3 m	Diarrheal disease (DD) & acute respiratory infection (ARI)	No impact in reducing DD, ARI prevalence & nutritional status	(Sampaio et al. 2013)

[a]*SF* serum ferritin
[b]*Hb* hemoglobin, *FEP* free erythrocyte proporphyrin, *IQ* intelligence quotient
[c]*STR* serum transferrin receptor
[d]*TfR* transferrin receptor, *CRP* C-reactive protein, *Zn* zinc
[e]*LPDR* Lao People's Democratic Republic

feed to the children by their careers (Yang et al. 2004; Zlotkin et al. 2005). The costs for processing and transportation of these products are also relatively inexpensive. The other optional ingredients can also be added according to the nutritional characteristics and nutrient deficiencies in different regions.

Micronutrient Sprinkles has been used to prevent IDA and the other micronutrient deficiencies in children under 5 years of age in developing countries (De-Regil et al. 2013). Several intervention trials to the children for 2 months or more using Sprinkles have been reported in China, Canada, Ghana, and other places (Jack et al. 2012; Yang et al. 2004; Adu-Afarwuah et al. 2007, 2008), which showed that Sprinkles can significantly reduce anemic prevalence through elevating hemoglobin levels, improving motor development, and having high compliance for this product, as well as easy to transport, carry, and well mix with the other CFs; however, its improving efficacy in growth development and SAM was very limited while using it alone because such products did fortify high qualitative protein and fat as multi-nutrient powder with food matrix and LNP (Adu-Afarwuah et al. 2007, 2008). One study has reported the effect of daily and once weekly Sprinkles for 14 weeks on hemoglobin, serum ferritin, erythrocyte protoporphyrin in whole blood, prevalence of iron deficiency in preschool children. Compared with a placebo control, once weekly supplement is effective same as daily supplement in improving iron status and reducing the prevalence of iron deficiency (Yang et al. 2004; Hyder et al. 2007); however, for severely to moderately anemic children, daily supplementation was more effective in elevating hemoglobin level and reducing IDA prevalence (Kounnavong et al. 2011).

The Issues of Safety Use on CFSs for Elder Infants and Young Children

The prevalence of malnutrition remains high in developing countries in infants and young children so that intervention using CFSs or home fortification of foods with multiple micronutrient powders would be an effective measure to reduce IDA and iron deficiency and improve growth and development in such areas. However, it should be particularly paid attention to the safety and quality control procedures to prevent contamination with toxins or pathogens and ensure the product to be stable and palatable over time during the shelf-life of products.

After natural disaster or in low-income areas, providing adequate food suitable for infant and young children feeding practices in food insecure environment may not be feasible because of lack of household access to promoted foods. Implementing CFSs intervention in such areas will involve two aspects which include hygiene and safety to meet the nutritional requirements. Hygiene is referring to the problems of traditional microbiology and safety of nutritional needs is related to prevent nutritional deficiencies due to poor compliance and avoid excessive intake due to overuse. Furthermore, issue of allergy should also be considered due to individual children sensitive to certain raw materials (such as gluten, peanut, etc.) in CFSs. Therefore, it should ensure adequate and safety for the targeted populations, that is, the supplementary quantity should be sufficient to prevent malnutrition and avoid excessive intake leading to adverse health effects.

After natural disasters or in low-income areas, the design intervention strategies on CFSs should consider the reasons behind choices by households to overuse or underuse the product and increase the likelihood of its appropriate utilization through appropriate propaganda and guidance for child's caregivers in the field sites. In addition, because most of CFSs are rich in nutrients and easily contaminated and spoiled which could lead to feeding injuries to infants and young children, too many products should not be distribute to the children every time, especially in those places where poor environmental conditions such as high temperature and high humidity, and no refrigerator or other cold storage facilities.

In summary, nutrient requirements of infants and young children have particularly high for their growth and development so that meeting their nutritional needs are challenging in settings where the ration is limited to a few food commodities, with little access to a diverse diet and bioavailable sources of micronutrients during and/or after a natural disaster or in the developing countries. Therefore, the nutritional interventions using CFSs will be most efficient in reducing IDA and/or preventing malnutrition and promoting adequate growth and development of infants and young children. However, many factors may affect the acceptability and thereby the beneficial effect of nutritional intervention of CFSs, such as cost, taste, texture, shelf-life, how they are used, as well as its nutrient composition; all of these should be considered when implementing nutrition intervention in such areas.

Policies and Protocols

Policies

In this chapter, we have described the importance of complementary food supplements for elder infant and young children after disasters. Based on China's successful experience on Ying Yang Bao (YYB) distribution in poor areas or affected-areas after natural disaster during recent 10 years, the policy and protocols should cover the following aspects:

Governmental Policies

- Central government domination should be the guarantee of successful promotion and distribution of CFSs, which include guarantees in legislation, technology, professionals, and funding. For example, the study on YYB was performed in 2001, a National food standard on CFSs was established in 2008, and such standard has been updated to China's National Food Safety Standard for CFSs (GB 22570 – 2014) in 2014.
- Assessing and monitoring the nutritional and health status of infants and young children after a natural disaster will provide basic information for the center and/or local government to implement intervention to the target vulnerable groups in affected areas.

- Center and/or local governments should prepare and store certain complementary food supplements for elder infant and young children for emergency at ordinary times.
- Government should regularly monitor and evaluate the safety and quality of complementary food supplements to prevent contamination with toxins or pathogens, and the company should ensure their products to be stable and palatable over time during the shelf-life of products.
- Government-leading interventions in improving nutrition status of infants and young children are conducive to maximizing in resource uses and working sustainably in promoting publicity.

Protocols

For the procedures and extent of intervention using complementary food supplements to the elder infants and young children after disasters, the protocol describes as follow:

- Basal evaluation and monitor on nutritional and health status of infants and young children will be important for finding nutrition issues and providing basic information to the local government to implement intervention to the target vulnerable groups after a natural disaster.
- Up to now, more than ten nutrition intervention projects using YYB to elder infants and young children have been carried out in poor rural areas or those areas affected by natural disaster.
- Re-evaluation will be useful to find the intervention efficacy and what kind of questions existed in those areas affected by natural disaster.
- Quality control system will be important involving survey methods, training investigators, and the quality of complementary food supplements providing for the target vulnerable group.
- Through systematic review and or meta analysis on the intervention studies, it would be useful for us to evaluate the intervention efficacy, experience, and existing problems and to help spread such intervention to the other places. For example, based on summing up the above experience, since 2012, Chinese government has launched a project named "improving children's nutrition status in poor rural regions" covering more than four millions of infants and young children aged 6–24 months in 341 counties from 21 provinces located in western and middle regions by the end of 2015.

Dictionary of Terms

- **Elder infants and young children** – The children are defined as elder infant after 6 months of birth and young children with 12–36 months after birth.
- **The period of complementary feeding** – The period during which other foods or liquids are introduced along with breast milk after 6 months of birth is considered as the period of complementary feeding.

- **Complementary food** – Any nutrient-containing foods or liquids other than breast milk given to elder infants and young children during the period of complementary feeding are defined as complementary foods.
- **Complementary food supplements** – Complementary food supplements used for elder infants and young children are fortified with multi-nutrients including multi-nutrient powder with food matrix, lipid-based nutrient supplements and corn-soy blend, and micronutrients Sprinkles.
- **Multi-nutrient powder with food matrix** – Multi-nutrient powder is a complementary food supplement with food matrix for infants and young children. High-quality protein and total energy as well as multi-nutrients are suitable for mild malnutrition children.
- **Corn-soy blend** – Corn-soy blend is micronutrient-fortified cereal-legume (corn and soy flours) mixtures generally used for low-income countries.
- **Lipid-based nutrient supplements** – Lipid-based nutrient supplements contains peanut butter, milk powder, cooking oil, sugar, and micronutrients, which includes ready-to-use supplementary food and ready-to-use therapeutic food.
- **Micronutrients Sprinkles** – Micronutrients Sprinkles is a complementary food supplements without food matrix and single-dose sachets containing iron and other multiple micronutrients, which are easily sprinkled onto any child's traditional complementary food in a powdered form for elder infants and young children prepared in the household.

Summary Points

- This chapter focuses on complementary food supplements (CFSs) used for elder infants and young children after disasters.
- Obvious characteristics of natural disasters are unpredictable and sudden, destructive, danger, and whole social emergency which make millions of people to be involved globally every year.
- Elder infants and young children's morbidity and mortality often dramatically increase in very short period since they are the most nutritionally vulnerable group due to poor or limited food choices after disasters.
- After a natural disaster, quickly assessing and monitoring the nutritional and health status of infants and young children will be important for providing basic information to the local government to make intervention.
- The practical experience of CFSs mainly includes multi-nutrient powder with food matrix, lipid-based nutrient supplements (LNS) and corn-soy blend (CSB), micronutrients Sprinkles without food matrix, etc.
- Home-based treatment to severe acute malnutrition will be better choice in most cases and complementary food supplement (CFS) can treat and prevent malnutrition in affected areas.
- Therefore the nutritional interventions using CFSs are most efficient in reducing IDA, preventing malnutrition, promoting adequate growth and development of infants and young children.

- Many factors may affect the acceptability and the beneficial effect of nutritional intervention of CFSs, such as cost, taste, texture, shelf- life.
- In those affected areas attacked by disasters, no single intervention measure can solve all complex nutritional problems that may exist in vulnerable groups.
- Final, we should pay attention to the safety and quality control procedures on CFSs to prevent contamination with toxins or pathogens and ensure the product to be stable and palatable during the shelf-life.

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Short-Term and Long-Term Effect of Exposure to Famine During Childhood on Human Health Status

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Caixia Dong and Shi-an Yin

Contents

Abstract

Famines are widespread, which generally result from or secondary to natural disasters. In most of famine cases, food supply systems were severely damaged or even completely stopped, major food shortages became a primary post-disaster or famine feature and the common characteristics were high prevalence of acute malnutrition and micronutrient deficiencies in infants and young children in very short period since they are the most nutritionally vulnerable subgroup due to poor food choices or low-nutrition food supply and decreased accessibility to certain foods rich in proteins, lipids, and micronutrients. The majority of epidemiological

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studies clearly show that maternal nutrition status before and during pregnancy has short-term and long-term impact through mediating fetal structure and physiology, growth and development, and future health status and susceptibility to nutrition-related NCDs at adulthood; and postnatal famine exposure in childhood, adolescence, or young adulthood also seems to raise risk for nutrition-related NCDs. This chapter reviewed the short-term impact-related common nutritional problems existed in infants and young children and the short-term intervention efficiency of timely improving nutritional status of infants and young children after famine, and the long-term effects of prenatal or postnatal exposure to the famine on adult diseases.

Keywords

Famine · Infancy · Children · Adulthood · Nutrition · Exposure · Human health · Noncommunicable chronic diseases · Short-term effect · Long-term effect

List of Abbreviations

ALP	Atherogenic lipid profile
CF	Complementary food
CFS	Complementary food supplement
CFs	Complementary foods
CFSs	Complementary food supplements
CHD	Coronary heart disease
DBC	Disturbed blood coagulation
DM	Diabetes mellitus
IDA	Iron-deficient anemia
MAM	Moderate acute malnutrition
NCDs	Noncommunicable diseases
NGOs	Nongovernmental organizations
PAD	Peripheral arterial diseases
SAM	Severe acute malnutrition
SGL	Standard glucose load
SR	Stress responsiveness

Introduction

Famines are widespread and frequent occurrences in human history, which generally are resulted from or secondary to natural disasters include severe droughts, floods and mudslides, earthquake, volcanic eruptions, tsunamis, etc. Such famines generally have obvious characteristics by sudden, destructive, danger, social and emergency which often affect millions of people globally every year (Nutrition 1993–2005). In most of famine cases, food supply systems were severely damaged or even completely stopped, major food shortages became a primary post-disaster or famine feature and the common characteristics were high prevalence of acute malnutrition and micronutrient deficiencies in vulnerable groups such as

elder infants and young children (Sun et al. 2013; Zhao et al. 2010a; Dong et al. 2014).

In those areas affected by severe natural disaster or famine, elder infant and young children's morbidity and mortality resulted from malnutrition often dramatically increased in very short period (Nutrition, 1993–2005) since they are the most nutritionally vulnerable subgroup due to poor food choices and decreased accessibility to certain foods rich in proteins, lipids, and micronutrients (Nishikiori et al. 2006; Wang et al. 2010a; Zhao et al. 2010b). Malnutrition will lead to susceptibility to preventable infectious diseases such as diarrhea and pneumonia and has an indirect association with the leading causes of death in young children (Walker et al. 2013). The majority of epidemiological studies clearly show that exposure to famine during prenatal life increases risk for metabolic syndrome; postnatal famine exposure in childhood also seems to raise risk for nutrition-related non-communicable diseases (NCDs). These findings demonstrate the hypothesis of fetal programming, which indicates that maternal nutrition status before and during pregnancy has short-term and long-term impact through mediating fetal structure and physiology, growth, and future health status and susceptibility to NCDs. Furthermore, postnatal environment, such as nutrition status (deficiency or excess) at infancy, has also been shown to induce powerful, lasting programming effects on growth and development trajectories, body composition, tissue differentiation and functional development, as well as long-term health, performance, and the risks for NCDs until old age. Even after controlling socioeconomic conditions and other factors in the home environment, malnutrition in early life had a negative impact on cognitive and behavioral functioning throughout childhood and adolescence. Short- and long-term effects of exposure to famine on health status during early life were shown in Fig. 1.

This chapter will review the short-term impact-related nutritional problems existed in infants and young children and the short-term intervention efficiency of timely improving nutritional status of infants and young children after famine, and the long-term effects of exposure to the famine before and during pregnancy on adult chronic diseases.

The Short-Term Effect on Nutrition-Related Diseases

Severe natural disaster or famine often occurs in the poverty and developing areas and affects millions of people worldwide each year. After a serious destructive event, the quantity and quality of daily supply foods markedly decreased and the general nutritional status of populations gradually deteriorated, especially in infants and young children because the nutritional quality of the food commodities provided in such situation might be insufficient to meet their nutritional needs, the prevalence of acute malnutrition and micronutrient deficiencies such as iron-deficient anemia would significantly increase and become more serious public health priority to be solved (Dong et al. 2013, 2014; Wang et al. 2010a; Zhao et al. 2010b; Eriksson 2007).

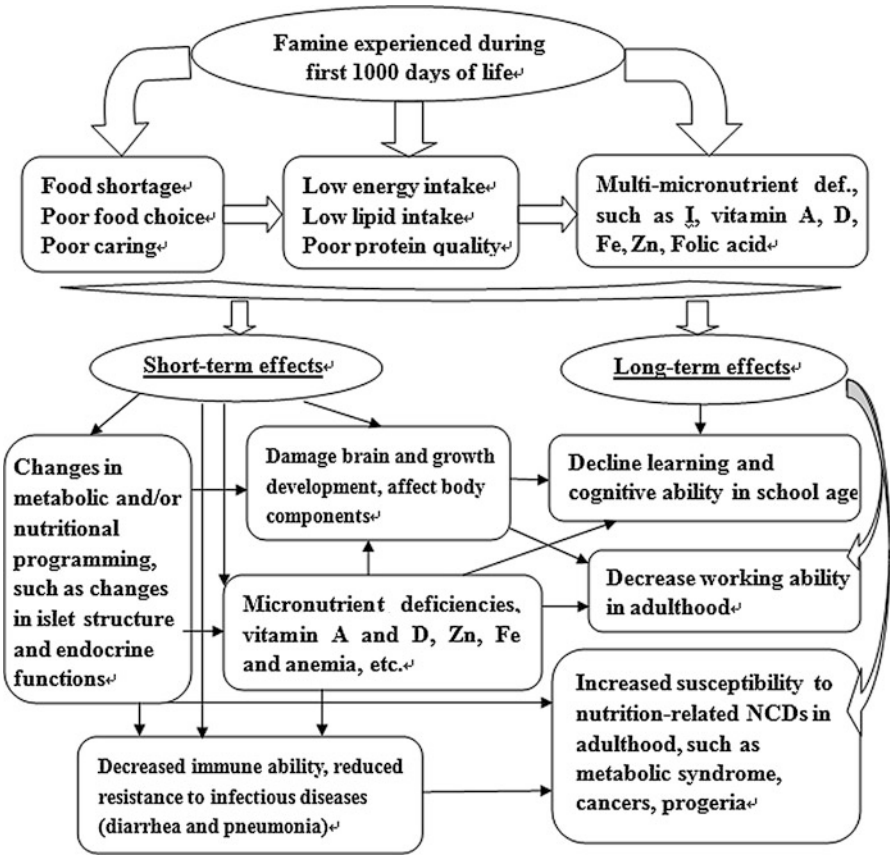

Fig. 1 Diagram of short- and long-term effects of exposure to famine on health status during early life

1. *Protein – energy malnutrition*. During and after famine, the prevalence of acute malnutrition including severe acute malnutrition (SAM) and moderate acute malnutrition (MAM) in young children may be significantly increased due to the shortage of food, food insecurity, poor sanitation, etc., and hunger was a contributing factor to the increased mortality due to infectious diseases and diseases of digestive system (Lumey and van Poppel 1995). For example, during Dutch famine (1944–45), the estimated number of deaths from undernutrition were 20,000–30,000 in Western Netherlands (Stein and Susser 1975; Trienekens 2000); the Chinese Great Famine (1959–61) had resulted in about 30 million deaths of children and adults due to severe protein-energy malnutrition (Smil 1999). "District food supply crisis" in southern Africa caused by the drought and widespread crop failures resulted in significant increase in acute malnutrition and mortality in some areas during 2001–2002 (Renzaho

2007). In drought-affected desert areas of western Rajasthan of India in 2003, the percentages of stunting and underweight of children aged 0–5 years were reported to be high up to 53% and 60% (Singh et al. 2006). The rates of underweight were respectively 15.6% and 9.1% and stunting percentages were 26.0% and 24.2% in Beichuan and Li counties of Sichuan Province of China after Wenchuan Earthquake on May 12, 2008, which indicated the presence of SAM (Wang et al. 2010a); the stunting prevalence of children aged from 2 to 5 years living in affected areas of Sichuan, Shanxi and Gansu Province of China was up to 13.6% after 11 months of Wenchuan Earthquake which showed that such situation was closely related to protein and energy malnutrition (Zhao et al. 2010b).

2. *Iron-deficiency and anemia (IDA)*. The elder infants and young children are at higher risk susceptible to iron-deficiency and IDA (Medicine, 2001). If foods rich in iron or supplements with iron could not be provided, these vulnerable groups would be more easily suffered from IDA (Rim et al. 2008). For example, in those areas affected by Wenchuan earthquake, the iron deficiency and IDA in children were reported to be higher prevalence (Zhao et al. 2010a, b; Wang et al. 2010a). Children suffered from anemia not only increase susceptibility to infectious diseases (Zhao et al. 2010a) but also will have a higher risk died of such diseases (Walker et al. 2013). After 4 months of Wenchuan Earthquake, the children aged from 6 to 23 months had a higher prevalence of anemia up to 49.6% and 78.8% in Beichuan and Li counties of Sichuan Province, and the highest prevalence was the infants aged from 6 to 11 months (Wang et al. 2010a). The average prevalence of anemia in children aged 0 to 24 months living in the affected areas of Sichuan, Shanxi, and Gansu Provinces of China was 45.7% after 11 months of Wenchuan Earthquake (Zhao et al. 2010b). It has been reported that the anemia prevalence of children long-term living in refugee camps in Africa was up to 60% and iron-deficiency was much higher with the range of 23% to 75% from four refugee camps (Seal et al. 2005).
3. *Vitamin A deficiency and decrease in resistance to infectious diseases*. It has been reported that the prevalence of vitamin A deficiency and marginal deficiency in children was generally quite high in most of the developing countries (Yin and Lai 2002; Akhtar et al. 2013; Sherwin et al. 2012). This would predict that young children were more vulnerable to have vitamin A deficiency during famine due to a single species of food and very limited food supply suitable for them. After 11 months of Wenchuan Earthquake, the prevalence of vitamin A deficiency was significantly increased (15.4%) in children aged 24–60 months living in affected areas from Sichuan, Shanxi, and Gansu Provinces of China which was significantly higher than that of national level (Dong et al. 2014). The results from international surveys indicated that vitamin A deficiency was much higher in children long-term living in refugee camps which was up to 20.5~61.7% (Seal et al. 2005).
4. *Vitamin D deficiency and rickets*. Vitamin D deficiency is associated with adverse effects in children such as growth failure and rickets, which should

adversely affect children's normal growth and bone development. Using serum $25(OH)_2D_3$ less than 12 nmol/L for vitamin D severe deficiency, 12~48 nmol/L for vitamin D deficiency, and 48~78 nmol/L for vitamin D marginal deficiency, vitamin D severe deficiency, deficiency, and marginal deficiency in children living in earthquake-affected areas from Sichuan, Shanxi, and Gansu Provinces of China were 1.5%, 61.8%, and 28.6%, respectively, and total of vitamin D deficiencies and marginal deficiency were over 90% after 11 months of Wenchuan Earthquake (Dong et al. 2014). The reasons resulted in such higher vitamin D deficiency or poor status in children in those areas were related to the lower intake of animal foods rich in vitamin D and no vitamin D supplements as well as lack of adequate sunlight exposure in winter and spring or rainy season which resulted in the decrease of vitamin D synthesis in body so that the young children living in these areas would have high risk for rickets (Zhu et al. 2012; Gordon et al. 2008).

5. *The other multi-micronutrient deficiencies*. After a natural disaster or lasting famine, the provided foods were very limited and low percentages of animal foods, fresh fruits, and vegetables in affected areas; the children were very easy to have zinc, vitamin B_2, vitamin B_{12}, vitamin C, and iron deficiencies which could affect children's appetite and diminished sense of taste, and manifest pica, angular cheilitis, glossitis, and other clinic symptoms as well as stunting, underweight, and nutritional anemia (Zhao et al. 2010a, b; Wang et al. 2010a; Dong et al. 2013). For example, during 2000 and 2003, scurvy outbreaks in Afghanistan were documented, although this country had a relative low incidence of acute malnutrition (3–12%) (Young et al. 2004). After 1 year of the Wenchuan Earthquake, the percentages of vitamin B_{12} marginal and deficiency of children aged 24–60 months were 8.6% and 10.6%, and the prevalence of zinc deficiency including marginal and deficiency was 65.5% in the affected areas (Dong et al. 2014). Lasting multi-micronutrient deficiencies resulted from famine would have the current and long-term effects on cognitive and behavioral development in children under 5 years. The brain is vulnerable to the effects of insults during critical periods of brain development from the second trimester of pregnancy until 2 years of age. Malnutrition experienced at these ages will have lifelong consequences that are not reversed by adequate nutrition.

As mentioned about, nutritional problems and overall deterioration mentioned above in the general health conditions that resulted from natural disasters or famine in young children were related to some public problems in food insecurity and/or social unrest and lack of complementary foods (CFs) suitable for them; many infants from 6 to 10 months of age without breastfeeding ate the same foods as adults as the families could not prepare infant's foods (Zhao et al. 2010a; Wang et al. 2010b). All of these should affect the nutritional and health status of children and make them facing high risk suffered from infectious diseases such as diarrhea and pneumonia which should significantly increase the risk for mortality (Zhao et al. 2010a, b; Nishikiori et al. 2006; Singh et al. 2006).

The Short-Term Intervention Efficiency of Timely Improving Nutritional Status of Elder Infants and Young Children During and/or After Famine

A common representative characteristics in public health would be the increasing risk of malnutrition and mortality by nutrition-related diseases in infant and young children after famine (Nutrition, 1993–2005; Nishikiori et al. 2006; Young and Jaspars 1995; Singh et al. 2006). These children were not only facing high risk of SAM due to food shortage and insecurity but also easily suffered from some nutritional and infectious diseases so that the synergistic effect would cause exponentially significant increase in mortality (Nishikiori et al. 2006; Singh et al. 2006). Therefore, effectively targeted intervention using CFSs to prevent and control nutritional deficiencies must be carried out. The intervention by CFS usage would quickly achieve the following benefits:

1. *Effectively improve children's nutritional status.* Providing CFS, micronutrient supplements or the other fortified foods for the targeted populations not only meet the energy and macronutrient requirements but also effectively improves the micronutrient status to prevent nutritional deficiencies because these micronutrients participate in the metabolic and transforming process of energy and macronutrients in the body. Taking comprehensive measures to improve the nutritional status of the young children in the affected areas will reduce or prevent the prevalence of malnutrition and mortality. The relief and assessment of the nutritional and health status of vulnerable populations carried out after Earthquake in Wenchuan showed the importance to provide efficiently nutritional support and protection for these subgroups through nutritional intervention using CFSs (Sun et al. 2011; Dong et al. 2013).
2. *Decrease the risk suffered from malnutrition and infectious diseases.* Under many natural disasters or after famine, due to the deterioration of living place and surrounding environment, food shortage, very irregular or uneven eating, and family member death, children in the affected areas would be in a very sensitive status for malnutrition and infectious diseases (Walker et al. 2013). In such situations, requirements for a variety of micronutrients would be significantly increased so that supplementing multiple micronutrients or providing CFSs as well as meeting macronutrient and energy needs would improve the general nutritional status and reduce or alleviate stress conditions and prevent infectious diseases, which is particularly important for protecting those vulnerable populations.
3. *Decrease mortality of elder infants and young children:* The period from 6 to 24 months of age is the most critical period because of their rapid growth and an increasing reliance on complementary food (CF) and or CFSs. In order to improve nutritional status and reduce the mortality, adequate food-based nutrition interventions using CFSs should be very important, since such interventions might play a key role in saving lives through their impact on the nutrition and health. Several strategies can be used to improve complementary feeding

practices. These include education related to nutrition and child care knowledge to mothers or caretakers designed to promote healthy feeding practices, provision of CFSs offering extra energy with or without micronutrient fortification, and increasing energy density of CFs. However, the usage of CFSs has been interested after famine or natural disaster in recent years (Sun et al. 2011; Lopez Boo et al. 2014; Zhang et al. 2016). Malnutrition of young children is preventable through effective nutritional intervention by implementing complementary feeding practices and giving CFSs or multiple micronutrient supplements (Dong et al. 2013; Wang et al. 2009, 2015). Frequently, a food supply and nutrition improving response, together with other public health interventions to reduce the risk of malnutrition, will be required in such situation (Young et al. 2004; Bhutta et al. 2013).

The Long-Term Effects of Prenatal, During or Postnatal Exposure to the Famine on Adult Diseases

The lasting effect of early life exposure to famine on fetus is predicted by the fetal origins hypothesis (Barker 1992), which postulated that nutritional deprivations during the fetal period could lead to higher prevalence of nutrition-related NCDs at adulthood, and such findings have been supported by several cohort studies based on Dutch famine and China's great famine. Poor maternal nutrition during gestation may contribute to restricted fetal growth, leading to increased susceptibility to nutrition-related NCDs in later life which is supported by animal studies that undernutrition during gestation is associated with reduced life span and increased disease susceptibility at adulthood, and these findings could be explained by the thrifty gene hypothesis (de Rooij et al. 2014). Historical severe famines, the Dutch famine (1944–45) and the Chinese great famine (1959–61), have provided a unique opportunity to study the long-term impact of human maternal nutrition status during specific periods of gestation on the risk for nutrition-related NCDs at adulthood (Lumey et al. 2011).

1. *Long-term persisting impact on child's subsequent growth and development*. Food supply shortage, simple species of food aid with low quality would be difficulty to meet the nutrient requirements for infants and young children during famine (Young and Jaspars 1995). Because these groups for nutrient requirement are usually higher than the general populations, they are much more susceptible to nutritional deficiencies and such adverse effects on their growth and development may continue for a long period of time and will increase the risk suffered from NCDs at adulthood. The findings from Chen and Zhou's 1959 birth cohort showed that on average the individuals who did not experience Chinese great famine born in the year 1958 would otherwise have grown 3.03 cm taller in adulthood, worked longer hours, and earned more income (Chen and Zhou 2007); the data from Li and An's study from the China Health and Nutrition Survey indicated that children with both parents born in the Great Famine were

significantly shorter by 0.37 SD (1.89 cm for boys and 1.78 cm for girls) compared to children with parents who have not experienced the mass starvation (Li and An 2015). Therefore, intervention through CFSs will be a benefit for preventing or minimizing irreversible adverse impact of malnutrition and anemia on development of cognitive as well as growth development and this beneficial effect shall be lasting for a long time (Grantham-McGregor and Ani 2001).

2. *Dutch famine cohort studies*: Dutch famine located in the Western Netherland occurred from October 1944 until the surrender of the German Forces on 7 May 1945, and the period of most intense starvation was February to April 1945. Based on a cohort study in Wilhelmina Gasthuis in Amsterdam from the Dutch famine (1944–45), the findings of long-term effects of prenatal exposure to the famine on NCDs at adulthood are shown in Table 1 (Roseboom et al. 2000, 2006; Painter et al. 2006a, b; Portrait et al. 2011). The results showed that exposure to famine in early gestation increased the risk for coronary heart disease (CHD), atherogenic lipid profile (ALP), disturbed blood coagulation (DBC), stress responsiveness (SR), and serum glucose levels 2 h after a standard glucose load (SGL) (Roseboom et al. 2000, 2006); the subjects at ages of 11–14 years with exposure to severe undernutrition would have higher risk for developing diabetes mellitus (DM), and peripheral arterial diseases (PAD) at ages of 60–70 in women (Portrait et al. 2011); the women exposure to famine in utero would have more risk for a history of breast cancer than unexposed women (Painter et al. 2006a); and any famine exposure at least 10 week duration during gestation was associated with elevated systolic and diastolic blood and hypertension prevalence in middle age (Stein et al. 2006).
3. *China's great famine cohort studies*: China's great famine (1959–61) was one of the worst human catastrophes during the twentieth century and affected all regions of a vast and diverse country, which provides a unique cohort case for studying the long-term effects of exposure to famine during fetal and childhood period on NCDs at adulthood (Chen and Zhou 2007). In the past decades, the findings from extensive epidemiologic or cohort studies indicated that early-life conditions, such as nutrition status, influence later risk of nutrition-related NCDs such as metabolic syndrome (Wang et al. 2017; Zheng et al. 2012; Li et al. 2010) as shown in Table 2. Undernutrition in early life may participate in the origins and development of abdominal obesity (Huang et al. 2010; Liu et al. 2017), insulin resistance and type 2 diabetes (Liu et al. 2016, Wang et al. 2010a), fatty liver disease (Wang et al. 2010b), hypertension (Yu et al. 2017), and hyperlipidemia (Li et al. 2010). The present findings also suggest that exposure to famine in early life had sex-specific association with MS and the adverse effects of malnutrition might extend beyond the "first 1000 days and last 9 years" (Wang et al. 2017).

   In addition, some studies also showed that exposure to the Chinese famine (1959–61) in early life (poor nutrition status) was related to the other adult chronic diseases such as higher prevalence of anemia (Shi et al. 2013), organ function damage in liver and kidney (Li et al. 2014), higher level of proteinuria in women (Huang et al. 2014), increased risk of arthritis (Xu et al. 2017) and osteoporosis in post-menopausal women (Kin et al. 2007), and prevalence of osteoporosis during early life were significantly higher in males than in females

Table 1 Long-term effects of prenatal exposure to the famine on adult disease in later life based on studies from the Dutch famine (1944–45) from a cohort study in Wilhelmina Gasthuis in Amsterdam

Authors	Surveyed age	Aim/objective of the study	Methods	Results
Roseboom et al. (2006)	50–58 years	To study the effects of restricted prenatal nutrition during different periods of gestation on adult diseases in later life	Trace 2414 people born around the time of the Dutch famine	Exposure to famine in early gestation had more CHD[a], ALP[b], DBC[c], increased SR[d], and more obesity
Ravelli et al. (1998)	50–58 years	Investigate glucose tolerance in subjects born around the Dutch famine	Trace 2155 live born singletons	Increase in glucose levels 2 h after a SGL[e] among exposed subjects, and highest in men and women exposed during mid and late gestation
Roseboom et al. (2000)	50–58 years	Assess the effects of maternal malnutrition during specific periods of gestation on PLP[f]	Trace 2155 live born singletons	The group exposed to famine in early gestation had higher ALP, LDL-HDL, TC, LDL-C and apolipoprotein B, lower HDL-C and apolipoprotein A
Painter et al. (2006a)	Up to age 61 years	Determine a possible link between prenatal exposure to famine and breast cancer risk in later life	Trace 475 women	Women exposed to famine in utero more frequently reported a history of breast cancer than unexposed women, unadjusted HR = 2.6, 95% CL: 0.9–7.7
Portrait et al. (2011)	60–76 years	Examine the effects of early life exposure to the Dutch famine on the prevalence of adult NCDs	Using data from the fifth cycle of the aging study Amsterdam	Exposure to severe undernutrition at ages of 11–14 years is associated with a higher probability of developing DM[g] and/or PAD[h] at ages of 60–76 in women

(*continued*)

Table 1 (continued)

Authors	Surveyed age	Aim/objective of the study	Methods	Results
Painter et al. (2006b)	50–58 years	Investigate a link between prenatal exposure to the Dutch famine and early onset of CAD[i]	Compared the age at onset and cumulative incidence of CAD between subjects exposed to the Dutch famine and unexposed subjects	Maternal nutrition in early gestation may play a role in the onset of CAD, HR = 1.8, 95% Cl: 1.0–3.8

[a]*CHD* coronary heart disease
[b]*ALP* atherogenic lipid profile
[c]*DBC* disturbed blood coagulation
[d]*SR* stress responsiveness
[e]*SGL* standard glucose load
[f]*PLP* plasma lipid profiles
[g]*DM* diabetes mellitus
[h]*PAD* peripheral arterial diseases
[i]*CAD* coronary artery disease

(Chen et al. 2016), which would be related to severe malnutrition resulted from famine during gestation and early period.

4. *The effect of exposure of famine in early life on mental health at adulthood*: The substance disaster and/or famine might be temporary but the spirit disaster and/or famine might influence the affected-area victims' life for many years and even longer time, and the famine or disaster changed the people's moral world in varying degrees. Numerous results from animal models, epidemiological surveys, and some intervention trials have shown that exposures' nutritional deficiency during critical periods (fetal or early life) of development can result in structural and metabolic alterations that would have lasting influences on mental and physical well-being.

The majority of study on maternal prenatal exposure to famine and risk for mental illness such as schizophrenia at adulthood or adult offspring are mainly based on the "Dutch famine of 1944–45" or "the Chinese great famine of 1959–61." The related studies have been ongoing since the 1970s using birth cohort of Dutch famine and emerging in recent years using birth cohort of Chinese famine. For example, exposed famine group at the first trimester of gestation had twofold increase in the incidence of schizophrenia and the prevalence of clinical central nervous system anomalies compared with unexposed group during the Dutch Hunger Winter of 1944/1945 (Hulshoff Pol et al. 2000). Through the 1987 Chinese National Disability Sample Survey, the post-famine cohort (1963–1965) had a higher risk (OR = 1.32, 95% Cl = 1.03–1.69) of developing schizophrenia than the pre-famine (1955–58) or famine cohorts (1959–62) in the rural population, and there was virtually no difference in schizophrenia risk between the pre-famine and the famine cohorts

Table 2 Long-term effects of prenatal exposure to the famine on adult disease in later life based on studies from the Chinese great famine (1959–61)

Authors	Surveyed time	Aim/objective of the study	Methods	Results
Wang et al. (2017)	2014-SPECT-China[a]	To explore exposure to Chinese famine during fetal and childhood period was related to MS[b] in adulthood	6445 subjects from SPECT-China were divided into fetal-exposed (1959–62), childhood-exposed (1949–58), adolescence/young adult-exposed (1921–48), non-exposed (1963–74)	Exposure to famine in fetal- and childhood-exposed women had significantly higher prevalence of MS with OR 1.47 and 1.80 ($P < 0.05$), but not in men
Li et al. (2011)	2002-CNNHS[c]	To examine exposure to Chinese famine during fetal life and early childhood is related to the risks for MS and effect of later life environment on such association	7874 adults born between 1954 and 1964 from 2002 CNNHS were divided into non-exposed, fetal-exposed, early childhood, mid-childhood, or late childhood exposed groups	Adults exposed to this famine during fetal life or early childhood had significantly higher risk for MS compared with non-exposed subjects
Zheng et al. (2012)	2008 annual physical examination	To determine exposure to Chinese famine during fetal life and early childhood is related to a greater risk of MS in later life	5040 subjects were categorized by birthday into control (1963–64), fetal-exposed (1960–61), and postnatal-exposed (1957–58) groups	Women in fetal- and postnatal-exposed groups had higher prevalence and risk for MS than in control group ($P < 0.05$)
Wang et al. (2012)	Mar–Sep 2010	To assess impact of exposure to Chinese famine during fetal and infancy period on the risk for hypertension at adulthood	12,065 adults (46–53 years) born 1957–64 from a cross-sectional health survey in Nanhai and Zhongshan Municipalities of Guangdong province	Exposure to the famine during the first trimester, during infancy, or both increased the risk of hypertension at adulthood
Huang et al. (2010)	1993–1996	To assess the impact of famine exposure on height, BMI, and hypertension at –32 years of age	35,025 women born in 1957–63 from China–US CPNTD[d] were divided into groups born before 1957–58, during 1959–61(the famine), and after 1962–63	Postnatal exposure during 2–3 years of life reduced height and increased BMI and hypertension, whereas exposure during pregnancy and infancy reduced BMI

(*continued*)

Table 2 (continued)

Authors	Surveyed time	Aim/objective of the study	Methods	Results
Li et al. (2010)	2002-CNNHS	Examine exposure to Chinese famine during fetal life and childhood with the risk for hyperglycemia and type 2 diabetes at adulthood	7874 rural adults born between 1954 and 1964 were from 2002 CNNH	Fetal exposure to the famine had higher risk of hyperglycemia at adulthood, and OR values were significantly different between groups from severe and less severe famine areas ($P = 0.001$)
Wang et al. (2016a)	Apr–Oct 2013	Investigate exposure to Chinese famine (1959–61) during fetal stage or childhood (0–9 years) is related to T2Dd[e] and hyperglycemia at adulthood	7801 retirees from Dongfeng-Tongji cohort were divided into late-, mid-, and early-childhood exposed, fetal exposed, and unexposed groups. 16.2% were exposed to Chinese famine and 71.7% were exposed to this famine during childhood	Exposure to the famine in childhood was related to increased risk for T2D in adult, particularly in women. Subjects experienced famine in middle childhood had a high hyperglycemia risk compared with unexposed group ($OR = 2.06$)
Wang et al. (2016b)	2014-SPECT-China	Explore the association between adult NAFLD[f] and fetal or childhood exposure to Chinese famine (1959–61)	5306 subjects from 2014 SPECT-China were divided into fetal-exposed (1959–62), childhood-exposed (1949–58), adolescence/young adult-exposed (1921–1948), and non-exposed (1963–74) groups	Fetal- and childhood-exposed women but not men had a higher prevalence of moderate-severe steatosis compared with non-expected group, which indicated that malnutrition in early life affected the development of adult NAFLD
Liu et al. (2017)	Qingdao, China in 2006	Evaluate the association between famine exposure during early life and obesity at adulthood	8185 subjects from cross-sectional surveys were divided by famine exposure into unexposed born in 1962–71, fetal/infant exposed born	Exposure to famine in early life was associated with increased risks of obesity and $obesity_{max}$[g] at adulthood. Compared with

(*continued*)

Table 2 (continued)

Authors	Surveyed time	Aim/objective of the study	Methods	Results
			in 1959–61, childhood exposed born in 1949–58, and adolescence exposed born in 1941–1948	unexposed group, OR values were 1.59, 1.42, and 1.86 for fetal/infant, childhood, and adolescence exposed, respectively
Wang et al. (2010b)	Chongqing, China in 2006–2008	Investigate an association between early nutritional status during the famine and the risk for overweight and obesity at adulthood	17,023 subjects from annual physical evaluation were categorized by birthday into childhood-exposed (1956–58), fetal-exposed (1959–61), and unexposed control (1962–64)	The groups of childhood and fetal exposed to famine had significantly higher body weight and BMI and lower height compared with unexposed control

[a]*SPECT-China* survey on prevalence in East China for metabolic diseases and risk factors
[b]*MS* metabolic syndrome
[c]*CNNHS* China National Nutrition and Health Survey
[d]CPNTD collaborative project for neural tube defect conducted by the US CDC and Beijing Medical University between 1993 and 1996
[e]*T2D* type 2 diabetes
[f]*NAFLD* nonalcoholic fatty liver disease
[g]$obesity_{max}$ obese at the highest weight

(Song et al. 2009). However, in another large mental health epidemiology survey conducted between 2001 and 2005, which included 4972 subjects born between 1956 and 1963, the potential impact of exposure to famine in utero and during the early postnatal life on adult mental illness was estimated and evaluated by difference-in-difference models. Compared with women born in 1963, women born during the famine years (1959–61) had higher risk of mental illness (OR = 2.08; Cl = 1.23–6.39) (Huang et al. 2013). These findings support that exposure to nutritional deficiency during fetal life may be a risk factor for developing mental illness such as schizophrenia, which might be related to an increase in clinical brain abnormalities resulted from aberrant early brain development (Table 3).

However, there are several limitations on such cohort studies related to undernutrition effects of prenatal exposure to the famine on NCDs in later life. Underestimating famine exposure or fuzzy time during gestation and associated outcomes related to NCDs in later life; lack of information on exact food intake and experienced duration of the Dutch famine or Chinese famine so that it is impossible to use the size of the effects in such study to estimate the importance of maternal nutrition for public health in prevention of NCDs; and using famine as a natural experiment in itself do not guarantee correct statistical inference about the

Table 3 The effect of malnutrition in early life on adult mental health based on studies from the Chinese great famine (1959–61)

Authors	Surveyed time	Aim/objective of the study	Methods	Results
Clair et al. (2005)	Wuhu and its surrounding counties in Anhui Province 1971–2001	Determine whether those who endured Chinese great famine (1959–61) had a higher risk of schizophrenia at adulthood	All psychiatric case records for the years 1971–2001 were examined and divided into born before (1959), during (1959–61), or after the famine years (1962)	Prenatal exposure to famine increased risk of schizophrenia in later adulthood, adjusted risk for schizophrenia increased from 0.84% in 1959 to 2.15% in 1960 and 1.81% in 1961
Xu et al. (2009)	Liuzhou and its surrounding counties in Guangxi AR[a] 1971–2001.	Test prenatal malnutrition and adult schizophrenia in a second Chinese population and also determine whether risk differed between urban and rural areas	All psychiatric case records in Liuzhou psychiatric hospital for the years 1971–2001 were examined; exposed birth years 1960–61, compared groups 1956–58 and 1963–65	Mortality-adjusted RR for schizophrenia was 1.5 for 1960 and 2.05 for 1961 compared with the other years and the absolute rate of schizophrenia was higher in rural than in urban
Song et al. (2009)	The 1987 CNDSS[b]	Test the hypothesis that prenatal exposure to famine increases schizophrenia risk at adulthood	Recruit 1,579,316 residents from all 29 provincial level administrative units in China including 3907 with a mental disorder and 2574 with schizophrenia; 294,365 subjects were born between 1955 and 1965, and 494 with schizophrenia	Urban population conceived and born during the famine had higher risk for schizophrenia at early adulthood, compared with the pre-famine and post-famine cohorts, and rural population had the highest risk
Huang et al. (2013)	ZJ, SD, QH, and GS[c] provinces 2001–2005	Investigate the potential impact of famine exposure in utero and during early postnatal life on mental illness at adulthood	4972 subjects born between 1956 and 1963, and divided into famine exposure in utero and early postnatal life from a series of epidemiological surveys on mental disorders of four provinces	Long-term consequences of early life famine exposure include both the selection of the hardiest and enduring deleterious effects of famine on those who survive in the risk for mental illness at adulthood

[a] *AR* autonomous region
[b] *CNDSS* Chinese National Disability Sample Survey
[c] *ZJ* Zhejiang, *SD* Shandong, *QH* Qianghai, *GS* Gansu

Fig. 2 What did they dream or think after natural disaster?

long-term health impacts of prenatal malnutrition when other analytical challenges remain unresolved (Xu et al. 2016).

In summary, during famine, the important task for healing the spiritual wounds and stabilizing the people's mood in the affected areas was the contact and communication to the outside and especially to the local and/or central government, which is important to include early and comprehensive interventions to reverse not only medical conditions resulting from malnutrition but also cognitive and behavioral deficits arising from famine. Young children what are their dreams or thinking about tomorrow's life should be considered for us during famine as the situation we can see after Wenchuan earthquake in Fig. 2. What can we do for them would be important priority task to be considered and solved for us.

Policies and Protocols

In this chapter we have described the short-term and long-term impact of exposure to famine during, prenatal or postnatal gestation on human health status. In some cases of short-term effect due to famine, malnutrition such as protein-energy and anemia, especially some diseases resulted from micronutrient deficiencies became more serious public health priority to be solved because this period was considered as a unique window of opportunity (the first 1000 days of life) to decide children's healthier and more prosperous future; long-term impact will focus on future health status and susceptibility to nutrition-related NCDs at adulthood. The findings based on epidemiological investigation, early intervention, and cohort studies on the effect of exposure to famine to human health have shown very clear implications for making public health policy and improving national nutritional status for the entire population, and investing the improvement of children's nutrition status in the early years of life has reached a consensus at international level and has been considered as one of the least investments and the biggest benefit for a country.

Dictionary of Terms

- **Famine** – When a country or region has "more than half of the population directly or indirectly facing death caused by hunger or dying situation" can be regarded as "famine" or when a country or region has doubles of the mortality rate due to severe food shortage and more than 20% of children suffered from malnutrition can be generally defined as "famine."
- **Dutch famine** – Dutch famine, located in the Western Netherland, occurred from October 1944 until the surrender of the German Forces on 7 May 1945, and the period of most intense starvation was February to April 1945.
- **China's great famine** – China's great famine (1959–61) was one of the worst human catastrophes during the twentieth century and affected all regions of a vast and diverse country.
- **Cohort study of exposure to famine** – The studies related to the effect of exposure to malnutrition during, prenatal or postnatal gestation due to famine were to observe the long-term effects on NCDs at adulthood.
- **Metabolic syndrome (MS)** – MS can be diagnosed with three or more of the following conditions based on the Chinese Diabetes Society: BMI $\geq$25.0 kg/m^2, serum triglyceride $\geq$1.70 mmol/L or drug treatment for elevated triglyceride, serum high-density lipoprotein cholesterol $<$0.90 mmol/L in men or $<$1.00 mmol/L in women or drug treatment for decreased high-density lipoprotein cholesterol, systolic blood pressure $\geq$90 mmHg or drug treatment for hypertension, fasting plasma glucose $\geq$6.1 mmol/L or drug treatment for diabetes.
- **Fetal origins hypothesis** – This hypothesis postulated that nutritional deprivations during the fetal period or early life could lead to higher prevalence of nutrition-related NCDs or increase the susceptibility to NCDs at adulthood.

Summary Points

- This chapter focuses on short-term and long-term effect of exposure to famine during early life on human health status and susceptibility to nutrition-related NCDs at adulthood.
- The short-term impacts include protein-energy malnutrition, iron-deficiency and anemia, vitamin A deficiency and decrease in resistance to infectious diseases, vitamin D deficiency and rickets, and other multi-micronutrient deficiencies.
- Malnutrition during early life will lead to susceptibility to preventable infectious diseases such as diarrhea and pneumonia and has an indirect association with the leading causes of death in young children.
- The long-term effects include the impact on child's subsequent growth and development, and the susceptibility to NCDs and mental health at adulthood.
- During and/or after famine, timely nutrition intervention to elder infants and young children can effectively improve their nutritional status and decrease the mortality resulted from malnutrition and infectious diseases.

- Dutch famine occurred in the Western Netherland from October 1944 until the surrender of the German Forces on 7 May 1945, and the period of most intense starvation was February to April 1945d.
- Dutch famine cohort studies intend to evaluate the relationship of exposure to famine during fetal and childhood period and the risk for NCDs in adulthood.
- China's great famine was one of the worst human catastrophes during the twentieth century and affected all regions of a vast and diverse country (1959–61).
- China's great famine cohort studies indicate that early-life conditions, such as nutrition status, influence later risk of nutrition-related NCDs such as metabolic syndrome.
- Exposure to famine in early life has impact on mental health at adulthood based on Dutch famine and China's great famine cohorts.

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Evidence for the Association Between Early Childhood Stunting and Metabolic Syndrome

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Abstract

Stunting or linear growth deficit is the most prevalent form of undernutrition globally and is internationally recognized as an important public-health indicator for monitoring health in populations. In 2015, 23.2%, or just under one in four children under the age of 5 worldwide had stunted growth and the number of children affected fell from 255 million to 156 million. Stunting has long-term effects on individuals and societies, including diminished cognitive and physical development, reduced productive capacity, poor health, and an increased risk of degenerative diseases such as diabetes. Metabolic syndrome is a multicomponent risk factor for cardiovascular disease and type 2 diabetes mellitus that reflects the clustering of individual cardiometabolic risk factors related to abdominal obesity and insulin resistance. Estimates have indicated that nearly one quarter of the world's adult population suffers from metabolic syndrome. The aim of this study was to investigate the association between childhood stunting and metabolic syndrome components in adulthood. A systematic review was undertaken to address this question. Eight relevant articles were located. In the majority of the studies, the metabolic syndrome components were measured in adolescence or early adulthood. An outcome measured in later adulthood was observed in only one study, and earlier onset of metabolic risks was observed in men who had a timing of early peak height velocity. The results in this literature indicated that the consequences of stunting were complex and likely to depend on local environment, diet, and developmental timing. On concluding this review, the evidence for the association between early childhood stunting and metabolic syndrome was unclear.

Keywords

Adult · Central obesity · Child · Cohort study · Dyslipidemia · Hypertension · Insulin resistance · Malnutrition · Metabolic syndrome · Prevalence · Stunting

List of Abbreviations

AACE	American Association of Clinical Endocrinology
AHA	American Heart Association
ALSPAC	Avon Longitudinal Study of Parents and Children
AO	Abdominal obesity
CD	Cardiovascular disease
CI	Confidence interval
COHORTS	Consortium for Health Orientated Research in Transitional Societies
EGIR	European Group for the Study of Insulin Resistance
HBP	High blood pressure
HDL-C	High-density lipoprotein cholesterol
IDF	International Diabetes Federation
IR	Insulin resistance
MetS	Metabolic syndrome

NCEP-ATPIII	National Cholesterol Education Program – Third Adult Treatment Panel
NHLBI	National Heart, Lung, and Blood Institute
T2DM	Type 2 diabetes mellitus
TG	Triglycerides
UNICEF	United Nations Children's Fund
WHO	World Health Organization

Introduction

Growth Faltering in Childhood: Definition of Stunting

Stunting or linear growth deficit is the most prevalent form of undernutrition all over the world and is internationally recognized as an important public-health indicator for monitoring health in populations. Moreover, children who suffer from growth retardation tend to be at higher risk of suffering illness and death. The percentage of children with low height-for-age reflects the cumulative effects of undernutrition and infections since birth, and even before birth. This measure, therefore, should be interpreted as an indication of poor environmental conditions and/or long-term restriction of a child's growth potential. Linear growth is the best overall indicator of children's well-being and stunting, measured by the child's length/height for the same age and sex, is defined as being minus 2 standard deviations below the value of the World Health Organization (WHO) Child Growth Standards median (WHO 2014a). Nearly half of all deaths in children under 5 years of age are attributable to undernutrition. This translates into the unnecessary loss of about three million young lives a year. Undernutrition puts children at greater risk of dying from common infections, increases the frequency and severity of such infections, and contributes to delayed recovery. In addition, the interaction between undernutrition and infection can create a potentially lethal cycle of worsening illness and deteriorating nutritional status. Growth failure begins in utero and continues for at least the first 2 years of post-natal life; poor nutrition in the first 1,000 days of a child's life can also lead to irreversible stunted growth, associated with impaired cognitive ability and reduced school and work performance (Black et al. 2013; UNICEF/WHO/Word Bank 2016). Growth failure in early life has profound adverse consequences over the life course on human, social, and economic capital (Hoddinott et al. 2013).

Stunting is one of the global nutrition targets for 2025 adopted by the World Health Assembly, in 2012 with the aim of reducing linear growth deficit by 40% by 2025 (WHO 2014a). According to De Onis and Branca (2016), this increased international attention has resulted from the greater significance of stunting as a major public health problem for five reasons: it affects large numbers of children globally; has severe short-term and long-term health and functional consequences; there is consensus regarding its definition and a standard to define normal human growth that is applicable everywhere; there is agreement on a critical time-frame from conception through the first 2 years of life – when linear

growth is most sensitive to environmentally modifiable factors; and it is a cross-cutting problem calling for a multisectoral response. Stunting results from a complex interaction of household, environmental, socioeconomic, and cultural influences that are described in the World Health Organization Conceptual Framework on Childhood Stunting (Stewart et al. 2013).

Worldwide Patterns and Recent Trends

In September 2016, United Nations Children's Fund (UNICEF), WHO, and World Bank Group released the 2016 edition of the joint child malnutrition estimates for the 1990–2015 period, representing the most recent global and regional figures. In 2015, 23.2%, or just under one in four children under the age of 5 worldwide had stunted growth. Having said that, overall trends are positive. Between 1990 and 2015, stunting prevalence globally declined from 39.6% to 23.2%, and the number of children affected fell from 255 million to 156 million. In developing countries this percentage was 25.4%. In 2025, the estimate of stunting in children under 5 years of age around the world is 19.1%; whereas, in children from low and middle income countries it is 20.8%. Africa has made only limited progress on stunting since 2000 (−17%) compared with other regions such as Asia (−36%), Latin America and Caribbean (−39%). Western Africa accounts for half of the increase in stunting in Africa. There were 4 million more stunted children in Western Africa in 2015 than in 2000. Progress on stunting within Asia has been uneven since 2000; rates in Eastern Asia have dropped by more than two thirds since 2000 (−69%), compared with Southern Asia, where stunting declined by less than one third during the same period (−30%). More data are needed to generate reliable estimates for Oceania. Based on available data, stunting rates in Oceania have been stagnant for the past 15 years, but confidence intervals are very large. Progress in Latin America and Caribbean is aligned with global goals. Improvements in malnutrition in the region are encouraging. The graphs show trends (1990–2025) in child malnutrition indicators for stunting global and by United Regions (UNICEF/WHO/Word Bank 2016) (Fig. 1).

The percentage of stunted children under 5 years of age, according to WHO Regions in 2015 is shown in Fig. 2. Three regions have stunting rates exceeding 25%.

In almost all countries that have available data, stunting rates are higher among boys than girls. While analyses to determine underlying causes for this phenomenon are underway, an initial review of the literature suggested that the higher risk for preterm birth among boys (which is inextricably linked to lower birth weight) is a potential reason for this sex-based disparity in stunting (UNICEF 2014).

Of all stunted children, 32.6% live in lower-middle income countries and 37.3% live in low-income countries. The other 7.6% of all stunted children live in upper-, middle- or high-income countries, with only 2.6% living in the latter (high-income) group. Percentage of stunted children under the age of 5, by country and income classification in the period from 2000 to 2015 is shown in Fig. 3 (UNICEF/WHO/Word Bank 2016).

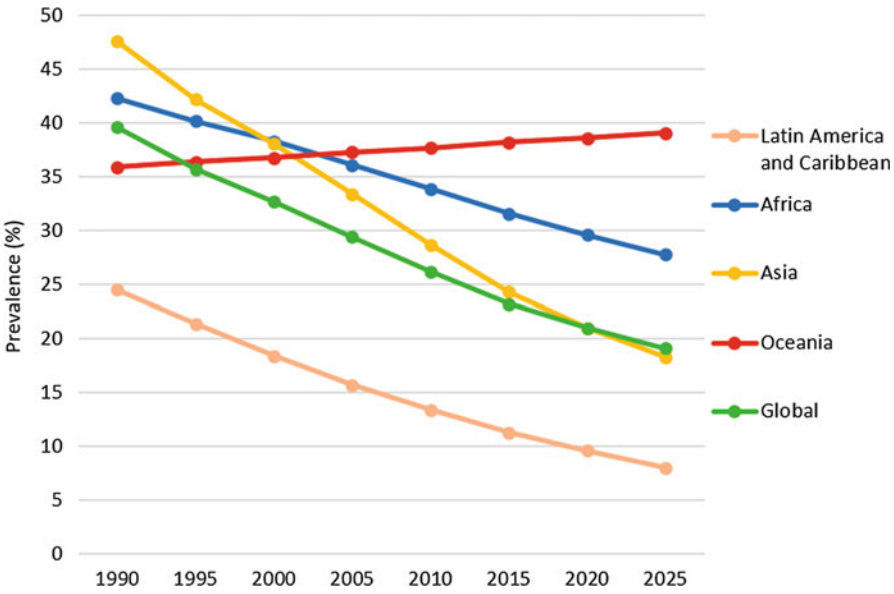

Fig. 1 Percentage of stunted children under 5 years of age, global and by United Regions, 1990–2025 (Source: United Nations Children's Fund, World Health Organization, World Bank Group joint malnutrition estimates, 2016 edition. Note: Oceania excluding Australia and New Zealand)

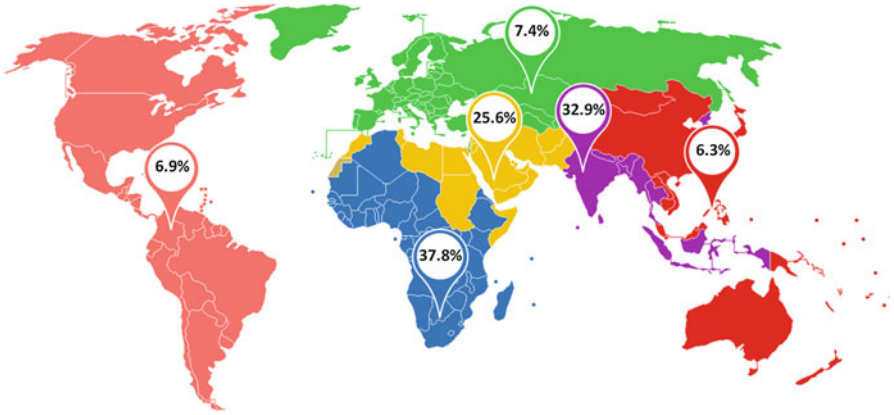

Fig. 2 Percentage of stunted children under 5 years of age, by World Health Organization Regions, 2015 (Source: United Nations Children's Fund, World Health Organization, World Bank Group joint malnutrition estimates, 2016 edition)

Consequences of Stunted Growth

Stunting is associated with increased morbidity and mortality from infections, in particular pneumonia and diarrhoea (Black et al. 2013; Prendergast and Humphrey

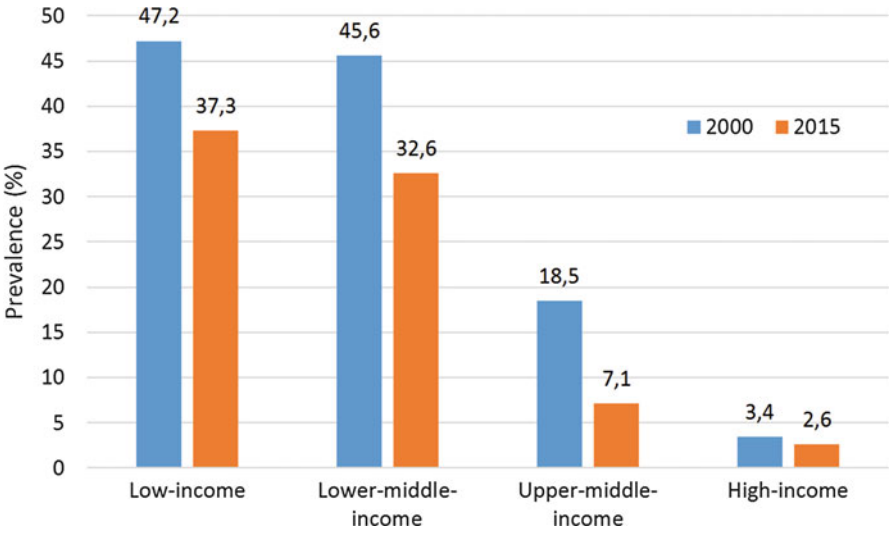

Fig. 3 Prevalence of stunted children under 5 years of age, by country income classification, 2000 and 2015 (Source: United Nations Children's Fund, World Health Organization, World Bank Group joint malnutrition estimates, 2016 edition)

2014), and it serves as a marker for multiple pathological disorders such as loss of physical growth potential, reduced neurodevelopmental and cognitive function, and an elevated risk of cardiovascular disease (CD) in adulthood (Prendergast and Humphrey 2014; De Onis and Branca 2016).

Definition of Metabolic Syndrome

Metabolic syndrome (MetS) is a multicomponent risk factor for CD and type 2 diabetes mellitus (T2DM) that reflects the clustering of individual cardiometabolic risk factors related to abdominal obesity (AO) and insulin resistance (IR). The defining components of metabolic syndrome that cluster together are AO, IR, high blood pressure (HBP), and dyslipidemia (Kaur 2014, O'Neil and O'Driscoll 2015; Benjamin et al. 2017).

Reports of clustering of metabolic risk factors date back to the early 1920s (Sarafidis, Nilsson 2006). However in 1988, Reaven observed that several risk factors (dyslipidemia, hypertension, hyperglycemia) tended to occur together; this set was named syndrome X (Reaven 1988).

Several groups have attempted to develop diagnostic criteria for the diagnosis of the MetS. The first attempt with the name MetS like diagnostic entity with defined criteria was made by a World Health Organization Diabetes Group (WHO) in 1998 (Alberti and Zimmet 1998); subsequently, the European Group for the Study of Insulin Resistance (EGIR) countered with a modification of the WHO definition in

1999 (Balkau and Charles 1999). In 2001, the National Cholesterol Education Program Adult Treatment Panel (NCEP: ATPIII) released its definition (Cleeman 2001). In 2003 the American Association of Clinical Endocrinologists (AACE) offered its views (Einhorn 2003) and in 2005 the International Diabetes Federation (IDF) proposed a new definition of the metabolic syndrome a single unifying definition (International Diabetes Federation 2006; O'Neil and O'Driscoll 2015). Although several different clinical definitions for MetS have been proposed, in 2009 the IDF; National Heart, Lung, and Blood Institute (NHLBI); American Heart Association (AHA); and others proposed a harmonized definition for MetS (Alberti et al. 2009) (Table 1).

This definition stated that obesity and IR were not pre-requisites for MetS but that three of the five components would suffice for a diagnosis of MetS, with the thresholds for measuring waist circumference requiring ethnic and nation specificity (Table 2).

Prevalence

The existence of varying definitions, which differ in small defining values, has impeded determination of the true prevalence of MetS; however, it has generally been accepted by all groups that there was increasing prevalence of MetS (O'Neil and O'Driscoll 2015). The IDF estimated that nearly one quarter of the world's adult population was suffering from MetS (International Diabetes Federation 2015). Data on the prevalence of MetS from several regions have been compared, defined by NCEP: ATPIII criteria (National Cholesterol Education Program 2002) (Table 3).

Components of Metabolic Syndrome

Abdominal Obesity

The link between obesity, poor health outcomes, and all-cause mortality is well established. Obesity increases the likelihood of diabetes, HBP, CD, stroke, certain cancers, obstructive sleep apnea, and osteoarthritis. There has been increasing obesity in all countries. In 2014, 39% of adults aged 18 years and older (38% of men and 40% of women) were overweight. The worldwide prevalence of obesity nearly doubled between 1980 and 2014. In 2014, 11% of men and 15% of women worldwide were obese. Thus, more than half a billion adults worldwide are classed as obese (WHO 2014b).

From the epidemiological point of view, the growing epidemic of obesity is connected with the increase in CD and MetS. Obesity can be defined as an increase in percentage of total body fat, in relation to a standard, which is reflected at cellular level by an increase in the number and/or size of the adipocytes. Much more importance has been given to the distribution of adipose tissue than to its volume. There is good evidence that associates AO with cardiovascular and metabolic risk,

Table 1 Criteria as set out by the different associations for MetS definition

Criteria	WHO (1998)	EGIR (1999)	NCEP:ATPIII (2001)	AACE (2003)	IDF (2005)	IDF + AHA/ NHLBI (2009)
Insulin resistance	High insulin level + two of the following:	Plasma insulin >75th percentile + two of the following:	Any three of the following:	Fasting plasma glucose 110–125 mg/dL	≥ 110 mg/dL	≥ 110 mg/dL
Abdominal obobesity	Waist to hip ratio >0.90 (male) and > 0.85 (female) Body mass index >30 kg/m^2	Waist circumference > 0.94 (male) and >0.80 (female)	Waist circumference >102 cm (male) and >88 cm (female)		Waist circumference ≥94 cm (Europid male) and ≥ 80 cm (Europid female), ethnicity specific values for other groups	Population and country - specific definitions
TG	≥150 mg/dL	≥150 mg/dL	≥150 mg/dL	≥150 mg/dL	≥150 mg/dL	≥150 mg/dL
TG HDL-C	<35 mg/dL (male) <39 mg/dL (female)	<39 mg/dL (male and female)	<40 mg/dL (male) <50 mg/dL (female)	<40 mg/dL (male) <50 mg/dL (female)	<40 mg/dL (male) <50 mg/dL (female)	<40 mg/dL (male) <50 mg/dL (female)
Blood pressure	≥140/90 mmHg	≥140/90 mmHg or hypertensive medication	≥130/85 mmHg	≥130/ 85 mmHg	≥130/85 mmHg	≥130/ 85 mmHg
Other	Microalbuminuria or albumin: creatinine ratio ≥30 mg/g	Fasting glucose ≥6.1 mmol/L	Fasting glucose ≥110 mg/dL			

Note: *WHO* World Health Organization, *EGIR* European Group for the Study of Insulin Resistance, *NCEP: ATPIII* National Cholesterol Education Program-Third Adult Treatment Panel, *AACE* American Association of Clinical Endocrinology, *IDF* International Diabetes Federation, *AHA* American Heart Association, *NHLBI* National Heart, Lung, and Blood Institute, *TG* triglycerides, *HDL-C* high-density lipoprotein cholesterol

Table 2 Ethnic-specific waist circumference thresholds for abdominal obesity

Ethnicity	Men	Women	Reference
Europid	≥ 94 cm	≥ 80 cm	IDF (2006)
Ethnic Central and South American	≥ 90 cm	≥ 80 cm	IDF (2006)
Middle Eastern/Mediterranean	≥ 94 cm	≥ 80 cm	IDF (2006)
South Asians, Chinese, and Japanese	≥ 90 cm	≥ 80 cm	IDF (2006)
Sub-Saharan African	≥ 94 cm	≥ 80 cm	IDF (2006)
United States	≥ 102 cm	≥ 88 cm	NIH (1998)

IDF International Diabetes Federation, *NIH* National Institutes of Health

Table 3 Prevalence of metabolic syndrome in the different geographical regions

Region (Reference)	Year publication	Prevalence of metabolic syndrome (%)
Asia-Pacific (Ranasinghe et al. 2017)	2017	11.9–37.1
Africa (Okafor 2012)	2012	12.5–62.5
Brazil (de Carvalho et al. 2013)	2013	29.6
Central America (Wong-McClure et al. 2015)	2015	30.3
Europe (Van Vliet-Ostaptchouk et al. 2014)	2014	24.0–78.2
India (Sawant et al. 2011)	2011	19.5
Latin America (Marquez-Sandoval et al. 2011)	2011	18.8–43.3
Mexico (Salas et al. 2014)	2014	54.8
Middle East (Sliem et al. 2012)	2012	13.6–45.5
South Asia (Aryal and Wasti 2016)	2016	26.1
United States (Liu et al. 2014)	2014	26.7

because of its high relationship with perivisceral fat. Intra-abdominal or visceral fat is a risk factor irrespective of IR, intolerance to glucose, dyslipidemia, and HBP, all of which are components of the MetS (Carr et al. 2004).

Several studies have shown that intra-abdominal fat, measured by the abdominal circumference was independently associated with each one of the criteria of the MetS and suggest that it may play a central role in the pathogenesis of MetS (International Diabetes Federation 2006; Pineda 2008).

Insulin Resistance

IR is a pathophysiological phenomenon in which the biological action of insulin is altered in the different tissues of the body and causes compensatory hyperinsulinemia. When the body cannot maintain this response, hyperinsulinemia – often associated with T2DM – develops; but in the opposite case, if hyperinsulinemia is sustained, a series of changes (mainly of the metabolic type)

occur, which increase the risk of CD. Most people with MetS show evidence of IR; its complications are major causes of death in most countries (Pineda 2008). People with T2DM are at higher risk of developing a number of disabling and life-threatening health problems than people without T2DM; such as serious diseases affecting the heart and blood vessels, eyes, kidneys, and nerves, in addition to increased risk of developing infections. In 2015, the global prevalence of diabetes was 8.8% in individuals between 20 the 79 years of age; the number of people with diabetes was 415 million (International Diabetes Federation 2015).

Dyslipidemia

In the MetS, dyslipidemia is characterized by the presence of low levels of HDL-cholesterol (HDL-C) and elevated levels of triglycerides (TG).

Dyslipidemia is another important feature that can be included in all the criteria mentioned above. Dyslipidemia associated with MetS is considered highly atherogenic. Low HDL-C and elevated TG are predictors of cardiovascular risk factors in patients with MetS. The combination of low HDL-C and elevated basal glycemia has been shown to be a predictor of coronary disease (International Diabetes Federation 2006; Pineda 2008).

Hypertension

Raised blood pressure is one of the leading risk factors for global mortality and is estimated to have caused 9.4 million deaths in 2010. A reduction in systolic blood pressure of 10 mmHg has been associated with a 22% reduction in coronary heart disease, 41% reduction in stroke in randomized trials, and a 41–46% reduction in cardiometabolic mortality in epidemiological studies. The global prevalence of raised blood pressure (defined as systolic and/or diastolic blood pressure $\geq$140/90 mmHg) in adults aged 18 years and over was around 22% in 2014. The proportion of the world's population with HBP or uncontrolled hypertension fell modestly between 1980 and 2010. However, because of population growth and ageing, the number of people with uncontrolled hypertension has risen over the years (WHO 2014b).

A meta-analysis of 242 studies (n = 1.5 million adults) reported that 32.3% (95% CI = 29.4–35.3%) of adults had hypertension, with the highest prevalence in the Latin America and Caribbean region. Hypertension prevalence estimates were highest across upper-middle-income countries (37.8%, 95% CI = 35.0–40.6%) and lowest across low-income countries (23.1%, 95% CI = 20.1–26.2%) (Sarki et al. 2015; Benjamin et al. 2017).

Metabolic Syndrome Characteristics Following Early Childhood Stunting

The systematic literature review included searches for articles in the PubMed, MEDLINE, LILACS, and SciELO databases. Various combinations using multiple search terms of Health Sciences Descriptors were used ("metabolic syndrome"; "stunting or height for age deficit"; "cohort studies") and a search was conducted for association between "metabolic syndrome and stunting." Articles published until February 2017 were considered. The reference lists were compared and the relevant articles selected by title and abstract. All the apparently relevant articles were obtained and reviewed by the authors. Of the articles read, 98 were selected for reference in the review and 65 were published in the last ten years. Of these, 27 titles were selected and eight abstracts were chosen for reading of the complete manuscript. Of all eight articles read, four were chosen for this review. In addition, four studies were identified in the references of the articles. These eight studies are presented in the following sections.

In a birth cohort study conducted in Southern Brazil (Grillo et al. 2016), the association between stunting at the age of 2 years and MetS components in early adulthood was investigated. Individuals with stunting showed significantly higher prevalence of altered HDL-C; however, when the analyses were adjusted for socioeconomic variables and maternal characteristics, this association vanished.

In another study (Sun et al. 2014) with a sample that consisted of 431 adults (213 males and 218 females) from the Fels Longitudinal Study, the subjects' childhood height data had been recorded to capture the age at peak height velocity, as well as sufficient serial MetS risk-factor data that had been collected later in life. The peak height velocity was around 13.5 years for boys and 11.5 for girls. Females who had early peak height velocity tended to develop more AO than girls who had a late peak. Boys who had early peak height velocity tended to have a higher risk for the MetS, with elevated levels of fasting plasma glucose, elevated TG, and low HDL-C.

A retrospective cohort study of school-aged children was conducted in Chile and the national individual identification number was used to link information gathered at school with perinatal data collected by the civil registry (Mardones et al. 2014). The lowest and highest categories of birth length were associated with a twofold increase in the risk of HBP and birth length categories below 50 centimeters seemed to increase the risk for IR when adjusting for fat mass, sex, and Tanner stage among Chilean school-aged children.

A birth cohort study from India investigated the linear growth and fat and lean tissue gain during discrete age periods from birth to adolescence, and the findings showed that linear growth (up to age 2 years) was unrelated to cardiometabolic risk factors (Krishnaveni et al. 2015).

The Avon Longitudinal Study of Parents and Children (ALSPAC) was established in 1990 when pregnant women, expected to deliver between April

1991 and December 1992, were enrolled in a cohort study. Around 14,000 live born children were followed up with questionnaires, and from the age of 7 years, at regular clinical visits to obtain data relative to anthropometric, behavioral, cardiovascular factors, and metabolic phenotypes. The conditional height growth was positively associated with systolic blood pressure in the 3230 boys and 3346 girls from ALSPAC, at 10 years of age (Jones et al. 2012).

In another birth cohort study from Southern Brazil (Menezes et al. 2012) the independent effects were investigated relative to weight and length/height gains in different periods during childhood on blood pressure and on measures of obesity and central fat distribution in adolescence. The findings showed that length/height gain increased systolic blood pressure at 14–15 year of age, but it was not associated when adjusting for body mass index in adolescence, suggesting that the increase in length/height without excessive weight gain was beneficial for the development of body composition.

Peak height velocity in infancy and metabolic outcomes at the age of 31 years was also studied in a population-based birth-cohort study of 3,778 Finns (1966–1998). Growth data were collected from communal health clinics and eight height measurements were obtained per person, on an average, from birth to age 2 years. A peak height velocity that was higher by 8 cm/year was positively associated with systolic and diastolic blood pressure, waist circumference, and a nonsignificant 13% higher risk of MetS ($P = 0.08$) that completely disappeared when adjusting for body mass index ($P = 0.32$). The positive associations were attenuated after adjusting for birth weight and body mass index at the age of 31 years but remained significant (Tzoulaki et al. 2010).

The association between linear growth and cardiometabolic risk factors was also investigated in the five prospective birth cohort studies included in the Consortium for Health Orientated Research in Transitional Societies – COHORTS (Adair et al. 2013). Faster linear growth was strongly associated with a reduced risk of short adult stature (age 2 years: 0.23, 95% CI = 0.20–0.52; mid-childhood: 0.39, 95% CI = 0.36–0.43) and elevated blood pressure (age 2 years: 1.12, 95% CI = 1.06–1.19; mid-childhood: 1.07, 95% CI = 1.01–1.13). Linear growth and relative weight gain were not associated with dysglycemia.

The relationship of birthweight (Würtz et al. 2016) or preschool weight (Graversen et al. 2014) with metabolic risk has been shown in birth cohort studies. However, less is known about the effect of height on MetS. In our review the effects of length/height measured during childhood occurring later in life were mainly assessed by means of blood pressure, and the results showed that there was a positive association between height gain and blood pressure that generally disappeared after adjusting for weight or body mass index. The effect on MetS was investigated in only one study (Tzoulaki et al. 2010). It is important to highlight that in the majority of studies, the components of metabolic syndrome were measured in adolescence or early adulthood. Outcome measured in

later adulthood was observed in only one study (Sun et al. 2014) and earlier onset of metabolic risks was observed in men who had an early timing of peak height velocity. On conclusion of this review, the evidence for the association between early childhood stunting and MetS was unclear.

Protocols

Stunting

In this chapter we have described that stunting or linear growth is the most prevalent form of undernutrition globally and is internationally recognized as an important public-health indicator for monitoring health in populations. Stunting often begins in utero and continues for at least the first 2 years of post-natal life. Stunting is identified by assessing a child's length or height (recumbent length for children less than 2 years old and standing height for children aged 2 years or older) and interpreting the measurements by comparing them with an acceptable set of standard values. There is international agreement that children are stunted if their length/height is minus 2 standard deviations below the values of the World Health Organization Child Growth Standards median for the same age and sex. The use of cut-off points is required to determine the limits of normality. Children are considered severely stunted if their length/height is minus 3 standard deviations below the values of the World Health Organization Child Growth Standards median for the same age and sex.

Metabolic Syndrome

The metabolic syndrome is a complex of interrelated risk factors for cardiovascular disease and diabetes. Although several different clinical definitions have been proposed for metabolic syndrome, according to the new harmonized metabolic syndrome definition, it is diagnosed when any three of the following five risk factors are present:

- Fasting plasma glucose: $\geq$100 mg/dL or undergoing drug treatment for elevated glucose
- HDL-cholesterol: <40 mg/dL in males or <50 mg/dL in females or undergoing drug treatment for reduced HDL-cholesterol
- Triglycerides: $\geq$150 mg/dL or undergoing drug treatment for elevated triglycerides
- Waist circumference: Ethnicity and country-specific thresholds can be used for diagnosis in the groups
- Blood pressure: $\geq$130 mm Hg systolic or $\geq$85 mm Hg diastolic or undergoing drug treatment for hypertension, or antihypertensive drug treatment in a patient with a history of hypertension

Dictionary of Terms

- **Stunting** – Length/height is minus 2 standard deviations below the values of the World Health Organization Child Growth Standards median for the same age and sex.
- **Metabolic syndrome** – Three abnormal findings out of five would qualify a person for the metabolic syndrome: elevated waist circumference, elevated triglycerides, reduced high-density lipoprotein cholesterol, elevated blood pressure, and elevated fasting glucose.
- **Abdominal obesity** – Excessive abdominal fat around the stomach and abdomen that has built up to the extent that it is likely to have a negative impact on health.
- **Insulin resistance** – A decreased sensitivity to the action of insulin. Conditions in which the increased amount of insulin are inadequate to induce normal insulin responses in insulin-sensitive tissues (liver, skeletal muscles, adipose tissues).
- **Dyslipidemia** – It is an abnormal amount of lipids (example, triglycerides, cholesterol, and/or fat phospholipids) in the blood.
- **Hypertension** – This, also known as high blood pressure, is a long term medical condition in which the blood pressure in the arteries is persistently elevated.

Summary Points

- Stunting or linear growth deficit is the most prevalent form of undernutrition globally and is internationally recognized as an important public-health indicator for monitoring health in populations.
- Factors that contribute to stunted growth and development include poor maternal health and nutrition, and inadequate infant and young child feeding practices and infection.
- The 1,000 days between a woman's pregnancy and her child's 2nd birthday offer a unique window of opportunity to build healthier and more prosperous futures.
- Growth failure in early life has profound adverse consequences over the life course on human, social, and economic capital.
- In 2015, 23.2%, or just under one in four children under the age of 5 worldwide had stunted growth, globally affecting 156 million children. In developing countries this percentage was 25.4%.
- Percentage of stunted children under the age of 5, by World Health Organization Regions, was found in Africa: 37.8%, South East Asia: 32.9%, Eastern Mediterranean: 25.6%, Europe: 7.4%, Americas: 6.9%, and Western Pacific: 6.3%.
- Metabolic syndrome is a multicomponent risk factor for cardiovascular disease and type 2 diabetes mellitus that reflects the clustering of individual cardiometabolic risk factors related to abdominal obesity and insulin resistance.
- The prevalence of metabolic syndrome in the different geographical regions ranged from 11.9% to 78.2%.

- For the systematic literature review, 98 articles were selected for reference in the review and 65 were published in the last ten years. Of these, 27 titles were selected and eight abstracts were chosen for complete reading of the manuscript.
- In the majority of the studies, the components of metabolic syndrome were measured in adolescence or early adulthood. Outcome measured in later adulthood was observed in only one study, and earlier onset of metabolic risks was observed in men who had an early timing of peak height velocity.
- The results in this literature indicated that the consequences of stunting were complex and likely to depend on local environment, diet, and developmental timing.
- On conclusion of this review, the evidence for the association between early childhood stunting and metabolic syndrome was unclear.

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Nutritional Status of the Elderly in an Arab Country in Social Transition: The Case of Lebanon

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Abstract

Lebanon is a small country located in the Middle East region which is undergoing rapid demographic transition. Among Arabic countries Lebanon has the fastest growing elderly population, and by the year 2025, older adults above 65 years are expected to represent more than 10% of the population. Nutrition is a key factor during the aging process and may help to prevent age-related diseases. However with increase in age, malnutrition is more frequent and might become an important health problem in the future. The prevalence of malnutrition among elderly people is particularly high in hospital environment and nursing homes compared to community settings, where mainly frail and dependent elderly are affected. Numerous factors are contributing to malnutrition such as age-related changes, social, physical, and psychological factors which are mostly acting in combination. The danger of malnutrition is related to its negative health consequences with increased risk of morbidity, functional decline, and decreased quality of life. Early detection and nutritional intervention may allow preventing negative health outcomes and increasing chance for healthy aging.

This review presents the results of several surveys mainly carried out in the community and institutional settings. The principle goal of these studies was to estimate the prevalence of malnutrition and identify factors associated with malnutrition. The importance of increased awareness and early detection of malnutrition is highlighted.

Keywords

Malnutrition · Nutritional status · Mini nutritional assessment · Nutritional assessment · Nutritional deficiencies · Nutritional risk · Weightloss · Elderly · Older adults · Lebanon

List of Abbreviations

BC	Before Christ
BMI	Body mass index
CI	Confidence interval
Ft	Feet
GB	Greater Beirut
GOHAI	General oral health assessment index
IOM	International organization for migration
Kg	kilograms
M	Meter
MMS	Mini mental state
MN	Malnutrition
MNA	Mini nutritional assessment
MOPH	Ministry of public health
ORa	Odds ratio adjusted
POQL	Poor oral quality of life
SES	Socioeconomic status
SRH	Self-reported health

Introduction

Lebanon is a small country (area 10,000 km^2 or 4000 square miles) which lies on the Eastern shores of the Mediterranean (Fig. 1). Bordered by Syria to the north and east and Israel to the south, the country is composed of a narrow western coastline, along which runs a central mountainous chain known as Mount-Lebanon, which culminates at 2500 m (8200 ft.). To the east of Mount-Lebanon lies the fertile inner valley of the Bekaa, separated from Syria by another chain, the Anti-Lebanon which culminates at Mount Hermon (2800 m or 9200 ft.).

Historically, the coastline had been settled since the Neolithic (6000 BC), and in the last millennium BC had witnessed the flourishing of the Phoenician civilization, in maritime trading cities such as Tyr, Sidon, and Byblos. For centuries, the coastline and the inner valley were obligated paths for invaders including the Egyptians, Assyrians, Babylonians, Persians, and Greeks. Less than a century after Alexander the Great's invasion of Phoenicia (circa 330 BC), the country became part

Fig. 1 Map of Lebanon (Central Intelligence Agency, the World fact Book. Accessed https://www.cia.gov/library/publications/resources/the-world-factbook/geos/le.html). The map presents Lebanon and its principal cities which are mainly located in the coastal area. Lebanon is bordered by Syria in the North and East and Israel in the South

of the Roman (later Byzantine) Empire and eventually adopted Christianity. The Arab conquest in the seventh century AD brought Islam to Lebanon. The two monotheistic religions have coexisted and interacted in ways which still contribute to the pluralistic cultural specificity of Lebanon today. The Ottomans who occupied the country in 1514 established a semiautonomous feudal system headed by the "Emir of Mount Lebanon," which would become the nucleus of the nation of Lebanon. After the fall of the Ottoman Empire (1918), Lebanon and neighboring Syria were placed by the Society of Nations under French Mandate, while the other Arab lands of the Levant (Iraq, Transjordan and Palestine) were placed under English Mandate.

Lebanon became independent in 1943, as a parliamentarian democratic republic, and joined the League of Arab States. Since then, Lebanon had to withstand a series of major crises: the influx of Palestinian refugees displaced by the creation of the state of Israel in 1948, a protracted civil war (1975–1991), during which Israel occupied 10% of the country (1978–2000) and Syria all the rest (1977–2005). Hardly had national sovereignty been restored that a new refugee crises started, now with Syrians escaping civil war in their country starting 2011. By 2014, refugees were believed to compose at least 30% of the population of Lebanon, estimated at 5.5 million (Ministry of Public Health [MOPH] 2014).

Amidst troubled times, Lebanon was nevertheless undergoing several important sociological, cultural, economic, and epidemiological transitions. During the decades preceding the civil war, the population of Lebanon was in majority rural, but trends toward urban migration had started to be noted. By 2015, about 90% of the population had been urbanized (International Organization for Migration [IOM] 2015). The highest concentration of the population is found now in the Greater Beirut (GB) area, which includes the capital city Beirut and its immediate suburbs. Located in the middle of the coastline, GB is the seat of government. It concentrates the oldest universities, most advanced tertiary care centers, major business offices, banking headquarters, a busy port, and the only civilian airport of the country. Urbanization has been accompanied by important changes in the composition of the Lebanese population. Nuclear families are replacing extended families, and exogamy replacing regional endogamy (Ferrante 2014). Throughout the last decades of the twentieth century, fertility had been declining. Up till the mid-1970s, the fertility ratio was still about four children per woman in reproductive age. This meant that the burden of caring and supporting aging parents was fairly distributed among siblings. It is now at less than two children per woman, very likely the lowest ratio in the Arab world. Improved health care and better socioeconomic conditions have created a transition to increased survival to later stages of life. Life expectancy is nearing now 80 years (MOPH 2014). Before the Syrian refugee influx, the country was graying rapidly, with consistent increase in the relative proportion of elderly persons. Projections suggested that the population over 65 years of age will constitute more than 10% of the population by the year 2025, mostly concentrated in urban areas (Sibai et al. 2015). Figure 2 shows the demographic pyramid of Lebanon for the year 2016 based on current estimations (US Census Bureau 2017).

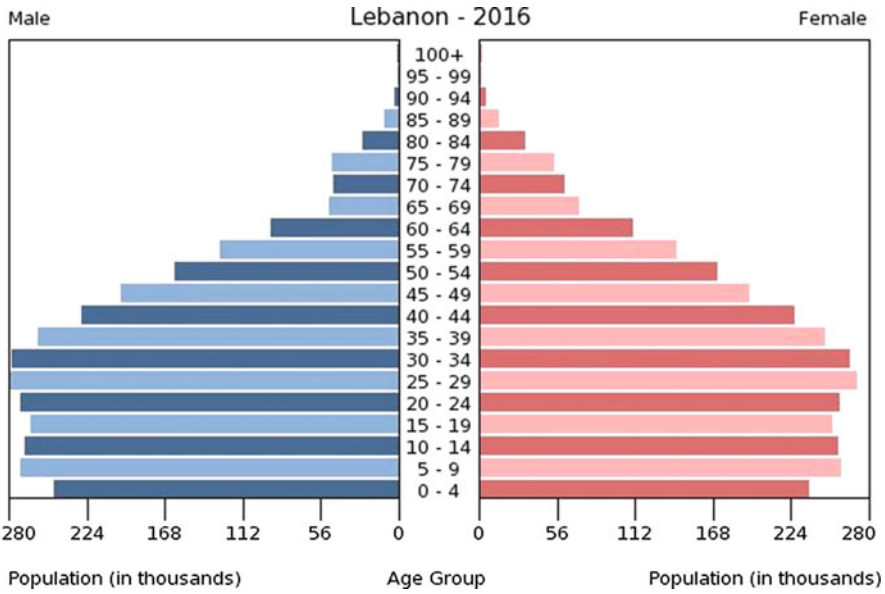

Fig. 2 Population Pyramid Lebanon 2016 distributed by gender (US Census Bureau 2017). Population pyramid of Lebanese population following estimations for year 2016, distributed by gender

Aging in Lebanon was not perceived as a major public health priority until the first decade of the twenty-first century. Strong family and community structures traditionally embraced seniors and kept them inserted within the social network. The civil war, often accompanied with forced displacement, and the difficult recovery period which followed, dismantled many neighborhood/village ties. Families have been affected by the massive emigration of the active members (Kasparian 2010). The case of elderly parents living alone in Lebanon, with no children within driving distance, has ceased to be exceptional. Concerned public agencies and stakeholders from the civil society are acutely aware of the low national level of preparedness in response to the increasing health and well-being needs of the aging population. In the early 2000s, the Ministry of Social Affairs established a special Office for Ageing. Nevertheless, special dispositions for the elderly are still scarce. Geriatric services and geriatricians are still rare, and the primary and secondary care systems do not generally cater to the specific problems of aging citizens. To make the situation worse, employees lose their health insurance through social security when they retire, at the exact moment when their need for care starts increasing. Few are able at that point to obtain an affordable private insurance. More than 2/3 of elderly persons have to rely on their families to be able to cover the cost of their chronic medications and hospitalizations (Abyad 2001).

Unfortunately for the aging population, the priority given to their issues in the early 2000s has been largely affected by the overwhelming and more immediately pressing

needs of the Syrian refugees, of whom at least 500,000 are infants and children. Despite this shift in visibility, geriatric issues have continued to generate research and calls for services (Haddad 2000). In particular, nutrition in that subpopulation has emerged as a concern because of the multiple implications of malnutrition on the overall health and well-being of older persons (Miller and Wolfe 2008).

Malnutrition is an underestimated and underreported problem in the elderly population as stated by several authors (Correia and Waitzberg 2003). It is one of the main public health challenges among geriatric populations in both developed and developing countries, and will become a growing concern with continued demographic trends (Zohoori 2001). Among elderly individuals, malnutrition refers in general to undernutrition which is the result of a chronic deficiency in food intake (Pirlich and Lochs 2001), and/or increased energy expenditure due to hypercatabolism frequently associated with acute disease and hospitalization (Visvanathan 2003). Depending on the definition used and the living situation, the prevalence of malnutrition shows a wide international range. For example in developed countries, the prevalence of malnutrition and risk of malnutrition may range from 4.2% to 27.4% among community-dwelling elderly, and 27.2% to 52.1% in elderly living in nursing homes (Cereda 2012). In developing countries, the phenomenon remains less explored although some papers suggest higher rates of malnutrition among elderly (Amirkalali et al. 2010; Rodríguez-Tadeo et al. 2012).

Social isolation is one important and overall recognized risk factor for malnutrition among elderly persons (Cacioppo et al. 2011). When the extended rural family was the predominant pattern in Lebanon, older women presided over the "mouneh" activity, which refers to the transformation of spring-summer food products into winter provisions. With the slow disappearance of large rural families, the isolated older persons still holding on in mountain villages are unable to secure those provisions and do not have an easy access to markets where all sorts of food products are always available (El Bcheraoui et al. 2015). Fortunately, the majority of older Lebanese lives now in urban and suburban areas. While access may still remain a problem for an older person even in urban areas, it is less acute than in rural remote regions. The main obstacle for good nutrition in this case becomes the affordability of nutritious food, which may be limited for seniors with poor financial conditions.

This chapter reviews evidence accumulated since the end of the civil war in 1991, concerning the nutritional status of the elderly Lebanese and its particular determinants. Papers have assessed the situation of urban and rural elderly persons, in open community as well as in institutions. The review will conclude with practical recommendations meant at improving the situation of elderly persons in Lebanon and in neighboring countries undergoing similar transitions.

Epidemiology of Malnutrition in the Geriatric Population of Lebanon

The overall epidemiology of malnutrition among the elderly population in Lebanon is characterized by its fragmentation and often by the small numbers of subjects involved. These gaps reflects the relatively lower interest that geriatric issues

generate so far in Lebanon, added to the overall misperception that malnutrition cannot exist in this country. Added obstacles to valid and comprehensive assessments include the difficulty to reach mentally competent elderly persons, and the limited freedom to participate when confronted with reluctance or opposition from their care-taking entourage (in the community) or the institution in which they live. Various surveys have also differed in the exclusion criteria selected, with most excluding more deteriorated elderly. This has regularly led to an under-estimation of risks, as demonstrated by the marked difference found in one study with less stringent exclusion criteria (see below). On the positive side, the methodology used in various assessments since the first decade of the twenty-first century has tended to be uniform and to rely heavily on the same Arabic version of the Mini Nutritional Assessment (MNA) which is the most used nutritional assessment tool among elderly independently of their living situation (Guigoz et al. 1996). This version, despite not being validated yet under international standards, has nevertheless the advantage of reducing interobservers variability in outcome assessment.

In various small and large studies, malnutrition in the elderly has so far been described using three parameters: the geographic distribution (urban versus rural), living conditions (community versus institutions), and health status. A summary of the studies is presented in Table 1.

Malnutrition by Geographic Distribution

Two population-based surveys were performed to evaluate the nutritional status of the noninstitutionalized, community-dwelling elderly population (Fig. 3). The first survey included a randomly selected sample of 1200 elderly individuals aged $\geq$65 years living in rural districts of Lebanon. The nutritional status of all participants was measured through the MNA, combined with anthropometric measures (Body Mass Index or BMI). Individuals on artificial nutrition were excluded. The overall prevalence of malnutrition and risk of malnutrition were 8.0% and 29.1% respectively. Both were more prevalent among females (9.1% and 35.3% respectively) than males (6.9% and 22.9% respectively) (Boulos et al. 2013).

A second survey conducted a few years later used largely the same methodology on a sample of older persons living in the Greater Beirut (GB) urban/suburban area. The survey included 905 elderly aged $\geq$65 years. Unlike the rural study, this survey excluded elderly with cognitive dysfunction. Malnutrition was less prevalent than in rural areas (2.8%), while risk of malnutrition was higher (45.5%). Furthermore, there were no significant differences in poor nutritional status (malnutrition or risk of malnutrition) by gender (Mitri et al. 2016).

Malnutrition by Living Conditions

A study published in 2003 was one of the first to compare the nutritional status of 100 community-dwelling elderly persons to 100 elderly living in institutional settings (Sibai et al. 2003). Results showed that the energy, calcium, zinc, magnesium,

Table 1 Summary of studies on nutritionals status of elderly subjects carried out in Lebanon. This table presents the methods and results of the surveys carried out in Lebanon between 2003 and 2016. Most studies were performed in community and institutional settings reporting estimates of malnutrition/risk of malnutrition as well as factors associated with malnutrition. All studies except one used the Mini Nutritional Assessment tool. Inclusion and exclusion criteria show variations among the different studies

Studies	Setting and participants	Inclusion/exclusion criteria	Measures	Main results
Boulos et al. (2014), (2017)	Community (rural) N = 1200[a]	Age ≥ 65y/terminal illness, artificial nutrition	MNA, SES, health, functional status, cognitive status, mood, frailty	MN = 8%, at risk = 29.1% MN was independently associated with lower income, comorbidities, chronic pain, depressive disorders, cognitive dysfunction, social isolation, and loneliness
Mitri et al. (2016)	Community (urban) N = 905[a]	Age ≥ 65y/ bedridden, cognitive impairment MMS <24	MNA, SES, health, functional status, mood, GOHAI	MN = 2.8%, at risk = 45.5% MN was independently associated with poor SRH, comorbidities, POQL, depressive disorders, and dependency
El Zoghbi et al. (2014)	Nursing home N = 111[a]	Age ≥ 65y/renal dialysis, MMS <14	MNA, SES, health, functional status, cognitive status, mood, frailty	MN = 12.6%, at risk = 48.7% MN was independently associated with frailty, depressive disorders, being sedentary, and cognitive decline
Doumit et al. (2014)	Nursing home N = 221[a]	Age ≥ 60y/MMS <20, terminal disease, being blind or deaf, admitted since <3 month	MNA, SES, health and functional status, mood	MN = 3.2%, at risk = 27.6%
Sibai et al. (2003)	Community and nursing home N = 200[a]	Age ≥ 65y/terminal illness, cognitive impairment, admitted since <3 month	SES, BMI, dietary intake (3 days), albumin	Community elderly >BMI > nursing home residents Energy, Ca, Zn, mg, Vit A, D, E below recommended intakes in both samples
El Helou et al. (2014)	Hospital N = 115[b]	Age ≥ 70y, MMS ≥ 24, able to communicate/ hypercatabolic state, artificial nutrition, malabsorption	MNA, GOHAI	MN: 6%; at risk = 37.4%; POQL associated with 2.84 fold increased risk of MN

(*continued*)

Table 1 (continued)

Studies	Setting and participants	Inclusion/exclusion criteria	Measures	Main results
El Osta et al. (2014)	Primary care clinic N = 201[b]	Age ≥ 65y, without dementia, psychiatric disorders, acute systemic disease and being independent	MNA, GOHAI	MN = 8.5%, at risk = 33.8% MN was independently associated with suffering from xerostomia, POQL and having less than four posterior functional units

Key: *MNA* mini nutritional assessment, *MN* malnutrition, *SES* socioeconomic status, *MMS* mini mental state, *GOHAI* general oral health assessment index, *SRH* self-reported health, *POQL* poor oral quality of life
[a]Randomized sample
[b]Convenience sample

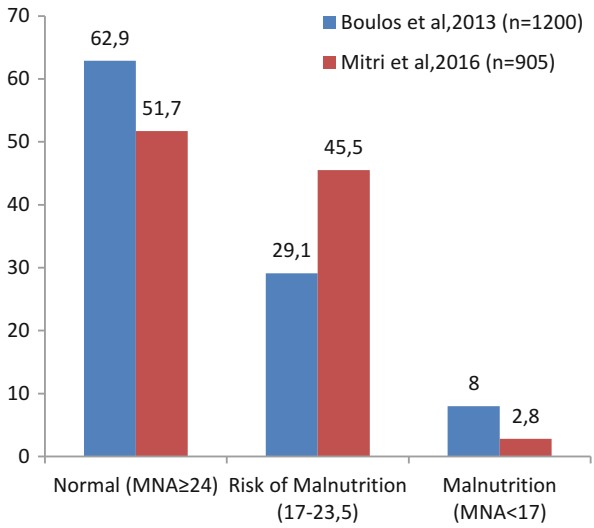

Fig. 3 Nutritional status among community dwelling elderly Lebanese. This figure shows the with prevalence of malnutrition and risk of malnutrition assessed through MNA among community dwelling elderly Lebanese living in rural (Boulos et al. 2013) and urban areas (Mitri et al. 2016)

alpha-tocopherol, and vitamin A and D intakes were all below the recommended levels in both groups. However, community-dwelling elderly had a higher mean BMI and waist circumference compared to the institutional residents. After adjusting for potential confounders, the risk of having a BMI above 30 kg/m^2 was significantly lower for those living in the institution compared with community dwelling elderly.

Two separate studies were subsequently carried out among the elderly living in long-term institutions (Fig. 4). A survey of 36 nursing homes outside the GB area (out of a total of 49 homes) was carried out between 2007 and 2009, on a sample composed of 148 women (67%), and 73 men (33%). Based on the MNA, the prevalence of malnutrition was 3.2% and the risk of malnutrition 27.6%, with no

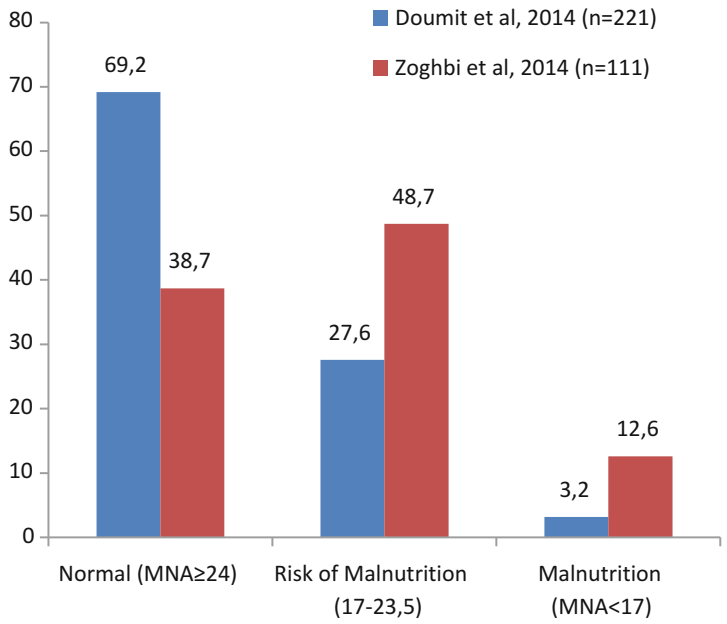

Fig. 4 Nutritional status among institutionalized elderly Lebanese. This figure shows the prevalence of malnutrition and risk of malnutrition assessed through MNA among elderly Lebanese living in nursing homes in Greater Beirut (El Zoghbi et al. 2014) and in other regions of Lebanon (Doumit et al. 2014)

significant difference between genders (Doumit et al. 2014). The other study conducted during 2012 reported the prevalence of malnutrition among the elderly living in three out of the five long-term geriatric institutions in GB. Only 111 persons could be assessed. Among those, the prevalence of malnutrition was estimated at 12.6% and the risk of malnutrition at 48.7% respectively (El Zoghbi et al. 2014). The higher estimates of malnutrition in this study compared to the previous one might be explained by selection bias in the former, where only those who scored above 20 on the Mini-Mental State (MMS) assessment of cognitive aptitude were included (see Table 1).

Distribution by Health Status

Elderly persons suffering from multiple chronic conditions are at high risk of acute exacerbations which is frequently followed by hospital admission. Among these acute care patients, malnutrition is a common and unrecognized problem which worsens mostly during hospital stay due decreased food intake, polymedication, lack of nutritional training, lack of assistance, and many other reason (Kubrak and Jensen 2007).

A small pilot study was performed in 2013 to assess malnutrition among hospitalized elderly in association with oral health. The study included 115 older adults

aged ≥70 years admitted to the largest public tertiary care hospital in Lebanon, located within the GB area. Among other findings, the prevalence of malnutrition among these hospitalized seniors, using the MNA, was 6%, with an additional 37.4% who were at risk of malnutrition (El Helou et al. 2014). The same objective was targeted by another survey of elderly aged ≥65 years drawn from two primary care clinics over a period of three consecutive months. Using the MNA, this survey of 201 older adults found a malnutrition prevalence of 8.5%, and a risk of malnutrition of 33.8% (El Osta et al. 2014).

Determinants of Poor Nutritional Status

Poor nutritional status is the results of multiple factors acting mostly in combination such as age-related physiological changes, poor socioeconomic condition, health-related problems, and psychological factors (Pirlich and Lochs 2001). Table 1 presents those factors that were associated with higher risk of malnutrition in our studies.

Socioeconomic and Life Style Factors

Poor socioeconomic status is a main risk factor for malnutrition (Pirlich and Lochs 2001; Timpini et al. 2011). Accordingly, among community dwelling rural Lebanese, reporting a higher monthly income was independently associated with a reduced risk of malnutrition (ORa 0.77; CI:0.61-0.97) (Boulos et al. 2014). Inversely, this relation was found neither among the urban community dwellers nor among institutional residents (El Zoghbi et al. 2014; Mitri et al. 2016). Furthermore, none of the performed surveys reported a significant association between educational status and malnutrition. As for physical activity, among institutionalized elderly living in GB, doing regular physical exercise was associated with a lower risk of malnutrition (El Zoghbi et al. 2014). In addition, among community living elderly, both social isolation and perceived loneliness were independently associated with an increased risk of malnutrition (Boulos et al. 2017).

Physical Health Status

Comorbidities and poor health status are often associated with decreased food intake and therefore can be considered as major risk factors for poor nutritional status (Kubrak and Jensen 2007). Similar finding were reported by Boulos et al. (2014) in the rural community survey where both variables, reporting more than three chronic diseases and suffering from chronic pain were respectively associated with a 1.2 fold and a 1.7 fold increased risk of malnutrition. Moreover, a poorer perception of general health and a higher number of chronic diseases were independently associated with an increased risk of poor nutritional status among older adults living

in GB. In this study, elderly suffering from disability were also at higher risk of malnutrition (Mitri et al. 2016).

In addition, among the nursing home population of GB (El Zoghbi et al. 2014), the authors found that frailty, a syndrome which recently received major attention in geriatric practice and research, was a significant correlate of nutritional risk.

Mental Health Status

Mental health problems, such as depression and dementia, are main issues among geriatric population and may affect food intake and nutritional status in various ways (Shatenstein et al. 2007). Accordingly, in both, the rural and the urban community survey, depressive disorders were associated with a 1.6 fold increased risk of malnutrition (Boulos et al. 2014; Mitri et al. 2016). Similar findings were described among residents of urban nursing homes (El Zoghbi et al. 2014). Moreover, among rural community dwellers and institutionalized urban elderly a significant association was found between cognitive decline, assessed through MMS, and malnutrition (Boulos et al. 2014; El Zoghbi et al. 2014). In the two other studies, elderly with dementia were initially excluded from the study sample.

Oral Health Status

Two studies focused specifically on the relation between oral health and nutritional status. Both found a significant relation between oral health related quality of life and poor nutritional status. For example, El Osta et al. (2014) reported a 2.9 fold increased risk of malnutrition in presence of poor oral health related quality of life among 201 outpatients. Similar findings were reported in urban community dwellers (Mitri et al. 2016). Furthermore, among outpatients, complaining about xerostomia and having less than four posterior dental functional units were associated with a higher risk of malnutrition (El Osta et al. 2014).

Recommendation and Future Research Perspectives

This review showed that among Lebanese elderly living in various settings, malnutrition is a common finding which might have negative effects on different health outcomes. These results highlighted the need for better awareness of malnutrition among the different parties which might be concerned by the health and well-being of elderly people. First, among health care professionals, who have to consider to detect malnutrition through simple available screening tools and to intervene as soon as possible. Second, among people caring about elderly in their homes or in institutions to understand that nutritional care is a major factor in disease prevention which contributes to health and quality of life. In Lebanon, community dispensaries depending on the Ministry of Social Affairs are implemented all over the country.

One of the first steps would be to integrate dieticians within the team of nurses and social workers in these centers with a special focus on nutritional screening and care for elderly individuals. In a second step, these dieticians have to educate employees of these centers to learn how to detect malnutrition through an easy-to-use screening tool and to give simple advices. If necessary elderly with poor nutritional status or special needs might be oriented toward the dietician. Furthermore nursing homes and hospitals should be targeted and those responsible must be convinced that nutritional care of elderly should become one of the main goals helping to improve the quality and efficiency of hospital and institutional care. In addition clinicians have to be equipped with simple non-time-consuming tools that may easily identify this frequently underdiagnosed condition. We suggest changes at the level of undergraduate studies curricula to increase elderly malnutrition knowledge and tools' use in future practice.

Another important issue is the access to health care among elderly in Lebanon. Unfortunately, upon retirement, Lebanese elderly lose their health insurance coverage and thus are often relying on the financial support of other family members. This may also be one of the reasons for limited access to dental care and therefore poor oral health, which may increase the risk of malnutrition (El Helou et al. 2014; El Osta et al. 2014). Another reason for decline in oral health might be the lack of knowledge about the importance of maintaining good oral health in order to guarantee adequate nutrition. In fact, poor oral health can affect food choices and consequently lead to a monotonous diet with risk of multiple nutrient deficiencies (Bailey et al. 2004). Unfortunately buccodental care is not only neglected by the individuals themselves but also by the clinicians, who mostly are not aware about the physiological changes which occur during normal aging and which may influence oral intake. Therefore integrating oral health into medical care of elderly patients would be of great importance. Finally, during their curriculum, future dentists have to be more educated regarding specific changes and oral health needs of elderly people.

In addition, there is still an important lack regarding malnutrition among hospitalized elderly. Studying the particular role of oral supplementation and enteral nutrition in reducing malnutrition, accelerating recovery and decreasing infectious diseases incidence is also necessary. Only a pilot study was performed reporting poor nutritional status among 43% of elderly without cognitive impairment (El Helou et al. 2014). More comprehensive data are needed through studies based on representative sample from hospitals in different regions. Also several tools, especially the MNA which is the most used screening tool among elderly needs to be validated among Lebanese elderly. MNA is already available in Arabic language and has been used in several studies in Lebanon; however cross-cultural validation or generation of culturally adapted new tools studies are still lacking.

Finally supplementary research is needed in the field of elderly nutrition. As a first step, studies estimating dietary intakes among elderly living in different settings have to be planned in order to assess the current energy and protein intake and to identify special deficiencies in micronutrients. Measurement of vitamins and minerals among elderly individuals would be also of interest, such as vitamin D, that is known to be deficient in the majority of the Lebanese population (Gannagé-Yared et al. 1998, 2000) as well as vitamin B9 and B12 who may influence cerebral function

and homocystein metabolism (Dangour et al. 2010). In addition, food habits and dietary patterns have to be explored with a special focus on traditional Lebanese dietary patterns as described by Jomaa et al. (2016), and its relation with health outcomes. As for the latter, longitudinal studies would be of high interest in order to better understand the way how eating habits may affect the process of aging and the appearance of chronic diseases. As for the traditional Lebanese diet, its protective effect on cardiovascular and other chronic diseases is still to be established, as it was demonstrated for other Mediterranean types of diet (Sofi et al. 2010). However some interesting results have recently be published regarding a possible protective effect of these type of diet on metabolic disorders associated with overweight and obesity (Matta et al. 2016).

As a final point, we suggest the establishment of research-oriented postgraduate studies and projects at the national level to clarify the points suggested above.

Policies and Protocols

Policies

Countries in demographic transition like most Arab nations must reorient services and resources to address the mounting proportion of aged citizens. Seniors' particular needs may be obvious, such as medical problems which require specialized health care departments with health professionals trained in geriatric care. Other needs are less salient but equally as crucial for the health and well-being of elderly persons. Of those, the threat of malnutrition requires a multisectoral approach aiming at avoiding the deterioration of the social insertion of older persons, and of their financial capacity to access nutritive food and adequate preventing care, most notably dental care. Full protective packages can use the abundance of young adults with no immediate employment path to create a "visitation" system which supports an older persons' autonomy within their own familiar settings as long as such an option can be safely obtained. In nations such as Lebanon with inadequate funding for social protection in postretirement ages, policies need to be drawn to help citizens invest in secure financial projects which can ensure an adequate income when needed.

Protocols

In this chapter we reported a summary of studies on malnutrition among Lebanese elderly within different setting. Nearly all these surveys except one were based on the MNA, a specific geriatric tool, used for nutritional screening and assessment among older adults. It is a simple and well-validated instrument developed by Guigoz et al. (1996) with a high specifity, sensitivity, and reliability. The MNA can be used in various settings and is recommended by many international clinical and scientific organizations (Vellas et al. 2006). It has been translated in more than twenty languages and is also available in Arabic language; it can be performed by a

variety of health professionals including doctors, dietitians, nurses, or research assistants (Vellas et al. 2006). The MNA contains 18 questions including anthropometric measures, mobility, cognition, dietary intake, and health condition. Based on the total score, the subjects were classified into three categories: malnourished (<17), at nutritional risk ($17\leq$ score <24), and adequate nutritional status (≥ 24) (Guigoz et al. 1996). The MNA has shown to predict adverse health outcome, functional decline, length of hospital stay, and mortality (Vellas et al. 2006).

One of the mentioned studies used a combination of anthropometric measures (BMI), albumin, and dietary intake (Sibai et al. 2003). BMI was calculated as weight (kg) divided by the square of height (m). Cut-off values were defined according to the World Health Organization categories: underweight (BMI <18.5), normal (18.5–24.9), overweight (25–29.9), and obesity (BMI >29.9). Anthropometric measures have the advantage of being easy to perform and inexpensive; however they may not be reliable indicators of nutritional status in the presence of edema or ascites and should therefore be combined with other markers (Harris and Haboubi 2005). Among biochemical markers, albumin has frequently been used in studies because of its ability to predict mortality. However the level of albumin is also influenced by inflammatory status, hydration, and physical activity, which limits the usefulness of this marker in elderly suffering from acute disease and/or reduced mobility (Harris and Haboubi 2005; Kuzuya et al. 2007).

Finally, dietary intake can be obtained through different ways. In the present study, a prospective 3-day food record was used including 1 weekend day. Food records needs motivation and compliance of the participants and have to be reviewed by a dietician for reliability and completeness. Daily intake of nutrients and energy were obtained through specific nutritional software and compared with recommended nutritional intakes.

Dictionary of Terms

- **Frailty** – A state of increased weakness and vulnerability toward serious health outcomes and disability as a result of age-related, physical, mental, and social changes during life course of elderly people.
- **Homocystein** – Homocystein is an amino acid derived from methionine which is a risk factor for cardiovascular and cerebrovascular disease; the level of homocysteine is influenced by vitamin B9, B12, and vitamin B6 status.
- **Hypercatabolism** – Excessive increase in basal metabolic activity with breakdown of body tissue due to various situations such as infection, burns, traumas, severe organ failure, etc. Hypercatabolism is often associated with anorexia and weightloss.
- **Nuclear families** – Families that include two parents and one or more children, in contrast to single-parent families and extended families (aunts, uncles, cousins, etc). Nuclear families are characteristic of modern societies.
- **Oral health–related quality of life** – Reflects individual's personal satisfaction with oral health and comfort when eating, speaking, and during social interaction.

- **Posterior dental functional units** – A measure to assess the functional masticatory status of the subject. Individuals who have less than four posterior dental functional units are considered to have an altered functional status.

Summary Points

- This chapter reviews the prevalence of malnutrition and associated factors among Lebanese elderly living mainly in the community and in institutions.
- The results show that poor nutritional status is an important concern among older adults in Lebanon.
- A high proportion of elderly were categorized as at risk of malnutrition, meaning that these people may benefit from corrective measures as far as they are recognized early.
- Screening for malnutrition has to be implemented among special risk groups (those suffering from comorbidities, disability, depressive disorders, poverty and those who are widowed and advanced in age).
- Dieticians need to be integrated into an interdisciplinary care team on both, community and institutional levels, to prevent early decline of nutritional intake and malnutrition.
- Special attention has to be given to oral health among elderly as it is a major factor influencing food intake and long-term health outcomes.

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Nutrients of Concern for Older People

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Abstract

Globally, the population is ageing, a process which is associated with a range of physiological changes linked to increased morbidity, with consequent increases in healthcare expenditure. Malnutrition is common in older adults due to a myriad of factors including physiological changes of ageing; thus, with growth in numbers, we can expect the burden of malnutrition and associated negative outcomes such as impaired immunity, loss of physical function, and independence to increase. Both malnutrition and the physiological changes of ageing can manifest in poor nutritional status, due to simple starvation, either through poor food intake or through changes in absorption and metabolism of nutrients in the body. Good nutrition is a modifiable factor which can help to minimize the effects of nutrient deprivation and reduce the impact of ageing on individuals and the community, as well as the healthcare budget. This chapter explains the importance of nutrition in the ageing population and present evidence of the consequences of malnutrition.

The older generation is a heterogeneous group who are quite different to younger people. Yet, there is a lack of specific nutrient reference values especially for adults in advanced age and recommendations are often based on evidence extrapolated from younger adults. Energy needs vary across age groups based on gender, age, and level of activity; however, in older adults, the variation is exacerbated by the physiological changes of ageing and inflammation from illness. Further, many of the assumptions behind calculations of energy requirements, often based on younger people, are not valid in the older population; thus, assessments may not be accurate. Protein requirements are known to be higher in this group, at the very least to attempt to preserve muscle mass, but there is insufficient research to be able to establish guidelines. Fat is an important energy source particularly for older adults whose appetite may be diminished. The evidence suggests that adults of advanced age eat fat in excess of recommendations; however, there are no age-specific guidelines to help interpret the implications of this overconsumption.

Generally, if older people eat a healthy balanced diet, they will meet micronutrient requirements. Micronutrient deficiencies can nevertheless arise from decreased food intake often due to the aforementioned physiological changes of ageing, poor health, and polypharmacy. Key micronutrients of interest in older adults are calcium, magnesium, selenium, zinc, and iron, vitamin B_{12}, vitamin D, and folate. Of particular concern is vitamin D as food sources are limited and exposure to sunlight is often reduced. Given the need for vitamin D for adequate calcium absorption and the roles of calcium in the body, not least for bone strength, older adults may benefit from routine vitamin D supplementation.

Keywords

Ageing · Energy · Fat · Health · Malnutrition · Micronutrients · Nutrients · Older adult · Prevalence · Protein

List of Abbreviations

AI	Adequate intake
AMDR	Acceptable macronutrient distribution range
BMR	Basal metabolic rate
COPD	Chronic obstructive pulmonary disease
EAR	Estimated average requirement
ESPEN	European Society for Clinical Nutrition and Metabolism
EUGMS	European Geriatric Medicine Society
g/kg	Grams per kilogram
HBV	Higher biological value
HRQOL	Health-related quality of life
IQR	Interquartile range
kg	Kilogram
MJ/d	Mega Joules per day
NHANES	National Health and Nutrition Examination Survey
NHMRC	National Health and Medical Research Council
NZ	New Zealand
RDA	Recommended dietary allowance
RDI	Recommended daily intake
RNI	Reference nutrient intake
UK	United Kingdom
USA	United States of America

Introduction

Meeting the nutritional needs of older people is an immediate challenge with life expectancy at its highest throughout many parts of the world. Not only is the number of older people ≥65 years increasing rapidly, but the population of adults of advanced age is expanding due to the steady rise in life expectancy and decrease in later life mortality. The European Commission's 2015 ageing report suggests that the percentage of those aged ≥80 years in the total population in Europe will increase from 5% in 2013 to 12% in 2060 (European Commission (DG ECFIN) and Economic Policy Committee (Ageing Working Group) (2015)).

Older adults are a heterogeneous population with unique nutritional needs and the process of ageing occurs at different rates in different people. Nutritional requirements of individuals may be influenced by a myriad of factors including reduced mobility and independence, financial constraints, higher rates of hospitalization, chronic diseases and disabilities, changes in body composition, sensory deficits, taste perception, and digestion and absorption of food, all of which place older adults at increased risk of nutritional deficiencies. Furthermore, the process of ageing affects nutritional needs; requirements for some nutrients may be reduced while requirements for others may be increased.

There is a close relationship between nutritional status and health in the general population. While the main health risk for younger population groups is weight gain

and being overweight, older adults are vulnerable to consuming too little energy with associated weight loss. The range of physiological changes that contribute to reduced food intake has been termed the "anorexia of aging" (Morley, 1997). Social, psychological, and medical factors may also negatively impact the desire to eat.

In general, healthy older people tend to consume smaller meals, fewer snacks, eat more slowly, and become satiated after meals more rapidly than younger people, and daily intake of food may decrease by an average of 30% between the ages of 20 and 80 years (Wurtman et al., 1988). Most of the age-related decrease in energy is a response to the decline in energy expenditure with age; however, for many older people, the decrease in energy intake tends to be greater than the decrease in energy expenditure with a resultant loss of body weight, particularly muscle mass.

The age-related changes which predispose older adults to a decrease in food intake may lead to malnutrition, especially in the presence of additional health and social risk factors (Wham et al. 2015). Consequently, malnutrition is widespread among vulnerable older adults especially in those who are institutionalized. In developed countries, it has previously been estimated that malnutrition is present in 5–30% of community-living older people, 23–60% of those who are hospitalized, and up to 70% of those living in residential aged care or nursing homes (Agarwal et al. 2013). Among community-living older people in Europe, it has been estimated that 20% of those aged between 75 and 80 years are malnourished (Ljungqvist et al. 2010). Along with its associated health complications, undernutrition is estimated to cost the European health and social care system approximately 120 billion Euros per year (Ljungqvist et al. 2010).

Malnutrition is associated with functional impairment, increased comorbidity (in relation to immune dysfunction, anemia, reduced cognitive function, and poor wound healing), delayed recovery from surgery, higher hospital and readmission rates, and mortality (Chapman 2006). Studies of older people also show a relationship between malnutrition risk and health-related quality of life (Kvamme et al. 2011; Johansson et al. 2009), which underpins the importance of optimizing the nutritional health of older people.

Good nutrition is a modifiable factor which may help to prevent health problems. Improvements in nutritional status can also allow for greater health expenditure to be directed toward keeping older people well for longer. Screening for malnutrition risk, a process which can identify factors related to nutritional status that could lead to malnutrition, can act as a preventative health measure. Validated nutrition screening tools provide a reliable method to identify those at high risk of malnutrition. Early identification and nutrition intervention is important because it can be difficult to reverse adverse effects, once malnutrition is established. In the absence of formal screening procedures, more than half of older adults at risk of malnutrition in various settings may not be recognized or referred for treatment (Elia et al. 2005). As early identification and intervention in malnourished older adults can improve clinical outcomes and reduce health care use, guideline recommendations for nutrition screening and assessment provide an important safeguard.

Understanding nutrients of concern for older people is critical for the prevention and management of chronic disease and other health problems. Policy is an important vehicle to implement actions for the improvement of nutrition. Country-specific food and nutrition guidelines are formulated to protect the health of populations in the context of other relevant policies and strategies. Dietary guidelines are based on the nutritional needs of a population; however, little is known about the dietary habits and nutritional status of the very old. Although adults of advanced age are physiologically different to their younger counterparts and are more prone to inadequacy of energy and certain nutrients than younger adults, there are no specific dietary guidelines for those aged ≥85 years. Generally, older people are underrepresented in nutrition surveys and data for older adults are aggregated for those ≥65 or 70 years with recommendations in many cases based on evidence from younger adults. Indeed, in some countries, dietary recommendations for healthy adults (≥19 years) are applied to older adults (≥65 years). The lack of nutrition surveillance data for older adults makes it difficult to establish their nutritional needs and to develop policies to protect this vulnerable group. Given the heterogeneity of older people, it has been suggested that future guidelines may need to identify the nutritional needs of older people in relation to their functional ability and morbidity (Suominen et al. 2014).

In this chapter, the nutritional needs of older people will be examined. Adequacy of food intake of adults of advanced age has been reported from longitudinal studies of ageing. More recently, the macronutrient and micronutrient intakes of the very old were investigated from three cohorts of advanced age from the UK and New Zealand (NZ) (Mendonca et al. 2016a, b; Wham et al. 2016a, b). Dietary information was collected using a repeated multiple-pass recall (2×24h recalls) in 793 85-year olds (302 men and 491 women) living in North-East England (the Newcastle 85+ study) and in 216 Māori and 362 non-Māori from the Bay of Plenty and Rotorua regions of New Zealand participating in Life and Living in Advanced Age: A Cohort Study in New Zealand (LiLACS NZ). The nutritional enquiry included in these cohort studies of ageing provides a preliminary assessment of nutritional status in the very old.

Energy

The energy needs of older adults vary widely according to gender, body size, and physical activity. Those with good health and physical function may have similar energy needs to younger adults. Typically, both lean body mass, total body water, and basal metabolic rate tend to decline with age, concurrent with body composition changes slowly over time, and body fat may increase proportionally. There is a decrease in skeletal muscle, smooth muscle, and muscle that affects vital organ function. This reduction in lean body mass, basal metabolic rate (BMR), and overall physical activity contributes to an overall reduction in the energy needs of older adults compared with younger people. Changes in body composition affect the body's metabolism, nutrient intake, nutrient metabolism, and overall nutrient requirements. As BMR declines proportionately with the decline in

muscle tissue, an older person's energy requirement per kilogram of body weight tends to be reduced.

Based on data from the US Institute of Medicine of the National Academies database for individuals aged between 20 and 100 years, a progressive decline in total energy expenditure and physical activity level with advancing age is evident (Roberts and Dallal 2005). Maintaining an adequate energy intake and weight can be a challenge especially in advanced age. Older people are less able than younger adults to make compensatory increases in their energy intake and are less able to regulate weight and therefore regain any lost weight.

Food intake in older people may be compromised due to taste changes, sensory deficits, and impaired sensory-specific society which leads to less variety-seeking behavior. Physiological changes in gastrointestinal function that occur with ageing may have an adverse impact on appetite and contribute to an overall decrease in food intake. Combined with poor dentition, chronic illness, and adverse social and psychological factors such as bereavement and depression, older adults tend to be less hungry than younger adults.

Among older adults showing characteristics of frailty, meeting energy requirements can be particularly difficult. Eating at least three meals a day and, where possible, energy- and nutrient-dense snacks, is especially important for the frail old. An overall decline in food intake may compromise dietary variety which is positively associated with nutritional quality and positive health outcomes. A lack of energy intake from food may lead to nutrient deficiencies and can augment functional decline which may contribute to further deterioration of health. Nevertheless, with adequate consumption of a variety of foods from the main food groups, older individuals can meet recommended macronutrient and micronutrient intakes and energy balance can be achieved.

Estimates of total energy requirements for older adults are problematic given the evidence to suggest that the desirable healthy weight range should be set higher for improved health outcomes and the potential that the current estimates are based on predictive equations that have not been validated in this age group and may therefore overestimate requirements as a result of the decline in muscle mass with age (NHMRC (National Health and Medical Research Council) 2006). In general, data on the energy requirements of people over 80 years are scarce. Among 87 octogenarians (mean age 82 $\pm$ 3.1 years) participating in the Health, Aging, and Body Composition (Health ABC) study, energy requirements based on doubly labelled water measures of total energy expenditure were 9.24 $\pm$ 1.57 MJ/d for men and 7.59 $\pm$ 1.41 MJ/d for women (Cooper et al. 2013).

Among octogenarians participating in three cohorts of advanced age in the UK and New Zealand, a wide range of energy intakes were reported. Median (IQR) energy intakes reported in the Newcastle 85+ study were generally low; 7.73 (6.36–9.20)MJ/d for men and 6.15 (5.09–7.25)MJ/d for women (Mendonca et al. 2016a) with only 20% of cohort meeting the EAR (UK) (Scientific Advisory Committee on Nutrition (SACN) 2011). Similar energy intakes were reported in LiLACS NZ: Māori men 7.45 (6.05, 9.13)MJ/d and women 6.06 (4.80, 7.21)MJ/d and non-Māori men 7.90 (6.70, 9.57)MJ/d and women 6.27 (7.50, 7.50)MJ/d

(Wham et al. 2016a). These dietary energy intakes also appear comparable to energy intake reported in previous European studies which included octogenarians. For example, median energy intakes (MJ/d) for men and women, respectively, were from the European Prospective Investigation into Cancer and Nutrition (EPIC) (The EPIC-Oxford Study, 2010–2014) 9.84 and 9.02 and Dutch National Food Consumption Survey (DNFCS) 2010–2012 (Ocke et al. 2013) 7.4 and 7.30.

Protein

Older adults have a higher requirement for protein compared to younger adults. However, older adults usually eat less, including less protein, but have higher protein needs to offset the reduction in muscle protein synthesis (anabolic resistance) as well as the elevated metabolism of inflammatory conditions such as chronic obstructive pulmonary disease (COPD) (Deutz et al. 2014). There is mounting evidence that the existing recommended dietary intakes (RDI) for protein are too low for older people (Bauer et al. 2013) and do not take into consideration age-related changes in metabolism and immunity (Clegg et al. 2013). Findings suggest that protein intake greater than the RDI can help older people to improve immune status and wound healing, as well as muscle mass, strength, and function (Wolfe et al. 2008).

Although there is insufficient longer term research with defined health outcomes to specify an optimal intake for protein, there is mounting evidence that increasing protein intake beyond 0.8 g/kg may enhance protein anabolism and help reduce the progressive loss of lean mass with ageing. Protein intake has been demonstrated to be an important determinant of muscle mass and function. Among a group of healthy older women with protein intake 0.45 g/kg bodyweight/day, muscle mass and strength decreased over a period of 9 weeks. By contrast, in women who consumed twice that amount of protein (0.92 g/kg bodyweight/day), muscle mass remained stable and muscle strength improved (Castaneda et al. 1995). It has also been demonstrated that chronic ingestion of the recommended dietary allowance (RDA) (USA) for protein results in reduced skeletal muscle size in weight-stable older adults with no change in muscle function (Campbell et al. 2001). Findings from the Health ABC cohort indicate lower energy-adjusted protein intake in 2066 healthy older adults aged 70–79 years is associated with a larger loss of lean body mass over a period of 3 years of observation (Houston et al. 2008) with a median protein intake between 0.7 g/kg (lowest quintile) and 1.1 g/kg (highest quintile) resulting in a reported loss in lean mass of 0.85 and 0.45 kg, respectively; i.e., 40% less decrease in lean mass over 3 years suggesting a clear linkage between protein intake and muscle change in older adults. Furthermore, data from the InChianti and the Women's Health Initiative cohort studies indicate that higher protein intake is associated with reduced risk of muscle strength loss and incident frailty (Beasley et al. 2010; Bartali et al. 2012).

Evidence from recent cohort studies suggests that observed weight-adjusted protein intake of octogenarians tends to meet respective nutrient reference values for people over 70 years but may be low when compared to newer recommendations

(1.0–1.2 g/kg/day to preserve and regain lean body mass and function) made by the PROT-AGE Study Group (Bauer et al. 2013). In the Newcastle 85+ study, the daily median (IQR) weight-adjusted protein intake for men was 1.04(0.81–1.32)g/kg and for women was 0.96(0.75–1.17)g/kg higher than the Reference Nutrient Intake (RNI) (UK) of 0.75 g/kg (Department of Health: Committee on Medical Aspects of Food Policy (COMA) 1991). Overall, 78.1 and 67.4% of men and women, respectively, had higher protein intakes than the RNI. In LiLACS NZ, the median weight-adjusted protein intake for Māori and non-Māori men was 1.05 and 0.98 g/kg/day, respectively; for Māori and non-Māori women 0.87 and 0.91 g/kg/day, respectively. Similarly, the reference standard was met by 66% of Māori and 73% of non-Māori women (EAR (Australia/NZ) 0.75 g/kg/day) and by 65% of Māori and 72% of non-Māori men (EAR 0.86 g/kg/day) (NHMRC (National Health and Medical Research Council) 2006).

Not only do older adults usually eat less protein compared to younger adults, they often consume less high biological value (HBV) animal protein such as meat (Gaffney-Stomberg et al. 2009) due to factors such as difficulty chewing, fear of increasing fat and cholesterol and cost and accessibility of these sources. Although muscle mass decreases in older people, the formation of muscle protein can be stimulated by HBV proteins, so it is prudent that an adequate protein intake is maintained. An adequate protein intake is especially important to maintain a healthy functional status and decrease the risk of prolonged infections that may lead to hospitalization. The pattern of protein intake may also be important to stimulate protein synthesis in older adults and there is some evidence that spreading protein intake evenly over meals may be beneficial (Bouillanne et al. 2013). However, further studies are needed to determine the optimal pattern of intake to improve muscle strength and function.

The optimal protein intake for older adults to maintain nitrogen balance and to preserve muscle mass and function remains to be ascertained. Recent recommendations from the European Society for Clinical Nutrition and Metabolism (ESPEN) suggest higher dietary protein intakes for older adults ($\geq$65 years) compared to younger adults (Deutz et al. 2014). It is suggested that diet should provide between 1.0 and 1.2 g protein/kg body weight/day for healthy older people (Cruz-Jentoft et al. 2010) and between 1.2 and 1.5 g protein/kg body weight/day for older people who are malnourished or at nutrition risk (Deutz et al. 2014). To limit age-related decline in muscle mass, strength, and function, resistance exercise training is also recommended (Lanza et al. 2008).

Notably, higher protein intakes are now recommended by the PROT-AGE Study Group appointed by the European Geriatric Medicine Society (EUGMS) (1.0–1.2 g protein/kg body weight/day for healthy older people, $\geq$1.2 g protein/kg body weight/day for active and exercising older adults, and 1.2–1.5 g protein/kg body weight/day for older adults who have acute or chronic disease (Bauer et al. 2013)). Similarly, in Norway, the Nordic Nutrition Recommendations suggest a safe intake of 1.2–1.5 g protein/kg body weight/day for healthy older people or approximately 15–20% of total energy intake (Pedersen and Cederholm 2014).

Fat

Based on the acceptable macronutrient distribution range (AMDR) (Australia/NZ), participants in both the Newcastle 85+ study and LiLACS NZ met or exceeded the minimum of 15% energy from fat proposed to ensure adequate consumption of total energy, essential fatty acids, and fat-soluble vitamins. Fat intake, as median percent energy was above the maximum AMDR range (20–35%), for participants in Newcastle is 36.8%, for Māori 38.5%, and for non-Māori 36.7% (NHMRC (National Health and Medical Research Council) 2006). These intakes are greater than reported for New Zealand adults aged 71+ years in the New Zealand Adult Nutrition Survey (33%) (University of Otago and Ministry of Health 2011). A lack of evidence for age-specific dietary recommendations for the oldest old hinders the interpretation for higher energy from fat but suggests that octogenarians eating patterns may differ from younger adults.

Micronutrients

Generally, an older adult who is eating well in terms of quality and quantity and is not experiencing or recovering from an acute illness will achieve an adequate intake of all micronutrients. While some micronutrients are required in larger amounts in older age, these amounts can be achieved within a healthy, well balanced diet which meets energy and macronutrient recommendations.

Micronutrient deficiencies tend to arise due to a reduction in food intake in response to a decline in energy needs with age. BMR and energy expenditure for physical activity may be reduced (Roberts and Rosenberg 2006) while vitamin and mineral needs remain unchanged or are increased (Zhu et al. 2010). Physiological changes may impact the metabolism of micronutrients and poor health and medications may compromise nutrient absorption. Eating habits affected by poor oral health and social isolation may further contribute to lower food intake and nutrient deficiency (Elmadfa and Meyer 2008). As micronutrient deficiencies are associated with adverse functional outcomes (Inzitari et al. 2011), they may impact the independence of older adults.

Calcium

Vital for bone health, calcium is consistently recommended in higher amounts for older compared to younger adults. Food sources high in calcium include milk, cheese, yoghurt, fortified foods (e.g., juice, breakfast cereal, breads), and some fish (e.g., sardines) and are recommended in favor of supplementation due to the unexpected finding that calcium supplementation was associated with increased cardiovascular events in osteoporosis trials (Reid et al. 2011).

Across various studies of older adults, dietary calcium intake does not meet dietary recommendations (Zhu et al. 2010). A similar shortfall of calcium intake was also observed for octogenarians in the Newcastle 85+ study median (IQR) 731 (554–916)mg and in LiLACS NZ for Maori 563 (424–778)mg and for non-Maori 702 (541–905)mg. More than 85% of octogenarians In LiLACS did not meet the EAR (Australia/NZ) for calcium (1100 mg). Calcium is not as well absorbed by the oldest age group and increased intake is needed. Novel ways of increasing dietary intake and intervention trials that study dose–response relationships to outcomes are needed as not enough is known about calcium requirements during ageing.

Magnesium

Magnesium requirements appear to change with age, but clear conclusions are absent to set higher requirements. Data from the National Health and Nutrition Examination Survey (NHANES) III showed a progressive decrease in daily magnesium intake with age (Ford and Mokdad 2003) with mean intakes for older men (225 mg) and women (166 mg) being well below the recommended daily allowance (RDA) (USA). A comprehensive review suggests that the dietary intake of magnesium is inadequate in elderly populations (Vaquero 2002), and this has previously been observed in older people in New Zealand (Horwath et al. 1992). In octogenarians participating in the Newcastle 85+ study, >20% of participants were below the lower NRI (UK) for magnesium (Mendonca et al. 2016b), and in Lilacs NZ, the EAR (Australia/NZ) for magnesium was not met by most (Wham et al. 2016b).

In older adults, magnesium is associated with physical performance (Veronese et al. 2014), adequate bone mineral density (Orchard et al. 2014), and inflammation (Chacko et al. 2010); hence, low intakes are of concern. Rich food sources of magnesium include nuts, legumes, whole grains, and most green vegetables, but despite a wide distribution in the food supply, older adults are less likely than younger adults to consume sufficient to meet their needs (Barbagallo et al. 2009).

Selenium

Selenium is an important antioxidant and contributes to a strong immune system and healthy thyroid function. It may also play a role in cancer prevention but this is an ongoing area of research. Good food sources of selenium include Brazil nuts and oily fish; however, the food content may depend on the local geographical area. Whole population estimates from NHANES do not show low intakes of selenium (Fulgoni et al., 2011; however, in the USA, frail older people have been found to be more likely to be deficient in selenium than other population groups (Smit et al., 2013). In the Newcastle 85+ study in the UK >20% of participants

had selenium intakes below the lower RNI (UK) (Mendonca et al., 2016b). Selenium intakes among octogenarians in LiLACS NZ were marginal with over two-thirds of participants falling below the EAR (Australia/NZ) (Wham et al. 2016b), similar to the NZ Adult Nutrition Survey for those aged over 70 years (University of Otago and Ministry of Health 2011). However New Zealand soils are low in selenium and New Zealand population blood selenium concentrations remain lower than those reported in other Western countries (Thomson 2004). Intakes in vulnerable older populations need to be examined in relationship to serum levels and outcomes over time to fully understand the significance of low intake.

Zinc

Zinc plays an important role in immunity but also has a role to play in wound healing and maintaining the senses of taste and smell, important for the enjoyment of food and optimal health of older adults. Zinc can be found in reasonable amounts in a range of foods including pulses, nuts and legumes, wholegrain cereals, and dairy products. Among octogenarians in LiLACS NZ, most men did not meet the EAR (Australia/NZ) for zinc intake (Wham et al. 2016b). Expressed on a per MJ food energy basis, zinc intake for all participants was 1.2 mg/MJ lower than for European men (aged 70+ y) in the Zenith study (Andriollo-Sanchez et al. 2005). Data on zinc status in normal ageing are lacking and the implication of low intake is unknown. An adequate intake of zinc nevertheless appears to be important for prevention of oxidative stress, immunity, and cognitive functions.

Iron

Iron plays a number of important roles in the body but is primarily concerned with transport of oxygen from the lungs to the cells of the body. Approximately, 70% of the body's iron is to be found in red blood cells in the form of hemoglobin. Iron in food is found as heme iron, from animal products, or non-heme iron, from non-animal-based products. Good sources include red meat, dark green leafy vegetables, and fortified breakfast cereals. Iron deficiency occurs in around two-thirds of the world's population with signs and symptoms being fatigue and decreased immunity. The most probable cause of iron deficiency anemia among older adults is inadequate dietary intake or blood loss from conditions such as ulcers, polyps, or intestinal cancer. There is no evidence that heme iron absorption is impaired in older adults. Iron deficiency in older adults is reported to be especially common over the age of 80 years (Fairweather-Tait et al. 2014). Prevalence of anemia in the USA has been reported to be approximately 10% at age 65 years and up to 60% in older adults in residential aged care.

Vitamin D

Older people are especially susceptible to vitamin D insufficiency due to reduced mobility, decreased sun exposure, and a decline in cutaneous synthesis of vitamin D with age (Heaney 2006). Unfortunately, food sources of vitamin D are limited (e.g., some fish, fortified foods, and liver) and while exposure to sunlight provides an endogenous source, disease or disability can prevent adequate exposure especially among the housebound or those in residential care. Most community-living octogenarians (>95%) in the Newcastle 85+ study had vitamin D intakes below the RNI (UK) (10 μg/d) (Mendonca et al., 2016b). Similarly in LiLACS NZ, the adequate intake (AI((Australia/NZ) for vitamin D (15 μg/d) was not met by >95% of participants (Wham et al. 2016b). To ensure adequate vitamin D status of older people, supplementation and food fortification may be needed; targeting those at high risk of insufficiency and providing vitamin D supplementation is likely the most cost-effective approach.

Vitamin B_{12}

Recommendations for dietary vitamin B_{12} intake are generally not different to younger adults. Increasing risk of deficiency among older people, despite adequate intakes, appears to be related to physiological changes in the gut with age that may prevent the release of vitamin B_{12} from natural food sources (McNulty and Scott 2008). Pernicious anemia is a further cause of deficiency in older people (Andres et al. 2004). Vitamin B_{12} is mostly found in animal products such as fish, poultry, meat, eggs, and dairy; hence, vegetarians may be at higher risk; however, it is increasingly found in fortified foods, particularly breakfast cereals. Vitamin B_{12} deficiency commonly manifests as fatigue, anemia, and depression which may be corrected through diet or supplementation.

Folate

Folate is found across a wide range of foods including fruit and vegetables, legumes and nuts, dairy, poultry, meat, and eggs. Despite being commonly found in the food supply and older adults not generally having any greater requirement than younger adults, folate can become a nutrient at risk if access and availability of fresh food is limited or there is a significant decline in appetite. Of particular concern for older people is that natural food folates found in foods such as green vegetables can be unstable under typical cooking conditions, and this can substantially reduce the folate content even before it is ingested. Folic acid found in supplements and fortified foods such as breakfast cereals is however a very stable and highly bioavailable form of the vitamin (McNulty and Scott 2008). Many developed countries mandatorily fortify grain flours, including wheat, with folic acid, e.g., Australia, USA, while others fortify voluntarily, e.g., NZ, to address public health

concerns related to folate insufficiency. This largely relates to neural tube defects; however, the older population is likely to benefit from the increased availability in the food supply. The primary clinical sign of deficiency is megaloblastic anemia, commonly manifesting as fatigue, weakness, and headache.

Dictionary of Terms

- Malnutrition is a broad term referring to both over- and undernutrition; however, in this context, it is exclusively used to describe undernutrition occurring as a result of inadequate nutrient intake, which may be due to insufficient food intake or illness.
- Māori are the indigenous people of Aotearoa, New Zealand comprising 14% of the total population and 2% of those aged over 80 years.
- Macronutrients are those which are required by the body in large amounts, and which contribute to energy intake, i.e., protein, fat, carbohydrate, alcohol.
- Energy (calories or kilojoules) is the fuel gained from macronutrients which is used by the body for all processes and activities including breathing, sleeping, thinking, and walking.
- Acceptable macronutrient distribution ranges (AMDR) are the recommended ranges for % energy from macronutrients required to reduce chronic disease risk, e.g., protein 15–25%, fat 20–35%, carbohydrate 45–65% (Australia/NZ).
- Nutrient reference values referred to throughout this chapter, e.g., RDA, RDI, AMDR, etc., are population-level recommendations based on thorough and systematic review of current scientific knowledge.

Summary Points

- This chapter reviews the current understanding of energy, macronutrient, and key micronutrient requirements for older people including the oldest old.
- Ageing is associated with a range of physiological and psychosocial changes which can result in poor nutritional health and consequently poor general health.
- Older people tend to be nutritionally vulnerable with advancing age as typically they eat less food, consume smaller meals, fewer snacks, and become satiated after meals more rapidly than younger people; daily intake of food may decrease by a third between the ages of 20 and 80 years.
- Understanding nutrients of concern for older people is critical for the prevention and management of chronic disease and other health problems.
- Dietary guidelines are based on the nutritional needs of a population; however, little is known about the dietary habits and nutritional status of the very old.
- Energy requirements vary with age, gender, body size, and physical activity. While healthy active older adults may have similar energy needs to younger adults, energy expenditure tends to decline progressively between the ages of 20 and 100 years, largely due to body composition changes of ageing.

- Meeting energy requirements can be challenging for older adults due to reduced food intake and hence a reduction in dietary variety.
- Older adults have higher protein requirements than younger adults due to reduced muscle protein synthesis rates and elevated metabolism from inflammatory conditions. There are inconsistencies in guidelines for protein intake in this population; recent European recommendations promote higher intakes up to 1.5 g protein/kg body weight.
- Fat is an important energy source particularly for older adults whose appetite may be diminished. While the evidence suggests that adults of advanced age eat fat in excess of recommendations, there are no age-specific guidelines to help interpret the implications of this over consumption.
- Healthy older adults who eat well in terms of quality and quantity will generally meet micronutrient recommendations.
- Micronutrient deficiencies tend to arise from reduced food intake occurring as a result of reduced energy requirements with age.
- Absorption and metabolism can be affected by ageing as well as polypharmacy, poor health, and social isolation.
- Micronutrients of concern in older adults are calcium, magnesium, selenium, zinc, iron, vitamin D, vitamin B_{12}, and folate with vitamin D of particular concern, as food sources are limited and exposure to sunlight is often reduced. Routine vitamin D supplementation may be necessary to achieve requirements.

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Part X

Micronutrients

Low Vitamin A Status and Diabetes: An Overview

78

Farzad Shidfar and Javad Heshmati

Contents

Abstract

The prevalence of chronic diseases is increasing around the world, and diabetes is the one with a significant prevalence. Environmental and genetic factors have been proposed in the pathophysiology of diabetes. Vitamin A is a micronutrient that plays a vital role in health. Vitamin A deficiency is very prevalent in low-income countries. Diets poor in vitamin A and its precursors lead to vitamin A deficiency (VAD). Vitamin A deficiency is commonly associated with ocular

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complications, delayed growth, and higher sensitivity to infections including respiratory infections, diarrhea, and measles.

Previous studies have shown that the concentration of vitamin A, plasma retinol, transthyretin (TTR), and retinol-binding protein (RBP) are low in the adults and children with type 1 diabetes mellitus (T1DM). Beside the role of VAD in the pathology of T1DM due to its effects on the immune system, VAD plays a role in type 2 diabetes mellitus (T2DM) through its effects on antioxidants and lipid metabolism. So, the possible mechanisms of the effect of vitamin A on the pathology of T2DM are categorized as oxidant radicals chelation, increased insulin sensitivity, and obesity and adipose biology.

VAD has shown to have a critical role in pathology of diabetes. VAD affects the pathology of T1DM and T2DM through several different mechanisms. VAD should be considered to be corrected, specifically in high-risk regions. Therefore, VAD should be surveyed and monitored in these regions. If VAD is epidemic in one region, deficiency correction policies should be implemented.

Keywords

Vitamin A · Deficiency · Diabetes · Xerophthalmia · Retinol · Retinol-binding proteins · Transthyretin · Immune system · Blindness · Radicals · Insulin sensitivity · Obesity

List of Abbreviations

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
ATRA	All-trans retinoic acid
BAT	Brown adipose tissue
C/EBPb	Enhancer-binding protein beta
IFN	Interferon
IFN-g	Interferon-gamma
IL-1	Interleukin-1
IL-10	Interleukin-10
IL-12	Interleukin-12
IL-2	Interleukin-2
IL-6	Interleukin-6
JNK	c-Jun N-terminal protein kinase
NK cells	Natural killer cells
NO	Nitric oxide
PI3K	Phosphatidylinositol 3-kinase
PKC	Protein kinase C
PPARg	Peroxisome proliferator-activated receptor gamma
9-cis-RA	9-cis-retinoic acid
RA	Retinoic acid
RALDH 1	Retinal dehydrogenase 1
RAR	Retinoic acid receptor
RBP4	Retinol-binding protein 4

ROS	Reactive oxygen species
RXR	Retinoid X receptor
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TLR4	Toll-like receptor 4
TNF-α	Tumor necrosis factor-α
TTR	Transthyretin
UCP-1	Uncoupling protein 1
UCP-2	Uncoupling protein 2
VAD	Vitamin A deficiency
WHO	World Health Organization

Introduction

Diabetes

The prevalence of chronic diseases is increasing around the world, and diabetes is the one with a significant prevalence. According to studies, the worldwide prevalence of diabetes in 2013 was 8.3%, i.e., 381.3 million persons, and based on estimations, this figure will increase to 8.8%, i.e., 591 million persons, in 2035 (Guariguata et al. 2014). A noticeable percentage is originating from developing countries. In fact, urbanization and lifestyle changes in developing countries have increased the prevalence of noncommunicable diseases (Forouhi and Wareham 2014). Diabetes mellitus has two types: type 1(T1DM) and type 2 (T2DM). T1DM, which is more prevalent in younger people, is caused by lack of insulin secretion and considered an autoimmune disease (Szablewski 2014). T2DM results from decreased insulin sensitivity in older individuals. T2DM accounts for more than 90% of cases and is more common in obese people with low physical activity. Both types of diabetes have many complications and affect the patient's life. Visual problems, heart and kidney disease, and diabetic ketoacidosis are common problems in diabetes (Chiang et al. 2014). In T2DM, eye complications, amputation, cardiovascular problems, and kidney complications are common (Althouse et al. 2014).

Environmental and genetic factors have been proposed in the pathophysiology of diabetes. Genetic factors have a more prominent role in T1DM. Immune cells attack beta cells in the pancreas (Zaccardi et al. 2015). In the past, it was believed that insulin resistance is the cause of T2DM and lack of insulin secretion from β cells is a secondary complication. However, recent studies indicate that glucose hemostasis as well as other regulatory mechanisms are adjusted by various factors, and regulation of the blood sugar level in a proper range is not only affected by glucose uptaking cells but is also affected by insulin secretion by beta cells (Kahn et al. 2014).

Genetic factors can affect insulin secretion from pancreatic β cells and insulin resistance in insulin receptor cells. Environmental factors, among which diet is the most important one, may also affect insulin secretion and insulin resistance. Diet and diet-related factors have the greatest impact on the prevalence of diabetes, especially

T2DM. In addition to the role of diet in the incidence of the disease, it may have a role in the prevention and treatment of diabetes (Korat et al. 2014). Controlling T1DM and T2DM is impossible without controlling food intake and weight loss (Ley et al. 2016). In comparison to other dietary factors which may have a role in the incidence of diabetes, the calorie intake is of importance. Therefore, obese people who have a higher calorie intake are more prone to diabetes. Moreover, macro and micronutrients of the diet have a great role in the incidence, control, and treatment of diabetes (Ley et al. 2014). In recent years, many researchers have considered a special role for micronutrients and conducted many studies in this regard (Pereira 2013). The reason is that many micronutrients are associated with carbohydrate metabolism and insulin. However, without any doubt, there is no consensus on the effect of micronutrients on the prevention and control of T2DM (Neumiller 2012). Among micronutrients, the role of vitamin A in the prevention and control of diabetes will be discussed in this section.

Vitamin A

Vitamin A is a micronutrient that plays a vital role in health. As a fat-soluble vitamin, it plays a significant role in visual health, and it is also important in immunity, epithelial and reproductive tissue health, and gene expression (Iskakova et al. 2015). Vitamin A deficiency causes many health problems among which xerophthalmia is well known. Xerophthalmia's manifestations include eye problems, increased susceptibility to infections, and growth retardation (WHO 2009). Human and animals are unable to produce vitamin A internally, therefore they should receive it from their diet. Vitamin A is available in diet sources as retinol, retinol esters, and previtamins like carotenoids that can convert to the active form in the body. Retinol and retinol esters come from animal foods, but previtamins are available in plant sources. There is debate about the value of these precursors; however, previous studies have shown that every 30 grams of these precursors is converted to 1 gram of vitamin A (Mahan and Raymond 2016). Vitamin A is stored in the liver in the usual form of retinyl ester, but in the circulation, retinol is transferred by a special protein carrier that obtains retinyl ester after its hydrolysis in the liver. Retinol-binding proteins (RBP) are the carrier proteins of vitamin A. They are also bound to transthyretin (TTR).

Vitamin A Deficiency (VAD)

Vitamin A deficiency is very prevalent in low-income countries. Diets poor in vitamin A and its precursors lead to vitamin A deficiency (VAD). Vitamin A deficiency is commonly associated with ocular complications, reduced growth, and higher sensitivity to infections including respiratory infections, diarrhea, and measles. Vitamin A deficiency is one of the important reasons for the increased burden of diseases and reduced growth and is also one of the main causes of blindness in African and South East Asian children (WHO 2009). Severe VAD causes

xerophthalmia, which is initially characterized by night blindness and eye problems followed by Bitot's spots, eventually resulting in corneal ulcers and injuries. According to the WHO, the prevalence of VAD in pregnant women in low-income countries is about 10–20%, and also 250–500 million children have become blind due to secondary effects of VAD, of whom 50% die 1 year after blindness. There is lack of evidence on the prevalence of VAD in adults and other age groups, but there is VAD in other age groups in areas where VAD is common in children and pregnant women. In addition to severe vitamin A deficiency complications, subclinical vitamin A deficiency affects many people in Africa and South East Asia.

Vitamin A Deficiency and Diabetes

Evidence supports declined serum levels of vitamin A in T1DM patients (Yosaee et al. 2016); however, in such patients, the data of the serum levels of vitamin A are mostly vague (Sasaki et al. 1995). In addition, some studies have reported limited changes in the level of vitamin A in the T2DM patients (Sasaki et al. 1995; Lu et al. 2000). Studies indicate that the majority of T1DM patients suffer from low levels of vitamin A. According to many studies, a sort of vitamin A shortage can be seen in the diabetic patients with malnourishment in comparison to malnourished control subjects (Via 2012). We conducted this study to assess the evidence on the effect of vitamin A in T1DM and T2DM.

Cross-sectional studies evaluating the relationship between the metabolomic profile and the risk of diabetes have shown contradictory results. Moreover, limited evidence supports this relationship (Wang et al. 2011). Retinol-binding protein 4 (RBP4), which is a soluble-binding protein, is a vitamin A carrier that may cause insulin resistance. Inflammation and obesity are major causes of diabetes. Several mechanisms are offered to describe the relationship between RBP4 and insulin resistance. There are two receptors in interaction with RBP4; retinoic acid 6 (STRA6) could activate JAK2/STAT5 cascade and pathways of the c-Jun N-terminal protein kinase (JNK) by inducing toll-like receptor 4 (TLR4) and plasma membrane protein, which is involved in insulin receptors components. Insulin resistance can be enhanced by both of these mechanisms (Cione et al. 2016). Therefore, the pathology of diabetes may have a strong relationship with vitamin A. In this regard, there are some reports of the effect of vitamin A deficiency in diabetes patients.

Relationship Between Vitamin A Deficiency and Type 1 Diabetes

One of the common autoimmune diseases is T1DM, which is identified as the condition of absolute or relative destruction of insulin-producing beta cells by T cells (Szablewski 2014; Maahs et al. 2010). Nowadays, the main therapeutic approach to type 1 diabetes is exogenous insulin administration (Hassan et al. 2012; Laing et al. 1999), which could have some side effects. Therefore, there is a

need for substitute methods for insulin therapy to prevent and treat this disease. It has been proven that one of the substitute preventive and treatment methods for insulin is the manipulation of the immune system by changing the course of the disease (Bougnères et al. 1990). Among different nutrients, vitamin A has the greatest effect on the immune system. Evidence suggests that VAD may cause T1DM by its effect on the acquired and innate immune system. Since the early twentieth century, vitamin A has been used to modulate the immune system (Green and Mellanby 1928; Green and Mellanby 1930). Nowadays, to obtain the normal function and regulation of the immune system, vitamin A and its derivatives in the diet are known to be necessary (Ross and Hammerling 1994). It is found that lack of vitamin A could cause insulin secretion failure by glucose simulation (Berdanier 2003). Moreover, cellular retinoic acid (RA), retinol-binding protein (RBP), and transthyretin (TTR) are observed at high levels in the islets of the rat pancreas (Berdanier et al. 2001; Chertow et al. 1987; Driscoll et al. 1997).

Currently, T1DM patients at risk of vitamin A and carotenoids deficiency could benefit from our knowledge of vitamin A metabolism and its role in diabetes (Olmedilla et al. 1997; Granado et al. 2003). Previous studies have shown that the concentration of vitamin A, plasma retinol, TTR, and RBP is low in the adults and children with T1DM (Kobbah et al. 1988; Basu et al. 1989; Nakamura et al. 2010). Lack of vitamin A appears to be a potential cause of type 1 diabetes by modulating the immune system. Research has shown that retinoids are an important factor for regulating the monocytic/macrophage function (Breitman et al. 1980; Geissmann et al. 2003; Jiang et al. 2003). Cytokines like TNF-α and substances like nitric oxide (NO) can be released from macrophages under activated conditions (Sherry and Cerami 1988). The secretion of most of the cytokines produced by macrophages, such as IL-1, IL-6, IL-12, and TNF-α, can be affected by RA (Mohty et al. 2003). A marked reduction in the mRNA level in TNF, regulation of the production of nitric oxide, and increased IL-1 production have been reported as the effects of all-trans-RA supplementation in some studies (Mehta et al. 1994). Therefore, the performance of this pathway can be disturbed by lack of vitamin A. Natural killer cells could also be affected by VAD. The basic role of vitamin A deficiency in NK cell-related activities has been demonstrated in young rodents (Ross 2012). The activity of NK cells and the production of IFN by spleen cells after mitogen stimulation are decreased by lack of vitamin A (Trinchieri 1989; Michael et al. 1989). The activity of NK cells is enhanced at high concentrations of retinoids according to in vitro and in vivo studies (Alter et al. 1978; Micksche et al. 1985; Santoni et al. 1985). However, this simulation process is not quite clear. Moreover, vitamin A insufficiency and the observed immunosuppression have a fascinating dynamic association (Bowman et al. 1990). The activity of the neutrophils is widely affected by VAD. Oxidized retinol and RA are required for differentiation of the neutrophils (Robertson et al. 1992; Tsai and Collins 1993). The genes modulated by RA receptors control the development of neutrophils in the bone marrow, and the RA in cultures speeds up neutrophil maturation (Maun et al. 2004; Ribeiro et al. 2003). The level of neutrophils and the superoxide-production capacity could be significantly recovered by vitamin A or RA treatments in calves and rats (Zhao and Ross 1995; Higuchi and

Nagahata 2000). The level of hyper-segmented neutrophils is significantly higher (67%) in rats with low vitamin A compared to the control group (Twining et al. 1997). Therefore, inconsistent information is available on the association of neutrophilic function and vitamin A in humans.

Evidence also indicates that VAD may cause T1DM by its effects on the acquired and innate immune system. The RA function in T cell biology was explained by Iwata et al. (Iwata et al. 2004) for the first time. Recently, studies have shown the effect of vitamin A deficiency on the immune competence of T cells (Villamor and Fawzi 2005). The results of many studies show the involvement of vitamin A in the modulation of IL-10 production. Th2 cells secrete IL-10 that produces pro-inflammatory cytokines like IFN-g and IL-2 (Leal et al. 2004).

There are a few mechanisms through which vitamin A adjusts the innate and adaptive immune responses, but immune disorders and subsequently T1DM occur following vitamin A deficiency. In addition, vitamin A is very important in the release of glucagon and insulin (Berdanier 2003) and is basically involved in glucose homeostasis in the body. The release of insulin in Langerhans islet cells is associated with activated vitamin A. For a perfect operation of the islets, vitamin A has an important role because of the existing RA-binding proteins in the pancreatic islet cells. The islet cells secrete the hormones which are more than likely regulated by RA. Islet cells act abnormally in secretion of hormone after exposure to graded glucose levels. VAD makes changes in the quality of the pancreatic tissue, possibly through increased digestion of the tissue by collagenase (Berdanier et al. 2001).

Potential Mechanisms of Vitamin A Effects on T2DM

Beside the role of VAD in the pathology of T1DM due to its effects on the immune system, VAD plays a role in T2DM through its effects on antioxidants and lipid metabolism. The possible mechanisms of the effect of vitamin A on the pathology of T2DM are located in three categories: oxidant radicals chelation, increased insulin sensitivity, and obesity and adipose biology.

1- Oxide Radicals Chelation

In diabetic patients suffering from hypoxia, retinol metabolites act as a scavenger of lipoperoxyl radicals at low oxygen pressures (Berry et al. 2012). Self-oxidation reaction uses retinol within lipid oxidation, and retinol is oxidized to 5,6-retinol epoxide as a result of overproduction of free radicals (Regazzetti et al. 2009). To release insulin in pancreatic islet cells, the K/adenosine triphosphate (ATP) channel must be opened as a prerequisite characterized by the ATP/adenosine diphosphate (ADP) ratio. It seems that disorders in the mitochondrial function can change the ratio of ATP/ADP and affect the insulin secretion response stimulated by glucose. ROS not only can nonspecifically damage biomolecules but can also reduce the membrane potential of the mitochondria. Theoretically, the respiratory chain is

irreversibly disconnected by a consequent leakage of superoxide anions and hydrogen peroxide from the electron transport chain amplifying the redox stress until damage (Roehrs et al. 2009).

Mitochondrial DNA is exceedingly near the production site of the ROS; therefore, its performance can be reduced. The radical superoxide activates the UCP-2 (uncoupling protein 2) which can lead to reduction in the ATP/ADP ratio, and consequently insulin secretory response is also decreased. Insulin secretion could be influenced by mitochondrial regulation caused by chelation of ROS that can also prevent cellular damage and adjust the endogenous activities of the scavenging enzymes, which are increased by oxidative stress (Korichneva et al. 2003). Retinol is considered a part of general cellular defense due to its capability to protect (even partly) the mitochondria against the oxidative damage (Robertson et al. 2003).

2-Insulin Sensitivity

Vitamin A could increase insulin signaling or stimulate the insulin release, which both affect insulin sensitivity. Activation of PI3K (phosphatidylinositol 3-kinase) stimulated by RA has a new mechanism in which RA-RAR-binding mediated conjunction is created between p110 (which is a catalytic subunit of PI3K) and a compound containing the regulatory subunits of p85 and PI3K, RAR, and some other unknown proteins. RA appears to have a role in the activation of PI3K by involving in regulation of the intracellular site of the signaling compound. According to the results obtained from binding experiments and analysis of the crystal structure, it has been shown that all-trans RA (ATRA) could conjunct to the PKC isozymes, resulting in reduced activity of protein kinase C (PKC) (Masiá et al. 2007; Finel et al. 2005). It has been shown that this effect is beneficial in diabetes and is involved in decreased insulin-signaling power through affecting insulin signaling and PKC. Protein kinase C also intensifies oxidative stress by activating NADH. Vitamin A could modulate the PKC activity and consequently increase insulin sensitivity through increasing insulin signaling and decreasing oxidative stress (Rolo and Palmeira 2006, Draznin 2006). A lipophilic ligand of the nuclear hormone receptor superfamily which can be associated with the regulation of the transcription, ATRA, and pancreatic glucokinase activity could cause a retinol-meditated release of insulin (Cabrera-Valladares et al. 1999).

3-Obesity and Adipose Biology

Of the potential action sites targeted for retinoids are the retinoic acid receptor (RAR) and retinoid X receptor (RXR) expressed in the adipose tissue (Jeyakumar et al. 2007). It has been shown that retinol levels are low in the human fat tissue (Hong et al. 2004). Numerous studies have demonstrated that differentiation of adipocytes, possible effect of vitamin A on the adipogenic, and thermogenic capacity of rodents could be prevented by RA at high concentrations in vitro and expression

of brown adipose tissue (BAT)-uncoupling protein 1 (UCP-1) gene in vivo (Jeyakumar et al. 2006). It has been reported that secretion of leptin could be inhibited by 9-cis-RA (9cRA), while it can increase glucose uptake and adipogenesis in mature fat cells (Hong et al. 2004). Differentiation of fat cells can be prevented by binding of 9cRA to RAR which halts the transcriptional role of CCAAT/ C/EBPb (enhancer-binding protein beta) (Regazzetti et al. 2009; D'Ambrosio et al. 2011). Increased intracellular retinal levels caused by lack of retinal dehydrogenase 1 (RALDH 1) is associated with decreased adiposity. Retinol is converted to RA by an enzyme. The retinol ability to bind could be predicted at least in part. Retinol disrupts the activity of PPARg due to suppressing receptor activation by binding RXR and PPARg, which emphasizes the main role of retinol as a transcriptional regulator (Frey and Vogel 2011). UCP-1 gene can be positively regulated by vitamin A and one of its famous metabolites, RA. Despite reduced ATP levels, glucose transportation in the skeletal muscle can be enhanced because of overexpression of murine UCP-1. Insulin resistance in diabetes caused by high-fat regimens could be improved by the expression of hepatic UCP-1 (Rolo and Palmeira 2006); moreover, increased expression of UCP-1 results in thermogenesis. It is possible to control obesity through a regulatory function, so that vitamin A supplements contribute to reduced adiposity and increased thermogenic activity through removal of adipose storage and enhancement of BAT-UCP-1 gene expression, for example, in WNIN/ Ob strain (Jeyakumar et al. 2007). One of the inhibitory ways for adipogenesis is probably the use of retinoic acid, so that the master regulator of PPARg can control retinoid metabolism, according to new investigations (Frey and Vogel 2011).

Conclusion

VAD clearly demonstrates having a critical role in pathology of diabetes. VAD affects the pathology of type 1 and type 2 diabetes through several different mechanisms. VAD should be certainly considered an important factor and addressed, specifically in high-risk regions. Therefore, VAD should be extensively surveyed and monitored in affected regions. If VAD is proven to be an epidemic in any one region, deficiency correction policies and procedures should be implemented. In high-risk regions which more than likely happen to be low-income regions as well, prevention is a much more viable and cost effective option.

Policies and Protocols

- Megadose vitamin A capsules (200,000 IU) prescribed 6-monthly have been given to exactly 80% of 1–5-year-old children in developing countries up to now and are accounted for the decrease in the 1–5-year-old mortality.
- However, the adequacy trials on which this is based were directed more than 20 years ago. A single program assessment that has been completed in 2004 (DEVTA in India, 1999–2004) demonstrated no effect on mortality. Vitamin A

capsules decrease the mortality of diarrhea and measles, which have forcefully dropped as the result of implementation of comprehensive inoculatious and oral rehydration programs. Therefore, this regimen does not probably have any significant effect.

- The 6-month course of vitamin A capsules does not reduce vitamin A deficiency. VAD can be decreased by higher intake of vitamin A at physiological levels, by dietary modification, enrichment, and repeated (daily or weekly) supplements. Unlike high-dose vitamin A capsules, good responses have been observed following these methods in reproductive-age women (Mason et al. 2014).

Dictionary of Terms

- **Diabetes** – A Metabolic disease which characterized by high blood sugar, dyslipidemia, and insulin resistance.
- **Vitamin A** – One of the essential fat-soluble vitamins that have several roles in body like vision, immune system modulation, and epithelial tissue function.
- **Vitamin A deficiency** – Lack of vitamin A in diet that cause several damage to body function, like ocular problems and immune system dysfunction.
- **Xerophthalmia** – A severe vitamin A deficiency that characterized by sever visual problems and increased sensibility to infections.
- **Insulin Resistance** – Decrease sensibility of body cells to insulin that make them to need more insulin to response.

Summary Points

- This chapter is about the effect of vitamin A in pathology of diabetes.
- Vitamin A deficiency causes several metabolic problems including an increased risk of diabetes.
- Vitamin A deficiency can cause type 1 diabetes through its effect on immune system function.
- Because of vitamin A role in protection of body from oxidative stress and inflammation, vitamin A deficiency can cause type 2 diabetes because of oxidative stress and inflammation.
- Vitamin A supplementation has been effective in preventing diabetes in vitamin A-deficient societies.

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Vitamin A's Role in the Regulation of Hepatic Glucose and Lipid Metabolism During the Transition from Fasting to Refeeding

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Yang Li, Rui Li, and Guoxun Chen

Contents

Abstract

The fact that the world population could reach 8.5 billion by 2030 indicates the challenges of maintaining global food and nutrition securities, especially in low-income countries worldwide. The functions of nutrients to support human health and prevent diseases have been gradually recognized through the survival process of humankind. Micronutrients such as vitamin A are essential

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for a variety of biochemical processes. The deficiency of a particular micronutrient is associated with certain symptoms. Depending on the length of time without food intake, humans and animals may experience fasting, starvation, and famine. This is accompanied by reduction of both macronutrients and micronutrients. This chapter is to summarize the role of vitamin A in the regulation of glucose and lipid metabolism and VA metabolism in the cycle of fasting and refeeding. We also would like to offer some future perspectives for vitamin A study.

Keywords

Fasting · Gene expression · Glucose · Lipid · Liver · Macronutrients · Metabolism · Micronutrients · Nutrition security · Refeeding · Retinoic acid · Vitamin A

List of Abbreviations

ALDH	Aldehyde dehydrogenase
CBP	Cellular retinol-binding protein
Gck	Glucokinase gene
Pck1	Cytosolic form of phosphoenolpyruvate carboxykinase gene
RA	Retinoic acid
RALDH	Retinaldehyde dehydrogenase
RAR	Retinoic acid receptor
RARE	Retinoic acid-responsive element
RBP	Retinol-binding protein
RDH	Retinol dehydrogenase
RXR	Retinoid X receptor
Srebp-1c	Sterol regulatory element-binding protein 1c
VA	Vitamin A
VAD	VA deficient
VAS	VA sufficient
ZL	Zucker lean
ZF	Zucker fatty

Introduction

Nutrition is the sum of processes of nourishing or being nourished in an organism. As an essential micronutrient, vitamin A (VA, retinol) is critical for the general health of the human body. The lack of VA leads to the development of diseases such as night blindness in humans. From a public health point of view, it is important to maintain the availability of VA to prevent the development of diseases in a population and an individual.

The Concept of Vitamin, the Discovery of Vitamin A, and Its Role in Public Health

The essentiality of VA to the health of an animal was not discovered due to its role in the treatment or prevention of night blindness. It was recognized by its activity to support animal growth. To discover the essential components in foods, synthetic diets made of purified ingredients were used to test their capability to support the growth or healthy status of animals for the goal of identification of hypothetical "vital amines" (Carpenter 2003). VA was found as a fat-derived factor supporting rat growth (Ross et al. 2012). When weaning rats (3 weeks of age) were fed a purified diet with fat extracted by ether, they only grew normally for about 40 days before the body weight gain stopped. The growth returned when VA-containing foods such as butter and egg yolk were included in the diet (McCollum and Davis 1913; Osborne and Mendel 1913).

As retinal, an oxidized product of retinol, takes part in the vision cycle, VA deficiency will lead to eye problems such as night blindness. In addition, VA deficiency may also increase mortality in children due to its role in immunity (Semba 2012). The lack of dietary VA intake is the major reason for the development of its deficiency. VA deficiency could also be observed in subjects with malfunctions of digestion and absorption in regions that have plenty of dietary VA sources (Hajar Al Binali 2014).

Due to the gradual growth of human population on this planet, food security and health problems have become challenges of human society for a while. Both Millennium Development Goals of United Nations Millennium Declaration in 2000 (Waage et al. 2010) and Sustainable Development Goals by United Nations General Assembly in 2015 (Lim et al. 2008) had pointed out these challenges. Hunger and malnutrition are major global health problems. For example, micronutrient deficiencies affect two billion population globally (Bhutta et al. 2013). According to the data collected in 1995–2005, 5.2 and 190 million preschool-age children might be affected with night blindness and at risk of VA deficiency (serum retinol concentrations <0.70 μmol/l), respectively (WHO 2009). It has been thought that 0.6 million deaths for children under 5 years old were due to VA deficiency (Black et al. 2008). A meta-analysis has indicated that significant reductions in mortality of children aged under 5 in VA deficiency regions were observed after its supplementation (Mayo-Wilson et al. 2011).

Molecular Pathways of Vitamin A Absorption and Signal Transduction

VA plays crucial roles in a variety of physiological functions ranging from vision to cell differentiation. Dietary molecules with VA activity are in two forms, preformed VA mainly as retinyl esters from animals and provitamin A as carotenoids from

plants (Ross et al. 2012). In the digestive tract, retinyl esters are hydrolyzed into retinol and fatty acids by lipases before they are absorbed into enterocytes (Harrison 2005). Provitamin A carotenoids such as β-carotene are absorbed into enterocytes without any modification. After absorption, β-carotene is converted into retinol eventually in both small intestine and liver cells (Lakshman 2004; Wyss 2004). Inside enterocytes, retinol is re-esterified with fatty acids to form retinyl esters again through the actions of lecithin-retinol acyltransferase and acyl-CoA-retinol acyltransferase. Retinyl esters and other dietary lipids are assembled into chylomicrons and delivered to other parts of the body. Retinyl esters in the chylomicron remnants are eventually taken up into hepatocytes for their uses or storage in the hepatic stellate cells (Blomhoff and Blomhoff 2006).

VA's metabolites, retinal and retinoic acid (RA), are the main mediators of its functions. Several enzymes and proteins are involved in the transport, production, and catabolism of retinoids. Retinol is oxidized to retinal (retinaldehyde). Then, retinal is further oxidized to RA (Blomhoff et al. 2006). These are processes catalyzed by enzymes (Duester 2000). RA mainly regulates gene expression in its targeted tissues and cells. This is achieved through the activation of retinoic acid receptors (RARs) and retinoid X receptors (RXRs)(Napoli 2011). These two members of nuclear receptor superfamily bind to retinoic acid-responsive element (RARE) and activate the transcription of RA-responsive genes (Evans 2005). Additional nuclear receptors have been suggested to mediate RA signals as well (Zhang et al. 2015a). One is hepatocyte nuclear factor 4α (Sladek et al. 1990). Another one is chicken ovalbumin upstream promoter-transcription factor II (Li et al. 2009), which interacts with RXRα to form heterodimer (Kliewer et al. 1992).

The Metabolic Changes During the Transition of Fasting and Refeeding

Depending on the length of time between two food ingestions, the subjects could be in the postabsorptive or fasting period that lasts several hours to a day or the starvation period that could last for days. Dramatic metabolic changes occur with the food ingestion in the body of the subjects. These include the switch of primary substrates for energy production and the changes of plasma levels of hormones responsible for the regulation of anabolic and catabolic states (McGarry et al. 1987).

The liver plays an essential role in the regulation of metabolic homeostasis. This is achieved, at least partially, through the regulation of expression levels of hepatic genes for glucose and lipid metabolism (Brown and Goldstein 2008). The transition of fasting to refeeding state is accompanied by the changes of the expression levels of genes, which are responsive to the stimuli of hormones such as insulin and glucagon. For instance, insulin increases the expression of genes involved in hepatic glycolysis, glycogenesis, and lipogenesis, and suppresses the expression of genes involved in gluconeogenesis, which correspond to the fed and fasted states, respectively. To facilitate the glucose usage in the fed state, insulin induces the expression of hepatic glucokinase gene (*Gck*), the first enzyme of hepatic glycolysis. At the

same time, insulin suppresses the expression levels of cytosolic form of phosphoenolpyruvate carboxykinase gene (*Pck1*) and glucose 6-phosphatase catalytic subunit, the enzymes involved in the early and late steps of gluconeogenesis, respectively (Hanson and Reshef 1997). To facilitate the hepatic lipogenesis in the fed state, insulin induces the expression level of sterol regulatory element-binding protein 1c (*Srebp-1c*), a master transcription factor that stimulates the expression of genes for the hepatic fatty acid biosynthesis (Horton et al. 2002). These genes are also regulated by glucagon, which activates protein kinase A pathway (Hardie and Carling 1997).

The Current Understanding of Roles of Vitamin A in Glucose and Lipid Metabolism

As early as 1937, the elevation of the hepatic VA content was observed in the liver samples of patients who died of diabetes, suggesting the role of VA in glucose metabolism (Moore 1937). Early studies had shown that the hepatic glycogen content was depleted in rats with VA deficient (VAD) status (Wolf et al. 1957). This depletion was not due to the reduction of food intake associated with the development of VA deficiency as rats in the pair-feeding group still had hepatic glycogen content. Our lab also reported the depletion of hepatic glycogen content in the liver of VAD rats recently (Zhang et al. 2012). Interestingly, rats fed a diet with excessive amount of VA had an increase of hepatic glycogen content (Singh et al. 1968).

VA status also affects the mRNA and protein expression levels of the hepatic glucokinase, the first enzyme for hepatic glycolysis (Li et al. 2016). In the liver, the *Gck* expression is reduced in fasting and increased upon refeeding. The induction and suppression of *Gck* mRNA level are attributed to the actions of insulin and glucagon, respectively (Iynedjian et al. 1989). In an attempt to identify lipophilic molecules with abilities to modulate the insulin-regulated gene expressions in hepatocytes, we have found that retinoids in the liver lipophilic extract synergized with insulin to induce *Gck* expression in primary rat hepatocytes (Chen et al. 2009). Retinoids synergized with insulin to induce the hepatic *Gck* expression through the activation of RAR/RXR (Chen et al. 2009). The levels of the hepatic *Gck* mRNA (Zhang et al. 2012) and glucokinase activity (Chen et al. 2009) in VAD rats were lower than that in VA-sufficient (VAS) animals.

Gluconeogenesis from endogenous noncarbohydrate sources is critical for the maintenance of plasma glucose level during fasting state. In the liver, the cytosolic form of phosphoenolpyruvate carboxykinase has been considered the first rate-limiting enzyme for gluconeogenesis (Hanson and Garber 1972). The hepatic *Pck1* expression is increased by fasting and diabetes and decreased by refeeding and insulin treatment. Glucagon, glucocorticoids, thyroid hormone, and RA induce hepatic *Pck1* expression, while insulin inhibits it (O'Brien and Granner 1996). Two RAREs have been identified in the hepatic *Pck1* promoter and have been shown to bind to several transcription factors (Scribner et al. 2007). We have shown that retinoids affect insulin-suppressed

Table 1 Comparisons of body and plasma parameters in fasting and VA deficiency

Parameters	Fasting	VA deficiency
Body weight	Reduced	Reduced
Fat mass	Reduced	Depleted
Liver mass (net)	Reduced	Reduced
Liver glycogen	Low	Low
Glucose	Low	Low
TAG levels	Low	Low
Insulin	Low	Low
Glucagon	High	Low
Leptin	Reduced	Low

Pck1 expression (Zhang et al. 2011). Interestingly, the hepatic *Pck1* mRNA level in Zucker lean (ZL) rats with VAD status is not reduced in comparison with that in VAS ZL rats (Zhang et al. 2012). However, VA deficiency reduced the hepatic *Pck1* mRNA in Zucker fatty (ZF) rats (Zhang et al. 2012).

VA status and retinoids also regulate lipid metabolism in animals. In rats, VA deficiency led to the loss of the carcass fat, but not cholesterol, with the reduction of body mass (Brown and Morgan 1948). RA such as isotretinoin (13-*cis* RA) has been used to treat patients with acne. Interestingly, significant portion of those patients developed hypertriglyceridemia (Bershad et al. 1985). In addition, RA-treated patients with acute promyelocytic leukemia gained body weight (Tallman and Kwaan 1992). The treatments of RA (Gerber and Erdman 1981)- and RXR-specific agonist (LG100268) (Davies et al. 2001) induced hypertriglyceridemia in rats. We have shown that retinoids synergized with insulin to induce the expression of *Srebp-1c* in primary rat hepatocytes (Li et al. 2011). The two liver X receptor binding sites (Chen et al. 2004) that mediate the insulin-induced *Srebp-1c* expression are also RAREs in the *Srebp-1c* promoter (Li et al. 2011).

In addition to the hepatic gene expressions, the VA status has been shown to affect insulin and glucagon secretions in pancreatic islets (Chertow et al. 1987, 1994). The glucose-stimulated insulin secretion is impaired in islets or the pancreas isolated from VAD rats, which can be restored after the replenishment of VA status in those animals. Table 1 shows the comparison of changes of body weight, fat mass, liver mass (net), liver glycogen, and plasma levels of glucose, plasma triacylglycerols, insulin, glucagon, and leptin in fasting and VA deficiency conditions.

VA Use and Metabolism in Different Cells

For their use and storage, retinoids and carotenoids are delivered to cells and tissues via multiple ways (O'Byrne and Blaner 2013). Retinol esters and carotenoids can be circulated in lipoproteins. In the postprandial state, retinyl esters in chylomicrons and their remnants are taken up by most cells and tissues. Retinyl esters and carotenoids may also associate with very-low-density lipoprotein, low-density lipoprotein, and high-

density lipoprotein. In the absence of food intake, retinol is mainly circulated in a complex containing retinol-binding protein 4 (RBP4), transthyretin and thyroid hormone. As the VA metabolite-regulating gene expression, RA is also present in circulation.

Retinoids and carotenoids are metabolized in a variety of cells (Ross et al. 2012). In enterocytes, retinol can be re-esterified into retinyl esters. Upon the entry, β-carotene is hydrolyzed into retinal, which is reduced by NADH-dependent retinal/retinaldehyde reductase to retinol. Retinol is then esterified by lecithin retinol acyltransferase to form retinyl esters, which are packed in chylomicrons for the transport to the lymph system and ultimately the circulation.

The liver is a significant site of VA storage and metabolism (Ross et al. 2012). Within hepatocytes, retinyl esters are hydrolyzed by retinyl ester hydrolase to retinol, which is then either esterified by acyl-CoA retinol acyltransferase as a free retinol or esterified via lecithin retinol acyltransferase if retinol is bound to cellular retinol-binding protein (CRBP). CRBP-bound retinol can be oxidized to retinal by NAD(P)H-dependent retinol dehydrogenases (RDHs) and then to RA by retinaldehyde dehydrogenases (RALDHs). RA binds to cellular RA-binding protein and then enters the nucleus to regulate gene expression. Further metabolism of RA occurs through the cytochrome P-450 enzyme system CYP26 in the liver and brain. In the liver, carotenoids undergo cleavage, incorporation into and release as part of very-low-density lipoprotein or other lipoproteins for storage or transport to other tissues. Locally, retinol can be transferred to the hepatic stellate cells for storage as retinyl esters in lipid droplets.

The adipose tissues also play a role in retinoid storage and metabolism (Bonet et al. 2012). Adipocytes take up circulating retinoid in the forms of chylomicron retinyl esters and retinol-RBP complexes, store it as retinyl esters with fatty acids, and convert retinol into RA for the regulation of adipogenesis. All adipocytes can account for 15–20% of the whole body retinoid stores in rats.

Early studies had shown that the secretion of insulin and glucagon was impaired in the pancreas isolated from VAD rats as reviewed in (Chen and Chen 2014). 9-cis RA has been observed in pancreatic β-cells and insulin secretion cells (Kane et al. 2010). We have shown that RA, but not retinal, induced *Srebp-1c* expression in INS-1 insulinoma cells (Chen et al. 2009; Li et al. 2011). Retinal can only induce *Srebp-1c* expression in INS-1 cells when RALDH1 was overexpressed, suggesting that pancreatic β-cells lack the enzymes oxidizing retinal to RA, such as RALDH1 (Li et al. 2012). The role of VA in the function of pancreatic β-cells needs further attention.

Current Understanding of Changes of the Enzymes Involved in VA Metabolism in the Development of Metabolic Diseases

To date, multiple RDHs and RALDHs (also known as aldehyde dehydrogenases, ALDHs) have been thought to be responsible for RA biosynthesis (Blomhoff et al. 2006). The alterations of VA metabolism have been associated with the metabolic diseases such as diabetes and obesity.

In the blood of patients with diabetes, the activity of ALDH1, an important enzyme to clear lipid peroxidation-derived aldehydes in red blood cells, was increased in comparison with that of the control subjects (Giebultowicz et al. 2014). The increase of ALDH1 activity seems to be associated with the severity of the disease and may be a compensatory effect against oxidative stress. In addition, the upregulation of RALDH1 (ALDH1A1) expression level has been observed in the kidney of *db/db* mice with decrease of RA level in the tissue, demonstrating the alteration of RA production in the diabetic state (Starkey et al. 2010). The increase of 13-*cis* RA has been considered as a consequence of increased *Raldh3* expression in pancreatic islets of high-fat diet-induced diabetic mice (Shimamura et al. 2010). Overexpression of *Raldh3* reduced insulin secretion but increased glucagon secretion in vitro, indicating that RALDH3 disrupts the balance between insulin and glucagon, and may induce β-cell dysfunction leading to the development of type 2 diabetes mellitus. These results indicate that RALDH3 is also associated with metabolic disease.

RALDH1 has been associated with obesity development and energy balance (Ziouzenkova et al. 2007). We have shown that both *Raldh1* mRNA and RALDH1 protein levels were elevated in the liver of ZF rats, which may cause the excessive RA production and result in higher *Srebp-1c* expression (Li et al. 2012). This may attribute to the elevated lipogenesis in ZF rats. Mice with deletion of *raldh1* gene (*raldh1*−/− mice) are resistant to high-fat diet-induced obesity and insulin resistance (Ziouzenkova et al. 2007). Originally, this was attributed to the reduction of peroxisomal proliferator-activated receptor γ activation in the presence of retinal in adipocytes. Alternatively, it was thought that the deficiency of *Raldh1* gene leads to an elevation of thermogenic program in white adipose tissue, a process that may promote energy dissipation (Kiefer et al. 2012). This may explain that *Raldh1*−/− mice display reductions of gluconeogenesis and hepatic lipogenesis (Kiefer et al. 2012). Later, it was suggested that RALDH1-derived RA promotes adipogenesis and RALDH1 deficiency impairs all-*trans* RA production, therefore decreasing adipogenesis (Reichert et al. 2011). The role of VA in the adipose tissues deserves further investigation.

It has been shown that rats fed a high-fat diet had an elevation of the hepatic RALDH activity and *Raldh1* mRNA expression, which are associated with downregulations of hepatic *Adh1* and *Rdh10* mRNA expression levels (Zhang et al. 2015b). This was attributed to the cholesterol intake accompanied by the feeding of the high-fat diet (Zhang et al. 2015b). Interestingly, the mouse *Raldh2* gene promoter contains a binding site for SREBPs (Wang et al. 2001). In addition, oxysterol induced *Raldh1* and *Raldh2* expression levels in the mouse liver and hepatoma cells via the possible activation of liver X receptors, which leads to the upregulation of SREBP-1c (Huq et al. 2006). There seems to be a link between cholesterol metabolism and RA production, a topic that remains to be determined.

Current Understanding of the Regulation of the Enzymes Involved in VA Metabolism in the Response to Physiological Changes

Theoretically, the rise and fall of the dietary amount of micronutrients should also send a signal to the body regarding the nutritional statuses. This signal should be integrated with the signals of macronutrients and hormones to contribute to the dynamic changes of the metabolism of the host.

As shown in Fig. 1, both insulin (a hormone) and RA (a metabolite of VA) can regulate the expression of genes critical for the hepatic glucose and lipid metabolism. We have shown that insulin synergizes with RA to induce *Gck* gene expression in primary hepatocytes (Chen et al. 2009). The RARE in the hepatic *Gck* promoter has been identified as a site interacting with multiple nuclear receptors (Li et al. 2014). We have also shown that insulin synergizes with RA to induce the *Srebp-1c* gene expression in primary hepatocytes, which is mediated by the two liver X receptor elements identified previously (Li et al. 2011). In addition, RA has been shown to attenuate the insulin-suppressed *Pck1* gene expression in primary hepatocytes (Zhang et al. 2011).

The cycle of fasting and refeeding is associated with the changes of expression levels of hepatic genes. These changes include the reduced expression levels of *Gck*, *Srebp-1c*, and *Fas*, and increased expression level of *Pck1* in the fasting state, which were reversed after refeeding as indicated in Table 2. Interestingly, the expression

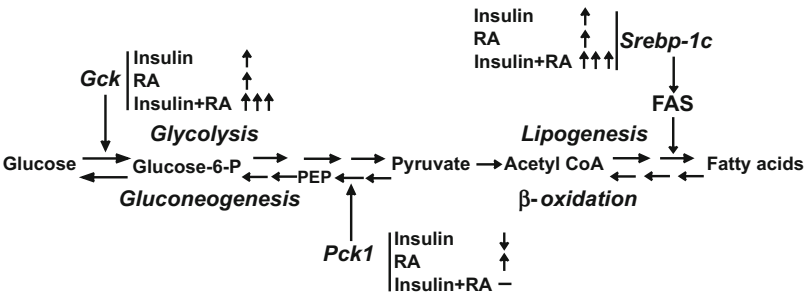

Fig. 1 Interactions of insulin and retinoic acid (RA) signaling in the regulation of critical genes for the hepatic glucose and fatty acid metabolism. Glucose is phosphorylated into glucose 6-phosphate by glucokinase (gene, *Gck*). Glucose 6-phosphate is converted into pyruvate via glycolysis. Pyruvate can be indirectly converted into phosphoenolpyruvate (PEP), a process that is mediated by phosphoenolpyruvate carboxykinase (gene, *Pck1*) in gluconeogenesis. Pyruvate is converted into acetyl CoA, which can be used for the synthesis of fatty acid via lipogenesis, a process mediated by fatty acid synthase (FAS). Acetyl CoA can also be derived from fatty acid through β-oxidation. FAS is induced by the activation of sterol regulatory element-binding protein 1c (gene, *Srebp-1c*), a gene that can be induced by insulin stimulation. RA synergizes with insulin to induce the expression levels of *Gck* and *Srebp-1c* genes. On the other hand, RA induces, and insulin reduces the expression level of *Pck1* gene. The presence of RA attenuated the insulin-reduced *Pck1* gene expression

Table 2 Comparisons of the changes of key representative hepatic genes of glucose and lipid in the cycle of fasting and refeeding

Genes	Fasting	Refeeding	VAS vs. VAD
Gck	Reduced	Increased	Higher
Pck1	Increased	Reduced	No change
Srebp-1c	Reduced	Increased	Higher
Fas	Reduced	Increased	Higher

levels of *Gck*, *Srebp-1c*, and *Fas*, but not *Pck1*, in the liver of VAD ZL rats experiencing the cycles of fasting and refeeding (Li et al. 2016) or fasted for 6 h (Zhang et al. 2012) were lower than that that of VAS ZL rats. This shows some similarities between VA deficiency and fasting as shown in Table 1. Indeed, the development of VA deficiency is associated with reduction of food intake (Chen et al. 2014; Zhang et al. 2012). It becomes reasonable when we observed that VA metabolism contributes to the regulation of the hepatic gene expression during the cycle of fasting and refeeding (Li et al. 2016).

It has been reported that refeeding after a 16 h fasting in mice resulted in the reduction of hepatic *Rdh1* and *Rdh10* mRNA levels and the reduction of hepatic RA content (Obrochta et al. 2015). This reduction of the gene expression can be observed in mice receiving the gavage of glucose or insulin injection after the fasting. The mechanism was attributed to the insulin-regulated activity of forkhead box protein O1 transcription factor, which regulated the hepatic *Pck1* gene expression (Obrochta et al. 2015). This insulin-reduced *Rdhs* expression can also be observed in cultured HepG2 cells (Obrochta et al. 2015). Unfortunately, when we analyzed the liver samples in VAS rats experiencing the cycle of fasting (48 h) and refeeding (for 6 h) (Li et al. 2016), no change of *Rdh2*, *Raldh1*, and *Raldh4* mRNA expression levels was detected (data not shown). Whether the refeeding time was not enough or too long for mRNA sample analysis or whether there is any change of the activities or protein levels of those enzymes remains to be determined.

Recently, we have observed changes of VA metabolic enzymes in another experimental setting related to fasting. VA deficiency leads to the reduction of food intake, which is similar to fasting state. Therefore, we have performed pair-feeding studies, in which ZL and ZF rats with VAS status consumed the same amount of the VAS diet in weight as the VAD diet consumed by VAD rats over an 8-week period of time (Chen et al. 2014). This caused a scenario, in which the VAS-pair-fed (VAS-PF) rats were in a fasted situation before they received their ration for the last day of the experiment. This was due to the reduced food intake of VAD rats in ad libitum state, which resulted in the lack of enough food for the VAS rats to consume over a 24-h period of time. Therefore, we created two groups of VAS-PF rats. The first group VAS-PF rats were allowed to consume as much VAS diet as they could (VAS-PF-AD group). The second group VAS-PF rats were fed the same amount of VAS diet in weight as that of VAD diet consumed by the VAD rats. To prevent the creation of a prolonged fasting state

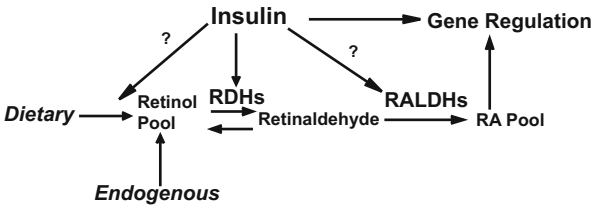

Fig. 2 Effects of insulin on retinol and retinoic acid (RA) pools for gene expression in hepatocytes. Retinol pool is derived from both dietary and endogenous sources. Retinol is reversibly oxidized into retinaldehyde by retinol dehydrogenases (RDHs), and retinal is irreversibly oxidized by retinaldehyde dehydrogenase (RALDHs) into RA. It has been shown that insulin can affect the expression levels of RDHs genes, which has the potential to regulate the sizes of retinol and RA pools. Whether insulin can regulate the entry of dietary retinol into cellular retinol pool and the activities or expression of RALDHs remains to be determined. RA and insulin work cooperatively to regulate gene expression in hepatocytes.

in VAS-PF rats, the VAS diet was divided into four equal portions and fed to them every 6 h (VAS-PF-AM). The VAS-PF-AD consumed more diet than the VAS-PF-4M rats during the 24-h period of time (Chen et al. 2014). The VAS-PF-AD ZL, but not ZF rats, had higher *Rhd2* and *Cyp26a1* mRNA levels than the VAS-PF-4M rats did. The VAS-PF-AD ZL and ZF rats had lower *Raldh1* mRNA level than the VAS-PF-4M rats did. The elevation of *Rhd2* and *Cyp26a1* expression levels suggests an increase of RA production as *Cyp26a1* is a very sensitive RA-responsive gene (Wang et al. 2002). As VAS-PF-AD rats consumed more VAS diet than VAS-PF-4M rats did, these changes of genes for VA metabolism and RA responses probably can be attributed to the effects of overfeeding on the VA metabolism in the liver.

We also have tested whether RA production is needed for the insulin-regulated hepatic gene expression (Li et al. 2017). We used streptozotocin to generate ZL diabetic rats with both VAD and VAS statuses and treated with vehicle control, insulin, RA, and RA + insulin for 3 h. The results show that insulin regulates the hepatic genes for glucose and lipid metabolism in VAD-diabetic rats, indicating that insulin functions in the absence of RA. However, RA still can modulate insulin-regulated gene expression in the liver of VAD-diabetic rats (Li et al. 2017).

As shown in Fig. 2, VA (retinol) in a hepatocyte can come from an endogenous pool or from a dietary source in a feeding status. During fasting, the VA from dietary source diminishes, which may change size of VA pool in hepatocytes. On the other hand, after feeding, the dietary VA enters the VA pool and contributes to the increase of the intracellular VA pool size. After a refeeding process, the dietary VA may be able to contribute to the cellular VA pool significantly. If insulin regulates the expression levels of genes involved in RA production, then these changes may indicate that there is a coordinative interaction between RA production and refeeding process. Theoretically, RA is dynamically produced and rapidly turned over inside a cell. At any moment, the cellular RA pool can be

from VA that derives from the dietary source or endogenously stored one. Since the plasma VA level is always maintained in a normal range for well-nourished individuals, the participation of dietary VA in the regulation of a hepatic gene expression can occur without any dramatic change of plasma VA levels.

Perspectives

VA was identified due to its ability to support the animal growth, an anabolic response. Famine, starvation, or any condition associated with reduction of food intake trigger catabolic states. The switch from fasting to feeding is accompanied by the changes of the expression levels of hepatic genes involved in glucose and lipid metabolism. The reduction of dietary VA as well as other macro- and micronutrients during the fasting should send the body signals to face the incoming catabolic changes. We have now begun to observe alterations of VA metabolism and its downstream RA signaling in response to the changes of feeding behavior. More studies are needed to define the role of VA in the fasting and starvation phase. This may help us to combat the food security and nutrient deficiency problems that may challenge human society when its population becomes larger and larger on this planet.

Policies and Protocols

The knowledge presented here has not been taken into policy considerations. However, the knowledge included here should be considered when policies are to be developed to deal with food or nutrition security. Many previous and current studies have demonstrated that vitamin A contributes to the glucose and lipid metabolism. Therefore, the ideal amount of micronutrients needed for the metabolism of macronutrients is something deserved to be considered. This will not only maximize the use of micronutrients in the prevention of nutrient deficiencies but also reduce the potential detrimental effects associated with overnutrition.

Dictionary of Terms

- **Glycolysis** – Glycolysis is the enzymatic pathway for the production of pyruvate from glucose.
- **Glucagon** – Glucagon is a catabolic hormone secreted from pancreatic α-cells to regulate metabolism.
- **Gluconeogenesis** – Gluconeogenesis refers to the enzymatic pathways for the production of glucose from noncarbohydrate precursors.

- **Insulin** – Insulin is an anabolic hormone secreted from pancreatic β-cells to regulate metabolism.
- **Lipogenesis** – Lipogenesis refers to the enzymatic pathways for the generation of lipids.
- **Nutrition** – Nutrition is the combined processes of nourishing an organism for the proliferation, growth, and aging across its life span.
- **Preformed vitamin A** – Preformed vitamin A refers to dietary molecules derived from animal products containing vitamin A activity such as retinol and retinyl esters.
- **Provitamin A** – Provitamin A refers to dietary molecules derived from plant products that can be used to generate retinol such as carotenoids.
- **Retinyl ester** – Retinyl ester is the retinol linked to a fatty acid through an ester bond.
- **Vitamin A** – Vitamin A is a micronutrient with activities derived from retinol or molecules leading to its production.

Summary Points

- Potential challenges of food security and nutrition due to the rise of human population on this planet require further understanding of the roles of nutrients in the control of human health.
- Diets provide both micronutrients with regulatory roles and macronutrients with energy for the body.
- The cycle of fasting and refeeding in the feeding behavior is associated variations of entries of micronutrients and macronutrients into the body.
- The liver is critical for the switch of fasting (a catabolic state) to refeeding (an anabolic state) in the metabolic homeostasis.
- The changes of hepatic gene expression levels are at least partially responsible for the switch of metabolic states.
- As an essential micronutrient, vitamin A was first discovered due to its role in the support of animal growth, an anabolic process.
- Recently, we have found that vitamin A status plays a role in the regulation of the hepatic glucose and lipid metabolism and body weight.
- The development of vitamin A deficiency in animals is associated reduction of body weight and expression levels of genes for glucose and lipid metabolism, very similar to the fasting or starvation.
- Vitamin A is oxidized into retinal and then to retinoic acid, which is the primary metabolite to regulate gene expression in cells.
- Insulin and retinoic acid work cooperatively to regulate expressions of genes involved in the hepatic glucose and lipid metabolism.
- Insulin may regulate the production of retinoic acid in hepatocytes.
- Vitamin A metabolism or retinoic acid production in hepatocytes may contribute the gene expression changes during the cycle of fasting and refeeding in the liver.

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Thiamine Deficiency and Poverty

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Abstract

Thiamine is a water-soluble vitamin, essential for normal cellular functions and energy production. As it is not synthesized by the human organism, it must be obtained by exogenous sources. A reduction in thiamine tissue concentration may interfere with numerous cellular mechanisms triggering off neurodegenerative processes with consequent changes in brain functions. Thiamine deficiency is common in certain groups like alcohol chronic users, pregnant woman, and people living in food insecurity situation. The low offer of thiamine for newborn and infants can compromise the correct maturation of the central nervous system and development of the cognition as explained by animal models studies. Thiamine deficiency during pregnancy and breastfeeding induces dysfunctions in the learning and consolidation of spatial abilities of offspring that can compromise the access of children living in food insecurity situations to formal job and education. Observed from this perspective, thiamine deficiency in vulnerable populations plays a more important role than the manifestation of clinical syndromes: the thiamine deficiency can be understood as a mechanism for perpetuating the poverty condition. Based on favorable risk/benefit profile, we recommend the fortification of easily accessible foods (such as wheat flour) with thiamine and oral replacement for alcohol dependent people, pregnant women, and infants living in situations of food insecurity.

Keywords

Thiamine · Deficiency · Poverty · Pregnancy · Prenatal nutrition · Breastfeeding · Child nutrition disorders · Malnutrition · Alcohol · Replacement

List of Abbreviations

WHO	World Health Organization
RDA	Recommended dietary allowances
THTR-1	Thiamine transporter 1
THTR-2	Thiamine transporter 2
ICD-10	International Classification of Diseases
AUDIT	Alcohol use disorders identification test
HPLC	High performance liquid chromatography
FAO	United Nations Food and Agriculture Organization
HFSSM	US Household Food Security Survey Measure
USDA	United States Department of Agriculture
CNS	central nervous system
GABA	gamma aminobutyric acid

Introduction

Vitamin B1, thiamine, reveals various biological functions and its deficiency can cause significant physical damage not only to the individual but also to the community. As the deficiency is related to social vulnerability situations, understanding the relation of thiamine deficiency with poverty may have repercussions on interventions with a high potential for changing the situation of the vulnerability of specific communities. Thus, this chapter seeks to show the biological functions of thiamine; to discuss the relationship of the deficiency in special situations, for instance, in the use of alcohol, during gestation, and breast-feeding; and to list these conditions with poverty and show health interventions.

Biological and Nutritional Aspects

Thiamine is a water-soluble vitamin, essential for normal cellular functions and energy production. Its chemical structure consists of a pyrimidine linked to thiazole by a methyl bridge; both fractions (pyrimidine and thiazole) are necessary for its biological activity. It is stable in pH acid, but becomes unstable in alkaline solutions, high temperatures, or when exposed to ultra violet light (Sardesai 2003; Bémeur and Butterworth 2014).

As it is not synthesized by the human organism, it must be obtained by exogenous sources through food and food supplements (Bettendorff 2012). It can also be produced by microbiota bacteria normal bowel, possibly by local nutrition of the colonocytes, as until now the contribution of this by human metabolism has not been proven.

It is found in the organism mainly in its phosphorylated forms: thiamine monophosphate, thiamine diphosphate, and thiamine triphosphate. About 80% of thiamine found in the body corresponds to thiamine diphosphate, its main active form; 10% is found in the form of thiamine triphosphate and the rest corresponds to free thiamine and monophosphate (Fig. 1) (Sardesai 2003).

Therefore, its presence is essential for the production of energy by the Krebs Cycle, lipid metabolism and glucose, the production of amino acids of the branch chain, and the production and maintenance of the myelin sheath. As well as this, it is necessary for the synthesis of the acetylcholine neurotransmitters, the acid aminobutryic gamma, and glutamate (Monograph, 2003). Its action on the pathways of glucose utilization has great importance on the central nervous system, which needs continuous supply of this nutrient. Thus, the neurobiological repercussions of thiamine deficiency have great clinical importance and will be prioritized in this chapter.

Food Sources

Thiamine is present in various food sources vegetable and animal. However, in larger quantities, it is found only in periocarp and cereal germ. Therefore, as a great amount is lost during its refinement process, many industrialized food stuffs are enriched with this nutrient (Sardesai 2003).

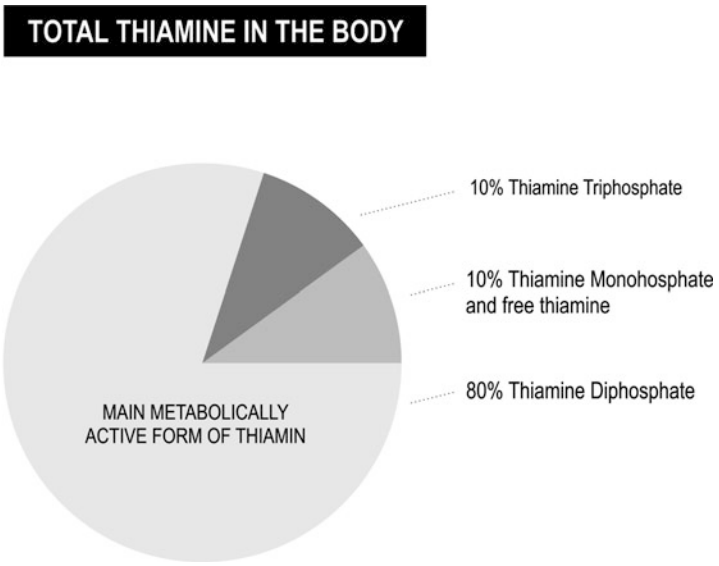


Fig. 1 Thiamine forms in human body. Approximate distribution of thiamine in human body (Source: Sardesai 2003)

Its natural sources include yeast, whole grains like wheat, oats and rice, meat (especially pork), green vegetables, fruit, roots, and legumes. It is not found in fat, oils, or sugar, and milk and derivatives are not considered good sources (Bettendorf 2012).

The breast milk of a well nourished mother supplies adequate quantities of thiamine. However, as the maternal diet is the main determinant of babies nutrition, those breast fed by mothers with lack of thiamine are at risk of developing the deficiency (Who 1999). Thiamine is stable for storage, but the way of preparing the food can change the final content.

Procedures that involve heating up, like prolonged boiling and milk pasteurization, can result in a considerable loss of vitamins, which are sensitive to high temperatures. There are also important losses in the discarded boiling water, since it is a water-soluble vitamin. That is why re-use of the water is recommended for the preparation of other food.

Dietary Recommendations

The recommended daily ingestion (RDA) is shown in Fig. 2 (IOM 2006).

Generally oral ingestion of high doses does not present adverse effects (Sardesai 2003). However, its intravenous administration may lead to respiratory depression and neuromuscular block. In human beings, the anaphylactic reactions are a rare complication of intravenous administration of thiamine (Bettendorff 2012).

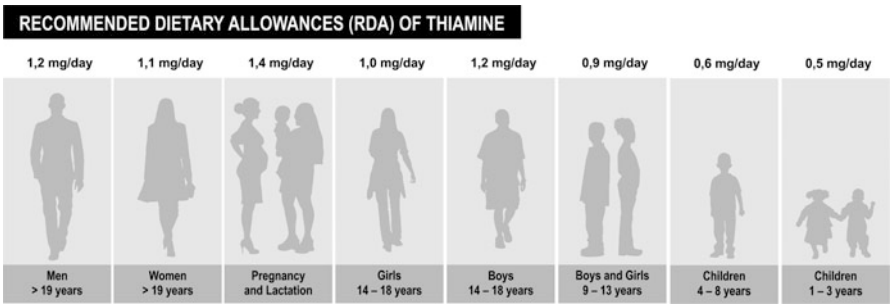


Fig. 2 Recommended Dietary Allowances (RDA) of thiamine by age range. Recommended dietary allowances represent the minimum amount of thiamine ingestion for a healthy person. These values may be insufficient in presence of some diseases (Source: IOM 2006)

Absorption and Metabolism

Adequate ingestion and intestinal absorption determine the availability of thiamine for the biological functions. Its absorption is measured by specific carriers, in a process regulated by innumerable factors and conditions, as what happens in relation to other water-soluble vitamins.

In foods of plant origin, most of the vitamins are found in a nonphosphorylated form. The opposite happens with vitamins of animal origin, in which the predominance of thiamine is in phosphorylated form (Sardesai 2003). To be absorbed, the phosphorylated forms are hydrolyzed by phosphatases in the intestinal lumen and converted into free thiamine, capable of penetrating the cell membrane from two mechanisms depending on the concentration.

1. In concentrations superior to 2 μm, thiamine is absorbed by passive diffusion.
2. In concentrations lower then 2 μm, it is absorbed by an active process depending on sodium and ATP, with the involvement of a specific carrier.

The carriers involved are THTR-1 and THTR-2 expressed in the small and large intestines expressed more significantly in the small intestine (Subramanya et al. 2010).

Following the intestinal absorption, the thiamine is carried in the blood to the liver, both to the erythrocytes and the plasma which are linked to proteins.

It is stored in low quantities, around 25–30mg, above all in the organs with high metabolic need, like the skeletal muscle, the heart, the brain, the liver, and kidneys. Figure 3 shows a simplified schematic of the process described above.

As thiamine is not stored in significant quantities, a continuous supplement of this vitamin is through food consumption, as in a state of insufficient intake, signs of deficiency may appear within 2–3 months (Who 1999) and in the case of absence, body reserves can run out in 2–3 weeks (Sechi et al. 2016).

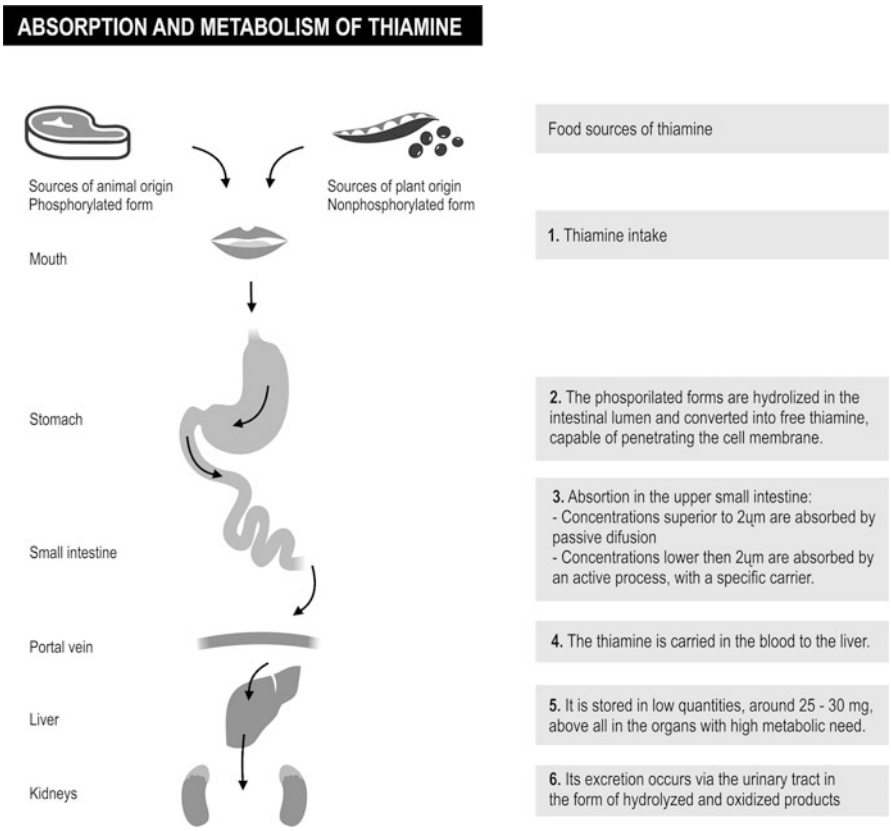


Fig. 3 Absorption and metabolism of thiamine. Simplified absorption and metabolism of thiamine in the human body (Source: Unpublished personal figure)

The main conditions of imbalance which can provoke its deficiency include: chronic use of alcohol, bad nutrition, loss of thiamine due to recurrent vomiting or bad absorption, an increase in the necessity for thiamine due to pregnancy or chronic illnesses, prolonged consumption in excess of carbohydrates, and gastrointestinal surgical procedures including gastrectomy and bariatric surgery (Sechi et al. 2016).

Clinical Thiamine Deficiency

Initial deficiency of thiamine can generate subjective complaints such as drowsiness, stomachache, constipation, weakness of the limbs, fatigue, postprandial plentitude, irritation, paresthesias, edema, palpitations, and lack of memory (Who 1999).

The symptoms can persist in this chronic state or as the deficiency progresses, more specific symptoms may appear, characterized by an acute condition with cardiac involvement and edema or peripheral neuropathy, due to the inhibition of a

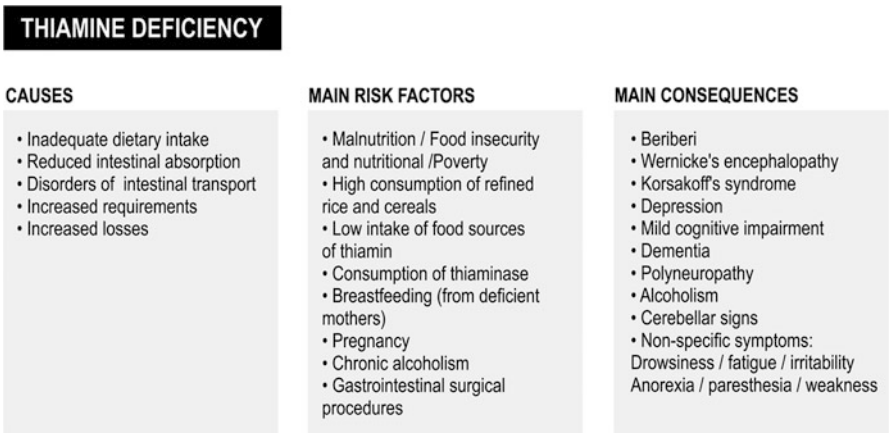


Fig. 4 Main causes, risk factors, and consequences of thiamine deficiency. The squares represent (from left to right) the main causes of thiamine deficiency, risk factors associated with this form of subnutrition, and the possible clinical outcomes (Source: Unpublished personal figure)

series of reactions in which thiamine dyphosphate acts as a coenzyme, thus, establishing Beriberi, one of the main diseases related to the major deficiencies of thiamine.

The World Health Organization (WHO) advises against using the term Beriberi substituting it for the term “thiamine deficiency with peripheral neuropathy” in cases of manifestations related to dry Beriberi, “thiamine deficiency with cardiopathy” referring to wet Beriberi and "thiamine deficiency with lactic acidosis” referring to Beriberi Shoshin (Who 1999).

Another related form of thiamine deficiency affects infants, breastfed by mothers with thiamine deficiency, usually asymptomatic. As the developing brain is more sensitive, symptoms appear acutely and mortality rates are high, with death occurring within a few days. The initial aspect is characterized by restlessness, crying, constipation and occasional vomiting, progressing with generalized edema, dyspnea, cardiac disturbance, gastrointestinal disorders, and oliguria (Who 1999).

Figure 4 summarizes the main causes, risk factors, and consequences of thiamine deficiency.

Poverty, Chronic Use of Alcohol, and Thiamine Deficiency

The Use of Alcohol and Thiamine

Among the biological consequences of the harmful use of alcohol, there is a high prevalence of the deficiency of complex B vitamins (Cook et al. 1998), of which thiamine is prominent. The chronic use of alcohol provokes a reduction in the absorption of thiamine, impaired use, increased metabolic demand, reduced hepatic stock, increase of urinary loss, as well as the usual inadequate global nutritional

intake (malnutrition) of these individuals. In relation to intestinal absorption, chronic alcohol consumption exerts a specific tissue effect, causing a significant inhibition of the absorption of thiamine through the brush and basolateral border membranes, associated to a decrease in the expression of the THRT-1 carrier in both membranes, apparently without affecting the carrier THTR-2 (Subramanya et al. 2010). It can be concluded that people who have problems with the use of alcohol present more health problems caused by the lack of thiamine.

The classification system of mental disorders "International Classification of Diseases" (ICD-10) categorizes the problems related to the chronic use of alcohol in abuse and dependence (ICD-10 2016). In general, abuse is characterized by the recurrent use of the substance leading to possible loss of capacity to deal with daily obligations, legal, social interpersonal and risk exposure, within a period of twelve months. Dependence is defined by the continued use of the substance, with a set of cognitive, behavioral symptoms characterized mainly by tolerance, withdrawal symptoms, and compulsion.

Screening individuals who make bad use of alcohol is justified by the increased risk of harmful consequences for their physical and mental health, as well as the problems caused to their families and society, since the patterns of dangerous consumption are of great importance for public health. Hence, diagnostic instruments that allow the health team to stratify the use are fundamental (Babor et al. 2001).

For the screening and detection of people with alcohol use, two instruments will be shown which have easy application and are extremely valid: CAGE (Fig. 5) and AUDIT (Fig. 6).

In the case of using CAGE, a result is considered positive when an affirmative answer is obtained for two or more questions. It is important to emphasize that the questionnaire does not diagnose dependence but rather demonstrates the high probability of the presence of the disorder, which should alert the interviewer to continue the approach, as these people would benefit from reducing or stopping consumption of alcoholic beverages (Ewing 1984). Recommendation of the use of CAGE lies in its high sensitivity and the possibility of any professional in the health area to apply it and start a closer relationship with the patient.

As thiamine plays a key role in the central nervous system, alcohol-induced deficiency can lead to a severe neurodegenerative process, culminating in neuronal death. In addition to thiamine deficiency, the direct neurotoxic effect of alcohol acts

Fig. 5 CAGE Questionnaire: questions and interpretation. Questionnaire for screening problems with alcohol use. Positive screening in CAGE questionnaire demands further diagnostic evaluation (Source: Ewing 1984, p. 1907)

AUDIT QUESTIONNAIRE

Question domain	Question	Content
Hazardous alcohol use	1	Frequency of drinking
	2	Typical quantity
	3	Frequency of heavy drinking
Dependence symptoms	4	Impaired control over drinking
	5	Increased salience of drinking
	6	Morning drinking
Harmful alcohol use	7	Guilt after drinking
	8	Blackouts
	9	Alcohol-related injuries
	10	Others concerned about Drinking
Result	**Score**	**Recommended course of action**
Questions have a score range of 0 – 4 points	0 - 8	Advice focused on the reduction of hazardous drinking.
	16 - 19	Brief counseling and continued monitoring
	20 or more	Further diagnostic evaluation for alcohol dependence

Fig. 6 AUDIT Questionnaire: questions, scores, and interpretation. Audit table contents are adapted from *World Health Organization – the alcohol use disorders identification test*. Full explanation and printable version of the test can be found at: http://www.who.int/substance_abuse/publications/audit/en/

in conjunction, revealing a higher prevalence of neuropsychiatric conditions in these patients (Bettendorff 2012). Thus, the neurodegenerative processes caused by alcohol use may provoke mild clinical cognitive, impairment, dementia, Wernicke's encephalopathy, or polyneuropathy (Zimatkin and Zimatkina 1996).

Approximately 50–80% of individuals in chronic use of harmful alcohol have, to some extent, cognitive functions impaired, which is sometimes not diagnosed (mild cognitive impairment that does not bring about functional problems, only diagnosed by neuropsychological tests; or dementia, with impact on the functional life of the patient). In these patients, diffuse atrophy of the central nervous system (enlargement of the ventricles and cortical reduction) and significant hippocampal atrophy can be observed. The clinical characteristics of impairment include limited learning capacity, coding, and

memory recall deficiency and altered visuospatial and executive functions (Bernardin et al. 2014). Patients who have cognitive alterations due to the use of alcohol seek treatment the least, making their condition worse. Therefore, a cognitive approach is an important health practice for the treatment of people with alcohol problems.

Polyneuropathy caused by chronic thiamine deficiency is characterized by being distal, sensorimotor, with greater involvement of the lower limbs in comparison to the superiors. Its onset is usually slow, with bilateral paresthesia, distal and painful. Distal motor weakness can lead to the illness known as "fallen foot" which is relatively common with variable muscle weakness in the lower limbs (Sechi et al. 2016).

Wernicke's encephalopathy occurs acutely and is characterized as triad: mental confusion, ophthalmoplegy, and ataxia, but not all elements are present at diagnosis (Bettendorff 2012). Its manifestation is commonly associated with alcohol abstinence, but it can also occur with people who are nonconsumers, for example with patients with gastric carcinoma (Zimatkin and Zimatkina 1996).

In these people, a significant reduction in the activity of thiamine-dependent brain enzymes is observed. Its neuropathology characteristic involves neuronal loss, microhemorrhages, and gliosis in the mammillary region and in the paraventricular periaqueductal (Thomson et al. 2009). Although this is common, it is often undiagnosed (Thomson et al. 2009).

Early treatment with high doses of thiamine permits a quick reserve of some cognitive and neurological deficiencies as well as preventing the development of structural lesions. To avoid further complications, thiamine must be administered prior to glucose replacement (Galvin et al. 2010).

If left untreated, Wernicke's encephalopathy may lead to death (20% mortality) or Korsakoff syndrome can develop which can be understood as chronicity, which is irreversible even with the use of thiamine (Sechi et al. 2016). Patients that develop Wernicke's encephalopathy because of other conditions not associated with the use of alcohol rarely evolve Korsakoff syndrome, indicating the synergistic effect of thiamine deficiency and alcohol neurotoxicity (Thomson et al. 2009).

Clinically, Korsakoff syndrome is characterized by recent memory impairment and affected individuals tend to repeat the same questions and fail to recognize people they know after the onset of the illness. In spite of mainly affecting the consolidation of recent memory, there may be an effect on past memory too. The individuals become disorientated and tend to invent situations and fill in memory gaps with false facts (Thomson et al. 2009).

The structural brain lesions found in Korsakoff's psychosis affect mainly the mammillary region, the mammillothalamic tract, and the anterior thalamus (Sechi et al. 2016), and the amnesia is probably due to the interruption of the diencephalic-hippocampal circuits (Thomson et al. 2009). That is, it is an extremely severe, dysfunctional condition that must be prevented with the replacement of thiamine for patients who develop Wernicke's encephalopathy.

In view of this, it is understood that for this group that consumes alcohol in a chronic and harmful way and that have an insecure food diet, the prophylactic use of thiamine must be considered, seeking to prevent all of the mentioned clinical disorders. In spite of the replacement of thiamine orally showing some controversy

concerning its effectiveness, such as the inhibition of intestinal absorption of thiamine by alcohol, a recommended practice for harm reduction (Galvin et al. 2010). In the case of prophylactic administration via parental, there are no studies evaluating its benefits nor evidence of effective dosing, but the biggest difficulty of administration, cost, and human resources may hinder its use.

Also, once people with alcohol problems present increased risk for other vitamins of B Complex, replacement of thiamine together with the other complex B vitamins necessary is recommended (Sechi et al. 2016).

In addition, knowing that the metabolism of thiamine and of magnesium are interdependent, it is fundamental that the levels of this micronutrient are monitored; this is because deficiency can cause thiamine tissue loss and reduction of enzymatic activity, as well as impairing the response to the administration of thiamine (Zieve 1969).

We suggest a simple and low cost approach to be applied in chronic alcohol users (Fig. 7), which could signal early the need for thiamine replacement, in order to prevent further harm to the health of these individuals.

The Use of Alcohol and Poverty

Although alcohol is a common denominator in the lives of many poor families, there are few strategies for addressing poverty in relation to its use (Samarasinghe 2014).

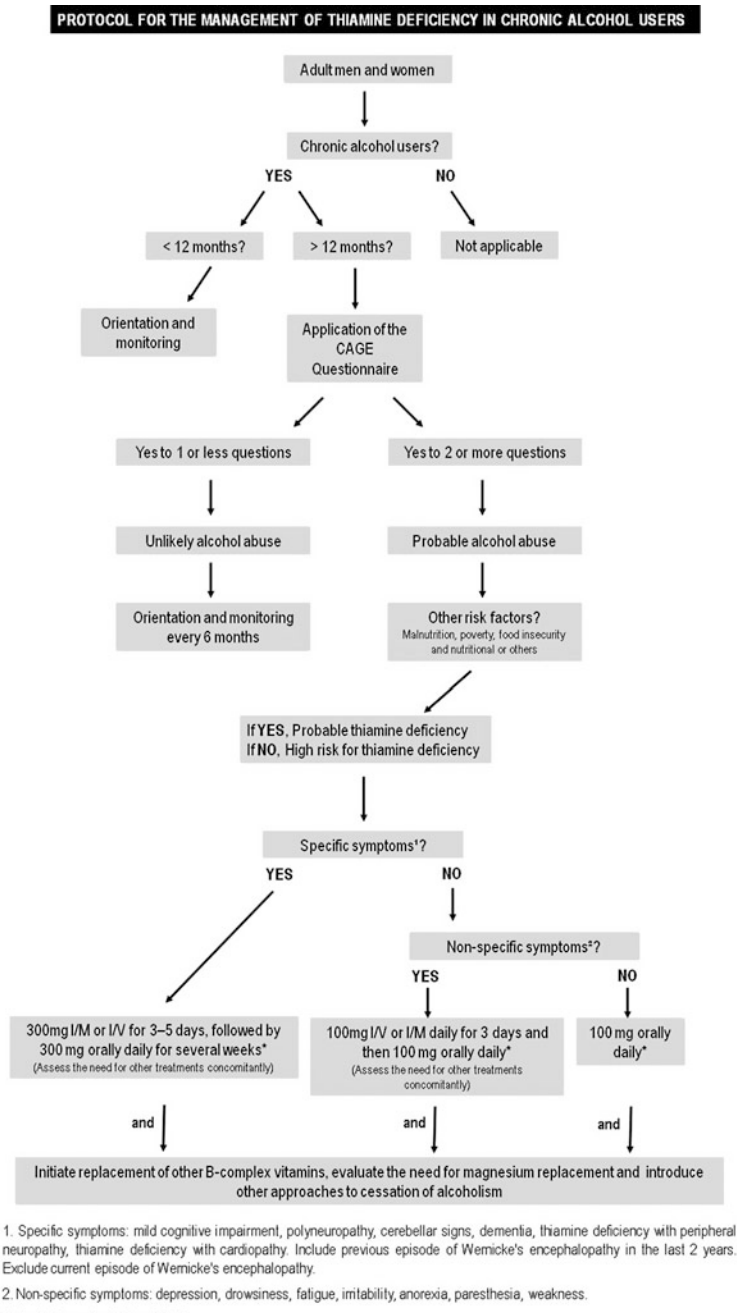
Alcohol can influence people's economic status, just as economic status can determine the use of alcohol. In the case of poor families, its use can have a great negative influence, since they are economically vulnerable and small changes can destabilize their daily economy, reducing their disposable income for basic necessities, such as food, being significantly responsible for poverty, and precarious health (Samarasinghe 2014).

Excessive use of alcohol contributes to prolonged social economic problems, interfering with education, health, and nutrition. It contributes to an increase in criminality, of domestic violence, overloads the health system, and is one of the main causes of incapacity.

It is therefore extremely important to investigate how poverty and chronic consumption of alcohol are interlinked, in order to take a new look at public policies, due to the fact that the reduction of poverty should not only focus on the increase of family income but in the way these families use the money available, as little as it may be (Samarasinghe 2014).

Even though of a lower socioeconomic level this does not mean that these people drink more than others, they seem to be more vulnerable to the consequences of alcohol consumption, due to lack of resources. They have less ability to avoid the adverse consequences of this behavior, making access to support health services difficult (Who 2014). The cause of thiamine deficiency, like poor alimentation (food), also neurobiological and behavioral can be explained, at least partially, the main deleterious impact is that the use of alcohol causes social vulnerability.

Approximately 30–80% of alcohol dependents have thiamine vitamin deficiency (Cook et al. 1998). This deficiency can make socioeconomic conditions worse for those who live in poverty. For example, the initial manifestations of deficiency can



1. Specific symptoms: mild cognitive impairment, polyneuropathy, cerebellar signs, dementia, thiamine deficiency with peripheral neuropathy, thiamine deficiency with cardiopathy. Include previous episode of Wernicke's encephalopathy in the last 2 years. Exclude current episode of Wernicke's encephalopathy.

2. Non-specific symptoms: depression, drowsiness, fatigue, irritability, anorexia, paresthesia, weakness.

* Adapted from: Latt, Dore, 2014.

Fig. 7 Suggested protocol for the management of thiamine deficiency in alcoholics. Suggested protocol for the management of thiamine deficiency in alcoholics. These individuals should be guided and monitored continuously, as well as the doses of thiamine administered and symptomatology reported individually (Source: Unpublished personal figure)

include irritability, fatigue, queasiness, and depression (Cook et al. 1998). These symptoms may harm the individual's family relationship, leading to a decrease in their productivity at work and an increase in the use of alcohol which, in the long term, can lead to neuropsychiatric impairment and the appearance of irreversible lesions, causing definite incapacitation.

Thus, it can be seen that in the face of a complex association between poverty and alcohol use, evaluation of nutritional aspect, specifically thiamine, becomes a fundamental health measure in vulnerable communities. The replacement of thiamine can represent a reduction in harm caused by the use of alcohol, diminishing the social impact of its use.

Pregnancy, Breastfeeding, and Thiamine

Pregnancy and Social Vulnerability

The state of pregnancy is responsible for the depletion of various metabolites essential for the maintenance of maternal homeostasis and fetal development (Black et al. 2008; Swaminathan et al. 2015).

This occurs due to an increase in metabolic demand for the formation of the fetus. Thiamine deficiency in pregnancy is related to an increase in morbidity and mortality of babies, especially if associated with other risk factors for malnutrition. As thiamine acts in metabolic processes fundamental for the Central Nervous System, its deficiency, from the neurobiological point of view, can cause neurodegenerative processes and consequent impairment in cognitive and emotional functions.

Thiamin deficiency is not a rare phenomenon in pregnancy and can occur in up to 27% of healthy pregnant women in the first 3 months of pregnancy, even as they receive adequate nutritional oral supplementation of this vitamin (Bakker et al. 2000; Baker et al. 2002). In part, this deficiency can be explained by the quick build-up of this vitamin and its metabolites on the fetal tissues (Bakker et al. 2000).

There is a relationship between groups with social vulnerability, that is, people living in extreme conditions and with thiamine deficiency. Groups of prisoners, people in refugee camps, and populations in situations of extreme poverty are more inclined to develop deficiency of this nutrient (Basoglu et al. 2006; Adamolekun 2010).

Thus, in vulnerable populations, thiamine deficiency in pregnancy is even more frequent: research done in a refugee camp showed that 58% of pregnant women had deficiency of this micronutrient, even after oral supplementation. In the population considered in this study, thiamine deficiency was the main cause of infant mortality (Mcgready et al. 2001), a fact that alerts us of the severe effects of malnutrition associated to pregnancy. It is emphasized that the methods of thiamine dosage and its concentration in the blood are difficult to be used in clinical practice, or to show indirect results, such as the evaluation by the activity of transketolase, or for being expensive as is the use of HPLC equipment. Therefore, it is soon realized that the identification of pregnant women in situations of social vulnerability is an important practice in order to diagnose thiamine deficiency in pregnancy.

Poverty and other social conditions of vulnerability are directly linked to food insecurity. The United Nations Food and Agriculture Organization (FAO) defines food security as "A situation that exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life" (FAO 2002). Thus, in general terms it can be assumed that food insecurity is caused by the absence of consistent, adequate food in sufficient quantity (Wight et al. 2014).

The measurement of food insecurity can be done by various methods, each one associated to specific limitations and advantages. Among these methods are included anthropometric evaluations, of household expenses and of food consumption, and national inquiries about the available caloric per capita. All of these methods, however, are indirect measures of the phenomenon, being done by the use of scales based on a food insecurity experience which is the only one in writing (Pérez-Escamilla et al. 2008). Of the scales available, the US Household Food Security Survey Measure Stands Out (HFSSM), whose development was coordinated by The United States Department of Agriculture (USDA) bringing with it, its main advantage, ample use, and validity in a wide range of languages and for various contexts sociocultural (Table 1) (Pérez-Escamilla et al. 2008; USDA 2012).

Scales such as the HFSSM provide objective parameters for screening at-risk families. The use of these tools may constitute an important health practice for the evaluation of groups in food insecurity situations especially, for pregnant women and children, increasing sensitivity of the screening programs, nutritional evaluation, and prenatal care. Not less important, its use enhances the stratification of necessary risks for the definition of public health policies, as well as allowing early intervention – before they become perceptible clinical manifestations and laboratory tests for malnutrition.

Thiamine Deficiency and Perpetuation of Poverty

Although clinical studies on the effects of maternal thiamine deficiency and their impacts on the cognitive development of a newly born baby are still scarce, there are solid indications that point to this phenomenon (Dias et al. 2013).

Thiamine plays a key role in neuronal physiology (Zhang et al. 2011). In CNS, in addition to the well-described metabolic function of thiamine as a cofactor of enzymes involved in glucose metabolism and in the synthesis of neurotransmitters, various researchers have suggested other roles nonmetabolic for thiamine. Among these functions, stabilization of the structure and function of the plasmatic membrane, participation in phosphorylate signal transduction paths, and action against agents which induce cytotoxicity can be highlighted (Bâ et al. 1996; Bâ 2008). In addition, studies suggest that thiamine may play a role in the modulation of iconic channels and nerve conduction (Ramakrishnan et al. 1999; Oliveira et al. 2007).

A reduction in thiamine tissue concentration may interfere with numerous cellular mechanisms triggering off neurodegenerative processes with consequent

Table 1 HFSSM score interpretation

For households with one or more children	
Raw score zero	High food security
Raw score 1 – 2	Marginal food security
Raw score 3 – 7	Low food security
Raw score 8 – 18	Very low food security
For households with no child present	
Raw score zero	High food security
Raw score 1 – 2	Marginal food security
Raw score 3 – 5	Low food security
Raw score 6 – 10	Very low food security

Source: Pérez-Escamilla et al. (2008), USDA (2012)
HFSSM = US Household Food Security Survey Measure. This scale composed by different sections based on the households characteristics: questions for all households, question for households with one or more children, and questions and adult referenced questions. Low and very low food security are positive screening for food insecurity

changes in brain functions (Martin et al. 2003). It is interesting to note that the neurogenerative process does not happen in a generalized way in the CNS, because there are brain regions more sensitive to the deficiency like thalamus, striated, and cerebellum. We infer that the greater sensitivity of neuronal areas reinforces the importance of thiamine in other biological processes in the CNS and not only as a coenzyme of carbohydrate metabolism.

Animal models demonstrate that thiamine deficiency during pregnancy and breastfeeding induces dysfunctions in the learning and consolidation of spatial abilities of offspring. This phenomenon is probably related to deficiencies in systems GABA and glutamate neurotransmitter (Dias et al. 2013). Furthermore, maternal thiamine deficiency is associated to motor development and also to memory formation of the offspring. There were losses in neurobiological processes such as cell proliferation, migration, and differentiation and synaptogenesis, axogenesis, and myelinogenesis (Bâ 2011). Therefore, thiamine deficiency during pregnancy and breastfeeding can cause damage to the formation and structuring of the baby's brain tissue, leading to cognitive impairment.

In this way, children of mothers who live in a situation of social vulnerability are subject to the risks of malnutrition and, consequently, of thiamine deficiency. It should be remembered that these children are exposed to hostile environments, with a low supply of nutrients and lack of psychosocial stimuli fundamental to their development. Born into these conditions, there is a commitment to the full development of their academic abilities being productive in the future. Children coming from this deprived environment can become adults unable to achieve social mobility, that is, to get out of poverty. In this scenario, it is understood that continuing in poverty is not only due to social factors, such as the lack of an educational system of quality, malnutrition, more specifically thiamine deficiency, constituting a determinant factor of poverty in communities with social vulnerability.

The Approach to Thiamine Deficiency During Pregnancy and Breastfeeding

The pharmacokinetics of thiamine and its response-curve to maternal supplementation is still not well established (Coats et al. 2013, Mcgready et al. 2001). The rapid growth of the fetal brain during the third trimester of pregnancy renders it vulnerable to inadequate nutrient supply (Drewett et al. 2001) and the deficiency of thiamine, in this manifestation of clinical and subclinical conditions of this hypovitaminosis. This scenario is aggravated by the maintenance of insufficient intake of this vitamin through breastfeeding, if maternal deficiencies are not corrected.

However, there are challenges to supplementing thiamine, aimed at treating the deficiency. It is proposed that the thiamin concentrated in maternal (human) milk should rise proportionally to the serum increase of the oral supplement. However, plasma levels of thiamine in newborns are not adequately corrected by short-term maternal supplementation even though such supplementation is able to normalize thiamine levels in the milk of these pregnant women (Coats et al. 2013). In this way, new born babies to mothers known to be deficient may need immediate replacement, even if they consume milk containing adequate levels of thiamine. Thus, direct replacement of thiamine for undernourished new born is recommended, considering that the recent neonatal or breastfeeding period is the most vulnerable to thiamine deficiency, in terms of adequate neurodevelopment of the offspring (Bâ 2005).

The current recommendations for nutritional interventions in the prenatal period by WHO are favorable to recommending a balanced diet and exercise during pregnancy for all women, besides the increase in caloric and protein intake in undernourished populations (Who 2016). However, "WHO" does not recommend maternal oral supplementation of thiamine or multivitamins and minerals in communities with social vulnerability (Who 2016). This fact is justified, primarily, by the small number of studies that are based on this replacement practice being safe and recommended. Therefore, there is a need for deeper investigation about the role of thiamine during pregnancy and breastfeeding, as well as the consequences for the woman and the baby, so that clinical replacement protocols are the best recommendation (Ramakrishnan et al. 1999).

(i) To identify the pregnant women in a risk situation by means of questionnaires and food insecurity evaluations, preferably adapted to and valid for the place and its reality.
(ii) To fortify nonperishable foods (such as flour), with thiamine. This is a simple public health measure, which is cheap and safe, for the prevention of the deficiency of vitamin B1, especially in populations where food insecurity exists (Harper 2006).
(iii) To begin oral replacement of thiamine in pregnant women proven to be vulnerable. Extend this supplement to the infants.

It is concluded then that there is a gap in the literature of systematic studies in which (i) the mothers are evaluated in the various stages of pregnancy in relation to thiamine deficiency and (ii) the impact of oral replacement in negative outcomes for

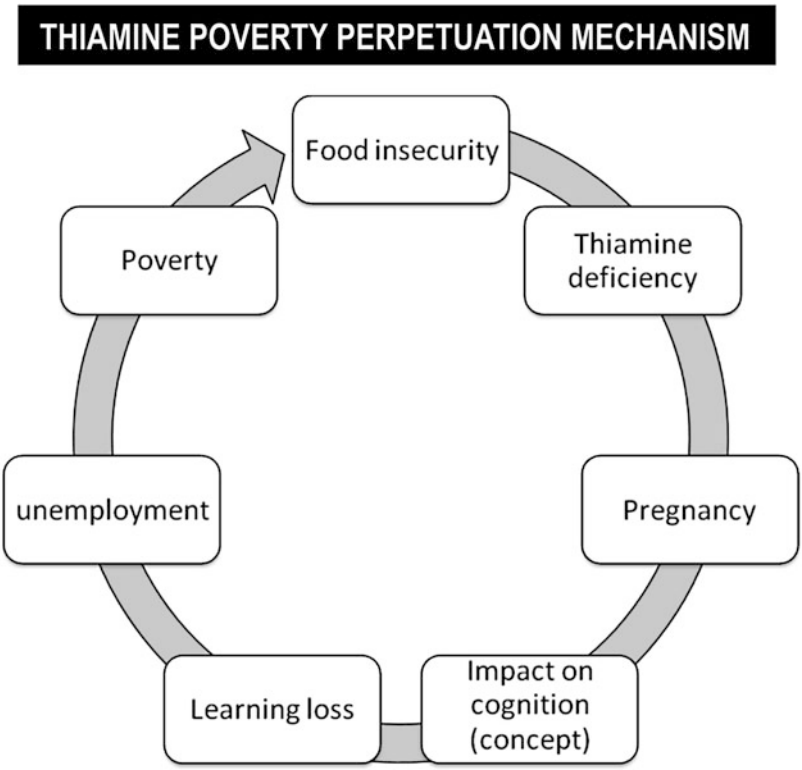


Fig. 8 Thiamine poverty perpetuation mechanism. Mechanism proposal for poverty perpetuation system associated with thiamine deficiency (Source: Unpublished personal figure)

pregnant women and the newly born. This need for studies is more noticeable for samples from populations of a low socioeconomic level, more vulnerable to multidimensional damage caused by malnutrition.

Finally, it is observed that the damage caused by thiamine deficiency goes further than its clinical manifestations they constitute, above all, a vicious cycle of perpetuation of the condition of poverty and vulnerability (Fig. 8).

Policies and Protocols

Public Policies for Prenatal Support and Fight Against Malnutrition and Alcoholism

As a way to minimize the negative impacts of thiamine deficiency in vulnerable populations, we recommend to adopt the following policies:

- Identification of potential vulnerable groups: refugees, people living in extreme poverty, exposed to urban violence, and children and pregnant women from low-income populations.
- Identification of adults, children, and pregnant women at food risk.
- Fortify nonperishable foods (such as wheat flour) with thiamine.
- Create and support programs to combat alcoholism with the support of local groups and initiatives.
- Invest in policies of social and labor reintegration to chronic alcohol dependents.
- Extend coverage of prenatal care programs to vulnerable and impoverished populations.
- Ensure adequate dietary intake for children and pregnant women in food risk.

Protocols for Food Insecurity Screening and Thiamine Supplementation in Children and Pregnant Women

- Identify food insecure situation through the Household Food Security Survey Measure (or adapted version of this questionnaire for local reality). The application of the questionnaire should take into account the presence or absence of children at home and their application instructions followed. Residents of a household should be considered food insecure if the score on the questionnaire is equal to or greater than 3.
- Consider extending thiamine replacement to mothers and infants at nutritional risk.

Protocols for Food Insecurity Screening and Thiamine Supplementation in People with Alcohol-Related Problems

- Screen all adolescents and adults, in all primary care consultations, for problems related to alcohol use through the CAGE or AUDIT questionnaire. Consider as positive screening those who answered yes to two or more questions in the CAGE or who obtained 8 or more points in the AUDIT.
- Include advice on the risks attributed to harmful use of alcohol in any primary care consultation of adolescents and adults positively screened by CAGE or AUDIT, regardless of the source population or other intervention proposals.
- Refer individuals with positive screening to alcohol-related problems for multi-disciplinary evaluation according to the reality of the local Health System.
- Initiate replacement of thiamine and other B-complex vitamins in all people with chronic alcohol-related problems at the doses indicated for the corresponding age group and sex.

Dictionary of Terms

- **Cytotoxicity** – Property of a substance, molecule, or microorganism that leads (by diverse mechanisms) to cellular destruction.
- **Food insecurity** – Inability to obtain adequate food to nourish the caloric, protein, and micronutrient demands needed by an individual.
- **Infant** – Period of life between 29 days and 2 years of age.
- Ionic Channels: Structures present in the wall of cell membranes that allows the passage of ions into or out of a cell.
- **Korsakoff's syndrome** – Disease caused by the evolution of untreated Wernicke's encephalopathy. It is characterized by irreversible structural brain lesions that lead to memory impairment and confabulations.
- **Micronutrient** – Necessary dietary substances in small amounts for the maintenance of homeostasis.
- **Plasma membrane** – Double phospholipid layer responsible for separating the interior from the cellular exterior.
- **Vulnerability** – Situation attributable to an individual, group, or population in which access to essential resources and rights is absent or severely compromised. This concept can be extended to people dependent on others to perform most of their activities or patients with certain physiological or pathological conditions that compromise their judgment or put them in a situation of fragility.
- **Wernicke's encephalopathy** – Neuropsychiatric disease caused by thiamine deficiency, especially in chronic alcoholics. It begins acutely and is characterized by the triad – ophthalmoplegia, ataxia, and mental confusion – and may or may not present itself with all of these.

Summary Points

- In this chapter, we presented the role of thiamine in the development of the central nervous system, its organic functions, and diseases related to its deficiency.
- Thiamine plays an important role in central nervous system maturation and consolidation of cognition, and its deficiency is particularly deleterious to chronic alcoholics, pregnant women, and infants.
- Pregnant women are more likely to develop thiamine deficiency than the general population, and this effect is more pronounced in those living in poverty.
- The role of thiamine deficiency in perpetuation of poverty status in vulnerable groups was observed.
- Despite the importance of this micronutrient, replacement programs are not yet well established in scientific literature.
- Based on favorable risk/benefit profile, we recommend the fortification of easily accessible foods (such as wheat flour) with thiamine and oral replacement for alcohol dependent people, pregnant women, and infants living in situations of food insecurity.

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Vitamin B6: Effects of Deficiency, and Metabolic and Therapeutic Functions

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Abstract

The vitamin B_6 vitamers include pyridoxine, pyridoxal, and pyridoxamine, as well as their phosphorylated forms such as pyridoxal phosphate, which is a key coenzyme for a surprising variety of enzymes involved in myriad aspects of metabolism. Vitamin B_6 also contributes to the synthesis of many neurotransmitters. Given this widespread role, it is not surprising that vitamin B_6 deficiency can induce many negative effects including convulsive seizures in infants, developmental delay, hypertension, and susceptibility to atherosclerosis. Conversely, the administration of vitamin B_6 vitamers, or the manipulation of vitamer-bound enzymes, has shown promise against cancer, parasitic diseases such as malaria, and Parkinson's disease. In this chapter, we examine the critical and broad role played by vitamin B_6 vitamers and their coenzymes in metabolism, with a focus on the detrimental effects of deficiency and their therapeutic potential.

Keywords

Vitamin B6 · Vitamers · Pyridoxal phosphate · Coenzyme · Neurological function · Seizure · Neuroprotection · Immunity · Diabetes

List of Abbreviations

5-HT	Serotonin
AADC	L-Aromatic amino acid decarboxylase
AGE	Advanced glycation end product
CBS	Cystathionine β-synthase
DA	Dopamine
DOPA	Dihydroxyphenylalanine
GABA	ɣ-Aminobutyric acid
GABA-T	GABA transaminase
GAD	Glutamic acid decarboxylase
Gc	Glucocorticoid
NAS	*N*-Acetylserotonin
NE	Norepinephrine
ODC	Ornithine decarboxylase
PL	Pyridoxal
PLP	Pyridoxal phosphate
PM	Pyridoxamine
PN	Pyridoxine

Introduction

The term vitamin B_6 refers to a group of naturally occurring pyridine derivatives including pyridoxine (PN, pyridoxol), pyridoxal (PL), and pyridoxamine (PM). Their phosphorylated derivatives are also included in this group, generically referred to as vitamin B_6 vitamers. PN specifically refers to the alcohol form, PL to the aldehyde form, and PM to the amine form. These forms of the vitamin B_6 vitamers could be converted to the key coenzymatic form pyridoxal phosphate (PLP) through the actions of an oxidase, a kinase, and a phosphatase (Fig. 1). Pyridoxal kinase activity increases during brain maturation. The activity of this enzyme in the red blood cells of African-Americans is approximately 50% lower than that of Caucasian Americans. An inverse relationship between the activity of the kinase and the concentrations of brain PLP and brain monoamines has been reported. Unbound PLP is hydrolyzed by an alkaline phosphatase. Much of the PLP in liver and muscle is protein bound, thus regulating the concentration of active, unbound PLP. There are more than 140 PLP-dependent enzymatic reactions distributed in all organisms comprising diverse groups such as the oxidoreductases, transferases, hydrolases, lyases, and isomerases. About 1.5% of the genes of prokaryotes encode PLP enzymes which participate in the metabolism of amino acids, carbohydrates, and lipids indicating the versatility of PLP-dependent enzymes. A detailed account has been provided (Dakshinamurti 1990) and recently reviewed (Dakshinamurti and Dakshinamurti 2014).

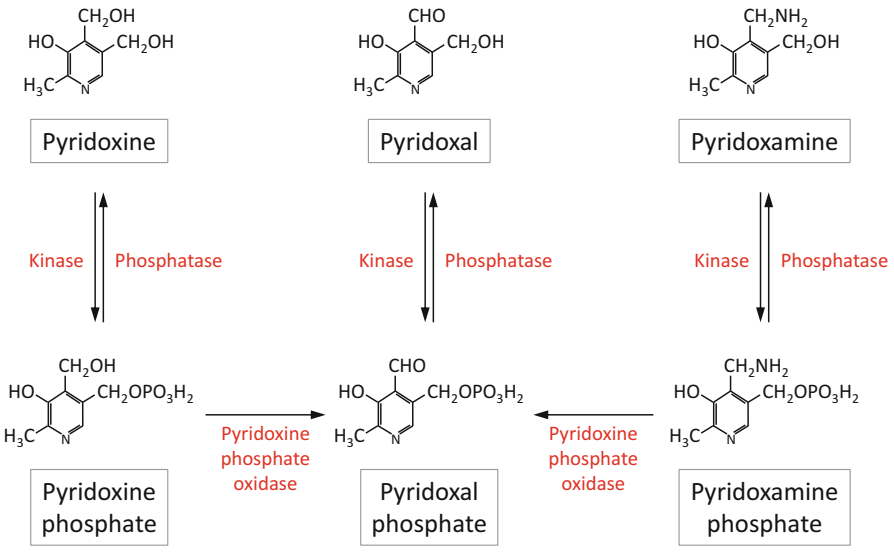

Fig. 1 Interconversion between vitamin B_6 vitamers. Pyridoxine, pyridoxal, and pyridoxamine are phosphorylated by a common pyridoxal kinase. The resulting phosphorylated forms of pyridoxine phosphate and pyridoxamine phosphate can be converted to pyridoxal phosphate by pyridoxine phosphate oxidase

PLP-Dependent Enzymes and Antioxidant Actions of PLP

PLP is the active coenzymatic form of vitamin B_6 and is the most versatile of all the coenzymatic form of vitamins found in all organisms. PLP-dependent enzymes are included in five of the six enzyme classes as defined by the Enzyme Nomenclature Committee of the International Union of Biochemistry and Molecular Biology. They act as coenzymes in enzymatic reactions involved in synthesis, degradation, and interconversion of amino acids, acting as oxidoreductases, transferases, hydrolases, lyases, and isomerases. Based on the fold type of the structural superfamilies, fold type I is the largest group, comprising the aspartate aminotransferase family. Fold type II is the tryptophan synthase family. Alanine racemase is fold type III, whereas the D-amino acid aminotransferase is type IV. PLP-dependent enzymes are classified into three groups depending on the site of elimination and replacement of the substituents. Reactions occurring at the α-carbon atom include transaminase, racemases of α-amino acid, amino acid α-decarboxylases, and enzymes catalyzing the condensation of glycine and the 2-β cleavage of β-hydroxy amino acids such as δ-aminolevulinic acid synthase, serine hydroxyl methylase, and sphingosine synthetase. Reactions occurring at the β-carbon atom of the substrate include serine and threonine dehydratase, cystathionine synthetase, tryptophanase, and kynureninase. Reactions occurring at the γ-carbon include homoserine dehydratase and γ-cystathionase.

Glycogen phosphorylase, another PLP-dependent enzyme, catalyzes the first step in the degradation of glycogen. The physiological role of phosphorylase in skeletal muscle is as an energy source, as approximately 2% of total soluble protein of muscle tissue is the enzyme phosphorylase. This enzyme is under regulatory control with AMP and IMP being activators and ATP and ADP being inhibitors. The involvement of the phosphate group rather than the carbonyl group is a novel feature of the role of PLP in the phosphorylase reaction. The phosphate group of PLP functions in the form of dianion as a proton donor-acceptor. PLP has an important role in maintaining the quaternary structure and conformation of phosphorylase. A reservoir function for PLP in muscle phosphorylase is also indicated.

Hydrogen sulfide (H_2S) is produced endogenously in organs such as the heart and the central nervous system. This endogenous production of H_2S depends upon PLP enzymes such as cystathionine β-synthase (CBS), cystathionine γ-lyase, and 3-mercaptopyruvate sulfotransferase. CBS is highly expressed in the hippocampus and the cerebellum. Along with nitric oxide and carbon monoxide, H_2S forms a triad of gaseous signaling molecules in the body. H_2S is involved in the regulation of intracellular signaling molecules such as protein kinase A, receptor tyrosine kinase, and mitogen kinase and in oxidative stress signaling. The L- and T-type calcium channels as well as potassium and chloride channels are regulated by H_2S. The release and function of neurotransmitters such as γ-aminobutyric acid (GABA), *N*-methyl-D-aspartate, glutamate, and catecholamines require H_2S as a signal. Thus, H_2S has a number of neuronal functions.

Recent evidence attests to the antioxidant properties of vitamin B_6 vitamers, comparing favorably to the well-established antioxidant vitamins such as ascorbic acid and the tocopherols. PN and PM inhibit superoxide radicals and prevent lipid peroxidation, protein glycosylation, and Na^+,K^+-ATPase activity. Hyperglycemia-induced oxidative stress is a significant cause of diabetic complications. PLP inhibits this oxidative stress as well as lipid peroxidation and protein oxidation. PN has a very high level of quenching of hydroxyl radicals.

Assay, Sources, Bioavailability, and Requirement

Much of the data currently available on the total vitamin B_6 content of foods is based on microbiological methods using the growth of *Saccharomyces uvarum* (ATCC 9080). Enzymatic and radioenzymatic methods have been used for the determination of PLP. The most commonly used methods are based on ion exchange or paired ion reverse-phase high-performance liquid chromatography with post-column derivatization (Dakshinamurti and Stephens 1969; Sharma and Dakshinamurti 1992a; Deitrick et al. 2001).

Vitamin B_6 vitamers and their phosphorylated derivatives are present in most foods. Glycosylated forms of PN are present in plant-based foods. PL and PLP are the major forms present in animal-based foods. The vitamin B_6 content of selected foods has been reported (Lecklem 2001). Vitamin B_6 vitamers and their phosphorylated derivatives are photosensitive. There is a loss of vitamin activity due to food processing techniques such as heat sterilization. This loss was responsible for the epidemic of seizures caused by vitamin B_6 deficiency in infants fed such formula diets (Coursin 1954).

The absorption of vitamin B_6 occurs after the hydrolysis of the phosphorylated and glycosylated forms in the lumen of the intestine. There is a specialized Na^+-dependent carrier-mediated system for the absorption of PN (Said 2004). Once absorbed, there is interconversion of the various forms of vitamin B_6 vitamers. The concentrations of PL and PLP in the erythrocyte are 2.6- and 1.8-fold higher than in blood plasma due to the higher affinity of PL to hemoglobin than to albumin. Similarly, PLP synthesized in the erythrocytes is bound with higher affinity to hemoglobin than to albumin. The kinase, oxidase, and transaminase are all present in the erythrocytes. In the muscle, vitamin B_6 is present mostly as PLP bound to glycogen phosphorylase.

The physiological requirement for vitamin B_6 depends on one's age, sex, body size, extent of physical activity, and protein intake in the diet. Oral contraceptive drug use is associated with many clinical side effects that are normally associated with pregnancy. The requirements for vitamin B_6 in women during pregnancy and lactation and of adolescents during the rapid phase of growth are high. The current dietary allowance (RDA) recommendations are set at 2.0 mg for adult males and females, 0.9 mg for children between 4 and 6 years of age, and 1.2 mg for children in the age group 7–10 years (National Research Council, Dietary Reference Intake Tables, The National Academies, Washington, D.C. 2005).

Vitamin B_6 Deficiency: Primary and Secondary

Impairment of somatic growth and a pellagra-like dermatitis are reported in all vitamin B_6-deficient animals. Anemia is reported in most vitamin B_6-deficient species. Ataxia, hyperacusis, hyperirritability, impaired alertness, abnormal head movements, and convulsions are seen in a variety of vitamin B_6-deficient species including humans. The widespread occurrence of vitamin B_6 deficiency-induced convulsive seizures in infants receiving heat-sterilized proprietary milk formulae has been referred to earlier (Coursin 1954). Treatment of these infants with PN resulted in a marked improvement in the wave form and normalization of the amplitude and frequency of their EEG.

Signs of vitamin B_6 deficiency caused by a primary dietary deficiency are rare in the developed world. However, many conditions are recognized in which a deficient condition is caused due to increased requirements or poor availability of the vitamin because of formation of inactive complexes between the vitamin and various drugs.

Vitamin B_6 deficiency has been recognized in pregnant women based on the tryptophan load test, as well as on the determination of vitamin B_6 vitamer levels. When maternal vitamin B_6 levels are low, the PLP levels of cord blood are significantly decreased. Premature infants have very low levels of plasma PLP at birth (Reinken and Mangold 1973). The use of oral contraceptive drugs is associated with clinical side effects that are similar to those associated with pregnancy and are related to hormone-induced changes in tryptophan metabolism.

A functional deficiency of vitamin B_6 has been recognized in uremic patients receiving hemodialysis (Dobbelstein et al. 1974). Resin-based phosphate binders lead to a greater loss of water-soluble vitamins. Isonicotinic acid hydrazide has been used for a long time in the treatment of pulmonary tuberculosis. Its use has been associated with signs of vitamin B_6 deficiency, and supplementation with 50 mg PN reversed this. Another antituberculosis drug, cycloserine, has similar effects on the vitamin B_6 status of patients receiving this drug. Penicillamine is used in the treatment of Wilson's disease and also for cystinuric patients to prevent the formation of urinary cysteine stones. These patients are prone to epileptic seizures which are corrected by the administration of PN (Smith and Gallagher 1970; Cohen 1969).

Neurobiology of Vitamin B_6

The crucial role played by vitamin B_6 in the nervous system arises out of the fact that the putative neurotransmitters dopamine (DA), norepinephrine (NE), serotonin (5-HT), and GABA, as well as taurine, sphingolipids, and polyamines, are synthesized by PLP-dependent enzymes. There is considerable variation in the affinities of the various apoenzymes for the cofactor PLP. In view of this, there is a differential susceptibility of various PLP enzymes to a decrease of PLP during vitamin B_6 depletion in animals and humans. The decarboxylation of glutamic acid, 5-hydroxytryptophan, and ornithine is considerably decreased in all vitamin B_6-deficient species (Fig. 2).

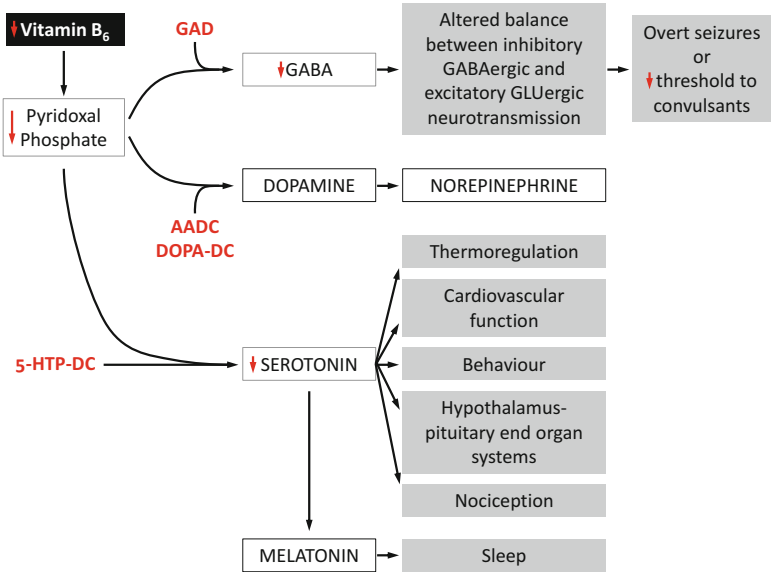

Fig. 2 Physiological consequences of vitamin B_6 depletion. Depletion of vitamin B_6 results in loss of the coenzyme form of pyridoxal phosphate/PLP, in turn decreasing synthesis of GABA and serotonin. These alterations have wide-ranging effects on normal physiology (*gray boxes*). Key enzymes are in *red text*

The enzyme L-aromatic amino acid decarboxylase (AADC) lacks substrate specificity and is considered to be involved in the synthesis of catecholamines and serotonin. There are many differences in the optimal conditions for enzyme activity including kinetics, affinity for PLP, activation and inhibition by specific chemicals, and differences in regional distribution of dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan decarboxylase activities. Nonparallel changes in brain monoamines in vitamin B_6-deficient rats have been reported (Siow and Dakshinamurti 1986; Dakshinamurti et al. 1976). Brain levels of serotonin were significantly decreased during vitamin B_6 deficiency, whereas DA and NE were not affected.

The neurotransmitter GABA is present almost exclusively in the nervous system of invertebrates and vertebrates. GABA is formed from glutamic acid through the action of glutamic acid decarboxylase (GAD) and is catabolized by GABA transaminase (GABA-T) to yield succinic semialdehyde. Both GAD and GABA-T are PLP enzymes. GABA is an inhibitory neurotransmitter, whereas glutamic acid is excitatory. GABA, GAD, and GABA-T are localized in areas of the brain that are inhibitory in function. In addition to the involvement of GABA in the etiology of convulsive seizures, abnormalities in the GABA-ergic neuronal pathways contribute to other CNS disorders such as depression, anxiety, and panic disorders. During a moderate deficiency of vitamin B_6, there are biologically significant decreases in the activities of the PLP-dependent GAD-65 isoform and AADC (5-HTP-DC) leading to decreases in neurotransmitters GABA and 5-HT. Under these conditions, DOPA

decarboxylase is not affected, resulting in no change or even an increase in catecholamines in the nervous system. Decreases in brain serotonin are implicated in physiological changes such as decreased deep-body temperature and altered sleep pattern with a decrease in deep slow-wave and REM sleep (Dakshinamurti 1982).

Neuroendocrinology of Vitamin B_6

The hypothalamus is one of the areas of the brain of vitamin B_6-deficient rats that exhibits significant decreases in PLP and serotonin compared with vitamin B_6-replete controls. There is no decrease in the content of dopamine and norepinephrine. The concept of the regulatory role of the hypothalamus through the neurotransmitters is generally accepted. The hypothalamus of normal animals has high concentrations of both serotonin and dopamine, which are antagonistic in their effects on pituitary hormone regulation (Dakshinamurti et al. 1988).

In determining the focus of the biochemical lesion leading to the hypothyroid state in the vitamin B_6-deficient animal, various possibilities such as primary with a defective thyroid gland, secondary with a defective pituitary thyrotroph, or tertiary with a defective hypothalamus were considered. The results are consistent with a hypothalamic type of hypothyroidism in the vitamin B_6-deficient animal, caused by a specific decrease in hypothalamic serotonin levels (Dakshinamurti et al. 1986).

The pineal gland transduces photoperiodic information and has a role in the temporal organization of various metabolic, physiological, and behavioral processes. Melatonin is the major secretory product of the pineal gland. In the pinealocyte tryptophan is hydroxylated to 5-hydroxytryptophan and decarboxylated to yield serotonin which is converted to *N*-acetylserotonin (NAS). NAS is converted to melatonin by hydroxyl-indole-*O*-methyltransferase. Melatonin synthesis is stimulated by β-adrenergic postganglionic sympathetic fibers from the superior cervical ganglion, which are stimulated in the dark. Melatonin levels in tissues and body fluids show both circadian and seasonal rhythms. Pineal levels of 5-HT and 5-hydroxyindoleacetic acid are significantly lower in the vitamin B_6-deficient rat, and treatment of the deficient rats with PN restored the levels of 5-HT, NAS, and melatonin to the levels seen in vitamin B_6-replete animals (Viswanathan et al. 1988).

The secretion of prolactin is regulated by both stimulatory and inhibitory factors of hypothalamic origin. Evidence based on the administration of serotonin precursors, agonists or antagonists, intraventricular administration of serotonin, and electrical stimulation of the raphe nucleus indicates that the central serotonergic projections to the hypothalamus are involved in the stimulation of prolactin secretion. Administration of PN to vitamin B_6-deficient rats results in a significant increase in plasma prolactin (Sharma and Dakshinamurti 1994).

The vitamin B_6 status of the individual has significant effects on the central production of serotonin and GABA, neurotransmitters that control pain perception, anxiety, and depression. High-dose PN, through its effects on neurotransmitters, has a favorable impact on dysphoric mental states (McCarty 2000; Russo et al. 2003).

Experimental Vitamin B_6 Deficiency in the Neonatal Rat: Seizures and Vitamin B_6

It was the general belief that if the nutrition of the mother was adequate for conception and maintenance of pregnancy, the intrauterine mechanisms for active transport and concentration would supply the necessary nutrients for the normal development of the unborn child. In view of this, it was of interest to produce and characterize vitamin B_6 deficiency in the very young rat. Sperm-positive female Holtzman rats were maintained on a vitamin B_6-supplemented diet during the first week of gestation and then divided into two groups. One was continued on the B_6-supplemented diet, and the other was fed a vitamin B_6-deficient diet until the delivery of the pups and also during the nursing period. There was a small but significant difference in the body weight of the pups between the two groups. Deficient pups had a significant decrease in brain PLP content. Related to this was the occasional finding, among the vitamin B_6-deficient group, of pups with spontaneous convulsions that became noticeable at about 3–4 days of postnatal age. These fits were characterized by a high-pitched scream followed by generalized convulsions of a few seconds' duration and repeated many times within a 1–3-min time period. The motility, perception, and alertness of the deficient neonates were inferior to those of the control pups. This was the first report of the production of congenital PN deficiency (Dakshinamurti and Stephens 1969). In view of the high mortality of the deficient pups, they could not be used in studies on the development of the central nervous system. In a further study, female rats were fed a vitamin B_6-deficient diet from the first postpartum day and the pups were fed the deficient diet from the day they were weaned until they were 5–6 weeks of age. The effects of deficiency on various electrophysiological parameters were examined. The bursts of high-voltage spikes during spontaneous EEG activity, as well as the spontaneous convulsions observed, reflect the decrease in cerebral GABA concentration in the deficient rats. The more complicated changes in cortical auditory evoked potentials in the vitamin B_6-deficient rats are the result of the retardation of normal ontogenetic development of the central nervous system of these rats (Stephens et al. 1971).

The thalamus acts as a relay station for various peripheral and central inputs to the cerebral cortex. Hence, the electro-responsiveness of thalamic ventroposterior lateral neurons in normal control and vitamin B_6-deficient adult rats in response to local administration of convulsants such as picrotoxin or pentylene tetrazole was studied. The extent of neuronal recovery following intrathalamic administration of either GABA or PN, or systemic administration of PN, was assessed using computerized EEG analysis. The results demonstrated an antiepileptic effect of exogenously applied GABA and PN on the thalamic ventroposterior lateral neuron. Neuronal recovery following PN administration is related to synthesis of GABA through activation of GAD (Sharma and Dakshinamurti 1992b; Sharma et al. 1994).

Pyridoxine: Dependency Seizures

PN dependency has been recognized as an inborn abnormality. Infants, generally soon after birth, have seizures that are resistant to the commonly used antiepileptic drugs and respond only to pharmacological doses of PN. It is a rare autosomal recessive disorder. In view of the prevalence of atypical variants of this disorder, it is generally under-recognized. A PN-dependent condition has to be considered in all children with intractable epilepsy up to 3 years of age (Gospe 2002). The unusual rhythmic in utero movements reported retrospectively by some mothers might represent fetal seizures (Clayton 2006). At present there is no biochemical test to confirm PN-dependent seizures: clinical diagnosis is the only mode of recognition. Response to PN monotherapy and recurrence of seizures upon withdrawal of treatment is the only confirmatory test. Such testing is fraught with difficulty due to ethical considerations. PN dependency is distinct from the PN-deficient state of infants reported earlier (Coursin 1954).

The intravenous administration of 50–100 mg PN results in a dramatic cessation of seizures in affected children. In some cases, the dose might be as high as 500 mg. The pharmacological requirement of PN is for life, and the required dose level can be titrated. An untreated PN-dependent condition results in delay in achieving milestones, developmental defects, as well as permanent brain damage (Alkan et al. 2004).

Recent studies indicate that increasing the dose of PN in PN-dependent children without seizures could improve their IQ, indicating a role for PN in normal brain development (Baxter 2003). Autopsy studies on PN-dependent seizure patients show elevated glutamate and decreased GABA levels in the frontal and occipital cortices (Alkan et al. 2003). Of the two isoforms of GAD, GAD-65 is PLP-dependent, and defective binding of PLP to the apoenzyme is suggested to be the cause of the decreased synthesis of GABA in these patients. There are other seizure conditions in which PN therapy finds a place. Infantile spasm in combination with diffuse electroencephalographic abnormalities is referred to as "West syndrome." Mental retardation is associated with this condition. Following reports of beneficial effects, initial treatment with high doses of PN is the established therapy in some European countries and in Japan (Pietz et al. 1993).

Neuroprotection by Vitamin B_6

Domoic acid, a rigid structural analog of glutamate, is a neuroexcitant. It was identified as the toxic contaminant of cultivated mussels responsible for the outbreak of acute food poisoning characterized by gastrointestinal and neurological symptoms (Teitelbaum et al. 1990). Acute intrahippocampal administration of picomole amounts of domoic acid led to EEG epileptiform seizure discharge activity. Domoic acid was 125 times more potent than kainic acid, a well-known neuroexcitant. Local administration of GABA or PN attenuated the seizure activity (Dakshinamurti et al. 1991). Following domoic acid injection, GABA levels decreased significantly in various brain regions. The direct application of GABA to the hippocampus of rats

exhibiting domoic acid-induced seizure activity resulted in suppression of spike discharges. PN had a similar, but slower effect (Dakshinamurti et al. 1993). It has been reported that serotonin functions in the stabilization of brain regional GABA-ergic neurons and in seizure control. The neuroprotective action of PN flows from its effect on the synthesis of both GABA and serotonin (Dakshinamurti et al. 2003). PN treatment, in association with the histidine deacetylase inhibitor sodium butyrate, significantly restored the age-related reduction in memory function. The restorative potential of PN on ischemic damage in the hippocampal CA1 region of Mongolian gerbils has been established (Yoo et al. 2012).

Vitamin B_6 and Hypertension

Hypertension is one of the major causes of chronic illness in western societies, where about 20–30% of the adult population have some degree of blood pressure elevation. For the majority of patients, the underlying cause has not yet been recognized and the condition is referred to as "essential hypertension." Various animal models have been used to study this hypertensive state. The moderately vitamin B_6-deficient rat has been introduced as an additional animal model to study hypertension (Paulose et al. 1988; Dakshinamurti and Lal 1992; Dakshinamurti and Dakshinamurti 2001). The possibility that the reversible hypertension in these animals was related to sympathetic stimulation was studied. The concentrations of both epinephrine and norepinephrine in plasma were threefold higher compared to controls. The turnover of norepinephrine in the hearts of deficient hypertensive rats was threefold higher as compared to controls. Treatment with PN returned both blood pressure and catecholamine levels to normal within 24 h, indicating that the lesion could be at the level of neurotransmitter regulation. Serotonergic neurotransmission in the central nervous system controls a wide variety of functions such as blood pressure, emotional behavior, endocrine secretion, and perception of pain and sleep. It is possible that the decrease in neuronal serotonin and the consequent changes in its receptors, particularly 5-HT_A, may cause hypertension in B_6 deficiency. The 5-HT_{1A} receptor agonists all have acute hypotensive effects in this rat model.

Pyridoxal Phosphate, Calcium Channels, and Cardiovascular Function

The end result of centrally mediated sympathetic stimulation is an increase in peripheral resistance which is reflected in elevation of both resting and stimulated vascular tone in the resistance arteries of the moderately vitamin B_6-deficient hypertensive rat. Elevated peripheral resistance is the hallmark of hypertension as seen in all models of hypertension. The increase in the tone of caudal artery segments from the hypertensive rat is calcium-dependent. The decrease in the tone following the addition to the medium of the calcium channel antagonist, nifedipine, indicates that increased peripheral resistance resulting from increased permeability of smooth

muscle plasma membrane to Ca^{2+} might be central to the development of hypertension in the vitamin B_6-deficient rat. Calcium influx occurs through plasma membrane Ca^{2+} channels that are voltage-operated or receptor-mediated. Voltage-sensitive Ca^{2+} channels open upon depolarization of the cell membrane, resulting in an inward movement of calcium ions. ATP is an extracellular nucleotide that mediates its effect via plasma membrane-bound P2 receptors. The slow L-type channel is the major pathway by which Ca^{2+} enters the cell during excitation for initiation and regulation of the force of contraction of cardiac and skeletal muscle. Vascular smooth muscle also contains L-type channels.

The possibility that in the vitamin B_6-deficient rat a higher concentration of cytosolic-free Ca^{2+} might be responsible for the higher tension in the vascular smooth muscle was studied. In the vitamin B_6-deficient hypertensive rat, radiolabeled $^{45}Ca^{2+}$ influx into the vascular smooth muscle was increased to twice that of the controls. The KCl-induced [Ca^{2+}] increase was significant in cardiomyocytes isolated from vitamin B_6-deficient hypertensive rats. A single injection of vitamin B_6 (10 mg/kg body weight) to the deficient animal completely reversed the KCl-induced changes in [Ca^{2+}] due to vitamin B_6 deficiency. Similar results were obtained in other diet-induced hypertensive animal models, as well as in genetically hypertensive animal models such as the Zucker obese rat (Lal and Dakshinamurti 1993, 1995). In further studies, the possibility that PN or more particularly PLP could directly modulate the cellular calcium uptake process was studied. Bay K8644, a dihydropyridine-sensitive calcium channel agonist, stimulated calcium entry into artery segments from control rats. PLP dose-dependently reduced the Bay K8644-stimulated calcium uptake by control artery segments (Lal et al. 1993; Dakshinamurti et al. 1998). The basal uptake of $^{45}Ca^{2+}$ by caudal artery segments from vitamin B_6-deficient hypertensive rats was at least twice the uptake by caudal artery segments from normal rats. The in vitro direct antagonism indicates the possibility that the calcium channel agonist Bay K8644, the calcium channel antagonist nifedipine, and PLP might all act at the same site on the calcium channel.

In a further study, the effect of PLP on the ATP-induced contractile activity of the isolated rat heart and the ATP-mediated increase in intracellular [Ca^{2+}] in freshly isolated adult rat cardiomyocytes, as well as on the specific binding of ATP to the cardiac sarcolemmal membrane, were examined to determine if PLP is an effective antagonist of ATP receptors in the myocardium. The infusion of ATP caused an immediate increase (within seconds) in LVDP, +dP/dt and –dP/dt. This effect was completely blocked in hearts pretreated with PLP for 10 min. The specificity of the effect of PLP was established (Wang et al. 1999).

Studies in humans have identified an independent association between low plasma vitamin B_6 concentration and a higher risk of coronary artery disease (Chang et al. 1999). In addition to the role of vitamin B_6 in atherosclerosis, other potential explanations include the role of PLP in platelet aggregation and the association between low PLP and inflammatory markers (Friso et al. 2005). Low plasma PLP concentration was inversely associated with major markers of inflammation and independently associated with cardiovascular disease risk (Friso et al. 2004). Our observations on the role of PLP in both major calcium channels for the

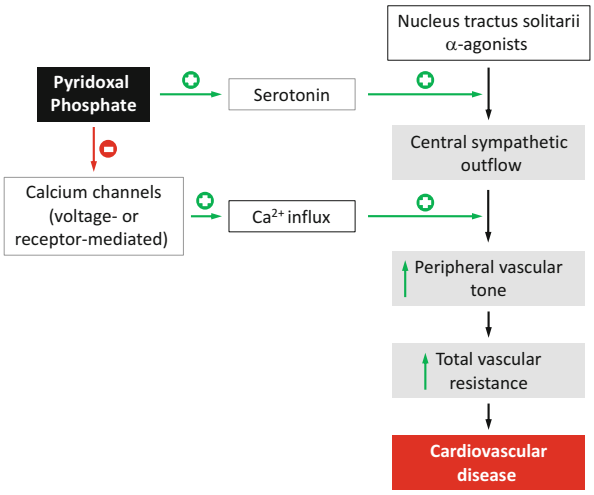

Fig. 3 PLP-mediated mechanisms leading to attenuation of hypertension. Adrenergic stimulation leads to increased peripheral vascular tone, increased total vascular resistance, and an increase in susceptibility to cardiovascular disease such as hypertension. Pyridoxal phosphate induces serotonin-mediated central sympathetic outflow, but also inhibits both voltage- and receptor-mediated calcium channels, reducing calcium influx and attenuating the increase in vascular tone, which may provide the mechanism for PLP-mediated cardioprotection

influx of extracellular calcium might proffer a viable biochemical explanation for the association between low PLP concentration and the risk for cardiovascular disease (Fig. 3). The increase in $[Ca^{2+}]$ in cardiomyocytes might contribute to heart dysfunction and increased myocardial infarction and explain the beneficial effect of vitamin B_6 in patients with hypertension and myocardial infarction (Dakshinamurti et al. 2000; Dhalla et al. 2000).

Cardiovascular Complications of Diabetes Mellitus: Inhibition of Advanced Glycation End Product Formation by Pyridoxamine

The reducing sugar glucose, which is elevated in the diabetic condition, can modify proteins through condensation of its aldehyde group with the ε-amino group of lysine residues, forming a Schiff base. This is referred to as the Maillard reaction and is dependent on the concentration of glucose, being exacerbated in the diabetic condition. The Schiff bases isomerize to intermediate ketosamine Amadori products including glycated hemoglobin (HbA_{1c}). Over a period of weeks and months, glucose-independent reactions including rearrangement, condensation, dehydration, polymerization, and fragmentation lead to a host of metabolites referred to as advanced glycation end products (AGEs). Lipid peroxidation of polyunsaturated fatty acids similarly produces advanced lipoxidation end products.

Histopathological evidence attests to the accumulation of AGEs in a variety of tissues such as the renal cortex, glomerular mesangium, and basement membrane. Evidence for the role of AGEs in vascular complications has been presented (Ahmed and Thornalley 2007). Experimentally, the injection of AGE precursors or AGE-modified proteins induces vascular damage similar to that seen in the diabetic condition. Various studies indicate that hyperglycemia is the most significant factor in the onset and development of vascular complications of diabetes, of which chronic kidney disease is a major component. Inhibition of the renin-angiotensin system with angiotensin-converting enzyme inhibitors and/or angiotensin II receptor blockers is the main pharmacotherapy for chronic kidney disease. In addition, they also block the formation of reactive carbonyl precursors of AGE as well as induced reactive oxygen species generation (Thomas et al. 2005). Of the various compounds that inhibit or correct individual steps in the pathophysiological sequence leading to vascular complications of diabetes, two stand out: PM and benfotiamine. PM is a member of the vitamin B_6 vitamer group. Benfotiamine is a lipid-soluble vitamin B_1 analog. Treatment with high-dose PM improved the urinary albumin/creatine ratios and fasting serum triglyceride and 3-deoxyglucosone. PM also prevents the accumulation of Nε-(carboxymethyl) lysine, nitrotyrosine, TGF-β1, and laminin-β1 in the kidney. The therapeutic potential of ACE inhibitors has been tested in clinical trials (Brenner et al. 2001). Studies using various animal models indicate that PM is effective in the treatment of diabetic nephropathy, retinopathy, and neuropathy (Chang et al. 2009; Chen and Francis 2012). The function of PM is not related to its role as a member of the vitamin B_6 vitamers. PM, in combination therapy, might find a use in the treatment of a wide range of chronic diseases in which oxidative stress, inflammation, and tissue damage lead to a chemical modification of proteins (Metz et al. 2003).

Vitamin B_6: Gene Expression and Anticancer Effect

In rats fed a diet adequate in vitamin B_6, the fraction of total PLP found in the nuclei of liver cells was 21%, and this increased to 39% in rats fed a vitamin B_6-deficient diet indicating a conservation of the vitamin in the nuclear compartment during deficiency, a situation analogous to the distribution of biotin in biotin-replete and biotin-deficient animals (Dakshinamurti 1997). PLP in the cell nucleus is protein bound. Cells grown in the presence of 5 mM PN have a decreased glucocorticoid-dependent induction of enzymes. Vitamin B_6 regulates the transcriptional activation of human glucocorticoid receptors in HeLa cells. This modulatory role on transcription is not restricted to the glucocorticoid receptor, but extends to all members of the steroid hormone receptorsuper family, leading to a decreased transcriptional response to various steroid hormones (Oka et al. 1995). PLP modulates gene expression through its influence on the functional interaction between the steroid hormone receptors and transcription factor NFI (Davis and Cowing 2000).

The growth of B16 melanoma in vitro was inhibited by 5 mM PN or PL. Treatment of mice with PL (0.5 g/kg body weight) reduced the growth of both

new and established B16 melanoma. PL supplementation was shown to reduce cell proliferation and DNA synthesis in both estrogen-dependent and estrogen-independent mammary carcinoma cell lines (Komatsu et al. 2003). The growth of MH-134 hepatoma cells transplanted into C3H/He mice was significantly reduced by the administration of large amounts of PN to mice. High dietary intake of vitamin B_6 has been shown to suppress herpes simplex virus type 2-tranformed cell-induced tumor growth in BALB/c mice (Jansen et al. 1999). Epidemiological studies indicate a reduced risk of lung and colorectal cancer in older men ingesting high doses of vitamin B_6 (Hartman et al. 2001; Larsson et al. 2010).

PLP was reported to be a strong inhibitor of DNA polymerases α and ε from a phylogenetically wide range of organisms, from protists, plants, insects, and fish to mammals. These polymerase classes are related to DNA replication. Treatment with pharmacological doses of vitamin B_6 suppressed the expression of the cell proliferation-related genes c-myc and c-fos in colon epithelium of mice treated with azomethane. PLP has been shown to inhibit DNA topoisomerases I and II, which are needed for strand separation, replication, and recombination. Inhibition of DNA topoisomerase arrests the cell cycle and induces apoptosis. PLP has been shown to be an effective inhibitor of many enzymes that have binding sites for phosphate-containing substrates of effectors, including RNA polymerase, reverse transcriptase, and DNA polymerase (Matsubara et al. 2003). Based on a meta-analysis of prospective studies, an inverse association between blood PLP levels and the risk of colorectal cancer has been reported (Larsson et al. 2010).

Oxaliplatin in combination with 5-fluorouracil is standard treatment for metastatic colorectal carcinoma. Peripheral sensory neuropathy is the dose-limiting side effect of this treatment. PN administration reduces oxaliplatin-induced neurotoxicity, thus allowing for more effective and less toxic treatment of such cancers. The preventive effect of vitamin B_6 on tumorigenesis might also derive from its strong antioxidant action (Garg and Ackland 2011; Jain and Lim 2001). These observations highlight the potential use of pharmacological doses of vitamin B_6 in cancer therapy.

Vitamin B_6 and Immunity

There is overwhelming evidence for the requirement of vitamin B_6 in antibody production. The thymus of vitamin B_6-deficient rats is depleted of lymphocytes, and deficient animals had impaired antibody formation following exposure to various antigens. Cell-mediated immunity is also impaired in vitamin B_6-deficient rats. A decline in immune response is a concomitant of the aging process in animals and humans with the most significant effect on cell-mediated immunity. PN supplementation led to lymphocyte proliferative response to both T- and B-cell antigens (Kwak et al. 2002). Diseases such as uremia and arthritis are associated with immunological abnormalities. Treatment of uremic patients with pharmacological doses of PN results in a significant increase in lymphocyte reactivity. Plasma PLP is significantly lower in patients with rheumatoid arthritis compared to controls matched for age, gender, race, and weight (Roubenoff et al. 1995). The plasma

levels of PLP inversely correlated with production of tumor necrosis factor by unstimulated peripheral blood mononuclear cells.

Glucocorticoids (Gc) are widely used in the treatment of inflammatory and autoimmune states. Long-term Gc use is associated with severe side effects. Vitamin B_6 does not interfere with Gc action in immune cells while selectively inhibiting the unwanted side effects of Gc receptor-dependent transactivation in nonimmune cells (Bamberger et al. 2004). PL and PLP suppress the expression of cytokine genes in macrophages, indicating the anti-inflammatory activity (Zhang et al. 2016).

Vitamin B_6 deficiency is widely prevalent among HIV-infected persons (Baum et al. 1991). The relationship of PLP to the activation of CD4 T cells by antigen-presenting cells has been studied. CD4 T cells are the mediators in the initiation and continuation of the immune response causing autoimmune diseases and allogenic transplant rejection. The CD4 glycoprotein is the characteristic surface receptor of all helper T cells. The extracellular part of the CD4 molecule is comprised of four domains, D1 to D4. CD4 binds to major histocompatibility complex MHC class II through the D1 and D2 domains. PLP binds very tightly to the D1 domain of CD4 and thus interferes with the CD4-MHC II interaction. Non-incorporation of CD4 into the activation complex could lead to T-cell apoptosis. The tight association of D1 and PLP would prevent protein-protein interaction of CD4 itself, its dimerization, and the interaction of the dimer with other molecules on the T-cell surface leading to apoptosis.

PLP has been shown to be an anion channel blocker in a variety of cells (Korchak et al. 1980). It has been suggested that PLP might have a role in the treatment of autoimmunity and in transplant rejection (Namazi 2003). As the interaction of HIV gp120 and CD4 occurs through the D1 domain, PLP may have an anti-HIV effect as well. High concentrations of PLP inhibit viral coat protein envelope glycoprotein binding and infection of CD4 T cells by isolates of HIV-1 in vitro (Guo et al. 1994). Thus, PLP might function as an immune stimulator, not only by increasing CD4 T-cell count but also by protecting uninfected CD4 T cells from infection by HIV-1 (Salhany and Stevenson 1996). These effects of PLP are seen at concentrations in the range 50–70 mM. The rapid hydrolysis of PLP by tissue nonspecific alkaline phosphatase is a problem in maintaining such high concentrations of PLP. Levamisole, an antihelminthic drug, is an inhibitor of alkaline phosphatase. Thus a combination of high PLP dose along with this inhibitor might maintain the required tissue concentration of PLP to afford protection of uninfected CD4 T cells against HIV-1.

Toxicity of Pyridoxine

Concern about the toxicity of PN was associated with the use of Bendectin (doxylamine plus PN) by pregnant women and the subsequent occurrence of birth defects in the offspring. Later studies have ruled out any teratogenic effect of PN (Check 1979). Concern about its toxicity resurfaced after reports of reversible sensory neuropathy in persons ingesting gram quantities of PN for long periods

extending to years. It is significant to note that the reported sensory neuropathy was reversible after stopping of the ingestion of these large amounts. This indicates no permanent structural damage to the nervous system. High-dose PN ingestion over several years has been in vogue for the treatment of various clinical conditions such as homocysteinemia, PN-dependent seizures, autism, and Down syndrome (Rimland et al. 1978). There have been no adverse effects associated with these treatments. A dose of 500 mg/day PN for up to 2 years was not associated with neuropathy (Bendich and Cohen 1990).

Pyridoxal Phosphate Enzymes as Drug Targets

PLP enzymes have been targets of therapeutic intervention. In Parkinson's disease, abnormal pulsatile stimulation of the striatal dopamine receptors leads to dysregulation of genes and proteins in the downstream neurons and, consequently, alterations in the neuronal firing patterns. This results in the motor complication associated with Parkinson's disease, and the treatment aim is to restore normal dopaminergic transmission of striatal synapse. Levodopa slows the progression of Parkinson's disease.

The PLP-binding enzyme ornithine decarboxylase (ODC) is the rate-limiting step in polyamine biosynthesis. Polyamines are DNA-binding agents regulating its conformation. Mammalian ODC is a downstream mediator of myc-regulated reactions and is upregulated in proliferating cells. It is implicated as an oncogene in multiple types of tumors. Inhibition of ODC suppresses tumor development. Hence, ODC is a promising anticancer target. Inhibitors of ODC such as difluoromethylornithine are being tested as anticancer agents (Wu et al. 2011; Seiler 2003).

PLP-dependent enzymes are potential targets for developing antiparasitic drugs. There are several PLP enzymes with high prevalence among the protozoan species. Enzymes of the sulfur-containing amino acid metabolic pathway are significant in terms of their distribution between hosts and their disease-causing parasites. The enzymes of the forward transsulfuration pathway are present in protozoan parasites, but lacking in their host. Another PLP enzyme, methionine ɣ-lyase, is important to the parasite for the production of propionic acid for energy metabolism and for the degradation of sulfur-containing amino acids. Host mammalian cells lack this enzyme, and hence it is a target for drug development against infection by protozoans such as *Trichomonas vulgaris* and *Entamoeba histolytica*. The halogenated methionine analog S-trifluoromethyl-L-homocysteine and its amide derivative are toxic to the parasite and have potential as antiparasitic drugs (Sato et al. 2010).

Of the parasitic diseases, the most devastating is malaria, with a worldwide annual death toll of more than a million people. It is transmitted by the mosquito. Xanthurenic acid is required for gametogenesis and fertility of the parasite *Plasmodium falciparum*. It is synthesized in the tryptophan degradative pathway through the PLP-dependent enzyme kynurenine aminotransferase. Hence, this enzyme is a target for the development of antimalarial drugs (Muller et al. 2009). African sleeping sickness is another widespread epidemic caused by *Trypanosoma*

brucei, which is transmitted by flies. ODC, a key enzyme in the pathway for the synthesis of polyamines, is a target enzyme for drug development. Alpha-difluoromethylornithine, an irreversible inhibitor of ODC, has been developed for the treatment of sleeping sickness (Krauth-Siegel et al. 2007).

Policies and Protocols

The prevalence of frank clinical symptoms of vitamin B_6 deficiency is rare in advanced countries. However, a relative deficiency is present in pregnant women and women on oral contraceptive steroids. Vitamin B_6-dependent seizures in infants and children, and the use of pyridoxine for their treatment, are well-recognized. However, newer information on the protective role of pharmacological doses of vitamin B_6 in the treatment and amelioration of a variety of chronic conditions needs to be disseminated, and policies in this regard are useful. RDA levels do not have any relevance to the protective role of vitamin B_6 in hypertension, cardiovascular diseases, nephropathy, retinopathy, neuropathy, and dysfunction of the immune system and as an adjuvant in cancer treatment. The required therapeutic doses are much higher. There is no toxicity associated with these dose levels. Given these considerations, hospital-level policies to consider vitamin B_6-based treatment as frontline or adjuvant treatment for such conditions should be evaluated.

Dictionary of Terms

- **Primary vitamin B_6 deficiency** – A deficiency in vitamin B_6 that is due to a lack of availability in the diet. Primary vitamin B_6 deficiency is relatively rare in the developed world.
- **Pyridoxal phosphate-dependent enzymes** – An enzyme whose activity depends upon the presence of pyridoxal phosphate as a coenzyme. Over 140 pyridoxal phosphate-dependent enzymes are known, and they play critical roles in the metabolism of amino acids, carbohydrates, and lipids.
- **Pyridoxine dependency** – A condition characterized by a requirement, typically lifelong, for administration of high levels of pyridoxine far in excess of normal daily requirements. Pyridoxine dependency can result in epileptic seizures that can only be alleviated by the provision of pyridoxine.
- **Secondary vitamin B_6 deficiency** – A deficiency in vitamin B_6 that occurs despite an adequate dietary supply. A secondary vitamin B_6 deficiency can occur in specific situations of vitamin B_6 loss, such as uremic dialysis patients, in whom water-soluble vitamin loss can be significant.
- **Vitamer** – Chemical compounds that exhibit the activity of a particular vitamin. These compounds typically have a similar chemical structure and can be used in vitamin-deficient individuals to provide vitamin activity.

Summary Points

- Pyridoxine, pyridoxal, and pyridoxamine and their phosphorylated derivatives are vitamers of the vitamin B_6 group. Pyridoxal phosphate is the most versatile of all coenzymatic forms of vitamins.
- There are over 140 PLP-dependent enzymes and they are present in all organisms. PLP enzymes participate in the metabolism of amino acids, carbohydrates, and lipids.
- Primary deficiency of vitamin B_6 is rare in developed countries. However, pregnant women and women on oral contraceptive steroids have a relative vitamin deficiency even on a normal intake of vitamin B_6. A functional deficiency of vitamin B_6 is also recognized in uremic patients and patients treated with drugs such as isonicotinic acid hydrazide, cycloserine, and penicillamine.
- Vitamin B_6 has a crucial role in neurobiology as the putative neurotransmitters, GABA, catecholamines, and serotonin are products of PLP-dependent decarboxylases. There are nonparallel changes in the catecholamines and serotonin in vitamin B_6 deficiency leading to a significant neurotransmitter imbalance.
- Maternal vitamin B_6 deficiency has a significant impact on neuronal development of the offspring. Congenital deficiency leads to seizures in the neonate and impaired neuronal development.
- Pyridoxine dependency is recognized as an inborn abnormality in children having intractable epilepsy which is not responsive to anticonvulsant therapy and responds only to pharmacological doses of pyridoxine.
- Pyridoxine affords neuroprotection against neuroexcitants such as domoic acid. This neuroprotection flows from its effect on the syntheses of GABA and serotonin.
- Vitamin B_6 deficiency is also associated with hypertension. Various animal models of hypertension respond to pharmacological doses of pyridoxine. In addition to its effect on serotonergic neurotransmission, PLP is a regulator of cellular calcium channels affecting both the voltage-mediated and the ATP-mediated Ca^{2+} channel transporters. High-dose pyridoxine is effective in the treatment of hypertension and cardiovascular diseases.
- Hyperglycemia is the most significant factor in the onset and development of vascular complications of diabetes leading to chronic kidney disease. Treatment with high-dose pyridoxine is effective in the treatment of nephropathy, retinopathy, and neuropathy.
- Vitamin B_6 regulates the transcriptional activation induced by steroid hormone receptors. Large doses of pyridoxine have an effect on tumorigenesis. PLP functions as an immune stimulator and protects uninfected CD4 T cells from infection by HIV-1.
- High doses of pyridoxine up to 500 mg/day over years have been used in the treatment of several chronic human conditions with no adverse effects.
- Pyridoxal phosphate-dependent enzymes such as ornithine decarboxylase are potential targets for developing antiparasitic drugs.

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Effects of Biotin Deprivation and Biotin Supplementation

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Abstract

A number of key carboxylase enzymes involved in metabolism depend upon the vitamin biotin for their structure and function. Humans and animals require biotin to be supplied in the diet. Biotin deficiency is relatively rare in the developed world and typically reflects consumption of egg white-enriched or ketogenic diets, whereas deficiency in underdeveloped countries is more likely to be due to biotin-poor diets. The effects of biotin deficiency are not typically life-threatening, but can lead to developmental delays in children, or hair and skin abnormalities in affected individuals. In contrast, biotin supplementation has been shown to provide salutary benefit in some individuals, such as diabetics. Here we examine the biochemistry of biotin, as well as the effects of biotin deficiency and supplementation, with a focus on human health.

Keywords

Biotin · Prosthetic group · Carboxylases · Multiple carboxylase deficiency · Glucose metabolism · Cell differentiation and proliferation · Pancreatic inflammatory proteins · Pharmacological effects

List of Abbreviations

ACC	Acetyl-CoA carboxylase
ATP	Adenosine triphosphate
HCS	Holocarboxylase synthetase
MCC	β-methylcrotonyl-CoA carboxylase
MCD	Multiple carboxylase deficiency
PAP	Pancreatitis-associated protein
PC	Pyruvate carboxylase
PCC	Propionyl-CoA carboxylase
PEPCK	Phosphoenolpyruvate carboxykinase
SMVT	Sodium-dependent multivitamin transporter

Introduction

Biotin is a water-soluble vitamin. Its discovery, elucidation of its structure, and delineation of its role in metabolism involved diverse investigations spanning several decades. Biotin was shown to be cis-hexahydro-2-oxo-1H-thieno [3,4] imidazole-4-valeric acid. The best known and understood role of biotin is as the prosthetic group of the biotin-containing enzymes – propionyl-CoA carboxylase (PCC), pyruvate carboxylase (PC), β-methylcrotonyl-CoA carboxylase (MCC), and acetyl-CoA carboxylase (ACC). The biotin-dependent carboxylase catalyzes an adenosine triphosphate (ATP)-dependent CO_2 fixation reaction in which biotin functions as a CO_2 carrier on the surface of the enzyme. These enzymes catalyze key reactions in gluconeogenesis, fatty acid biosynthesis, and amino acid catabolism. In addition, biotin has been shown to participate in other cellular events such as transcriptional and translational regulation. This review examines the role of biotin in metabolism, biotin deficiency, biotin dependency, as well as the non-prosthetic group functions of biotin.

Structure and Biosynthesis

du Vigneaud showed that the previously known requirement of various bacteria for pimelic acid was satisfied by biotin. Later studies provided direct evidence for the incorporation of pimelic acid as a unit in the biosynthesis of biotin, indicating a two-stage biosynthetic pathway involving the synthesis of pimelate and its incorporation in the biotin bicyclic ring structure (Lin and Cronan 2011). Dethiobiotin, a sulfur-free analog of biotin, is the direct precursor of biotin during its biosynthesis in microorganisms. Most microbes, plants, and fungi synthesize biotin, whereas for animals and humans, it is a required growth factor. The last four steps in the biosynthesis of biotin are conserved in biotin-producing microorganisms. Of these, two enzymes – 8-amino oxononanoate synthase and 7,8-diaminopelargonic acid aminotransferase – are pyridoxal-5-phosphate-dependent enzymes (Mann and Ploux 2011). Several inhibitors of these two enzymes have been used for the design of herbicides and antibiotics. The addition of biotin to food, feed, and cosmetic products creates a large demand which is met by chemical synthesis. In view of the high environmental burden of these processes, current efforts are directed toward the improvement of microbial biotin production. The major portion of biotin in animal and plant sources is protein bound. Biocytin (biotinyl lysine) is released upon the enzymatic digestion of biotin-containing proteins. It is cleaved by the enzyme biotinidase into biotin and lysine (Fig. 1).

Absorption and Transport

Biotin is widely distributed in foodstuffs, although in low concentrations. Egg yolk, liver, nuts, and legumes are rich in biotin. Most of the biotin in foods such as meat and cereals is protein bound. Enzymatic hydrolysis in the gastrointestinal

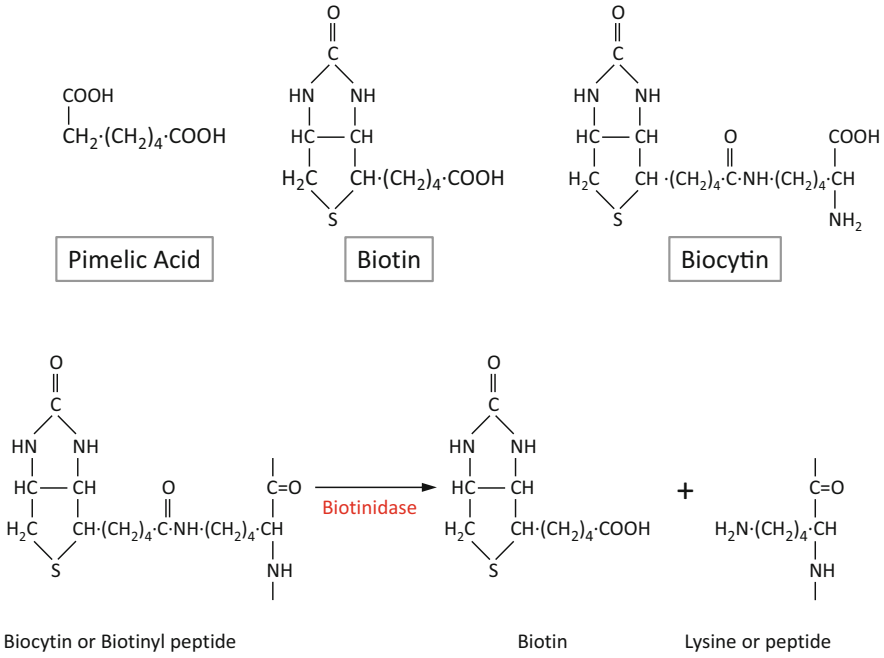


Fig. 1 Structure relationship of biotin and congeners

tract releases biocytin (or biotinyl peptides) rather than free biotin. Biotinidase activity is present in pancreatic and intestinal secretions as well as in brush border membranes. The uptake of biotin by human cell lines is saturable (Dakshinamurti and Chalifour 1981; Chalifour and Dakshinamurti 1982). The uptake of biotin and biocytin in rat jejunal segments is biphasic (Dakshinamurti et al. 1987). At concentrations below 50 nM, the saturable uptake mechanism would make enough biotin available to the animal. This saturable component uses a sodium-dependent multivitamin transporter (SMVT) having affinity for pantothenic acid, lipoic acid, as well as biotin. The rarity of primary biotin deficiency in humans is consistent with this. The late-onset type of multiple carboxylase deficiency (MCD) was shown to be due to the lack of a system for absorbing biotin in the nanomolar range, and in turn due to the lack of biotinidase. Biotinidase is the only protein in brush border membranes and the only protein in human serum that binds to biotin (Chauhan and Dakshinamurti 1986, 1988). Based on the above studies, it was suggested that biotinidase in vivo functions in the transport of biotin (Dakshinamurti and Chauhan 1989). It has been shown that in cases of late-onset MCD, patients lack the system for absorbing nanomolar biotin and respond only to pharmacological biotin doses, indicating that only the saturable portion of the biotin transport system is defective (Roth et al. 1982; Roth 1985).

Biotin Assay

Microbial growth assays using microorganisms such as *Lactobacillus plantarum*, *Lactobacillus casei*, *Ochromonas danica*, or *Saccharomyces cerevisiae* have traditionally been used to measure biotin content. Avidin-binding assays measure the ability of biotin to compete with radiolabeled (^{3}H or ^{14}C) biotin for binding to avidin (Dakshinamurti et al. 1974; Dakshinamurti and Allan 1979). Avidin-binding assays using electrochemical or bioluminescence detection or a double-antibody technique have been published (Terouanne et al. 1989; Thuy et al. 1991).

Biotin Requirement

There is no reliable estimate of the biotin requirement of various human groups. The Food and Nutrition Board of the National Research Council has prescribed adequate intakes for various age groups (National Research Council, Dietary Reference Intakes Tables, The National Academies, Washington, D.C., 2005).

Biotin as Prosthetic Group of Biotin Enzymes: Role in Metabolism

The best known and understood role of biotin is as the prosthetic group of biotin-containing enzymes, the carboxylases. Biotin acts as a vector for the transfer of the carboxyl group by biotin enzymes which include PCC, PC, MCC, and two isoforms of acetyl-CoA carboxylase (ACC1 and ACC2) in mammals. Biotin enzymes are present in prokaryotes and eukaryotes, with phylogenetic analysis indicating an ancient evolutionary origin. Although the enzymes catalyze very different reactions, they share common active site features and mechanisms of action. These enzymes control key steps in gluconeogenesis, branched-chain amino acid catabolism, fatty acid synthesis, and fatty acid oxidation in specific tissues, thus underscoring their key roles in intermediary metabolism (Fig. 2).

Biotin-dependent carboxylases function in two steps – biotin carboxylase and carboxyl transferase. The biotin carboxylase component catalyzes the MgATP-dependent carboxylation of the N1 of the biotin cofactor using bicarbonate as the CO_2 donor. Biotin is linked covalently through an amide linkage to the ε amino group of a specific lysine in the biotin carboxyl carrier protein by a protein ligase. In the second step, the carboxyl transferase catalyzes CO_2 transfer from carboxybiotin to the carboxyl group acceptor. The substrates are coenzyme A esters such as acetyl-CoA, propionyl-CoA, and 3-methylcrotonyl-CoA. Pyruvate serves directly as a substrate (Tong 2013).

As most food sources are low in their biotin content, which is usually protein bound, higher organisms have developed an efficient biotin recycling mechanism to ensure adequate supply of biotin for normal metabolism. Biotin taken up by the cell is covalently attached to various apocarboxylases by holocarboxylase synthetase

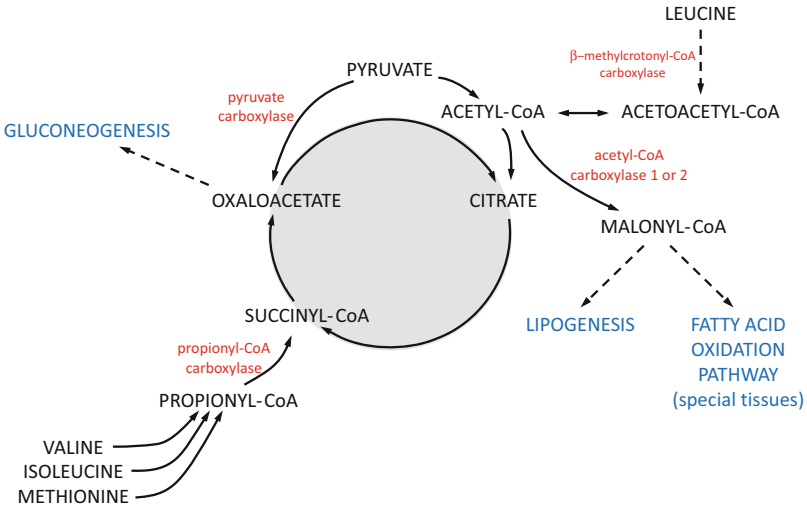
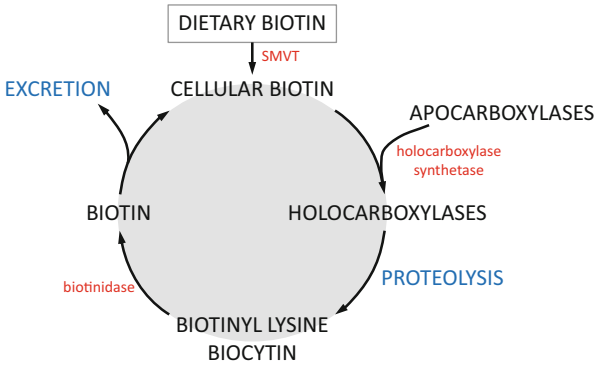


Fig. 2 Role of biotin enzymes in intermediary metabolism

Fig. 3 The biotin cycle



(HCS). During the turnover of biotin enzymes, the biotin peptide or its further degradation product biocytin is cleaved by intracellular or plasma biotinidase, thus regenerating biotin which is recycled for the synthesis of the new biotin holocarboxylase (Fig. 3). Given this efficient recycling mechanism, the biotin requirement of the organism is low. However, mutations in either biotinidase or HCS are lethal as they lead to MCD.

Acetyl-CoA Carboxylase

ACC catalyzes the ATP-dependent carboxylation of acetyl CoA, leading to the formation of malonyl-CoA. In mammals, there are two isoforms of ACC: ACC1 is expressed in lipogenic tissues such as liver, adipose tissue, and lactating mammary

gland. The product malonyl-CoA is the building block to extend the chain length of fatty acids by two carbon increments, catalyzed by fatty acid synthase. Long-chain fatty acids are incorporated into complex lipids such as triglyceride and phospholipid. ACC1 is regulated by hormones and dietary and nutritional state. ACC1 is stimulated by citrate and inhibited by long-chain acyl-CoA and by phosphorylation of the enzyme. Mammals have another isoform – ACC2 – which is associated with the outer mitochondrial membrane and is expressed in the heart, skeletal muscle, and liver. The product of ACC2, malonyl-CoA, is an inhibitor of carnitine palmitoyltransferase-1, involved in the transport of long-chain fatty acyl-CoAs into the mitochondria for β-oxidation. ACC2 knockout mice show elevated fatty acid oxidation and increased energy expenditure (Abu-Elheiga et al. 2001).

Propionyl-CoA Carboxylase

In the catabolic pathways of odd-chain fatty acids, PCC is crucial for the catabolism of branched-chain amino acids isoleucine, threonine, and valine as well as methionine. PCC catalyzes the conversion of propionyl-CoA to methylmalonyl-CoA which in turn enters the tricarboxylic acid cycle via succinyl-CoA. Mammalian PCC is a mitochondrial enzyme which is activated by monovalent cations (K^+, NH_4^+). Inherited deficiency of PCC leads to propionic acidemia (Dakshinamurti and Chauhan 1988).

3-Methylcrotonyl-CoA Carboxylase

MCC catalyzes the conversion of 3-methylcrotonyl-CoA to 3-methylglutaconyl-CoA and is involved in the catabolism of leucine and isovalerate. Deficiency in the activity of this enzyme results in 3-methylcrotonyl-glycinuria, an inborn error of metabolism.

Pyruvate Carboxylase

PC catalyzes pyruvate conversion to oxaloacetate, with a crucial role in gluconeogenesis in the liver and the kidney where it catalyzes the first step in the synthesis of glucose from pyruvate. It is also present in lipogenic tissues and participates in fatty acid synthesis by transporting acetyl groups via citrate and reducing groups via malate, from the mitochondria to the cytosol. It has an anaplerotic role in the formation of oxaloacetate, replenishing intermediates of the tricarboxylic acid cycle that have been used up for the synthesis of glucose, fatty acids, and amino acids. A rare autosomal recessive metabolic disorder resulting in deficient PC activity has been reported with multiple clinical manifestations.

With the exception of ACC1, the biotin carboxylases are mitochondrial enzymes. Biotin deficiency affects mitochondrial metabolism and function significantly.

Oxidative phosphorylation is impaired in biotin-deficient rat liver mitochondria (Dakshinamurti et al. 1970). A role for biotin in maintaining mitochondrial complex IV and heme metabolism has been reported (Atamna et al. 2007).

Biotin deficiency affects the level of tricarboxylic acid cycle intermediates. PC has an anaplerotic role, and its deficiency decreases the production of succinyl-CoA. MCC deficiency leads to methylcrotonyl-CoA accumulation in the mitochondria where it reacts with glycine, depleting its content in the mitochondrial matrix. Succinyl-CoA and glycine are the precursors of heme synthesis. Thus, biotin deficiency, through depletion of these intermediates, leads to a decrease of mitochondrial complex IV, a functional indicator of heme deficiency, decreasing ATP production (Atamna et al. 2007). The significant role of biotin in energy metabolism is indicated by the sparing of the brain and the heart from the effects of general biotin deficiency, thus protecting these organs at the expense of organs such as the liver and kidney (Pacheco-Alvarez et al. 2004; Velazquez-Arellano et al. 2008). Because of the relative insensitivity to biotin deficiency, cardiac tissue is able to maintain ATP synthesis.

Biotin Deficiency

Hair thinning with loss of color, periorificial skin rash, hypotonia, lethargy, and developmental delay are the clinical sequelae of pronounced biotin deficiency in human adults and infants. Early reports of these clinical findings were the result of feeding individuals an egg white diet. The egg white protein avidin binds to free biotin, rendering it unavailable for absorption. Diet-induced biotin deficiency, with its unique clinical signs, is rare; however, milder conditions of deficiency can occur.

Measurement of serum biotin and the urinary excretion of biotin and its metabolite bisnorbiotin have been used as measures of the biotin status of individuals, but they are fraught with uncertainty. Indices based on the metabolic function of biotin have been found to be more reliable as indicators of biotin status. Among these are the urinary excretion of metabolites in biotin-mediated steps in metabolism and the activation index of biotin enzymes. Urinary excretion of 3-hydroxyisovaleryl carnitine and 3-hydroxyisovaleric acid is increased in early biotin deficiency due to a decrease in activity of the biotin enzyme MCC (Stratton et al. 2011). PCC activity in peripheral blood lymphocytes has been shown to be an early and more sensitive indicator of marginal biotin deficiency than urinary excretion of 3-hydroxyisovaleric acid. Malnutrition in developing countries is a major cause of multiple vitamin deficiencies. In the developed world, biotin deficiency is related to genetic factors leading to biotin dependency. However, conditions of prolonged parenteral nutrition lacking biotin supplementation, or the use of infant formulas lacking biotin, have been shown to result in biotin deficiency (Sato et al. 2016; Wakabayashi et al. 2016). Long-term treatments with a heterogeneous group of anticonvulsants have been shown to result in biotin deficiency due to impaired biotin absorption and salvage

by the kidney (Mock and Dyken 1997). A low-carbohydrate, high-fat "ketogenic" diet is used to treat drug-resistant epilepsy and has been shown to induce a relative biotin deficiency (Yuasa et al. 2013). A marginal deficiency of biotin is common in normal human pregnancy. The frequency of such deficiency during the first trimester is of concern due to the teratogenicity of biotin deficiency.

Biotin Dependency

Single Carboxylase Deficiency

The incidence in infants of organic acidemia has been investigated and characterized as due to the lack of one or more of the biotin carboxylases (Sweetman and Nyhan 1986). Inherited disorders of individual biotin carboxylases have been reported. These patients do not respond to pharmacological biotin doses. PCC deficiency causes elevated concentrations of propionic and lactic acids in blood and increased levels of their secondary metabolites such as 3-hydroxypropionic acid, 2-methylcitrate, and propionyl glycine excreted in the urine.

Multiple Carboxylase Deficiency

In MCD, the patient exhibits deficiencies of all three mitochondrial carboxylases – PC, PCC, and MCC. Two distinct types of MCD are recognized based on the age of onset and the nature of clinical presentation (Sweetman and Nyhan 1986). A deficiency of HCS is generally regarded to be the prime biochemical lesion in the neonatal type of MCD. The beneficial response of the affected infant to large doses of biotin administered prenatally to the mother suggests a defective HCS with a high K_m for biotin (Roth et al. 1982). Incidentally, this was the first instance of successful prenatal treatment of this condition. The prenatal treatment followed by postnatal biotin supplementation of the child resulted in the total avoidance of any effects of biotin deficiency in the child.

The late-onset (or juvenile) form of MCD is associated with low serum biotin values and is associated with defective biotin absorption due to biotinidase deficiency. Biotinidase, along with HCS, participates in the cellular biotin cycle (Fig. 3). Biotinidase deficiency is an autosomal recessive inherited metabolic disorder. Untreated individuals develop neurological and cutaneous symptoms as well as myelopathy. These symptoms can be prevented if the biotin treatment is started at birth or before symptoms develop. Newborn biotinidase screening has been instituted in many countries worldwide (Wolf 2011, 2015). A case of biotin dependency due to a defect in biotin transport was reported. In this child, biotin dependency was not the result of biotinidase, holocarboxylase, or biotin deficiency but the result of a genetic defect in a biotin transport protein distinct from the SMVT.

Biotin-Binding Proteins

Besides the carboxylases in which biotin is attached covalently to the apocarboxylase, there are a group of proteins which bind biotin non-covalently. Both avidin, the biotin-binding protein of raw hen egg white, and streptavidin, a bacterial protein, have exceedingly high affinities for biotin (K_m of 10^{-15} M). This is the strongest known non-covalent binding between a protein and a small molecular weight ligand. Other biotin-binding proteins include biotinidase, biotin HCS, biotin antibodies, and nuclear biotin-binding protein, with progressively lower affinities for biotin.

Monoclonal antibodies to biotin have been prepared using keyhole limpet hemocyanin (Dakshinamurti et al. 1986; Dakshinamurti and Rector 1990). One of the four clones isolated produced antibody of high affinity that bound both free and haptenic biotin antigen as well as biocytin. The high affinity between biotin and avidin has been utilized in various areas of biological research. Of much significance is the use of avidin-biotin complex for the localization and evaluation of cell surface receptors. The use of monoclonal biotin antibodies facilitates these studies.

Avidin-biotin technology, based upon the strong interaction between avidin or streptavidin and biotin, has been applied in biology, medicine, and technology. Even biotin covalently bound to a protein is available for binding by avidin or streptavidin. The various chemical and enzymatic biotinylation procedures have contributed to the extensive use of the avidin (streptavidin)-biotin technology (Dundas et al. 2013). The monoclonal anti-biotin antibody has been shown to be an excellent substitute for streptavidin-based immunoassay systems.

Non-prosthetic Group Functions

Biotin Requirement for Cultured Cells and for Cell Differentiation

A requirement for biotin was demonstrated for HeLa cells, human fibroblasts, and Rous sarcoma virus-transformed baby hamster kidney cells (Chalifour and Dakshinamurti 1982; Bowers-Komro and McCormick 1985). Mammalian cultured cells under conditions such as serine starvation, when they do not get growth signals, come to a halt in the quiescent, non-growing G0 state. Normal cells in G1 arrest due to serine starvation do not incorporate [^{3}H]-thymidine into DNA, but do so as soon as serine is restored. However, biotin-deficient cells, under similar conditions, do not incorporate [^{3}H]-thymidine even when serine is restored. When biotin is restored to the medium, the biotin-deficient cells, after a lag, start incorporating thymidine into DNA, with a stimulation of protein synthesis during this lag.

The 3T3-L1 mouse fibroblast cell line can differentiate into an adipose cell type. When the cells reach confluence and start to differentiate, they greatly increase the rate of triglyceride synthesis. This parallels the coordinate increase in the activities of the key enzymes of lipogenesis and correlates with the rise in the nuclear run-off transcription rates for the mRNA during differentiation. This process of

differentiation can be accelerated by increasing the amount of serum in the culture medium, or by adding insulin or biotin to the culture medium.

Biotin deficiency in mice changes the subpopulation of spleen lymphocytes and decreases their proliferative response to concanavalin A. Under conditions of biotin deficiency, the involution of the thymus is accelerated and thymocyte maturation is arrested, indicating that a specific stage in T cell maturation is sensitive to biotin deficiency.

Development of the Palatal Process

Congenital malformations occur in domestic fowl maintained on a biotin-deficient diet. At mid-gestation, biotin-deficient embryos weighed less than normal embryos and had external malformations such as micrognathia and micromelia. There was a marked decrease in the size of the palatal process due to altered proliferation of the mesenchyme. The development of the palatal process in culture was investigated (Watanabe et al. 1995). After 72 h of organ culture, more than 40% of the explants from normal (biotin-repleted) mouse embryos were in stage 6 of development, which dropped to only 6.5% for embryos cultured in biotin-deficient medium. Administration of biotin to biotin-deficient dams 24 h prior to removal of the embryo resulted in over 50% of explants at stage 6 of development when cultured in a medium containing 10^{-7} M biotin. There was no detrimental effect on any of the organic acid intermediates or their secondary metabolites on palatal closure of the explants when these compounds were added to the organ culture medium, indicating the continuous requirement for biotin during proliferation of the mesenchyme, perhaps for the synthesis of growth factors during organogenesis. As a marginal deficiency of biotin in normal human pregnancy is quite common, the teratogenic effects of such deficiency are a matter of concern.

Biotin and the Reproductive System

In mammals, spermatogenesis is dependent primarily on testosterone, which is produced in the Leydig cells and acts on the tubular cells of the seminiferous tubules to drive spermatogenesis. Testicular and serum levels of testosterone are decreased in the biotin-deficient rat (Paulose et al. 1989). Biotin deficiency was accompanied by a significant sloughing of the seminiferous tubule epithelium in these rats. Treatment of biotin-deficient rats with gonadotropins or biotin increases the levels of serum testosterone. Even when the testosterone levels are maintained high in biotin-deficient rats by testosterone implants, the increase in serum testosterone does not result in normal spermatogenesis. In contrast, the administration of biotin alone or with testosterone to biotin-deficient rats leads to normal spermatogenesis. This suggests that biotin is involved in the formation of local testicular factors that are required in addition to testosterone and follicle-stimulating hormone, for the normal interaction among Leydig, Sertoli, and peritubular cells.

The effects of diets with varying biotin contents on the estrus cycle, estradiol and progesterone serum levels, ovarian morphology, uterine mRNA abundance of estradiol and progesterone nuclear receptors, as well as estradiol-degrading enzymes in the liver of BALB/cAnNHsd female mice were studied (Baez-Saldana et al. 2009). Biotin deficiency was associated with reduction in ovary weight, arrested estrous cycle, and significant changes in ovarian morphology. Biotin deficiency decreases serum insulin growth factor-1 concentration, which might account for the effects observed in ovarian follicles in biotin-deficient mice.

Immunological and Inflammatory Functions

Cytokines, secreted by immune cells in response to antigen stimulation, bind to target cell receptors to activate intracellular signaling cascades controlling cellular processes such as growth, proliferation, and apoptosis. The expression of genes encoding the cytokine interleukin-2 and its receptor correlates with the biotin status of human lymphoid cells (Wiedmann et al. 2003). The effect of biotin on the metabolism of cytokines might underlie its role in immune function. The activities of various cell signals such as biotinyl-AMP, SP1 and SP3, nuclear factor NF-κB, and receptor tyrosine kinase are biotin dependent. Biotin deficiency upregulates tumor necrosis factor-α production, and biotin excess downregulates it (Kuroishi 2015). As this factor has an important role in the pathogenesis of inflammatory disease, the possibility of treating inflammatory diseases with biotin would be a fruitful area of investigation.

Biotin and Glucose Metabolism

The initial and rate-limiting step in the metabolic utilization of glucose is its phosphorylation by glucokinase, whose activity is influenced by dietary, nutritional, and hormonal states of the animal. Hepatic glucokinase is decreased in biotin-deficient rats fed with either high or low carbohydrate diets, and administration of insulin or biotin restored glucokinase activity to normal levels (Dakshinamurti and Cheah-Tan 1968a, b). Biotin also played a role in the precocious development of glucokinase in young rats (Dakshinamurti and Ho Chong 1970). In all these studies, enzyme activity correlated with protein synthesis. Pharmacologic levels of biotin increased glucokinase activity levels in biotin-repleted animals (Dakshinamurti 2005). Hepatic glucokinase activity in biotin-injected starved rats increased threefold compared with starved rats. The relative amount of glucokinase mRNA in the liver of biotin-injected starved rats increased fourfold over the levels seen in normal-fed rats and about 20-fold of that seen in starved rats not receiving biotin injection. Glucokinase induction was marked and rapid and correlated with increased glucokinase enzyme activity. In “run-on” transcription assays using isolated liver nuclei, biotin administration to the whole animal increased glucokinase gene transcription sevenfold, an effect that was not due to an increase in overall transcription efficiency

as the transcription of the β-actin gene was unaffected (Chauhan and Dakshinamurti 1991).

In both fasted and diabetic rats, hepatic phosphoenolpyruvate carboxykinase (PEPCK) activities are markedly increased. Refeeding a high-carbohydrate diet to fasted rats decreased PEPCK mRNA due to repression of PEPCK gene transcription by insulin. Three hours after biotin administration to starved rats, hepatic PEPCK mRNA levels decreased to 15% of the levels seen in non-biotin-injected starved rats. The effect of biotin paralleled the effect of insulin in these animals. In "run-on" transcription experiments using isolated liver nuclei, biotin suppressed hepatic PEPCK mRNA by 55% at 30 min after biotin administration. The inhibition is dominant over other stimulatory effects (Dakshinamurti and Li 1994). There are many similarities between biotin and insulin in their actions on enzymes of glucose metabolism. Both induce a key glycolytic enzyme and repress PEPCK, the key gluconeogenic enzyme. Further experiments indicate that biotin repressed the gluconeogenic genes through a pathway independent of insulin signaling (Sugita et al. 2008).

Biotin stimulated glucokinase activity and mRNA expression after a short-term treatment in cultured pancreatic β cells. Biotin also stimulated glucokinase activity and insulin secretion in rat islets in culture. Islet glucokinase activity and mRNA are reduced by 50% in the biotin-deficient rat. Insulin secretion in response to glucose was also impaired in islets isolated from biotin-deficient rats (Romero-Navarro et al. 1999). Biotin deficiency also affects pancreatic islet morphology. Besides defects in insulin sensitivity, there was disruption of islet architecture with an increase in the number of α cells in the islet core. Biotin deficiency promoted hyperglycemic mechanisms (Larrieta et al. 2012). Administration of pharmacological concentrations of biotin to normal rodents enhanced insulin secretion and the expression of genes and signaling pathways that favor islet function and augmented the proportion of β cells by enlarging islet size (Lazo de la Vega-Monroy et al. 2013).

Biotin starvation reduces glucose consumption and energy production in three different eukaryotes, despite the fact their phylogenetic lines diverged more than a billion years ago. In these biotin-starved organisms – yeast, nematode, and rat – the genomic expression corresponded to scant glucose conditions, pointing to a strongly selected role of biotin in the control of carbon metabolism (Ortega-Cuellar et al. 2010; Velazquez-Arellano et al. 2011). This concept is strengthened by the similarities between biotin and insulin across species. In genetically diabetic kk mice and rats, biotin improved glucose tolerance. Similar results were also observed in humans with type I or type II diabetes (Singer and Geohas 2006). A combination of biotin and chromium picolinate improved glycemic control in poorly controlled diabetics receiving standard antidiabetic therapy (Albarracin et al. 2008).

A significant proportion of radioactive biotin injected into chicks or rats localized to the nuclear fraction of cells (Dakshinamurti and Mistry 1963b). During progressive biotin deficiency, the nuclear biotin content was relatively conserved, whereas there was a marked depletion of cytoplasmic and mitochondrial biotin content. A biotin-binding protein from rat liver nuclei has been isolated (Dakshinamurti and Chauhan 1990). This, along with other evidence on the incorporation of amino acids

into protein, indicated that biotin was involved in genetic regulation of protein synthesis (Dakshinamurti and Mistry 1963a). Later it was shown that all five histone classes extracted from human lymphocytes contained biotin (Stanley et al. 2001). HCS was distributed primarily in the nucleus of HeLa, Hep2, and fibroblasts with only a minor component in the cytoplasm (Narang et al. 2004; Gravel and Narang 2005). Thus, a dual role for HCS was established: its traditional role in the biotinylation of apocarboxylases and a novel role in the attachment of biotin to histones. HCS appears to interact with the methyl-CpG-binding domain protein 2 and also histone methyl transferase, thus creating epigenetic synergies between biotinylation and methylation events (Gravel and Narang 2005).

Biotin Induction of Pancreatitis-Associated Proteins

Recently, the effect of biotin repletion on the pancreas using a rat model of chronic biotin depletion was studied (Dakshinamurti et al. 2015). Animals fed with egg white-supplemented chow for 6 weeks were subsequently returned to a normal chow diet and given a single dose of biotin, or remained on the egg white diet for 1 week. Pancreatic gene expression was then analyzed by microarray.

Gene ontology analysis of the results revealed global gene expression changes in the pancreas of biotin-repleted rats compared to those with biotin depletion that, collectively, indicated a rapid induction of reparatory mechanisms upon restoration of biotin (Fig. 4) (Dakshinamurti et al. 2015). Notably, transcripts related to inflammation and fibrosis, such as mast cell protease 1 precursor, mast cell chymase 1, collagen Iα1, and collagen Vα1, were elevated by two- to threefold in the biotin-depleted pancreas. In contrast, transcripts associated with pancreatic endocrine/exocrine function were significantly increased by biotin repletion, including glucagon, islet amyloid polypeptide, and α-amylase 1A, as well as regulatory transcription factors including hepatocyte nuclear factor 6, hepatocyte nuclear factor 3β, and pancreas-specific transcription factor 1a. Further, evidence of the induction of a pancreas repair program following biotin repletion was noted. Pancreatitis-associated proteins 1 (PapI/Reg3b) and 2 (PapII/

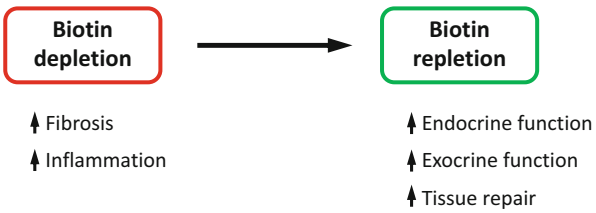


Fig. 4 Biotin repletion induces pancreatic repair. Following 6 weeks of biotin depletion, rats were maintained on a biotin-deficient diet, or were switched to a standard chow diet for 1 week and provided with a single injection of biotin (10 mg/kg IP). DNA microarray and quantitative PCR analysis of pancreas mRNA transcripts revealed gene expression changes indicative of tissue damage, inflammation, and fibrosis in biotin-deficient rats, compared to upregulation of endocrine and exocrine function and evidence of tissue repair following biotin repletion

Table 1 Expression of pancreatic repair transcripts following biotin depletion and repletion (Reproduced with permission from Dakshinamurti et al. 2015. Expression levels noted for acute pancreatitis are derived from Dusetti et al. 2000)

AffyID	Gene name	Gene symbol	BR/BD (fold)	Acute pancreatitis
1368238_at	Pancreatitis-associated protein 1 (PapI)/ regenerating islet-derived 3 beta	Reg3b	+34.3	+13.3
1387930_at	Pancreatitis-associated protein 2 (PapII)/ regenerating islet-derived 3 alpha	Reg3a	+14.9	+8.5
1367581_a_at	Osteopontin/secreted phosphoprotein 1	Spp1	+2.5	+3.6

Reg3a) are C-type lectins implicated in repair processes in numerous tissues; notably, they are induced by inflammation, and the loss of PapI, PapII, and the related PapIII by gene knockdown results in exacerbation of induced acute pancreatitis, with increased inflammatory cell infiltration (Closa et al. 2007; Vasseur et al. 2004; Zhang et al. 2004). A dramatic increase in PapI and PapII expression by 34- and 15-fold, respectively (Table 1), was noted similar to reports of Pap upregulation in acute pancreatitis (Dusetti et al. 2000); quantitative PCR analysis of pancreatic total RNA confirmed the microarray data, showing both transcripts increased in expression by 200-fold in response to biotin repletion. Given this robust response, biotin was assessed for the ability to independently alter Pap gene expression. Treatment of AR42J pancreatic acinar cells with biotin for 48 h induced significant five- to sevenfold increases in PapI and PapII expression (Dakshinamurti et al. 2015). Another transcript implicated in tissue repair, osteopontin/secreted phosphoprotein 1/Spp1, was also induced in the pancreas following biotin repletion, as well as in AR42J cells in response to biotin, but exhibited much lower levels of induction.

Such changes in Pap expression clearly reflect a potent induction; however, it pales compared to the changes observed in the biotin-repleted pancreas. This may simply reflect in vitro versus in vivo differences, however, as others noted that inflammation itself induces Pap gene expression (Closa et al. 2007). Given the microarray evidence of inflammation in the biotin-depleted pancreas, it is possible that restoration of biotin synergistically activates a Pap-mediated repair program in conjunction with the inflammation-driven mechanism.

Collectively, this data suggests that biotin deficiency results in pancreatic inflammation and fibrosis, possibly impairing its normal endocrine and exocrine functions. These results correlate with earlier reports of low biotin levels in diabetic patients and improved blood sugar control by biotin plus chromium picolinate. Biotin therefore appears to play an important role in normal pancreatic health and function. This data also suggests that much of the damage caused by biotin loss can be relatively rapidly reversed, concomitant with the activation of damage repair pathways, at least during the time period tested in this study (6 weeks). This is encouraging for individuals who may suffer acute or chronic biotin deficiency and furthermore suggests that biotin may be of benefit to diabetics in general, an avenue for further research.

Pharmacologic Effects: Biotin and Biotin-Binding Proteins

Animal studies have shown that biotin deficiency results in impaired glucose tolerance and decreased glucose utilization. Various clinical studies have indicated an inverse correlation between serum biotin levels and fasting blood glucose. The hypoglycemic effect of pharmacological doses of biotin has been reported in both type 1 and type 2 diabetic patients (Albarracin et al. 2008).

A common feature of metabolic syndrome is insulin resistance. It is also associated with hypertension. The pharmacological effect of biotin on hypertension was studied in the stroke-prone spontaneously hypertensive rat strain (Watanabe-Kamiyama et al. 2008). Biotin was beneficial: even a single dose decreased systolic blood pressure, an action that might be related to the effect of biotin as a soluble guanylate cyclase activator (Spence and Koudelka 1984; Singh and Dakshinamurti 1988). Biotin regulates the expression of several genes through activation of this cyclase (De La Vega and Stockert 2000).

ACC1 is a key lipogenic enzyme. In mammalian lipogenic tissues, this enzyme is regulated by dietary, nutritional, and hormonal conditions. Allosteric activation by citrate, feedback inhibition by long-chain fatty acids, reversible phosphorylation, and gene regulation of enzyme synthesis are the mechanisms regulating enzyme activity of ACC1. ACC1 is a cytosolic enzyme. In contrast, ACC2 is expressed mostly in the heart and skeletal muscle mitochondria where its product, malonyl-CoA, potently inhibits fatty acid oxidation. Long-chain fatty acids are converted by carnitine palmitoyltransferase into acyl carnitines for transport into the mitochondria, a process inhibited by ACC2-produced malonyl-CoA (Tong 2005; Tong and Harwood 2006). ACC2 knockout mice showed decreased malonyl-CoA content in skeletal and cardiac muscle, with increased fatty acid oxidation and reductions in total body fat, plasma free fatty acids and glucose, and tissue glycogen. These animals are protected from diet-induced diabetes and obesity (Oh et al. 2005). Because of its unique position in metabolism, the inhibition of ACC1 would inhibit de novo fatty acid production in lipogenic tissues and inhibition of ACC2 would inhibit fatty acid oxidation in the skeletal muscle and heart. This would have a favorable effect on cardiovascular risk factors associated with diabetes, insulin resistance, obesity, and the cluster of conditions referred to as the metabolic syndrome. A specific inhibitor of ACC2 would have therapeutic implications and is an area of intense current research.

One of the most devastating rice pathogens in the world is the fungus *Magnaporthe oryzae*, which depends on biotin for its growth (Skamnioti and Gurr 2009). Tamavidin1, an avidin-like biotin-binding protein from the mushroom *Pleurotus cornucopiae*, restricts the growth of *M. oryzae*. The possibility of reducing the availability of biotin to *M. oryzae* in rice by expression of the gene that encodes for tamavidin1 was investigated (Takakura et al. 2012). The positive results indicated a role for this strategy to engineer disease-resistant plants through the pathogen's auxotrophy. Biotin-binding proteins expressed in transgenic plants are

insecticidal to a wide variety of insects and, thus, useful in transgenic crop protection. Although their role in the protection of food crops such as rice has been established with some caveats, their use in protection of non-food crops such as fiber, forestry, and biofuel crops is boundless (Christeller et al. 2010).

Policies and Protocols

To our knowledge, policies on the provision of biotin in the diet, either for deficient individuals or as an adjunct to treatment of disease, are currently lacking. Biotin deficiency is not associated with short- or long-term threats to human life and furthermore is relatively rare, although occurrence is more likely in underdeveloped nations. A strong regulatory policy framework is thus unlikely to be needed, such as daily dietary requirements, particularly since such requirements remain poorly defined. Instead, it may be better to promote education of the benefit of biotin. Pregnant women may be biotin deficient and risk developmental delays in their children. In this case, government policies to promote monitoring of biotin status during pregnancy may be warranted. Government policies to promote education of pregnant women on the risks of biotin deficiency may also be useful. Likewise, those on long-term anticonvulsant therapy are likely to be biotin deficient, requiring supplementation.

Emerging evidence that biotin supplementation is beneficial in certain diseases such as diabetes suggests that it may be prudent to consider the development of policies at the point of care establishing the parameters of such supplementation. Further study in this area is strongly recommended, since biotin supplementation may provide a very cost-effective approach to helping manage diabetes and related conditions.

Dictionary of Terms

- **Avidin –** A protein found at high concentration in egg white, as well as other sources, which binds to biotin with high affinity.
- **Biotin** – A water-soluble vitamin that serves as a prosthetic group for multiple carboxylase enzymes. An alternate name for biotin is vitamin H.
- **Biotinidase** – An enzyme that cleaves covalently bound biotin from biotin-containing molecules. Biotinidase is the only known biotin-binding protein in human plasma.
- **Carboxylase** – An enzyme for transferring carboxyl moieties from a donor to an acceptor molecule.
- **Sodium-dependent multivitamin transporter (SMVT)** – A saturable transporter responsible for biotin uptake at the nanomolar level in the gut. SMVT is also able to bind to and transport pantothenic acid and lipoic acid.

Summary Points

- Biotin, a water-soluble vitamin, has a crucial role in the metabolism of carbohydrates and amino acids as well as in the synthesis and oxidative metabolism of lipids.
- Biotin acts as a prosthetic group for a number of key metabolic carboxylases.
- Humans and other animals obtain biotin from the diet, whereas many other organisms are capable of synthesizing biotin endogenously.
- While dietary guidelines for biotin in humans have been published, the precise requirement for biotin in the diet remains poorly defined.
- Biotin deficiency can cause alopecia, skin rash, lethargy, hypotonia, and, in biotin-deficient infants or children, developmental delays.
- Diet-induced biotin deficiency is rare, but can occur in response to excessive consumption of egg whites due to the biotin-binding capacity of avidin, due to chronic consumption of biotin-poor diets, or following long-term consumption of a ketogenic diet.
- Normal human pregnancy is associated with a biotin-deficient state; patients who have been on long-term anticonvulsant therapy are also biotin deficient.
- In addition to its role as the prosthetic group of the carboxylases, biotin has significant non-prosthetic group functions affecting cell proliferation, differentiation, glucose metabolism, and the inflammatory state.
- Pharmacological doses of biotin have profound ameliorative effects on conditions such as diabetes and inflammation.
- Biotin repletion studies in biotin-deficient rats suggest that biotin is capable of inducing the expression of reparative factors such as the pancreatitis-associated proteins.

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Vitamin B12 Deficiency and Impact on MRI Morphometrics: Association Between Cognitive Impairment and Neuroimaging Findings

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Abstract

Vitamin B12 deficiency has been associated with various neuropsychiatric symptoms, and it has also been reported to be a reversible cause of dementia. In addition to myelopathy and hematological changes, increasing evidence suggests an association between low vitamin B12 status and cognitive impairment. The negative impacts of low vitamin B12 status on cerebrum range from subclinical microstructural changes to an overt debilitating state associated with permanent cerebral volume reduction. Therefore, prompt diagnosis of cognitive syndromes related to vitamin B12 deficiency is of paramount clinical importance, since this can significantly alter the prognosis and treatment approach. In addition, the impact of low vitamin B12 status can vary in terms of clinical phenotype and disease severity, resulting in further challenges in clinical practice. This raises the urgent need for other objective tools with sufficient reliability and reproducibility in addition to the existing serology profile survey. By incorporating structuralized cognitive and magnetic resonance imaging evaluations, the cognitive profiles and neuronal substrates vulnerable to vitamin B12 deficiency in the context of neurobiochemistry basis can be further explored. This chapter will provide an overview of the current knowledge from studies aiming on cognitive and magnetic resonance imaging evaluations, and discuss supportive evidence in related fields.

Keywords

Vitamin B12 · Cognition · Magnetic resonance imaging · Mild cognitive impairment · Alzheimer's disease · Dementia · Subacute combined degeneration · Vitamin B12 Deficiency neuropsychological syndromes · Diffusion tensor imaging · Cognitive syndromes related to vitamin B12 deficiency

List of Abbreviations

AD	Alzheimer's disease
DTI	Diffusion tensor imaging
Hcy	Homocysteine
holo-TC	Holotranscobalamin
MCI	Mild cognitive impairment
MMA	Methylmalonic acid
MRI	Magnetic resonance imaging
TC	Transcobalamin
VBDNS	Vitamin B12 deficiency neuropsychological syndromes
Vit.B12	Vitamin B12

Introduction

Vitamin B12 (Vit.B12) is regarded to be a vitally important nutrient that has a significant impact on human health. In humans, vitamin B12 is required for two enzymatic reactions: the conversion of methylmalonyl-coenzyme A to succinyl

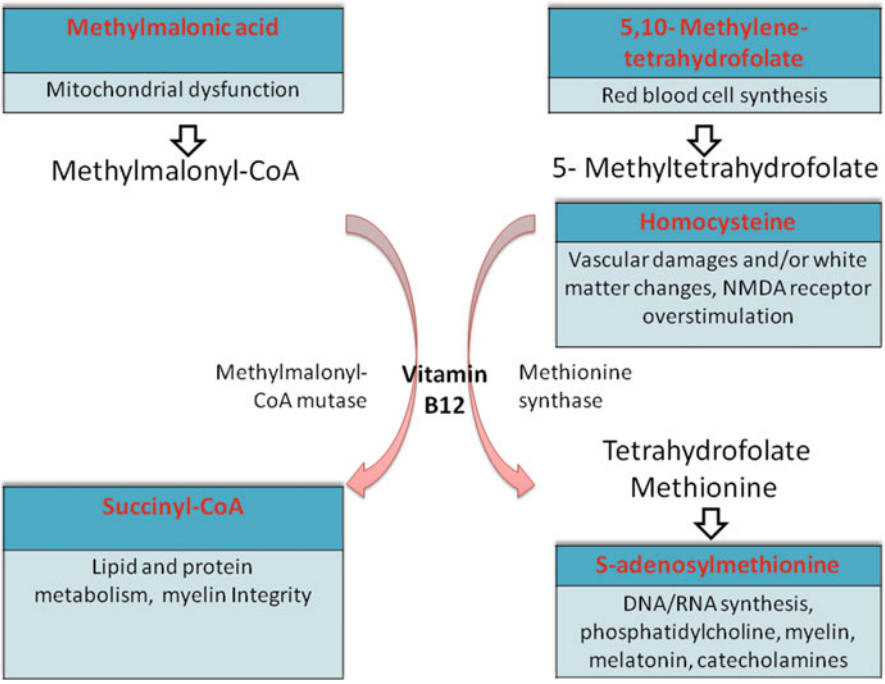

Fig. 1 Biochemistry pathway and cellular impact of vitamin B12 deficiency. In human, two typified major coenzyme B12-dependent enzyme are identified. Methylmalonyl-CoA mutase (the *left half* pathway) is regarded to be necessary for proper myelin synthesis and is not affected by folate supplementation. Contrarily, methionine synthase (the *right half* pathway) transfers a methyl group from 5-methyltetrahydrofolate to homocysteine, thereby generating tetrahydrofolate and methionine. In vitamin B12 deficiency, an increased homocysteine level and the trapping of folate as 5-methyl-tetrahydrofolate, from which tetrahydrofolate (the active form of folate) cannot be recovered, are expected. Current opinion suggests that vitamin B12 deficiency to be defined in terms of the serum concentration of vitamin B12, and of homocysteine and methylmalonic acid, two components of the vitamin B12 biochemistry pathways. *NMDA* N-methyl-D-aspartate, *DNA* deoxyribonucleic acid, *RNA* ribonucleic acid

coenzyme A, and the conversion of homocysteine (Hcy) to methionine. Through its unique biological properties, it is involved in several steps critical for hematopoiesis and myelination (Fig. 1). The clinical presentations related to Vit.B12 deficiency are polymorphic and of varying severity. Hematoneurological dissociations are common (Lindenbaum et al. 1988; Stabler et al. 1990; Tu et al. 2017). In the hematological system, Vit.B12 deficiency has been associated with megaloblastic anemia and dysplastic bone marrow (Stabler 2013). From a neurological aspect, except for subacute combined degeneration, low Vit.B12 status has been associated with various neuropsychiatric symptoms, and it is regarded to be a reversible cause of dementia (Stabler 2013). Cognitive impairment appears to be one of the most common Vit.B12 deficiency neuropsychological syndromes (VBDNS) (Table 1; Tu et al. 2017). As the negative impacts of low Vit.B12 status on cognition can

Table 1 Neuropsychiatric presentations of patients with vitamin B12 deficiency

Cognitive complaints[a]	Polyneuropathy
Dementia[a]	Cranial neuropathy
Anxiety[a]	Myelopathy
Cerebrovascular disease	Seizure
Depression	

Data adapted with permission from Tu et al. (2017).
[a]Denotes most commonly seen features.

range from subclinical neuronal metabolic derangement to an overt debilitating state associated with permanent cerebral structural changes, prompt recognition of Vit. B12 deficiency is of paramount clinical importance. Although increasing evidence supports that a low Vit.B12 status contributes to cognitive impairment, it remains frequently overlooked in clinical practice due to its diverse clinical presentations. On the basis of bioabsorption, several etiologies have been identified (Lachner et al. 2012):

1. Food-cobalamin malabsorption (e.g., atrophic gastritis and chronic metformin/antacids users)
2. Autoimmune (e.g., pernicious anemia and Sjögren's syndrome)
3. Surgical (e.g., gastrectomy and ileal resection)
4. Decreased intake or malnutrition (e.g., a vegetarian diet and alcoholism)
5. Intestinal malabsorption (e.g., chronic pancreatitis and inflammatory bowel disease)
6. Increased demand (e.g., pregnancy and lactation)
7. Genetic (e.g., transcobalamin II deficiency)
8. Food-cobalamin malabsorption is likely to be the leading cause of Vit.B12 deficiency, especially in elderly patients (Andrès et al. 2008).

With increasing research related to the biochemical pathway of Vit.B12 deficiency, several biochemical indicators reflecting Vit.B12 status have been identified. Elevated Hcy and methylmalonic acid (MMA) are generally regarded to be more sensitive metabolic indicators of Vit.B12 deficiency than total plasma Vit.B12 (Hvas and Nexo 2005). However, both measurements can be influenced by renal insufficiency, and elevated Hcy may also reflect a lack of folate as well as vitamin B6 (Joosten et al. 1993), thereby limiting their specificity. Another candidate marker, holotranscobalamin (holo-TC), represents the biologically active fraction of Vit.B12 (~20% of total Vit.B12) that can be delivered to all cells of the body and is as a more specific and sensitive marker for functional Vit.B12 status (Hvas and Nexo 2005). Likewise, transcobalamin (TC) saturation (the ratio of holo-TC over total TC) also represents another index that can be used to quantify the biologically active level of Vit.B12. These aforementioned indicators can be used as tools in diagnosis, evaluation, and follow-up, especially for those with hematological presentations. However, a considerable number of patients still have preferential neurological system involvement and/or discordant recovery between neurological and hematological

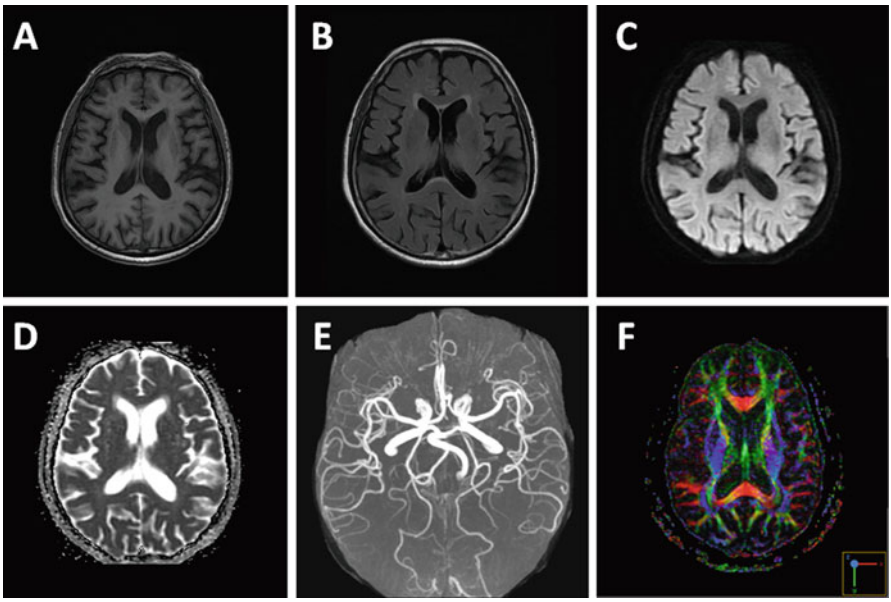

Fig. 2 Clinical and research applications of brain magnetic resonance imaging. There has been increased use of several sequences and protocols of brain magnetic resonance imaging in clinical practice. (**a**) Gradient echo T1-weighted imaging (3D fast-spoiled gradient): A sequence for visualizing detailed brain structure and volumetric assessment. (**b**) T2 FLAIR: A sequence commonly used for detecting structural damages, such as white-matter hyperintensities. A sequence for FLAIR is a special inversion recovery sequence with long inversion time, which results in removing signal from cerebrospinal fluid from the resulting images. (**c**) Diffusion-weighted imaging: A sequence sensitive in detecting highly cellular tissue and ischemic changes by measuring the random Brownian motion of water molecules within a voxel of tissue. (**d**) Apparent diffusion coefficient: A tool enabling quantification of the degree of diffusion of water molecules through different tissues. (**e**) Magnetic resonance angiography: A tool for visualization of intracranial artery pathology by creating an image of blood flow in the vessels. (**f**) Diffusion tensor imaging (displayed in color coding): A novel tool for delineating and quantifying cerebral microstructural changes. *FLAIR* Fluid attenuation inversion recovery

systems. Therefore, cognitive and neuroimaging assessments may be more pertinent tools to evaluate relevant neuropsychiatric symptoms related to Vit.B12 deficiency. In general, cognitive tests are used to assess the intelligence performance of subjects in a structuralized and standardized manner. Neuroimaging studies can mirror the degree and extent of brain parenchymal and even microstructural changes through quantification and descriptive methods. For example, there are various sequences in conventional magnetic resonance imaging (MRI) which can provide information related to brain pathology and volume changes (Fig. 2). Additionally, several other novel protocols have also been proven to detect microstructural changes within regions that are of normal appearances in conventional MRI (Fig. 2). The following text will detail evidence and opinions from current research.

Vitamin B12 Deficiency, Cognition, and Magnetic Resonance Imaging Findings

Impact of Vit.B12 Deficiency on Cognition

Associations Between Vit.B12 Dietary Intake and Cognitive Performance

The results of research on the association between Vit.B12 dietary intake and cognitive performance are diverse (Table 2). A meta-analysis (Doets et al. 2013) of three studies (5254 elderly people aged 49–93 years) for the relative risk of Alzheimer's disease (AD) (431 cases) with a duration of 3.9–9.3 years revealed no association between Vit.B12 intake and the incidence of AD (Corrada et al. 2005; Morris et al. 2006; Nelson et al. 2009). The same study (Doets et al. 2013) also found no significant association between Vit.B12 intake and global cognition. However, other studies reported an association between slower global cognitive declines in elderly people with a high Vit.B12 intake (Morris et al. 2005). Associations between total Vit.B12 intake and performance on naming, nonverbal memory recall, and constructional praxis were also observed in patients with AD (Kim et al. 2014). Furthermore, studies on the association between Vit.B12 intake and domain-specific cognition have also reported inconsistent results. Some studies have reported apossible relationship between dietary Vit.B12 intake and constructional praxis (Tucker et al. 2005), while others have reported associations with nonverbal memory and abstract reasoning (La Rue et al. 1997). In a double-blind, randomized placebo-controlled trial which investigated whether daily supplementation with high doses of oral Vit.B12 alone or in combination with folic acid had any beneficial effects on cognitive function in the elderly with mild Vit.B12 deficiency (a serum Vit.B12 level 100 ~ 200 pmol/L, or a serum Vit.B12 level 200 ~ 300 pmol/L *and* a plasma MMA concentration $\geq$ 0.32 μmol/L *and* a serum creatinine concentration $\leq$ 120 μmol/L), Vit.B12 supplementation with or without folic acid led to improvements in memory function, but not in the domains of sensorimotor speed or executive function (Eussen et al. 2006). Paradoxically, both the treatment and placebo groups in this study showed improvements in memory function. Moreover, the improvement in the placebo group was even better than that in the Vit.B12 group, further questioning whether Vit.B12 supplementation can provide cognitive benefits.

Studies investigating the relationship between Vit.B12 dietary intake and cognitive performance often demonstrate similar positive effects from other targeted nutrients in the same cohort. A link between Vit.B12 and cognitive performance may therefore be less concretized when considering other consumed nutrients as confounders in addition to interpersonal variations of Vit.B12 bioabsorption. Therefore, serological tests may provide more direct evidence from a biological aspect.

Association Between Vit.B12 Status and Cognitive Performance

Several studies have reported no association between serum Vit.B12 itself and the incidence of dementia, AD (Crystal et al. 1994; Wang et al. 2001; Ravaglia et al. 2005; Kivipelto et al. 2009), or global cognitive function (McCracken et al. 2006; Clarke et al. 2007). However, one study suggested the potential value of holo-TC, an

Table 2 Selected articles supporting negative impact of vitamin B12 deficiency on cognition

References	Vitamin B12	Global cognition/ specific cognitive domain	Cognitive assessment tools	Participants	Major positive findings	
					Results	Conclusions
La Rue et al. (1997)	Intake	Memory, constructional praxis, and abstract reasoning	Logical memory and visual reproduction (WMS), Rey-Osterrieth complex figure test, and abstraction scale from the Shipley-Hartford intelligence test	137 healthy elderly participants (community residents aged 66–90 y, followed for 6 years)	Past dietary Vit. B12 intake↑: visual memory recall and abstract reasoning↑($P < 0.05$) B vitamin supplement users: Rey-Osterrieth copy ($P < 0.05$), Rey-Osterrieth recall ($P < 0.05$), and abstraction ($P < 0.01$) tests↑	Higher past vitamin intake (including Vit. B12) is related to better visuospatial recall and/or abstraction performances
Morris et al. (2005)		Global cognition	Averaged z score of MMSE, East Boston tests of immediate and delayed recall, and symbol digit modalities test	3718 healthy elderly participants (aged ≥65 y)	Overall: N.S. Note: a significant interaction between total intake of Vit. B12 and older age (P for interaction = 0.009), and the rate of cognitive decline for eighties differ by Vit. B12 intake	High intake of Vit. B12 among the old participants (an average 80-year-old) may have slower cognitive decline
Tucker et al. (2005)		Global cognition	MMSE	321 healthy elderly men (mean age of 67 y at baseline, followed for 3 years)	N.S.	Declines in constructional praxis are significantly associated with the dietary intake of Vit. B12
		Memory, language, constructional praxis, and working memory	The CERAD battery (including word list memory, verbal fluency, and constructional praxis), backward digit span, and a spatial copying task from the VMI		Baseline dietary intake of Vit. B12↑: Spatial copying↑($P < 0.05$).	

(*continued*)

Table 2 (continued)

References	Vitamin B12	Global cognition/ specific cognitive domain	Cognitive assessment tools	Participants	Major positive findings	
					Results	Conclusions
Eussen et al. (2006)		Attention, constructional praxis, sensorimotor speed, memory, and executive function	Digit span, figure of Rey, finger tapping, motor planning, word learning, trail making test, Raven's Coloured progressive matrices, Stroop test, similarities (WAIS-R), word fluency	195 elderly participants (aged ≥70 y; MMSE >19; CDR = 0, 1 or 2)	All groups had improvement in memory function, with greater extent in the placebo group than in the group who received Vit. B12 alone ($P = 0.0036$). Time × treatment interaction for the domain of memory was significant ($P = 0.0142$)	Oral supplementation with Vit. B12 did not improve cognitive function
Kim et al. (2014)		Global cognition	MMSE	100 MCI; 100 AD; 121 elderly participants (aged ≥60 y)	In AD subjects: total Vit. B12 intake↑: MMSE score↑($P = 0.01$)	Vit. B12 intake is associated with cognitive function in AD patients
		Memory, language, and constructional praxis	CERAD battery (including word list memory, verbal fluency, constructional praxis, constructional recall, and Boston naming test)		In AD subjects: total Vit. B12 intake↑: Boston naming test ($P = 0.028$), constructional recall test ($P = 0.012$), and constructional praxis test ($P = 0.013$) scores↑	

Miller et al. (2003)	Status	Global cognition	Modified MMSE (3MSE)	1789 elderly participants (aged ≥60 y)	Homocysteine↑: 3MSE↓on considering demographic and biochemical (folate, Vit. B12, and creatinine) variables	Homocysteine level predicts cognitive function independently
		Memory, language, semantic knowledge, abstract thinking, attention, and visuospatial function	Word-list learning, object naming, picture association, verbal conceptual thinking, verbal attention span, and pattern recognition tasks		Homocysteine↑: picture association, verbal attention, and pattern recognition ↓on considering all demographic and biochemical variables	
Tucker et al. (2005)		Global cognition	MMSE	321 healthy elderly men (mean age of 67 y at baseline, followed for 3 years)	N.S.	Low Vit.B12 and high homocysteine concentrations predict decline of memory and constructional praxis
		Verbal memory, language, constructional praxis, and working memory	The CERAD battery (including word list memory, verbal fluency, and constructional praxis), backward digit span, and a spatial copying task from the VMI		Baseline plasma Vit.B12 ($P < 0.05$)↑ Hcy ($P < 0.001$)↓: Spatial copying score↑.Hcy ($I < 0.05$)↓: Recall memory↑	
McCracken et al. (2006)		Global cognition	MMSE	84 nondemented elderly participants (aged >69 y)	MMA↑: MMSE scores↓independent of age and education ($P = 0.007$). Vit.B12 or holoTC: N.S.	MMA but not total Vit.B12 is associated with lower general cognitive function and particularly with worse performances of praxis and language
		Orientation, language, attention, memory, praxis, calculation, abstract thinking, and perception	CAMCOG		MMA↑: CAMCOG scores of ideational praxis ($P < 0.05$) and language comprehension ($P < 0.05$) and expression ($P < 0.01$)↓	

(*continued*)

Table 2 (continued)

References	Vitamin B12	Global cognition/ specific cognitive domain	Cognitive assessment tools	Participants	Major positive findings	
					Results	Conclusions
Clarke et al. (2007)		Global cognition	MMSE	2741 healthy elderly participants (mean aged 75.7 y) at baseline and 653 survivors (mean aged 73.6 y) at 10-year follow-up	holoTC↑2 x: 30% slower rate of cognitive decline. Hcy or MMA levels↑2 x: >50% more rapid cognitive decline	Low Vit. B12 status, evident by associated biochemical markers, is associated with more rapid cognitive decline
Feng et al. (2009)		Global cognition	MMSE	539 adult participants (aged ≥55 y; MMSE ≥21) at baseline and 247 at follow-up after 38 months on average	A significant interaction between Vit. B12 and *APOE* ϵ4 was found for MMSE score, digit span backward longest sequence, and RAVLT immediate recall. On taking *APOE* ϵ4 status into account, better performance in the latter 2 tests was only associated with Vit.B12 in *APOE* ϵ4 carriers	*APOE* ϵ4 status may modulate the association between Vit. B12 and cognitive function
		Memory, attention, and working memory	RAVLT; digit span			

Tangney et al. (2009)		Global cognition	Averaged z score of MMSE, East Boston tests of immediate and delayed recall, and symbol digit modalities test	516 healthy elderly participants (mean aged 80 y) followed for 6 years	MMA↑: faster rates of cognitive decline ($P = 0.004$). Vit.B12↑: slower rates of cognitive decline ($P = 0.005$)	Serum MMA and Vit. B12 concentrations may be important risk factors for cognitive decline
Hsu et al. (2016)		Global cognition	MMSE	34 patients with Vit. B12 deficiency (serum level $\leq$ 250 pg/ml; mean aged 62.91 y; CDR $\leq$ 1); 34 healthy adults (mean aged = 65.21 y)	Patients with Vit.B12 deficiency: MMSE scores↓($P < 0.05$)	Vit.B12 deficiency is associated with a global cognition decline with language, orientation, and working memory selectively impaired
		Attention, orientation, memory, language, constructional praxis, abstract thinking, and working memory	CASI		Patients with Vit.B12 deficiency: Language ($P < 0.01$), orientation ($P < 0.01$), and mental manipulation ($P < 0.05$)↓	

WMS Wechsler memory scale, *Vit. B12* vitamin B12, *MMSE* Mini-mental state examination, *N.S.* No significance, *CERAD* The consortium to establish a registry for Alzheimer's disease, *VMI* The developmental test of visual-motor integratio, *WAIS-R* Wechsler Adult Intelligence Scale – revised, *CDR* Clinical dementia rating scale, *MCI* Mild cognitive impairment, *AD* Alzheimer's disease, *Hcy* homocysteine, *CAMCOG* Cognitive section of the Cambridge mental disorders of the elderly examination, *MMA* Methylmalonic acid, *holoTC* Holotranscobalamin, *RAVLT* Rey auditory verbal learning test, *APOE* apolipoprotein E, *CASI* Cognitive abilities screening instrument.

active form of Vit.B12, in attenuating the risk of AD (Hooshmand et al. 2010). Such clinical observations reflect the fact that serum Vit.B12 level does not represent the precise Vit.B12 status, and that it may be insufficient to evaluate biochemical utilization at a cellular level. To further elucidate the association between Vit.B12 level and cognitive performances, serum or plasma biochemical indicators reflecting Vit.B12 status (e.g., holo-TC, TC saturation, Hcy, and MMA) have attracted increasing attention. Interestingly, several studies have shown that a higher serum Vit.B12 concentration at baseline was associated with a better global cognitive reserve (Feng et al. 2009; Tangney et al. 2009), while a higher MMA level (Clarke et al. 2007; Tangney et al. 2009) or lower holo-TC (Clarke et al. 2007) were associated with more rapid global cognitive decline. Many studies have also addressed domain-specific effects related to Vit.B12 status by evaluating executive function (Tucker et al. 2005), working memory (Feng et al. 2009), attention (Feng et al. 2009), verbal memory (Feng et al. 2009), and constructional praxis (Tucker et al. 2005). In a study by Miller et al., a cohort with a high prevalence of low Vit.B12 status (plasma Vit.B12: $\leq$ 200 pg/mL in 6.5% of the participants, and 200–300 pg/mL in 16.4%), serum Hcy level was found to be correlated with global cognitive performance as well as performance in multiple cognitive domains including memory, attention, and visuospatial ability (Miller et al. 2003). Moreover, patients with predefined low serum Vit.B12 level have been reported to have defective language function, orientation, and mental manipulation (Hsu et al. 2016). However, other studies have not shown any remarkable relationship between Vit.B12 level and memory (La Rue et al. 1997; Ravaglia et al. 2005; Mooijaart et al. 2005), executive function (La Rue et al. 1997), attention (Mooijaart et al. 2005; Hsu et al. 2016), processing speed (Mooijaart et al. 2005), constructional praxis, verbal fluency, or abstract thinking (Hsu et al. 2016).

The inconclusive results in these studies may be due to variations in time frame, sample size and characteristics, diagnostic criteria (cut-off values in serology panels), and variable tools used to assess cognitive ability, in addition to the complex nature of the absorption process of Vit.B12. The preliminary results await further corroboration. However, as cognitive deficits are thought to be the consequence of neuronal dysfunction and/or damage, neuroimaging findings may provide additional information to determine the clinical impact of low Vit.B12 status.

Impact of Vit.B12 Deficiency on Changes in Brain MRI

Several brain MRI findings could be identified among the patients with Vit.B12 deficiency, including volume reduction, white matter changes, vascular occlusion, and even microstructural changes (Figs. 3, 4, 5, and 6). Several studies have recruited different populations or clinical phenotypes to investigate the association between brain parenchymal changes and low Vit.B12 status (Table 3). As hemato-neurological dissociations exist and the associations between serum biomarkers and cognition are diverse, some patients with cognitive syndromes related to vitamin B12 deficiency

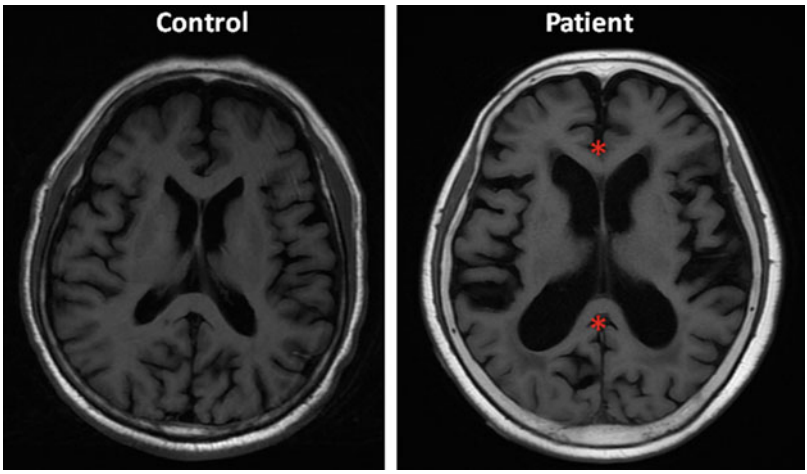

Fig. 3 Brain atrophy related to vitamin B12 deficiency. Example of a 77-year-old female patient with cognitive complaints for 2 years. Notice the pronounced ventricular dilatation and proportionate cortical atrophy in comparison with the images of age-matched control, obtained at approximately the same level. Notable thinning of the corpus callosum, either within the genu or splenium portion, is noted on axial T1-fluid attenuation inversion recovery images (*asterisk*) (Repetition time = 2000; echo time = 20)

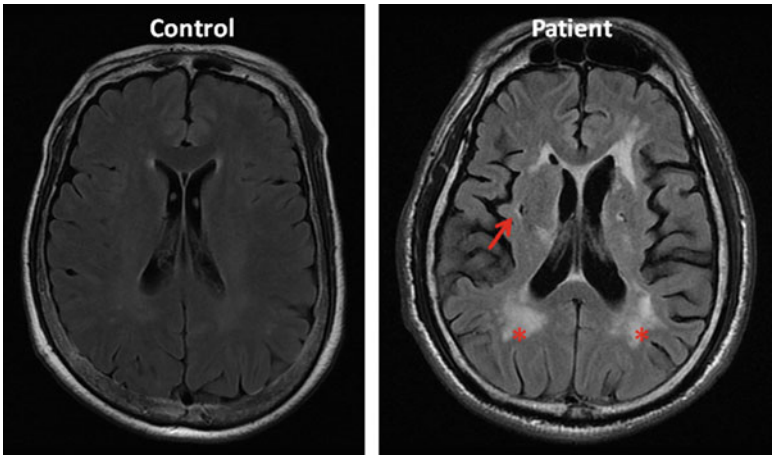

Fig. 4 White-matter hyperintensities related to vitamin B12 deficiency. Example of a 61-year-old, 6-year-education level, male patient with dull response for 1 year. Although he had no stroke history and his performance in Cognitive Abilities Screening Instrument was rated to be of borderline value (75/100), profound pathology was identified on axial T2-fluid attenuation inversion recovery images. Notice multifocal patchy areas of high signal in periventricular and deep white matter (*asterisk*) in comparison with images of age-matched control. There are several other small, discrete foci of hypointensities within bilateral basal ganglia, compatible with lacunes pathology (*arrow*) (Repetition time = 9000; echo time = 120)

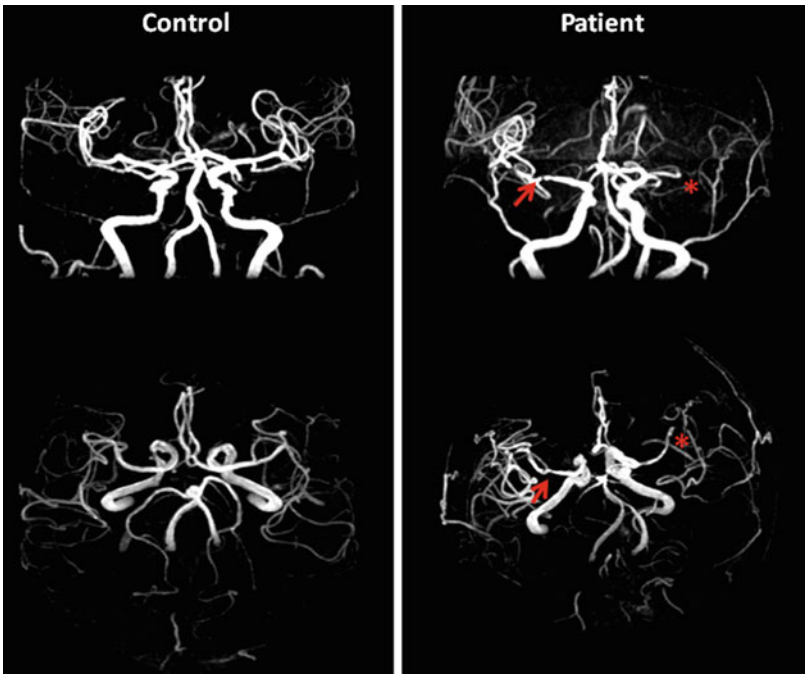

Fig. 5 Intracranial artery stenosis and occlusion precipitated by vitamin B12 deficiency. Example of an 86-year-old female patients with multiple recurrent stroke history. Although she had a history of hypertension and diabetes mellitus, both diseases were well controlled (baseline systolic blood pressure = 120 mmHg; glycated hemoglobin = 5.8%). Notice the absence of blood flow at proximal portion of the left middle cerebral artery (*asterisk*) in comparison with images of age-matched healthy control. Additionally, there is a narrowing of the right middle cerebral artery (*arrow*). Although there are still some other risk factors which may contribute to stroke events, intracranial artery stenosis/occlusion is frequently identified among patients with vitamin B12 deficiency (*upper row* frontal view, *lower row* bird's-eye view)

may demonstrate pure neurological symptoms in the absence of hematological changes. In clinical practice, their neurological complaints may also predate hematological derangements such as macrocytic anemia. The strength of studies incorporating MRI is to examine the negative impact of Vit.B12 deficiency on the brain through visualizing macro- and micro-structural changes. Through neuroimaging studies, issues related to the existence of domain-specific impacts related to Vit.B12 deficiency can be further clarified, as localization of relevant pathology may provide valuable information. Although this seems to be a potentially promising approach to explore the fundamental pathogenesis of cognitive syndromes related to vitamin B12 deficiency, several aspects should be considered with regard to interpretation. In addition to variables related to clinical status and demographic data, the neuroimaging protocols applied should be carefully reviewed, as not all provide spatially specific information.

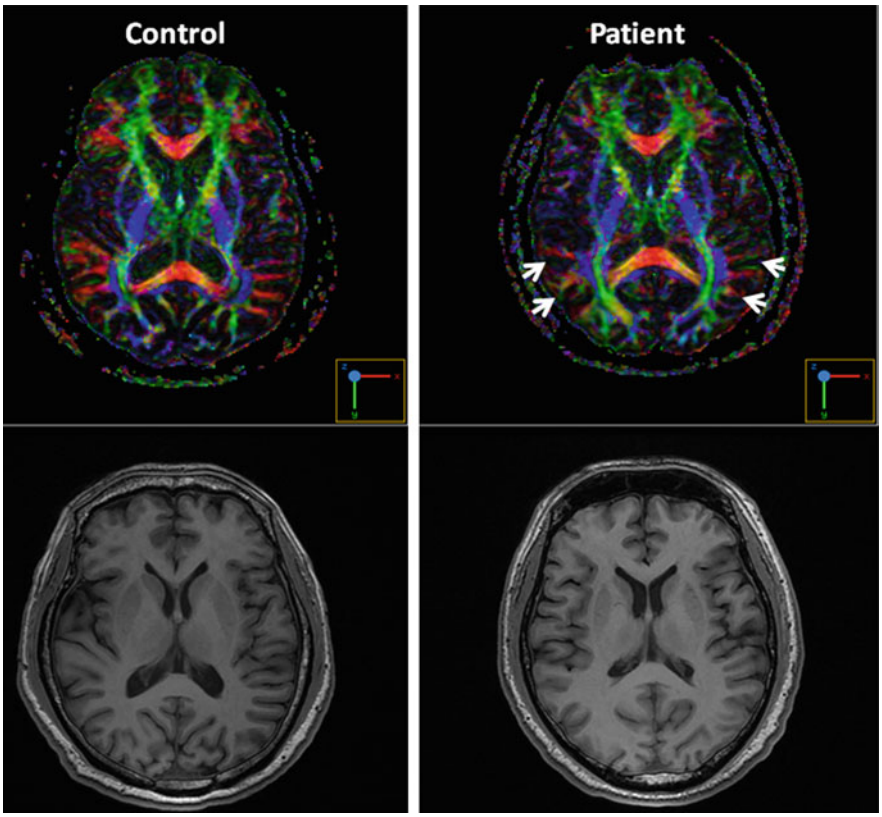

Fig. 6 Early cerebral microstructural changes related to vitamin B12 deficiency. Example of a 50-year-old, 10-year-education level, male patient with subtle cognitive complaints. His performance in Cognitive Abilities Screening Instrument was normal (93/100). Notice the loss of directionality within various association fibers on color-coded diffusion tensor images (*arrows*) compared with age-matched healthy control (*upper row*: repetition time = 8325; echo time = 83; diffusion weighting factor b = 1000 sec/mm2, plus one b = 0 image). In conventional MRI, his brain parenchyma is visually regarded to be normal in size (*lower row*: 3D fast-spoiled gradient; repetition time = 8; echo time = 3)

Healthy Elderly Subjects

A low Vit.B12 status has been associated with brain atrophy (Vogiatzoglou et al. 2008; Tangney et al. 2011), damage to the white matter (de Lau et al. 2009; Tangney et al. 2011), and gray matter volume reduction (Erickson et al. 2008) in healthy elderly people. Research recruiting cognitively normal subjects is of value to elucidate the impact of Vit.B12 status on occult brain lesions and/or cognitive decline in the context of a longitudinal study design.

In a longitudinal study by de Lau et al., a poor Vit.B12 status as assessed by measuring levels of Vit.B12, MMA, holo-TC, and TC saturation was significantly associated with a greater severity of white-matter lesions, particularly within

Table 3 Selected articles supporting negative impact of vitamin B12 deficiency on brain magnetic resonance imaging

References	Clinical phenotype	Methods	Major findings	
			Magnetic resonance imaging findings	Conclusion
Vogiatzoglou et al. (2008)	Healthy elderly	SIENA	Vit.B12 and holoTC↑: whole brain volume↑	Low Vit.B12 status in elderly subjects without dementia at baseline is associated with an increase rate of brain atrophy
Erickson et al. (2008)		Voxel-based morphometry	Intake of Vit.B12↑: gray matter volume↑. Effects were observed in the bilateral superior parietal lobules	Greater Vit.B12 intake is associated with protective effect on gray-matter volume
de Lau et al. (2009)		Semi-quantitative scoring for periventricular white matter lesions	Poorer Vit.B12 status (Vit.B12↓/holoTC↓/TC saturation↓/MMA↑): white-matter lesions↑(particularly those within periventricular regions/ in a concentration-related manner)	A concentration-related association between low Vit.B12 status and the severity of white-matter lesions, especially periventricular white-matter lesions, is identified and hypothesized to be related to myelin integrity damages
Tangney et al. (2011)		White-matter hyperintensity segmentation and total brain volume assessment	Hcy↑, MMA↑, cystathionine↑, 2-methylcitrate↑: total brain volume↓. Hcy↑: white matter hyperintensity volume↑	Poor Vit.B12 status is a risk factor for brain atrophy and white matter hyperintensity formation

Smith et al. (2010)	Mild cognitive impairment	SIENA	Users of Hcy-lowering B vitamins (folic acid, vitamins B6 and B12): brain atrophy rate↓(more favorable in participants with Hcy >13 μmol/L)	The brain atrophy rate in Pts with mild cognitive impairment can be slowed by treatment with Hcy-lowering B vitamins
Blasko et al. (2012)		Semiquantitative scoring for periventricular white-matter lesions	Users of Vit.B12 or folate: periventricular and deep white-matter hyperintensities↓	Vit.B12 or folate consumption may contribute to lower extent of white-matter hyperintensities formation
Köbe et al. (2016)		DTI (voxel-based analysis)	Pts with low-normal Vit.B12: mean diffusivity of the hippocampus↑ (mainly in the cornu ammonis 4 and dentate gyrus region)	Impaired microstructure integrity of the hippocampus, mainly in the cornu ammonis 4 and dentate gyrus region, is noted
Gupta et al. (2014)	Subacute combined degeneration	Voxel-based morphometry and DTI (tract-based statistics)	Compared with controls, the Pts: gray and white volumes: N.S. fractional anisotropy↓, mean and radial diffusivity↑in multiple brain regions	White-matter microstructural changes are seen in Pts with Vit.B12 deficiency
Roy et al. (2015)		DTI (tract-based statistics) and pseudo-continuous arterial spin labelling	Pts with Vit.B12 deficiency (pre- and posttreated state) had altered cerebral blood flow and fractional anisotropy values in various brain regions as compared with controls.	Vit.B12 supplement therapy can normalize cerebral microstructural changes. Microstructural recovery lags behind cerebral blood flow and cognition recovery at 6 weeks post therapy

SIENA Structural image evaluation using normalization of atrophy, *DTI* diffusion tensor imaging, *Vit.B12* vitamin B12, *N.S.* No significance, *MMA* methylmalonic acid, *holoTC* holotranscobalamin, *TC* transcobalamin, *Hcy* homocysteine, *Pts* patients.

periventricular areas, in a concentration-related manner (de Lau et al. 2009). These associations remained essentially unchanged after adjusting for potential clinical confounders. Although the associations appeared to be weakened after incorporating plasma Hcy and folate into the adjustment model, the association between TC saturation and periventricular white matter lesions remained robust and statistically significant. Of note, these associations occurred over a range of Vit.B12 levels and were not confined to the concentrations usually used to define deficiency. However, another cross-sectional study reported that higher levels of Vit.B12-related markers (i.e., serum Hcy, MMA, cystathionine, and 2-methylcitrate concentrations), but not serum Vit.B12 itself, were significantly associated with decreased total brain volume (Tangney et al. 2011). Interestingly, Hcy concentration was the only Vit.B12 marker that was associated with the volume of white matter hyperintensity (Tangney et al. 2011).

In contrast to the studies discussing a connection between Vit.B12 status and neuroimaging findings, one cohort study reported a positive association between dietary intake and brain structural changes (Erickson et al. 2008). In this study ($n = 32$), a greater total intake of Vit.B12 (diet plus supplementation) was reliably correlated with greater gray matter volume within bilateral superior parietal lobules. However, this effect was driven by the supplements, and the positive association with Vit.B12 was negated when only examining dietary intake. The authors did not identify any variables related to global gray or white matter. Of note, data of Vit.B12 consumption in this study were derived from 3-day food records/diaries (Erickson et al. 2008), and future studies incorporating serological data are warranted.

Subjects with Mild Cognitive Impairment (MCI)

MCI is an intermediate stage between the expected cognitive decline of normal aging and the more serious decline of dementia. Current opinion suggests that such transitional findings can also be observed from a neuroimaging aspect. That is, neuronal dysfunction and microstructural changes tend to predate overt brain volume loss by a considerable period of time. Therefore, sensitive MRI protocols are usually required to detect subtle changes in terms of this early disease process. For example, volumetry can provide information on brain volume in an overall or segmented method. Diffusion tensor imaging (DTI) usually includes parameters such as mean diffusivity and fractional anisotropy, and can delineate microstructural damage within regions that are of normal appearance in conventional MRI. Due to the fact that MCI is associated with the risk of dementia (Mufson et al. 2012), evaluating neuroimaging in these patients in association with Vit.B12 status provides the opportunity to determine whether Vit.B12 status has an additional impact on pre-existing (or ongoing) neurodegenerative processes.

By collecting information on both intake and status of Vit.B12 from a subset of a randomized double-blind controlled trial, Smith et al. suggested that patients over 70 years of age with MCI had a 29.6% lower rate of brain atrophy after 2 years of supplementation with high doses of vitamin B6, folic acid, and Vit.B12 compared with a placebo (Smith et al. 2010). Moreover, the rate of atrophy in the participants taking B vitamins was more than five times slower than in those taking the placebo. In addition, when focusing on the relationship between biological status and brain

atrophy, the rate of atrophy was significantly associated with changes in Hcy and inversely with changes in holo-TC and TC saturation, but not cystathionine (a marker of vitamin B6 status). The authors therefore concluded that the rate of brain atrophy in elderly patients with MCI can be slowed by treatment with homocysteine-lowering B vitamins, especially folic acid and Vit.B12 (Smith et al. 2010). In another study recruiting amnestic MCI patients, a novel finding suggested that the mean diffusivity of the hippocampus was higher in patients with low-normal Vit.B12 concentrations than in those with high-normal Vit.B12 concentrations (median split: 304 pmol/L) (Köbe et al. 2016). Comparing the volume of the total hippocampus and its subfields between these dichotomized groups, however, revealed unremarkable findings. These findings support that low Vit.B12 status has a detrimental impact on microstructural changes within the hippocampus before obvious volume reduction (Köbe et al. 2016).

However, patients with MCI are likely to be of heterogeneity from a pathological aspect (Mufson et al. 2012), which may explain the somewhat inconsistent findings of these studies. Current research also highlights the disagreement regarding the impact of Vit.B12 status across different stages of cognitive impairment. A cross-sectional study using data on 1095 participants (760 cognitively healthy individuals, 130 with MCI, and 205 with AD) found that Vit.B12 status was not significantly different between clinical groups after controlling for basic demographic confounders. Rather than group differences, a significant gender difference (higher Vit.B12 levels in females) was identified across all clinical groups (Faux et al. 2011). Likewise, a prospective study which retrospectively evaluated vitamin intake reported that serum levels of Hcy and Vit.B12 measured at baseline or at 5 years were not associated with dementia conversion rate, although users of Vit.B12 or folate had a lower grade of periventricular hyperintensities and a lower grade of deep white matter lesions compared to non-users (Blasko et al. 2012).

Subjects with Subacute Combined Degeneration

The patients with subacute combined degeneration (mostly in the 30s to 50s) appears to be younger than those with MCI, and the main findings of brain MRI are either microstructural or cerebral blood flow changes (Gupta et al. 2014a; Roy et al. 2015). Traditionally, therapeutic strategies in patients with subacute combined degeneration are mainly focused on gait performance related to myelopathy and polyneuropathies. Evaluating brain MRI among these patients can alert clinicians to the possibility of microstructural and/or functional changes within the cerebrum which tend to be ignored in patients with pronounced motor/sensory deficits. Gupta et al. investigated diffuse modification of DTI parameters in patients with subacute combined degeneration whose conventional MRI and gray/white matter volume appeared to be normal (Gupta et al. 2014a). Through a tract-based spatial statistics method, nearly global reductions in fractional anisotropy were noted. In addition, widespread increases in mean and radial diffusivity values, although predominantly within the right hemisphere, were also observed. Axial diffusivity was the only unchanged DTI parameter compared to the controls. Taken together, the authors suggested that demyelination rather than axonal loss is the major pathologic substrate in Vit.B12

deficiency. Similarly, another cohort study conducted by Roy et al. showed a global reduction in fractional anisotropy using tract-based spatial statistics (Roy et al. 2015). With the assistance of pseudocontinuous arterial spin labeling, the authors further identified significant alterations in cerebral blood flow values in both gray and white matter regions, and concluded that this technique could be an earlier predictor of complete recovery than DTI. These findings suggest the reversibility of metabolic dysfunction related to Vit.B12 deficiency, and that such damage occurs beyond the white matter tracts.

In summary, the multifaceted findings of MRI observations associated with Vit. B12 deficiency suggest that its pathology may vary according to clinical phenotypes, and that no single mechanism is responsible for cognitive decline related to Vit.B12 deficiency.

Evidence Through Incorporating Cognitive and Brain MRI Evaluations

In view of a priori knowledge suggesting the critical role of Vit.B12 in maintaining myelin integrity, lipid/protein metabolism, and the pathogenesis of vascular damage, it is reasonable that variable pathology relevant to low Vit.B12 status exists in neuroimaging evaluations (Fig. 7). A cross-sectional study also corroborated that Vit.B12 status affects the brain through multiple mechanisms such as brain volume reduction, increased white matter hyperintensity, and cerebral infarcts (Tangney et al. 2011). Identifying the clinical impact of these MRI findings would be an interesting issue, as the results may provide fascinating information on neuronal substrates with cognitive implications among patients with cognitive syndromes related to Vit.B12 deficiency (Table 4).

A cohort study which compared cognitive performance and morphometric indices of brain MRI between 34 pairs of consecutive patients with Vit.B12 deficiency and demographically matched controls indicated that the patients had global cognitive decline with selective impairment in language, orientation, and mental manipulation (Hsu et al. 2016). Preferential atrophy in frontal regions, evidenced as a larger frontal horn ratio, was the main neuroimaging feature in their study. In addition, among numerous morphometric indices significantly correlated with cognitive performance, the bicaudate ratio appeared to be the best index on the basis of its strong association with global cognition and related cognitive domains. The results seemed to imply that frontal regions are most vulnerable to damage related to low Vit.B12 status. On the basis of the robust cognitive association with preferential frontal region atrophy, the authors concluded that dysfunction of frontosubcortical circuits is the fundamental pathogenesis related to Vit.B12 deficiency (Hsu et al. 2016). These findings appear to be compatible with clinical observations, in which dysexecutive syndrome is a common presentation among the repertoire of cognitive syndromes related to vitamin B12 deficiency.

To date, two studies have assessed the clinical relevancy of DTI, although they used different assessment protocols and clinical phenotypes (Gupta et al. 2014a; Köbe et al.

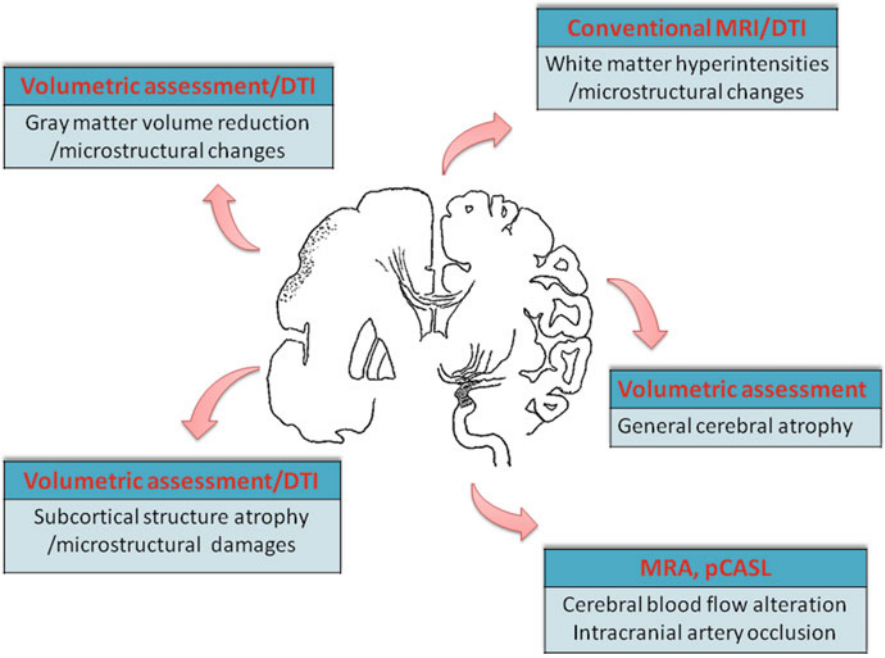

Fig. 7 Illustration picture of pathology related to vitamin B12 deficiency within the cerebrum. Several pathologies within human brain have been associated with low vitamin B12 status in magnetic resonance imaging research. Major findings and imaging protocols are labeled along with each representative pathology. *MRI* magnetic resonance imaging, *DTI* diffusion tensor imaging, *MRA* magnetic resonance angiography, *pCASL* pseudo-continuous arterial spin labeling

Table 4 Currently cited neural substrates related to low-vitamin B12 status by incorporating cognitive and brain magnetic resonance imaging findings.

References	Imaging protocol	Cohort characteristics	Neural substrates for cognitive decline
Tangney et al. (2011)	White-matter hyperintensity and total brain volume assessment	Community-dwelling participants versus controls	White-matter hyperintensity and cerebral infarcts Total brain volume reduction
Gupta et al. (2014a)	DTI (tract-based statistics)	Subacute combined degeneration versus control	Global microstructural changes in white matter
Köbe et al. (2016)	DTI (voxel-based analysis)	Pts with low-normal (<304 pmol/L) versus high-normal Vit.B12	Impaired microstructural integrity of the hippocampus
Hsu et al. (2016)	Morphometric index	Pts with Vit.B12 deficiency (≤250 pg/ml) versus control	Atrophy within frontosubcortical circuits and temporal region

DTI diffusion tensor imaging, *Pts* patients, *Vit.B12* vitamin B12.

2016). Gupta et al. evaluated patients with subacute combined degeneration with tract-based statistics (Gupta et al. 2014a), and Köbe et al. assessed patients with MCI using voxel-based analysis (Köbe et al. 2016). The former study, in which diffuse changes in diffusion parameters were identified, showed significant correlations between fractional anisotropy and radial diffusivity with tests related to visuospatial capacity and visuomotor speed (Gupta et al. 2014a). In comparison, Köbe et al. originally focused on segregated regions of the hippocampus and found that the microstructural integrity assessed by mean diffusivity in the cornu ammonis 4 and dentate gyrus region partially mediated the negative effect of low Vit.B12 status on memory performance (Köbe et al. 2016).

In summary, global changes in volume reduction or microstructural integrity reflect global cognitive decline and visuospatial capacity/visuomotor speed in patients with Vit.B12 deficiency. Some studies have addressed that frontal and temporal regions are associated with certain domain-specific cognitive deficits, however further studies are needed to assess the generalizability of these results. In contrast to volume reduction, the microstructural changes may imply that white matter integrity disconnection is an important neurobiological consequence related to Vit.B12 deficiency during the very early stage.

MRI as a Potential Tool to Predict or Monitor Therapeutic Responses

Several studies have investigated the possible factors related to therapeutic responses (Table 5). Milder dementia severity (Nilsson et al. 2000), elevation or certain range of MMA (Stabler et al. 1990; Hvas et al. 2001), and hyperhomocysteinemia (Stabler et al. 1990) have been associated with a better prognosis. In a cohort study aimed at identifying potential laboratory and neuroimaging biomarkers related to therapeutic responses, a greater load of total white matter hyperintensities, especially those defined within deep white matter regions, were associated with a poor prognosis (Tu et al. 2017).

A DTI study reported significant changes in diffusivity parameters and neuropsychological test scores between controls and patients with Vit.B12 deficiency in the pre- and posttreatment phases. In comparisons between the patients during the pretreatment phase and the controls, multiple regions of decreased fractional anisotropy and increased mean diffusivity were noted. These results are compatible with the defective formation of myelin sheaths, a consequence related to abnormal fatty acid formation and impaired methylation of myelin basic protein expected in patients with a low Vit.B12 status. In comparisons between pre- and posttreatment phases, the authors suggested that the corpus callosum and fornix were the most important tracts showing reciprocating changes in DTI parameters from the controls to the patients in the posttreatment phase (Gupta et al. 2014b).

Current articles describing therapeutic responses in patients with VBDNS are mainly confined to functional imaging or electrophysiology. The reversal of hypofrontality in single-photon emission computed tomography (Tu et al. 2015, 2016) has been reported to be responsible for clinical improvements after treatment.

Table 5 Possible factors in cognitive syndromes related to vitamin B12 deficiency that may predict therapeutic response.

Reference	Favorable	Less favorable
Stabler et al. (1990)	Hyperhomocysteinemia	Normal serum homocysteine level
Stabler et al. (1990) Hvas et al. (2001)	Elevation or certain range of methylmalonic acid	Normal serum methylmalonic acid level
Nilsson et al. (2000)	Milder dementia severity	More severe dementia severity
Tu et al. (2017)	Lesser load of total and deep white matter hyperintensities (MRI)	Greater load of total and deep white-matter hyperintensities
Tu et al. (2016)	Relatively intact frontal perfusion (SPECT)	More severe hypofrontality

MRI magnetic resonance imaging, *SPECT* single-photon emission-computed tomography.

Consistent with these findings, another study also identified reversible changes of prolonged P3 latency of evoked potentials in patients with VBDNS (Kalita et al. 2013). The P3 wave is an evoked potential involved in cognitive control and decision making, and the majority of signals are regarded to originate from frontal (and parietal) regions (Hall et al. 2015). Both studies highlighted that frontal regions are important neuronal substrates vulnerable to low Vit.B12 status, and that reversibility of the affected regions was suggestive of metabolic alterations in neuronal or myelin function. Intriguingly, a cohort study using novel MRI techniques (i.e., DTI and pseudocontinuous arterial spin labeling) demonstrated significant recovery both in microstructural changes and cerebral blood flow, in line with a complete reversal of neuropsychological scores at 6 weeks post therapy (Roy et al. 2015). As MRI is clinically feasible and avoids exposure to radiation, future studies incorporating MRI into assessments of neurobiological changes after Vit.B12 treatment would be of clinical value.

Supplementary Evidence from Other Neuroimaging and Electrophysiological Tools

As subtle neuronal dysfunction in the brain may precede overt structural changes in VBDNS, it is believed that functional evaluation may provide information fundamental to the underlying pathogenesis. A predominant decrease in postcentral cerebral blood flow with better preserved central and prefrontal flow has been described in patients with Vit.B12 deficiency with heterogeneous dementia subtypes and superimposed delirium (Nilsson et al. 2000). However, other single-photon emission computed tomography studies have indicated metabolic changes within frontotemporal regions and deep nuclei may account for the pathogenesis of cognitive syndromes related to Vit.B12 deficiency (Tu et al. 2015, 2016). Specifically, hypoperfusion within thalamus/basal ganglia/temporal regions and additional hypofrontality may contribute to psychiatric and cognitive symptoms, respectively. Moreover,

the extent and severity of hypofrontality may be associated with therapeutic responses, highlighting the role of frontosubcortical networks in cognitive syndromes related to Vit.B12 deficiency (Tu et al. 2016). Consistent with these findings, another study focusing on electrophysiological assessments reported clinical relevance at baseline and 3–6 months following treatment (Kalita et al. 2013). These sequential follow-up results suggested the role of P3 latency, which improved in accordance with clinical observations and was correlated with several subsets of neurobehavioral assessment. The authors hypothesized that P3 latency may be a surrogate marker for myelin function, and that a lower P3 amplitude may be the result of neuronal loss. On the basis of the significant correlation between P3 latency and Mini-Mental State Examination/Luria's three-step-test/motor speed and precision test, the authors suggested that their findings were primarily due to cortical and subcortical dysfunction (Kalita et al. 2013).

Conclusion

Although there is still a certain degree of disagreement regarding the causality between low Vit.B12 status and cognitive impairment, the associations are gradually being elucidated by integrated information from biochemical indicators, structuralized cognitive tests, and advanced neuroimaging evaluations. A review of the current literature indicated that global cognitive decline, with or without domain-specific impairment, may be a neurological consequence related to Vit.B12 deficiency. Consistent with these findings, many studies focusing on MRI observations have suggested that global alterations in cerebral blood flow, microstructural changes with certain reversibility, and reductions in brain parenchymal volume can be identified which vary according to the different clinical phenotype. On the basis of the current review, global disconnection syndrome, impaired frontosubcortical networks, and variable pathological changes within frontotemporal regions underlie the pathogenesis of cognitive syndromes related to vitamin B12 deficiency. Several recent studies have investigated issues related to regional spatial specificity related to Vit.B12 deficiency (e.g., frontosubcortical networks and frontotemporal regions), which is an interesting issue that remains to be confirmed in future study. With advances in MRI techniques and the clinical feasibility, its use in monitoring therapeutic responses and early identification of cognitive syndromes related to Vit.B12 deficiency can be expected.

Policies and Protocols

Vitamin B12 deficiency has been associated with cognitive impairment of various severity ranging from mild cognitive impairment to dementia. The role of the government in this important issue should focus on several aspects, including enhancing dietary supplements, implementing public education, and identifying those at risk of vitamin B12 deficiency. This chapter presents an overview of the

cognitive impact of vitamin B12 deficiency and its relevance to brain magnetic resonance imaging findings. Due to the increasing body of evidence supporting the negative impact of a low vitamin B12 status on cognition, policies focused on the prompt recognition of patients with vitamin B12 deficiency should be considered. Consistent with the results of epidemiological studies, our review emphasizes the importance of cognitive and magnetic resonance imaging evaluations in assessing elderly patients at potential risk of vitamin B12 deficiency (e.g., a vegan diet, cognitive complaints, and the chronic use of metformin/proton pump inhibitors/H2 blockers). In addition, both cognitive and magnetic resonance imaging evaluations have been validated to be potential tools in monitoring therapeutic responses across different clinical phenotypes related to vitamin B12 deficiency. Of note, magnetic resonance imaging appears to be major bottleneck in assisting the timely diagnosis of cognitive syndromes related to vitamin B12 deficiency, as it offers opportunity to detect subtle cerebral structural and perfusion changes that are frequently associated with vitamin B12 deficiency. Accountability for integrating medical resources and public health education should rest with the Department or Ministry of Health of individual countries.

Dictionary of Terms

- **Bicaudate ratio** – The minimum intercaudate distance divided by colinear skull internal diameter. Larger bicaudate ratio value could reflect greater extent of frontal subcortical region volume reduction.
- **Cognitive syndromes related to vitamin B12 deficiency** – A spectrum of cognitive disorders incorporating a wide range of disease severity, ranging from mild cognitive impairment to overt dementia.
- **Diffusion tensor imaging** – A magnetic resonance imaging-based technique which makes it possible to quantify anisotropy of water diffusion in different cerebral regions and structures. For example, the microstructural integrity of white matter tracts could be measured through variable sets of parameters. The location and orientation of specific fiber tracts could be tracked and visualized through color-coded diffusion tensor imaging and tractography.
- **Domain-specific effect** – Preferential involvement of certain cognitive domains, manifesting as more profound deficits from structuralized cognitive assessments.
- **Dysexcutive syndrome** – A neurological condition where patients have trouble with complex thinking and reasoning tasks, such as difficulty in applying previous knowledge to the new event, or losing track of conversations by other unexpected interruptions.
- **Frontal horn ratio** – The maximal frontal ventricular width divided by colinear skull internal diameter. Larger frontal horn ratio value indicates greater extent of frontal region volume reduction.
- **Frontosubcortical circuits** – Several segregated circuits connecting basal ganglia/thalamus with selected cortical areas within the frontal lobe. Except for

motor function, many of these circuits govern nonmotor function, including execution, motivation, and emotional/behavioral control.

- **Hemato-neurological dissociation** – A condition in which disproportional involvement between hematological and neurological systems is related to a low vitamin B12 status.
- **Pseudocontinuous arterial spin labeling** – A novel technique enabling the perfusion measurement (cerebral blood flow) in brain without the need of intravenous administration of contrast medium.
- **Vitamin B12 deficiency neuropsychological syndromes** – Neuropsychological disorders presumably relevant to a low vitamin B12 status, such as cognitive impairment, dementia, stroke, myelopathy, polyneuropathy, anxiety, and depression.

Summary Points

- A growing body of evidence supports the association between vitamin B12 intake/status with cognitive performance. Although the results of research are diverse, several lines of evidence highlight the negative impact on global cognition and possibly domain-specific impairments related to vitamin B12 deficiency.
- The results of research focusing on changes in magnetic resonance imaging associated with vitamin level vary according to the clinical phenotype. Overall, relevant pathology may range from alterations of cerebral blood flow, changes in microstructure with certain reversibility, to overt reductions in brain parenchymal volume. These findings have been identified globally, either within gray or white matter. Further studies are needed to confirm the preferentially involved regions.
- Research incorporating both cognitive and magnetic resonance imaging evaluations with regard to vitamin B12 status has found that (i) global changes in volume reduction/microstructural integrity with global cognitive decline and visuospatial capacity/visuomotor speed, (ii) microstructural integrity in the hippocampus with memory performance, and (iii) dysfunction of frontosubcortical circuits as part of the fundamental pathogenesis of cognitive syndromes are related to vitamin B12 deficiency.
- Several studies using novel magnetic resonance imaging techniques such as diffusion tensor imaging and pseudocontinuous arterial spin labeling have demonstrated changes consistent with recovery of cognition. Related clinical applications may be considered for the potential benefits in monitoring therapeutic responses and, possibly, the early detection of cognitive syndromes related to vitamin B12 deficiency.
- Clinico-radiological data derived from published articles suggest that a milder severity of dementia, elevation or certain range of methylmalonic acid, hyperhomocysteinemia, fewer deep and total white matter hyperintensities in magnetic resonance imaging, and lesser hypofrontality in single-photon emission

computed tomography are associated with a favorable prognosis with vitamin B12 supplement treatment.

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Vitamin D Deficiency and Fertility: An Overview

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Bianca Schröder-Heurich and Frauke von Versen-Höynck

Contents

Abstract

In recent years the interest in vitamin D is steadily growing. Numerous scientific publications and health guidebooks for the public have been published. Besides the well-known effects on calcium and bone metabolism, a positive impact of the sunshine vitamin on human wellbeing and health has been proposed. A high prevalence of vitamin D deficiency according to established optimal values has been described, although no increase of the classical diseases associated with vitamin D deficiency, e.g., rachitis and osteomalacia, has been observed. Besides infants and the elderly, populations at risk include women of childbearing age.

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The discovery that human tissues with a role in reproductive function, e.g., ovaries, endometrium, and placenta, express the vitamin D receptor and enzymes involved in vitamin D metabolism, has provoked studies on the role of vitamin D in reproductive health. While observational studies suggest an association between low vitamin D status and impaired reproductive outcomes, there are only few high quality randomized clinical trials available that could prove causality. This chapter summarizes the current knowledge on vitamin D metabolism, epidemiology, and treatment of vitamin D deficiency with focus on women's reproductive health.

Keywords

Vitamin D · Metabolism · Classification · Reproduction · Fertility · Menstruation · PCOS · Endometriosis · Assisted reproduction · Vitamin D deficiency

List of Abbreviations

aOR	Adjusted odds ratio
AFC	Antral follicle count
AMH	Anti-mullerian hormone
CI	Confidence interval
DBP	Vitamin D-binding protein
7-(DHC)	7-Dehydrocholesterol
DGE	German Nutrition Society
FSH	Follicle stimulating hormone
25(OH)D	25-Hydroxyvitamin D
$1,25(OH)_2D$	1,25-Dihydoxyvitamin D
IL-8	Interleukin-8
IR	Insulin resistance
IOM	Institute of Medicine
IVF	In vitro-fertilization
LH	Luteinising hormone
PCOS	Polycystic ovary syndrome
PTH	Parathyroid hormone
RCT	Randomized controlled trial
RDA	Recommended dietary allowance
RXR	Retinoic acid receptor
VDR	Vitamin D receptor

Introduction

Since the early 2000s, a wealth of scientific articles about vitamin D and its role for human biology have been published. The "sunshine" vitamin is involved in various functions in the body. Increasingly, scientific investigations are revealing the importance of vitamin D and its role in health and disease prevention. For the time being, it is known

that vitamin D is involved in immunomodulation processes, cell growth, differentiation, and hormonal balance. The secosteroid and prohormone is getting converted to its active metabolite, 1,25-alpha$(OH)_2$D through multiple converting steps in the body. By binding to its high-affinity receptor, activation of transcription occurs, which causes certain target genes to be activated or inhibited. The relationship between vitamin D and the reproductive system was established after detecting 1-alpha-hydroxylase and the vitamin D receptor (VDR) to be expressed in many female organs like placenta, ovary, endometrium, and decidua. Since vitamin D deficiency has been detected increasingly over the past decades among all racial groups and globally, a large proportion of the female population in their reproductive age is affected. Therefore, this chapter is focusing on the novel findings on vitamin D in the area of human reproduction, fertility, and disease prevention.

Metabolism and Synthesis of Vitamin D

Vitamin D is a group of fat-soluble vitamins, with vitamin D_2 and vitamin D_3 being the most common forms. Vitamin D is absorbed from food (vitamin D_3 [cholecalciferol], from animal sources, or from plant and fungi sources (vitamin D_2 [ergocalciferol]), in the form of food supplements (either vitamin D_2 or D_3) or through the self-synthesis in the skin (vitamin D_3) (Unholzer et al. 2017). Cholecalciferol, a prohormone and the physiologically most important representative, is formed from 7-dehydrocholesterol (7-DHC) by the action of UV-B rays in the keratinocytes of the skin (Unholzer et al. 2017) (Fig. 1). For its role in various processes in the body, cholecalciferol bound to its transport protein (vitamin D-binding protein (DBP)) is shuttled via the blood into the kidney, where it is metabolized into calcidiol (25(OH)D) (Unholzer et al. 2017) (a table of terms used in reference to vitamin D is given in Table 1 (modified after Unholzer et al. 2017)).The main steps of vitamin D metabolism include a multi-step hydroxylation process performed by cytochrome P450 mixed-function oxidases (CYPs). The enzymes responsible are located in the endoplasmic reticulum (e.g., CYP2R1) or in the mitochondria (e.g., CYP27A1, CYP27B1) and metabolize vitamin D_2 or D_3 into 25$(OH)D_2$ or D_3 (Bikle 2014).

25(OH)D (calcidiol) is the major circulating and storage form of vitamin D in the body and serves as a marker of vitamin D status, while most of all circulating 25(OH)D is 25$(OH)D_3$ (Unholzer et al. 2017). 25(OH)D is transported via its binding protein to the kidneys where it gets further hydroxylated by CYP27B1 (1-α hydroxylase), resulting in the biological active metabolite 1,25$(OH)_2$D (Christakos et al. 2010) also known as calcitriol. Calcitriol is released into the blood and exerts its effects through a change in gene expression. While calcitriol is produced according to its specific needs in the body, its own degradation occurs to the inactive metabolite calcitroic acid via CYP24A1 (Christakos et al. 2016; Unholzer et al. 2017). The impact of the active vitamin D on cell functions is influenced by the bioavailability at the target sites and highly dependent upon DBP concentrations and its polymorphisms, which can vary among ethnicities.

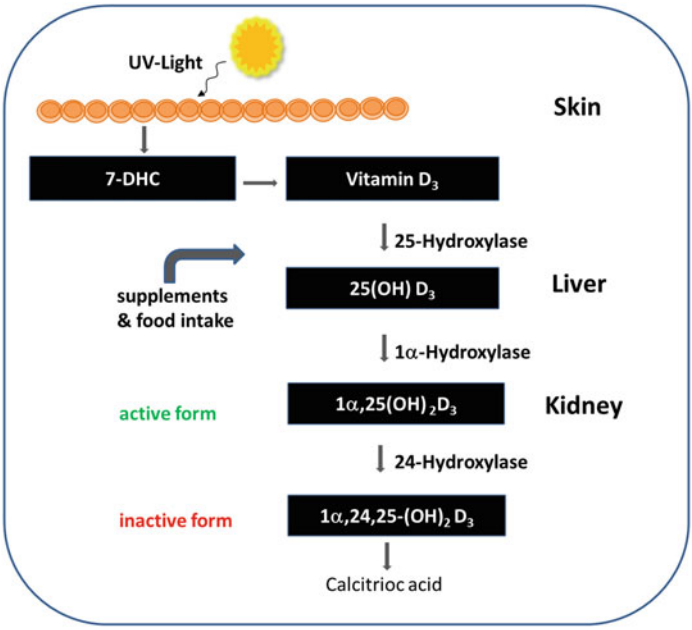

Fig. 1 Metabolism and synthesis of vitamin D. The formation of active $1,25(OH)_2D$ is a multi-step hydroxylation process. First, cholecalciferol (vitamin D_3) is formed in the skin after sufficient sunlight exposure from 7-DHC. After hydroxylation in the liver to calcidiol (25(OH)D), it gets metabolized in the kidney to the biological active form $1,25(OH)_2D$ (calcitriol). $1,25(OH)_2D$ is inactivated and degraded through further hydroxylation to calcitrioic acid

Table 1 Vitamin D metabolites, enzymes, and function. Terms of metabolites, function and food supplements used in reference to vitamin D (Modified after Unholzer et al. 2017, with permission of Springer Nature)

Terms	Affiliation and Function
Vitamin D (Calciferol)	Group of secosteroids; regulation of calcium balance
Cholecalciferol (vitamin D_3)	Prohormone; activated 7-DHC and precursor of calcitriol
Ergocalciferol (vitamin D_2)	From plant and fungi sources
Calcidiol (25(OH)D)	Storage form; reference molecule for vitamin D status in serum
Calcitriol ($1,25(OH)_2D_3$)	Biologically active form of vitamin D_3
Ercalcitriol ($1,25(OH)_2D_2$)	Biologically active form of vitamin D_2
Calcitroic acid	Inactive form; major terminal deactivation product of calcitriol
Vitamin D oral supplement (either vitamin D_2 or D_3)	Treatment of vitamin D deficiency or prevention (preferred vitamin D_3)

Table 2 Classification of serum 25(OH)D levels. The optimal vitamin D serum level lies between 50 and 100 ng/ml. Values below 20 ng/ml are considered deficient, values above 100 ng/ml can be toxic. To convert to nanomoles per liter multiply by 2.496 (Modified after Grant and Holick 2005, with permission from the Publisher)

Serum 25(OH)D concentration		Classification
<20 ng/ml	<50 nmol/l	Deficient
20–32ng/ml	50–80 nmol/l	Insufficient
50–70 ng/ml	125–175 nmol/l	Optimal
32–100 ng/ml	80–250 nmol/l	Sufficient
>150 ng/ml	>325 nmol/l	Intoxication

Determination of Vitamin D Status

Vitamin D deficiency is still a large and global problem of the human population. Depending on the geographic location, populations in the northern latitudes, older people, children, as well as women in their reproductive period are potentially at high risk for vitamin D deficiency.

Reference intake values for an adequate vitamin D status are incongruent and despite years of research it is still not clearly defined how high an optimal vitamin D level should be to have an overall preventive effect. The determination of a serum concentration of 25(OH)D is the accepted approach to identify individual vitamin D status (Holick 2009). To date, according to different investigators, a 25(OH)D serum level in the range between 30 and 50 ng/ml can be regarded as adequately and physiologically sufficient. Values below 20 ng/ml are considered as deficient, 21–29 ng/ml as insufficient (Grant and Holick 2005; Holick 2009) (Table 2). Measurement of serum 25(OH)D concentrations is not subject to a uniform standard procedure. At present, enzyme-linked immunosorbent assays, high-performance liquid chromatography, liquid chromatography–mass spectrometry, and radioimmunoassays are generally used, which, due to different detection limits and variabilities, make a direct comparison of the values challenging (Thierfelder et al. 2008; Unholzer et al. 2017). Therefore, development of a uniform reference test for determination of serum 25(OH)D concentration is urgently necessary.

Supplementation of Vitamin D and Prevention of Deficiency

Vitamin D deficiency may occur from inadequate intake or insufficient exposure to sun light. Synthesis of vitamin D mainly takes part in the skin after sufficient sunlight exposure (Böhles et al. 2011) and therefore is dependent on the season, daytime, and geographic factors. Other factors are the uncovered skin area, length of time staying outside, as well as the skin pigmentation which gets additionally influenced by decreasing age (Loomis 1967; Need et al. 1993; Holick 2002; Böhles et al. 2011; German Nutrition Society (DGE) 2012).

Table 3 Food sources of vitamin D_3. Vitamin D is found in a limited number of foods. Therefore, food supplementation or the intake of vitamin D supplements helps increase serum concentrations (USDA Food Composition Databases 1889)

Food sources of vitamin D	Measure	Vitamin D (IU) per measure
Cod liver oil	1 tablespoon	1360
Herring (Atlantic, pickled)	1 cup	158
Mackerel (jack, canned, drained solids)	1 oz	83
Salmon (pink, canned, drained solids)	3 oz	493
Swordfish (cooked, dry heat)	3 oz	566
Milk (lowfat, fluid, 1% milk fat, protein fortified, with added vitamin A and vitamin D)	1 cup	98
Yoghurt (plain, whole milk, 8 g protein per 8 ounce)	6 oz	3
Cheese, cheddar	1 oz	32
Cheese, camembert	1 oz	5
Cheese, feta	1 cup, crumbled	24
Egg (whole, cooked, fried)	1 Large	40
Orange juice (fortified with vitamin D), (varies among labels)	1 cup	137
Margarine (fortified)	1 tablespoon	60

The exogenous intake of vitamin D, which accounts for about 10%, is influenced by the consumption of certain foods or dietary supplements (Böhles et al. 2011). Vitamin D is found only in few foods naturally, which includes oily fish, liver, and margarine (enriched) (D-A-CH 2008; Böhles et al. 2011) (Table 3) (USDA Food Composition Databases 1889).

In some countries, e.g., the US and Canada, certain foods such as milk, some breakfast cereals, and breads are enriched with vitamin D (Institute of Medicine 1999), while food supplementation is mostly prohibited in Europe (Böhles et al. 2011). The daily intake recommendations for vitamin D, available in many countries of Europe and the USA (e.g., World Health Organization/Food and Agriculture Organization, Institute of Medicine, German Nutrition Society) are not uniform (Lanham-New et al. 2011; Ross 2011). The Food and Nutrition Board at the IOM established a recommended dietary allowance (RDA) for vitamin D which is defined as "average daily level of intake sufficient to meet the nutrient requirements of nearly all (97–98%) healthy people" (Institute of Medicine 2010). The Recommendation of the Associations for Nutrition of German-speaking Countries for daily intake is much higher (Table 4) (Institute of Medicine 2010; German Nutrition Society (DGE) 2012). These recommendations are mainly based on dietary intake and because of the many variable factors, which influence the synthesis of vitamin D, a standard for recommended adequate intake for all ages, sexes, and races is hard to define (Institute of Medicine 1999). Therefore, intake reference values from different countries could be eventually too low to maintain healthiness.

Table 4 Recommended daily allowance values of vitamin D_3. The RDA values of the IOM and the DGE depend on age or health status (e.g., pregnancy) (Institute of Medicine 2010; German Nutrition Society (DGE) 2012).

	Institute of Medicine (IOM), US	German Nutrition Society (DGE), D
	Recommended intake [μg/d]	Recommended intake [μg/d][a]
Infants 0–12 months	10	10
Children 1–13 years	15	20
Adolescents 14–18 years	15	20
Adults 18–65/70 years	15 (adults under 70)	20 (adults under 65)
Adults above 65/70 years	20	20
Pregnant women 14–50 years	15	20

[a]Estimated value while endogenous synthesis is lacking

Vitamin D and the Female Reproductive System

In northern countries, a peak in conception rates in the summer has been observed. Therefore, it seems likely that an association between vitamin D and fertility exists (Rojansky et al. 1992). The detection of the VDR in granulosa and cumulus oophorus cells of the human ovary (Perez-Fernandez et al. 1997) and enzymes involved in vitamin D metabolism in several female reproductive tissues (Thill et al. 2009; Parikh et al. 2010), e.g., the endometrium and fallopian epithelial cells (Agic et al. 2007), the decidua and placenta (Agic et al. 2007), and the pituitary gland, supports this assumption. Further, enzymes involved in vitamin D metabolism, e.g., 1-alpha hydroxylase, are expressed in female reproductive tissues indicating that locally the active $1,25(OH)_2D$ can be synthesized (Vigano et al. 2006; Fischer et al. 2009; Tamblyn et al. 2017). It has been proposed that the ovary is a target organ for $1,25(OH)_2D$ (Dokoh et al. 1983). In conjunction with estradiol, it labilizes lysosomes to weaken the tunica albuginea and to enhance ovum release during ovulation. Steroid hormone production (progesterone, estradiol, and estrone) of the ovary and placenta as well as the synthesis of chorionic gonadotropin and placental lactogen expression in human syncytiotrophoblasts is regulated by the vitamin (Barrera et al. 2008). In addition, $1,25(OH)_2D$ alters the expression and activity of the estrogen biosynthesis catalyzing enzyme P450 (Sun et al. 1998) and affects anti-mullerian hormone (AMH) signaling and steroidogenesis in human cumulus granulosa cells, both pathways with importance for folliculogenesis (Merhi et al. 2014).

Vitamin D and Assisted Reproduction

Utilization of assisted reproductive technologies (ART), especially *in vitro* fertilization (IVF), is growing (Kamphuis et al. 2014). IVF is now being used not only by infertile couples but also by couples carrying single gene mutations who use IVF

with pre-implantation embryo biopsy and transfer of unaffected embryos to the uterus (Berger and Baker 2014). ART allows the separate evaluation of the impact of vitamin D deficiency on various steps of reproduction from folliculogenesis to embryo implantation.

In several observational studies, serum 25(OH)D levels were highly deficient in women seeking medical help for couple's infertility (Pagliardini et al. 2015). In a cohort of 1,072 women in Northern Italy, 11% of women reached a sufficient level in this prospective cross-sectional study (Pagliardini et al. 2015). At a higher latitude (50°N) in Germany, vitamin D insufficiency affected 81–93% of women presenting for infertility treatment ($N = 312$) (Dressler et al. 2016). As with the previous study BMI and limited exposure to sun were associated with an increased risk of vitamin D deficiency.

The relationship between vitamin D serum levels and IVF outcomes has been investigated with controversial results. One of the first studies that investigated IVF success and vitamin D status in 10 women found an association of raised estradiol levels during gonadotrophin-induced ovarian stimulation and a significant increase of serum $1,25(OH)_2D$ ($r = 0.787$, $p < 0.001$) (Potashnik et al. 1992; Wehr et al. 2010). Several studies focused on 25(OH)D levels in follicular fluid as an independent predictor to success of an IVF-cycle. While Ozkan et al. showed higher pregnancy and implantation rates across tertiles of 25(OH)D in 84 infertile women undergoing IVF (Ozkan et al. 2010), two prospective studies with 101 and 82 women could not confirm these findings (Anifandis et al. 2010; Aleyasin et al. 2011). No significant differences in pregnancy rates and embryo quality were found between patients with low (<20 ng/ml) and moderate (20–30 ng/ml) 25(OH)D follicular fluid levels. Women with high vitamin D follicular fluid levels (>30 ng/ml) had even lower pregnancy rates and embryo quality (Anifandis et al. 2010; Aleyasin et al. 2011).

Rudick et al. observed a relationship between serum 25(OH)D levels and implantation, clinical pregnancy, and live birth rates in 188 women (Rudick et al. 2012). These findings were significant in non-hispanic whites but did not apply to the Asian ethnicity (Rudick et al. 2012). The same group demonstrated a positive association between vitamin D status and clinical pregnancy rate among recipients of oocyte donation (Rudick et al. 2014). This observation is supportive of an effect of 25(OH)D levels on ART outcomes possibly due to a mediation of endometrial receptivity. Women with 25(OH)D > 20 ng/ml ($n = 181$) had a higher chance of obtaining top quality embryos, higher implantation (1.91 [95% confidence interval (CI): 1.20–3.05, $P = 0.006$]) and clinical pregnancy rates (adjusted odds ratio (aOR) 2.15 [95%]) compared to those with levels < 20 ng/ml ($n = 154$) (Paffoni et al. 2014). In a retrospective cohort of 368 women, vitamin D deficiency measured seven days' prior blastocyst transfer, appeared as an independent predictor of lower clinical pregnancy rates (Polyzos et al. 2014). When the analysis was restricted to women undergoing elective single embryo transfer (274 patients), vitamin D deficiency was still independently associated with pregnancy rates [OR (95% CI) 0.56 (0.33–0.93), $P = 0.024$]. In a larger sample of 517 analyzed cycles, this finding was not confirmed (Franasiak et al. 2015). Vitamin D levels from serum samples obtained on the day of

ovulation trigger in the fresh IVF cycle were analyzed. In this cohort with extended embryo culture, blastocyst biopsy for comprehensive chromosome screening and subsequent euploid embryo transfer vitamin D levels were unrelated to ongoing pregnancy rate (Franasiak et al. 2015). However, in a recent systematic review and meta-analysis of five studies, a deficient vitamin D level was related to lower live birth rate (relative risk (RR) 0.76, 95% CI 0.61–0.93) but not to a lower clinical pregnancy rate (RR 0.88, 95% CI 0.69–1.11) (Lv et al. 2016). Although a biologically plausible effect of vitamin D on reproductive tissues is very likely, it is still controversial whether vitamin D levels are reliable predictors of ART outcomes. Current evidence relies on heterogeneous results of small cohort studies while findings from RCTs are not yet available.

Vitamin D and Ovarian Reserve

AMH is an ovarian reserve marker and produced in the granulosa cells of the ovaries. While the AMH gene promoter contains a vitamin D response element, it is suggested that vitamin D might be involved in the regulation of the gonadal status. When granulosa cells were directly incubated with the active $1,25(OH)_2D$, changes in AMH receptor expression and downstream signaling were noted. In this experimental set-up direct effects on AMH levels were studied eliminating issues of vitamin DBP that affects the bioavailability of the active hormone in the circulation (Merhi et al. 2014). In a cross-sectional study including 388 premenopausal women with regular menstrual cycles, the authors observed a positive independent association of 25(OH)D levels with AMH in women aged 40 years and older ($N = 141$) (Merhi et al. 2012). In a prospective cross-sectional study of 283 infertile women starting their first cycle of infertility treatment serum AMH and vitamin D were determined and antral follicle count (AFC) measured on the second or third day. In contrast to the previous study, no significant association was observed between AMH levels or AFC and vitamin D concentrations, even after controlling for relevant co-variants (Drakopoulos et al. 2017). Thus, it is unclear from the available data if vitamin D deficiency is associated with lower ovarian reserve in reproductive aged women.

Vitamin D and Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is among the most common endocrine disorders affecting women of reproductive age and contributes to sub- and infertility. Clinical characteristics include hyperandrogenism, menstrual disturbances, and polycystic ovaries on ultrasound. The associated ovarian dysfunction is noticeable by oligo-/anovulation. While many, but not all, women with PCOS are overweight or obese, insulin resistance (IR) and dyslipidemia are core pathophysiologic features of this syndrome. However, a substantial number of lean women affected by PCOS have IR as well, independent of obesity (Dunaif et al. 1989). There is increasing evidence

that vitamin D affects insulin and glucose metabolism (Scragg et al. 2004; Liu et al. 2009). Vitamin D deficiency therefore has been proposed as the possible missing link between IR and PCOS. This assumption is supported by the finding that the active VDR regulates genes that are important for glucose and lipid metabolism as well as blood pressure regulation (Bouillon et al. 2008).

Several studies suggest associations between VDR polymorphisms and the development of PCOS and IR. Most of them had only modest sample sizes (Chiu et al. 2001; Mahmoudi 2009; Ranjzad et al. 2010; 2011; Wehr et al. 2011). Possible explanations of the role of VDR variants in the pathogenesis of PCOS include effects on luteinizing hormone, sex hormone binding globulin levels, and testosterone (Ranjzad et al. 2010; Wehr et al. 2011).

In a systematic review to gain insights into the association between vitamin D, BMI, and IR, 29 eligible trials with only one randomized controlled trial have been analyzed. Although univariate regression analyses revealed vitamin D to be a significant and independent predictor of IR in both PCOS and control women, the significance disappeared after adjustment for BMI in women with PCOS (Krul-Poel et al. 2013). Due to the heterogeneity of the available studies and the lack of randomized trials, it is currently hard to draw a definite conclusion about a causal relationship between vitamin D status and metabolic disturbances in PCOS.

Vitamin D and Menstrual Cycle

Animal data from knock-out mice models for 1-alpha hydroxylase, the enzyme converting vitamin D to its active form, show delayed puberty, anovulation, and irregular menstrual cycles (Panda et al. 2001; Dicken et al. 2012). Human data about vitamin D levels in the menstrual cycle and an association with irregularities are sparse and partially contradictory.

A study of 33 women included 202 serum samples collected at different time points in the follicular phase of the menstrual cycle found no differences in mean levels of 25(OH)D, free 25(OH)D, and bioavailable 25(OH)D (Franasiak et al. 2016). Several observational studies described a mid-cycle rise in the serum level of human $1,25(OH)_2D$ with a near doubling of its concentration compared to early follicular levels (Pitkin et al. 1978; Gray et al. 1982; Buchanan et al. 1986). This finding, however, was not confirmed by other studies (Baran et al. 1980; Muse et al. 1986). The mid-cyclic peak was not associated with changes of serum calcium levels or other markers of bone health and not found in women on oral contraceptives (Gray et al. 1982; Tjellesen et al. 1983). Therefore, it was suggested that the mid-cycle endogenous estrogen increase induces the rise of 25(OH)D (Buchanan et al. 1986). As the vitamin D converting enzymes are found in human endometrium, the stage of menstrual cycle must be considered when serum concentrations of $1,25(OH)_2D$ are analyzed. While 25(OH)D appears to be stable in the follicular phase, the measurement could be reliably performed during that time window of the menstrual cycle.

In a recent population-based study, lower levels of 25(OH)D were associated with irregular menstrual cycles of late reproductive-aged women (Jukic et al. 2015). However, the same group performed a community-based, cross-sectional study of 1,102 African American women. In this population a doubling of 25(OH)D serum levels was associated with half the odds of having long menstrual cycles (aOR 0.54, 95% CI 0.32–0.89) but not with the occurrence of short (aOR 1.03, 95% CI 0.82–1.29) or irregular (aOR 1.46, 95% CI 0.88–2.41) menstrual cycles (Jukic et al. 2016).

A role for vitamin D has been suggested in primary dysmenorrhea, as vitamin D inhibits synthesis of prostaglandins and VDR is located in the human uterus (Lerchbaum and Obermayer-Pietsch 2012). The first RCT investigating the effect of a single loading dose of vitamin D (300,000 IU) versus placebo on primary dysmenorrhea observed an inverse correlation of 25(OH)D levels with pain score as well as a significant reduction of pain in the vitamin D group with the greatest reduction in women with severe pain at baseline (Lasco et al. 2012). Although in another RCT the mean pain intensity in women with primary dysmenorrhea was lower in both the calcium-alone (1,000 mg calcium) and calcium-vitamin D (1,000 mg calcium + 5,000 IU vitamin D_3) groups compared to placebo, the difference was statistically significant only in the calcium alone group (Zarei et al. 2016).

Vitamin D and Endometriosis

Endometriosis is a chronic and painful disease affecting up to 10% of women in their reproductive age (Eskenazi and Warner 1997). It leads to benign growths of endometrial cells outside the uterine cavity, such as in the pelvis, fallopian tubes, and ovaries. Endometriosis is strongly associated with female infertility (Eskenazi and Warner 1997).

Various studies suggest that there is a link between vitamin D and endometriosis. The endometrium represents a target tissue of vitamin D since it expresses the VDR as well as special vitamin D metabolizing enzymes. A dysregulation of vitamin D, vitamin D metabolites, and specific enzymes was observed in different studies of patients with endometriosis. The expression of 1α-hydoxylase and the VDR are increased in endometric tissue compared to normal endometrium (Agic et al. 2007). As part of a prospective cohort study with 1,385 endometric patients, a decrease of serum 25(OH)D in patients with the disease was observed (Harris et al. 2013). In contrast, serum levels of 25(OH)D have also been reported to be higher (Somigliana et al. 2007) or showed no difference (Agic et al. 2007) in patients suffering from endometriosis compared to healthy controls. Faserl et al. have found higher serum DBP levels in women with endometriosis in a cross-sectional study including 56 cases and 20 controls (Faserl et al. 2011). In the past years, *in vivo* and *in vitro* studies have been published suggesting a role of inflammation in endometriosis leading to the shift of anti-inflammatory treatments of the disease. Nevertheless, some results were found to be ambiguous (Ahn et al. 2015). Since these findings

remain conflictive, further epidemiologic studies and clinical trials are needed to investigate this complex relationship and underlying mechanism of endometriosis and vitamin D.

Summary and Conclusions

In recent years, several studies suggest that vitamin D modulates female reproductive biology due to the expression of VDR and 1α-hydroxylase in reproductive tissues. This is supported by a regulation of steroidogenesis of sex hormones by the vitamin. A growing body of literature proposes that an individual's vitamin D status may adversely impact reproductive functions (Fig. 2). Observational studies suggest a regulatory role of vitamin D in pathophysiological aspects of PCOS and endometriosis. Vitamin D might play a favorable role in IVF success and further beneficial effects include an improvement of primary dysmenorrhea following vitamin D supplementation and a possible association of high vitamin D levels with better ovarian reserve in women of late reproductive age. However, convincing evidence demonstrating a causal link between vitamin D and the pathophysiology of the diseases is still lacking. Most available studies are observational with an uneven distribution of populations, small numbers of participants, and different protocols for supplementation studies. Findings of observational studies must be confirmed by high quality randomized and interventional trials to obtain a better understanding of the underlying mechanisms and causality. Vitamin D supplementation is advised in the general population and in women planning on starting a family.

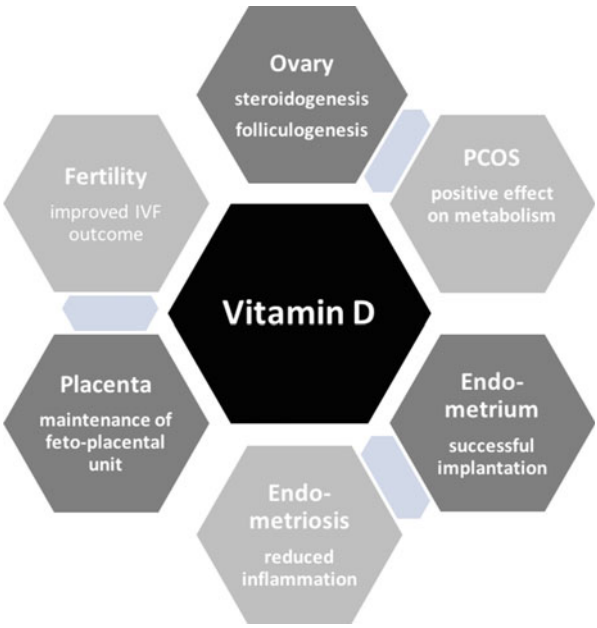

Fig. 2 Vitamin D in reproductive biology. Vitamin D status is associated with reproductive tissue and outcomes. A positive effect of vitamin D on reproductive health, fertility and on the metabolism of women suffering from PCOS and dysmenorrhea has been reported in observational studies

Policies and Protocols

- Researchers and clinicians should agree on a uniform method to measure serum 25(OH)D concentrations. For this purpose, not only a uniform method (e.g., enzyme-linked immunosorbent assay or high performance liquid chromatography) but also a uniform procedure for the collection and processing of the samples should be carried out. The serum should be worked up within a specific time frame and stored under uniform conditions until testing. It is recommended to store the samples in a biobank which meets the uniform technical and practical requirements and which underlies quality controls. This will help with the interpretation and comparison of study results in the future.
- In addition to the uniform determination of the vitamin D status, a uniform procedure to control the effects of vitamin D therapy to increase vitamin D levels to a normal range should also be established. For this, patients should take vitamin D supplements long-term under observation because medication is very different and vitamin D deficiency has to be treated over a very long period of time.
- The measurement of serum 25(OH)D has hitherto been the only method for determining vitamin D deficiency or sufficiency in the blood of patients. A further method or parameter for determination should be developed which can also be used as standard by the patient himself for the daily determination of vitamin D status, for example, via measurement in the urine or via skin.
- Awareness should be raised in the society about the relevance of a sufficient vitamin D status by physicians and clinical staff. Especially in groups at high risk, like pregnant women or the elderly, regular supplement intake can prevent vitamin D deficiency.
- Large scale randomized controlled trials (RCTs) need to be performed to prove a causal effect of vitamin D on reproductive health outcomes.

Dictionary of Terms

- **Active vitamin D** – There is an active form of vitamin D, called calcitriol (1,25-dihydroxyvitamin D), in the body that is converted from the inactive form (25-hydroxyvitamin D (25-(OH)D)). The active form is needed to fulfill vitamin D function in human cells.
- **Inactive vitamin D** – The inactive form of vitamin D is a precursor of the active form. It is used to determine a human's vitamin D status as it is measured in the blood.
- **Bioavailability** – If the active form of vitamin D can exert its actions in the body depends on the available free active vitamin D. A major proportion of the active vitamin D is bound to proteins in the blood (e.g., vitamin D-binding protein (DBP) and albumin) and therefore not readily available.
- **Vitamin D status** – The individual vitamin D status is determined by the measurement of 25(OH)D in the blood serum by various methods.

- **Vitamin D deficiency** – Serum levels of 25(OH)D below 20 ng/ml are considered as deficient.
- **Vitamin D insufficiency** – Serum levels of 25(OH)D of 21–29 ng/ml are in the insufficient range.
- **Vitamin D sufficiency** – A 25(OH)D serum level in the range of 30–50 ng/ml is considered physiologically sufficient.

Summary Points

- A uniform standard method to measure 25(OH)D concentrations to determine a person's vitamin D status does not exist, what makes a direct comparison of vitamin D levels between studies challenging.
- Vitamin D insufficiency and deficiency have a high incidence in reproductive age women around the world.
- Although the optimal level of vitamin D serum concentrations is still discussed among researchers, the currently widely accepted range for sufficient levels lies between 30 and 50 ng/ml.
- Fortified foods or vitamin D supplements are a cost-efficient and safe way to increase serum vitamin D levels.
- Vitamin D receptor and metabolizing enzymes are present in reproductive tissues and cells suggesting paracrine/autocrine functions of the vitamin.
- An effect on steroid hormone synthesis (progesterone, estradiol, and estrone) and hormones related to pregnancy (placental lactogen, chorionic gonadotropin) has been reported in in vitro studies.
- Vitamin D status is suggested to be associated with reproductive outcomes.
- A positive effect of vitamin D on the metabolism of women suffering from PCOS, the outcome of in vitro fertilization, and dysmenorrhea has been reported in observational studies.
- Available data are not consistent and limited due to methodological, ethnic, and racial differences and small sample sizes.
- In order to determine causality and prove benefits of vitamin D in the prevention of diseases and therapeutic measures, further large-scale randomized controlled trials (RCTs) must be performed and uniform clinical guidelines are needed.

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Vitamin D Assessment in Older Adults

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Abstract

Vitamin D is required for adequate calcium and phosphate metabolism, maintenance of bone structure, and maximum muscle function. Older adults, however, are at risk of inadequate vitamin D levels due to decreased skin synthesis, decreased metabolism in the kidneys, decreased gut absorption, and comorbid medical conditions. A person's vitamin D status is assessed by measuring the blood level of 25-hydroxyvitamin D. There is no generally accepted criterion for vitamin D deficiency and insufficiency. Serum 25OHD thresholds <25 nmol/l is defined as deficient, 25 – 50 nmol/l as insufficient, and >50 nmol/l as adequate for vitamin D. Vitamin D_3 is recommended as the vitamin D preparation of choice for the treatment of vitamin D deficiency. Oral administration of vitamin D is recommended over intramuscular administration. A guideline for the treatment of vitamin D deficiency and insufficiency in older adults has been suggested. Compliance to prescribed medications is also a major factor for consideration in older adults. Individualized vitamin D therapy focusing on knowledge of the elderly and/or their carers about their medications and health may help improve adherence.

Keywords

Vitamin D deficiency · Vitamin D insufficiency · Older adults · Cholecalciferol · 25-hydroxyvitamin D · Metabolism · Community dwelling · Hospitalized · Nursing home

List of Abbreviations

1,25(OH)2D	1,25-dihydroxy vitamin D; calcitriol
25OHD	25-hydroxyvitamin D; calcidiol; calcifediol
BMD	Bone mineral density
IOM	Institute of Medicine
IU	International Units
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
nmol/l	Nanomoles per liter
NOS	National Osteoporosis Society
PTH	Parathyroid hormone
RCT	Randomized controlled trial
RNI	Required Nutritional Intake
SACN	Scientific Advisory Committee on Nutrition
UV	Ultraviolet
Vitamin D	Calciferol (either D2 or D3)
Vitamin D_2	Ergocalciferol
Vitamin D_3	Cholecalciferol
WHO	World Health Organisation

Introduction

Vitamin D is obtained mainly from sunlight and from diet. It is required for adequate calcium and phosphate metabolism, maintenance of bone structure, and maximum muscle function. Its deficiency causes osteomalacia in adults and rickets in children. Older adults in this context refers to individuals from the age of 65 years and above. This group includes healthy community-living older adults, acutely unwell and hospitalized or living in a residential or nursing home. This population is prone to a higher risk of chronic diseases and nutritional deficiencies which can negatively affect their quality of life. In this chapter, the assessment of Vitamin D in older adults in relation to its sources and benefits, prevalence of Vitamin D deficiency in older adults, and strategies for its management are discussed.

Sources and Biochemistry of Vitamin D

Vitamin D can be made in the skin from exposure to sunlight. This is the major source of Vitamin D. Solar ultraviolet (UV) B radiation (wavelength, 290–315 nm) penetrates the skin and converts 7-dehydrocholesterol in the skin to previtamin D_3, which is rapidly converted to vitamin D3 (Holick 2006a, 2007). Vitamin D_3 also known as cholecalciferol is one of the two dominant forms of vitamin D, the other being Vitamin D_2 also known as ergocalciferol which is manufactured by invertebrates and plants after exposure to ultraviolet radiation (McGreevy and Williams 2011). In this chapter, the term vitamin D refers to both D_2 and D_3 unless indicated otherwise. Season, time of day, length of day, cloud cover, smog, skin melanin content, and sunscreen are among the factors that affect UV radiation exposure and vitamin D synthesis (IOM 2010).

Vitamin D is also obtained from diet and nutritional supplements. Though few foods naturally contain or are fortified with vitamin D, most oil-rich fish such as salmon, mackerel, and herring contain vitamin D_3 as well as egg yolk, red meat, liver, and some fortified breakfast cereals and fat spreads (Ovesen et al. 2003; Holick 2007; Nair and Maseeh 2012; Finglas et al. 2015; Bolland et al. 2016; Spector and Levy 2016). Both vitamin D_2 and D_3 are used to fortify milk, bread, and multivitamins in the United States. The fortification of milk with vitamin D beginning in the 1930s has made rickets a rare disease in the United States (Wharton and Bishop 2003). In Europe, vitamin D_3 is almost exclusively used for multivitamins and food fortification (Holick 2005).

Vitamin D from the skin and diet is converted to the active form in two steps. The first is hydroxylation in the liver by the enzyme vitamin D-25-hydroxylase (25-OHase) to 25-hydroxyvitamin D (25OHD). The second is further hydroxylation of the 25OHD in the kidneys by the enzyme 25-hydroxyvitamin D-1α-Hydroxylase (25(OH)D-1-OHase) to form the biologically active form of vitamin D, 1,25-dihydroxyvitamin D (1,25(OH)2D) (Holick 2006b, 2007).

Vitamin D intake is expressed in International Units (IU) or in micrograms (μg). One microgram of vitamin D3 is equivalent to 40 IU (SACN 2016). For this chapter, intake will be expressed as IU.

The Older Adults

Older adults refer to individuals aged 65 years and above. This is the chronological age accepted by most developed countries as the definition of elderly or older person as it is roughly equivalent to retirement age in most developed countries (WHO 2002). In relation to location, older adults could refer to healthy community-dwelling older adults, the acutely unwell and hospitalized older adults, or nursing home/institutionalized adults.

Older adults are at risk of inadequate vitamin D levels (Lips 2001; Durvasula et al. 2010). With advancing age, there is a decreased capacity to synthesize vitamin D3 in the skin. It has been shown that there is a decrease in the concentration of 7-dehydrocholesterol in the epidermis in old compared with young individuals and a reduced response to UV light, resulting in a 50% decrease in the formation of previtamin D_3 (MacLaughlin and Holick 1985; Gallagher 2013). Again, vitamin D metabolism in the kidneys is impaired. As renal function declines with age, there is a decrease in the activity of the kidney enzyme 25(OH)D-1-OHase that converts 25OHD into 1,25(OH)2D. Serum 1,25(OH)2D levels, therefore, decrease as a result of an age-related decline in renal function (Tsai et al. 1984; Gallagher 2013). Also, gut absorption of vitamin D is also reduced with aging (Barragry et al. 1978). Finally, older adults are more likely to spend more time indoors either because of frailty or being institutionalized, leading to reduced exposure to sunlight (Lips 2001; IOM 2010; Nair and Maseeh 2012; SACN 2016; Spector and Levy 2016).

Other comorbid conditions in older adults like malabsorption syndromes, obesity, smoking, chronic liver disease, chronic kidney disease, and use of certain medications like anticonvulsants, antiretrovirals, and corticosteroids increase the risk for vitamin D deficiency. Table 1 lists the risk factors for vitamin D deficiency (McGreevy and Williams 2011).

Based on the SACN report, the Required Nutritional Intake (RNI) for the UK older adult population recommendation for vitamin D is 400 IU per day (Table 2). In UK for community-dwelling older adults, the mean daily vitamin D intake from dietary sources and dietary supplements was 204 IU in men and 208 IU in women. This mean intake makes up 51% of their RNI. For institutionalized older adults, mean daily vitamin D intake was 156 IU for men and 136 IU for women, taking into account all sources. This daily intake of vitamin D was 39% of the RNI for men and 34% for women (SACN 2016).

Role of Vitamin D in Older Adults

Vitamin D is essential for bone health maintenance in older adults (Jansen 1950; Boucher 2012). It is required for calcium, phosphorus, and bone metabolism. It promotes calcium and phosphorus absorption from the bowel and enables

Table 1 Risk factors for vitamin D deficiency in older adults (Copyright permission obtained from author; McGreevy and Williams 2011)

Risk factors for vitamin D deficiency in older adults
Advanced age
Institutionalized or home-bound
Use of sunscreen with sun protection factor >15
Heavily pigmented skin
Smoking
Obesity
Malabsorption syndromes
Renal or liver disease
Medications like antiepileptics (phenytoin, phenobarbital), cholestyramine, orlistat, corticosteroids, and antiretrovirals

Table 2 Conversion of International Units (IU) to micrograms (μg)

Formula	40 IU = 1 μg
Example	204 IU = 204 ÷ 40 = 5.1 μg

mineralization of newly formed osteoid tissue in bone (Holick 2007). Low vitamin D as well as calcium leads to poorly mineralized (or weak) bones causing osteomalacia (Holick 2006a, 2007). This presents with severe aching in bone and muscles, marked proximal muscle weakness making standing up and walking difficult, and painful and a marked "waddling" gait. This increases the risk of frequent falls, thereby increasing the risk of fractures (Bischoff-Ferrari et al. 2005). Treatment with vitamin D is curative (Boucher 2012).

Also, a decrease in the absorption of dietary calcium and phosphorus also results in an increase in parathyroid hormone (PTH) levels (Lips 2001; Nair and Maseeh 2012). PTH-mediated increase in osteoclastic activity creates local foci of bone weakness and causes a generalized decrease in bone mineral density (BMD), resulting in osteopenia and osteoporosis (Nair and Maseeh 2012). The primary risk factors for low BMD, osteoporosis, and osteopenia include vitamin D insufficiency, inadequate calcium intake, and lack of exercise (Grant and Holick 2005).

Supplementation with vitamin D (800 IU daily or more in older people) reduces rates of bone loss over time (Boucher 2012). Among postmenopausal women and older men, supplements of both vitamin D and calcium result in small increases in bone mineral density throughout the skeleton. They also help to reduce fractures in institutionalized older adults, although the benefit is inconsistent in community-dwelling individuals (IOM 2010).

Vitamin D plays an important role in muscle function (Holick 2007). The skeletal muscles express nuclear vitamin D receptors, which promotes vitamin D-directed protein synthesis. Vitamin D is therefore required for maximum function with Vitamin D deficiency being expressed as muscle weakness (Holick 2006a, b, 2007). The secondary hyperparathyroidism from vitamin D deficiency by itself may also exert a negative influence on muscle function (Joborn et al. 1989). The impaired muscle function and muscle weakness from vitamin D deficiency are

reversible following vitamin D supplementation (Glerup et al. 2000). In a recent meta-analysis, however, looking at the role of vitamin D supplementation in maintaining or improving muscle strength and mobility in community-dwelling older persons, there was no improvement in muscle strength after the administration of vitamin D with or without calcium supplements (Rosendahl-Riise et al. 2017). Vitamin D treatment still has a role in institutionalized older adults with proven deficiency (Spector and Levy 2016).

Nonmusculoskeletal health outcomes of vitamin D have been shown in observational studies in conditions such as breast cancer, colorectal cancer, cardiovascular disease, inflammatory bowel diseases, diabetes, metabolic syndrome, dyslipidaemia, rheumatoid arthritis, chronic pain, multiple sclerosis, Alzheimer's disease, cognitive decline, Parkinson's disease, mood disorders including depression, respiratory infections, tuberculosis, bronchiectasis, systemic lupus erythematosus, and in all-cause mortality, cardiovascular mortality, and cancer mortality (Mosekilde 2005; Holick 2007, McGreevy and Williams 2011; Bolland et al. 2014, 2016). However, the results from systematic reviews of RCTs of vitamin D supplementation have been inconsistent (Bolland et al. 2014, 2016).

Policies and Protocols

Vitamin D Assessment Methodology

A person's vitamin D status is assessed by measuring the blood level of 25(OH)D (Holick 2006a, 2007; Aspray et al. 2014). It is the logical metabolite for assessment, believed to reflect medium term levels of substrate for vitamin D metabolism (Aspray et al. 2014). It reflects vitamin D supply from cutaneous synthesis and diet, has a long half-life in the circulation (about 2 – 3 weeks), and is not under tight homeostatic control (SACN 2016). Serum concentration of 25(OH)D is expressed as nanomoles per liter (nmol/l) or nanograms per milliliter (ng/ml); 1 ng/ml is equivalent to 2.5 nmol/l. For this chapter, concentration will be expressed as nmol/l.

The gold standard assay method is liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Zerwekh 2008; IOM 2010; Aspray et al. 2014; Osuafor et al. 2016). Alternative assays for evaluation of vitamin D status include serum 1,25 (OH) 2 vitamin D, PTH, and markers of bone turnover. These are not recommended for routine use (Aspray et al. 2014).

Vitamin D Deficiency in Older Adults

There is no generally accepted criterion for vitamin D deficiency. This, together with differences in serum vitamin D measurement, can make comparisons between different reports problematic (Hirani and Primatesta 2005).

The Endocrine Society Task Force on Vitamin D stated that individuals should be identified as vitamin-D deficient at a serum 25OHD level of 50 nmol/l and sufficient

at >75 nmol/l to maximize the effect of vitamin D on calcium, bone, and muscle metabolism (Holick 2011). However, the overall evidence suggests that risk of poor musculoskeletal health is increased at serum 25OHD concentrations below ~20–30 nmol/l. The Scientific Advisory Committee on Nutrition (SACN) maintains a threshold of 25 nmol/l to define the concentration below which risk of vitamin D deficiency increases (SACN 2016).

The National Osteoporosis Society (NOS) published a practical clinical guideline on the management of vitamin D deficiency in adult patients with, or at risk of developing, bone disease (Aspray et al. 2014). Based on this guideline, the serum 25OHD thresholds define <30 nmol/l as deficient; 30 – 50 nmol/l as inadequate in patients with osteoporosis, fragility fractures, malabsorption, or on steroids; and >50 nmol/l as sufficient. This is in agreement with the thresholds set by the Institute of Medicine (IOM) based on evidence from two systematic reviews (IOM 2010; NOS 2013).

For this chapter and the treatment algorithm, serum 25OHD thresholds <25 nmol/l is defined as deficient, 25 – 50 nmol/l as insufficient, and >50 nmol/l as adequate.

Vitamin D insufficiency affects almost 50% of the population worldwide (Nair and Maseeh 2012). Its prevalence is highly variable ranging from 28% to 100% of healthy adults and 70 – 100% of hospitalized adults in Europe (Passeri et al. 2003).

In older adults, the prevalence of vitamin D deficiency ranges from 20% to 55% in institutionalized adults in the United Kingdom (Hirani and Primatesta 2005) and community dwelling older adults in Ireland (Hill et al. 2006; McCarthy et al. 2006; Osuafor et al. 2016). Representative residents from the SENECA study of older Europeans aged 75 – 76 years old showed 36% of men and 47% of women were vitamin D deficient (Van der Wielen et al. 1995).

Patients living in nursing homes and in homes for the elderly had plasma 25OHD levels of 9 – 37 nmol/l in Europe compared with 53 – 45 nmol/l in the United States and 26 – 40 nmol/l in Australia (Lips 2001). In Denmark, 7% of postmenopausal women have vitamin D deficiency and 40% have insufficiency while 80% of elderly over 65 years have vitamin D insufficiency (Brot et al. 2001; Larsen 2002).

Regarding inpatient populations, as many as half of older adults in the United States with hip fractures could have serum 25(OH)D levels <30 nmol/l (Cranney et al. 2007), while a study in Singapore showed a vitamin D deficiency prevalence of 57.5% and vitamin D insufficiency of 34.5% in elderly patients admitted to hospital with hip fractures (Ramason et al. 2014).

Treatment Modality

Vitamin D_3 is recommended as the vitamin D preparation of choice for the treatment of vitamin D deficiency. This is because vitamin D_2 does appear to have quicker clearance than vitamin D_3 and lower tissue bioavailability. Vitamin D_2, however, may be preferred by vegetarians and those who wish to avoid animal-derived products (Romagnoli et al. 2008; Heaney et al. 2011; NOS 2013).

Oral administration of vitamin D is recommended over intramuscular (IM) administration. This is because the IM route has an unpredictable bioavailability, slower onset of repletion, and additional administration burden in comparison to oral preparations (Romagnoli et al. 2008; NOS 2013).

In the past, it was advocated that a single large dose (300,000 IU or higher) of vitamin D might lead to sustained correction of vitamin D deficiency and potentially avoid adherence problems with regular lower dose supplementation. However, more reports have suggested that large doses of vitamin D given intermittently are ineffective and might actually increase fracture risk (Smith et al. 2007; Sanders et al. 2010; NOS 2013).

Figure 1 shows a guideline for the treatment of vitamin D deficiency in older adults while Fig. 2 shows a guideline for the treatment of vitamin D insufficiency in older adults. Both protocols show the need for initial assessments of dietary habits, use of medications that may interfere with vitamin D metabolism and comorbid disease states mentioned earlier. Initial bloods investigations will also include bone, liver, renal profiles, and PTH level. Any abnormalities noted at this point will require the advice from an expert who may be a geriatrician, endocrinologist, nephrologist, or a specialist in bone health. If no abnormalities are found, then treatment can be commenced as per the guideline. This is only a guideline, and in all cases, it is recommended that expert advice should always be sought at every step in the assessment and treatment of older adults with vitamin D deficiency or insufficiency.

Monitoring and Assessment of Efficacy of Treatment

It is known that vitamin D treatment can unmask previously undiagnosed primary hyperparathyroidism (Hannan et al. 2004; NOS 2013). In order to monitor this outcome, adjusted serum calcium levels should be measured. Reassuringly, a report by Wagner et al. has shown that replacing vitamin D in mild primary hyperparathyroidism is safe and effective and does not increase calcium to dangerous levels (Wagner et al. 2013; Osuafor et al. 2016). According to the NOS, however, adjusted serum calcium levels should be measured within 1 month after the administration of the last loading dose of vitamin D. If hypercalcemia is observed at this time, further vitamin D supplementation should be stopped prior to investigation of the hypercalcemia (NOS 2013).

Due to confounding effects of UV exposure in the summer months, dose response to vitamin D supplementation shows considerable variability in different studies. When confined to winter levels, a daily supplement of 800–1000 IU cholecalciferol will cause an increase in 25OHD of 24 – 29 nmol/l (NOS 2013). Hence, the NOS guideline recommends that routine monitoring of serum 25OHD is unnecessary. It may however be appropriate in patients with symptomatic vitamin D deficiency or malabsorption and where poor compliance with medication is suspected, and this should be done not earlier than 3 months (NOS 2013; Aspray et al. 2014). Unnecessary retesting of vitamin D leads to increased laboratory expenditure, and it is recommended that hospitals work in conjunction with the laboratory and/or

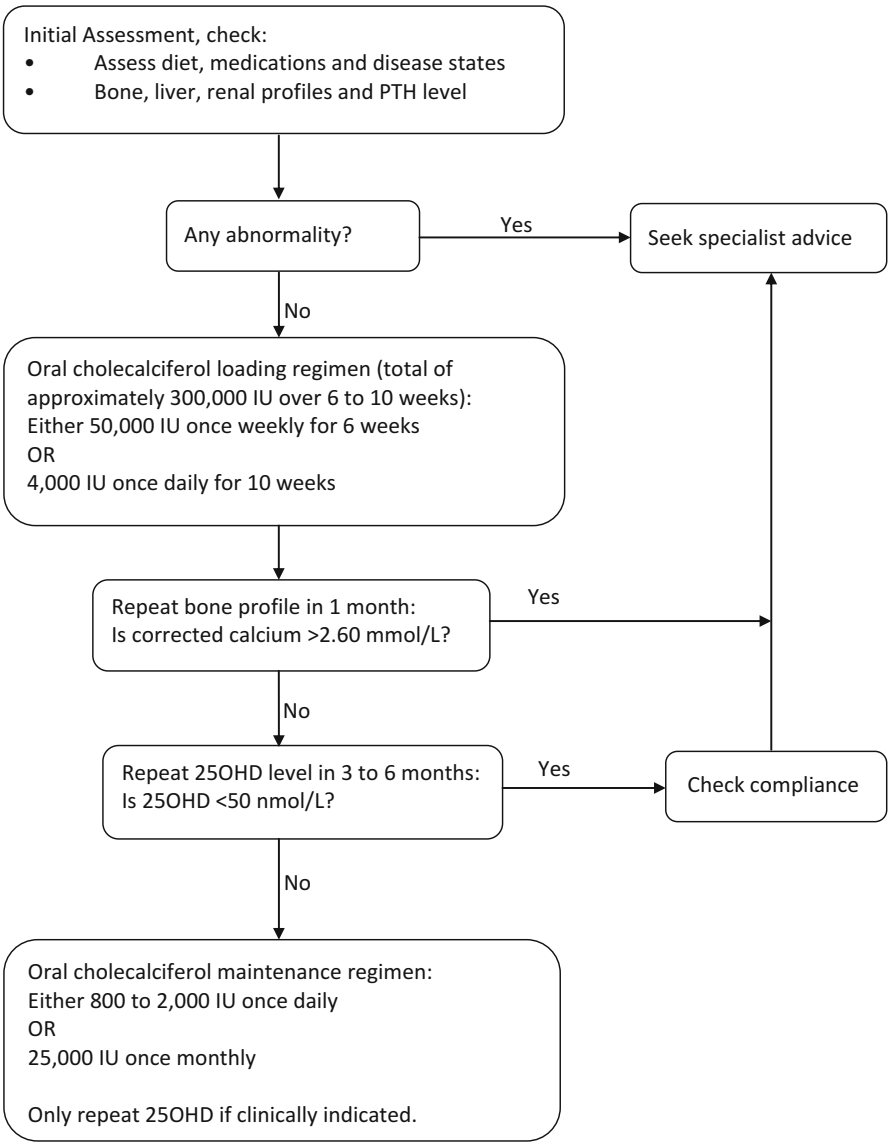

Fig. 1 Guideline for the treatment of vitamin D deficiency in older adults (Serum 25OHD <25 nmol/l)

information technology service to draw up policies regarding vitamin D retesting in patients known to their service (Osuafor et al. 2016).

Compliance to prescribed medications is also a factor for consideration as adherence to medications has always been an issue among the elderly (Yap et al. 2016). Extent of noncompliance in the elderly is estimated to vary from 40% to

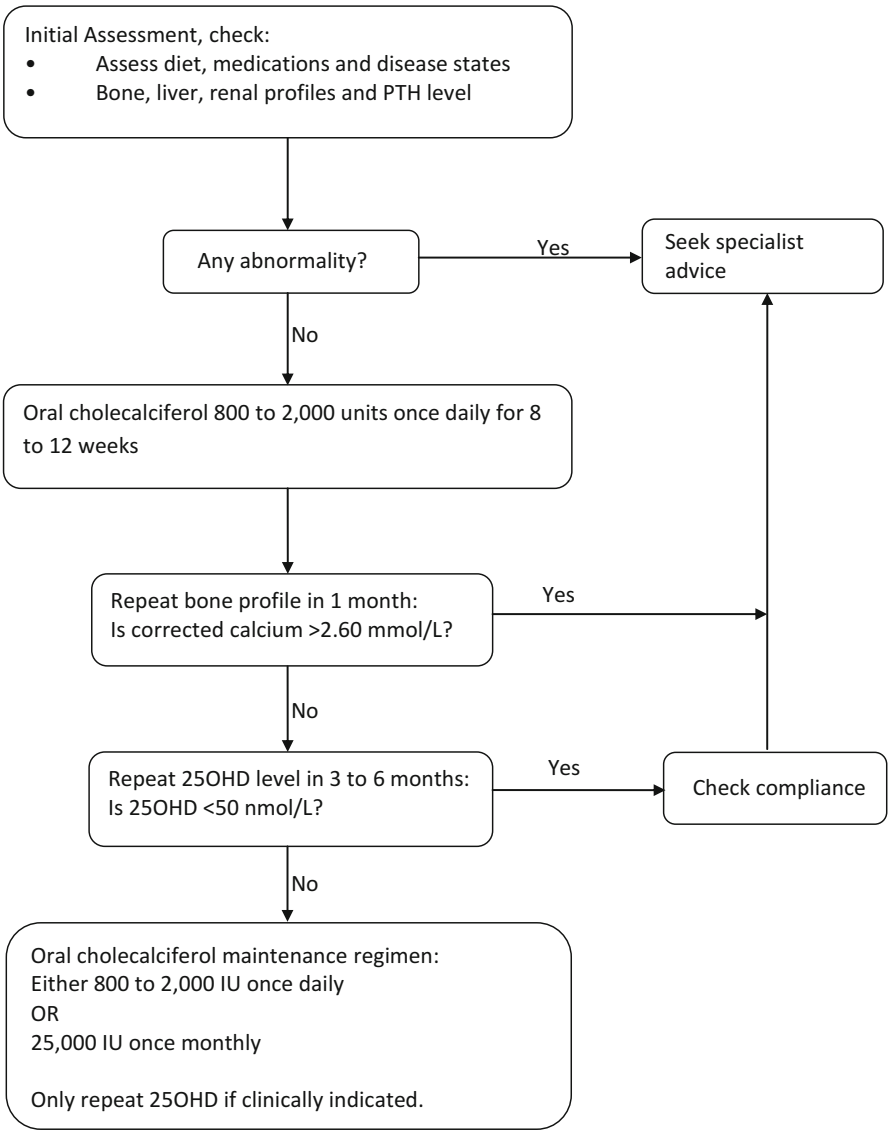

Fig. 2 Guideline for the treatment of vitamin D insufficiency in older adults (Serum 25OHD: 25 – 50 nmol/l)

75% (Salzman 1995). Overuse of prescribed medications is clearly associated with adverse drug reactions. Excessive vitamin D intake has been shown to have toxic effects (Vieth 2006). Vitamin D toxicity results in hypercalcemia caused by increased intestinal calcium absorption and mobilization of calcium from the bone which in turn can result in deposition of calcium in soft tissues, diffuse

demineralization of bones, and irreversible renal and cardiovascular toxicity (Jones 2008; SACN 2016). Reassuringly, the dosing regimen of vitamin D is unlikely to result in toxicity (NOS 2013).

The most common noncompliant behavior of the elderly appears to be underuse of the prescribed drug (Salzman 1995). An individualized therapy focusing on a patient's knowledge about his medications and health and use of a pharmacy-led medication reconciliation service may help improve adherence (Yap et al. 2016). In situations where the individual's cognition is a concern, as is the case often with frailer older adults, education of their carers on the patient's medications and health may help improve adherence.

Dictionary of Terms

- **1α-hydroxycholecalciferol** – A synthetic analogue of 1,25-dihydroxyvitamin D.
- **1,25-dihydroxyvitamin D (1,25(OH)2D)** – The main active metabolite of vitamin D in the body. Produced in the kidney from 25-hydroxyvitamin D.
- **7-dehydrocholesterol (7-DHC)** – A sterol found in the skin of humans and animals that is converted to vitamin D3 by the action of UVB rays in sunlight.
- **25-hydroxyvitamin D (25(OH)D)** – A metabolite of vitamin D produced in the liver from vitamin D. Circulates in the blood and is a marker of exposure to vitamin D, reflecting vitamin D supply from cutaneous synthesis and the diet.
- **Alfacalcidol** – Another name for 1α-hydroxycholecalciferol
- **Bone mineral density (BMD)** – The density of bone mineral in a skeletal unit (g/cm^3). When measured by single or dual-energy X-ray techniques it represents the mass of bone mineral measured within a scanned area (g/cm^2) and is not a true density measurement.
- **Bone profile** – Panel of tests for serum concentrations of albumin, calcium, corrected calcium, and phosphate.
- **Cholecalciferol** – Another name for vitamin D_3.
- **Reference Nutrient Intake (RNI)** – The nutrient intake that will be adequate to meet the needs of 97.5% of the population.
- **Fortification** – Addition of nutrients to foods during the manufacturing process.
- **Half-life** – Time required for concentration of a substance in the body to decrease by half.
- **Hypercalcemia** – A raised serum calcium concentration usually greater than 2.65 mmol/l.
- **Hyperparathyroidism** – Overactivity of the parathyroid glands that results in high concentration of parathyroid hormone. This leads to weakening of the bones through bone resorption to release calcium from the bone.
- **Liver profile** – Panel of tests for serum concentrations of alanine transaminases, aspartate transaminase, alkaline phosphatase, and gama-glutamyl transaminase.
- **Older adults** – Individuals above the age of 65 years.
- **Osteoclast** – Bone cell that resorbs bone tissue as part of the processes of skeletal development, maintenance, and repair.

- **Osteomalacia** – A skeletal disorder that develops as result of vitamin D deficiency. Causes severe aching in bones and muscles and muscle weakness. Caused by impairment in mineralization phase of bone remodeling, resulting in a lower ratio of bone mineral to osteoid than normal. Kidney or liver damage, which interferes with vitamin D metabolism, can also cause osteomalacia.
- **Osteoporosis** – A progressive skeletal disorder characterized by reduced bone strength due to loss of bone mass and deterioration in microarchitecture of bone, where the ratio of bone mineral to osteoid is normal. Leads to increased bone fragility and risk of fracture.
- **Parathyroid hormone (PTH)** – Hormone secreted by the parathyroid glands. Involved in regulation of calcium homeostasis.
- **Renal profile** – Panel of tests for serum concentrations of sodium, potassium, chloride, urea, and creatinine.
- **Rickets** – A disorder of the growth plates of infants and children that affects skeletal development. The most common cause is insufficient vitamin D (vitamin D deficiency rickets), calcium, or phosphate during growth, which leads to pain, softening and weakening of the bones, and characteristic deformities of the long bones.
- **Vitamin D_2** – Also known as ergocalciferol. Formed in fungi and yeast by UVB exposure of the steroid, ergosterol.
- **Vitamin D_3** – Also known as cholecalciferol. Synthesized in skin of humans from 7-dehydrocholesterol by the action of solar UVB radiation.

Summary Points

- Vitamin D is required for adequate calcium and phosphate metabolism, maintenance of bone structure, and maximum muscle function.
- Older adults whether healthy community-living older adults, acutely unwell and hospitalized, or living in a residential or nursing home are at risk of low levels of vitamin D.
- Vitamin D plays a significant role in musculoskeletal outcomes and probably plays a role in other nonmusculoskeletal conditions.
- A person's vitamin D status is assessed by measuring the blood level of 25-hydroxyvitamin D.
- We define serum 25OHD thresholds <25 nmol/l as deficient, 25 – 50 nmol/l as insufficient, and >50 nmol/l as adequate.
- Vitamin D insufficiency and deficiency has a worldwide prevalence.
- Oral Vitamin D_3 is a recommended choice for the treatment of vitamin D deficiency and insufficiency.
- Protocols have been proposed for the treatment of vitamin D deficiency and insufficiency in older adults. Expert advice should be sought at every step of the assessment and treatment.
- Compliance to prescribed medications is an ongoing concern in older adults and hence replacement requires monitoring.

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Congenital Vitamin E Deficiency

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Hamza El Hadi, Roberto Vettor, and Marco Rossato

Contents

Abstract

A growing body of literature has begun to delineate the unique and potent biological properties of vitamin E involved in cardiovascular disease, cancer, chronic inflammation, and neurodegeneration. Vitamin E was initally proposed as a dietary factor essential in preventing embryonic mortality and soon after considered as lipid-soluble antioxidant that inhibits lipid peroxidation by scavenging reactive oxygen species. Recent mechanistic studies indicate that vitamin

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E possesses functions that are independent of its antioxidant ability and mainly modulate cell signal transduction and gene expression. Despite overt vitamin E deficiency is rare, most commonly it can be found in children with inherited abnormalities that prevent the absorption or maintenance of normal blood concentration of vitamin E. This review summarizes the genetic disorders associated with congenital vitamin E deficiency. In parallel, it provides a brief overview on the historical, metabolic, and functional aspects of vitamin E.

Keywords
Vitamin E deficiency · Genetic disorders · Malabsorption · Oxidative stress · Humans

List of Abbreviations

ABL	Abetalipoproteinemia
AD	Autosomal dominant
apo	Apolipoprotein
AR	Autosomal recessive
AVED	Ataxia with vitamin E deficiency
CEHC	Carboxyethyl hydroxychromanol
CFTR	Cystic fibrosis transmembrane conductance regulator
CMRD	Chylomicron retention disease
COX	Cyclooxygenase
FFA	Free fatty acids
FHBL	Familial hypobetalipoproteinemia
HBL	Hypobetalipoproteinemia
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
LDL-C	Low-density lipoprotein-cholesterol
MIM	Mendelian Inheritance in Man
MMPs	Matrix metalloproteinases
MTP	Microsomal triglyceride transfer protein
NADPH	Nicotinamide adenine dinucleotide phosphate
PKC	Protein kinase C
ROS	Reactive oxygen species
TTPA	α-tocopherol transfer protein gene
VLDL	Very low-density lipoprotein
α-TTP	α-Tocopherol-transfer protein

Introduction

Since its discovery, research recognized potential ability of vitamin E to prevent chronic diseases and clinical syndromes. Particularly this was supported by a number of clinical conditions believed to have an oxidative stress component such as diabetes (Evans et al. 2002), cardiovascular (Rimm et al. 1993), neurodegenerative

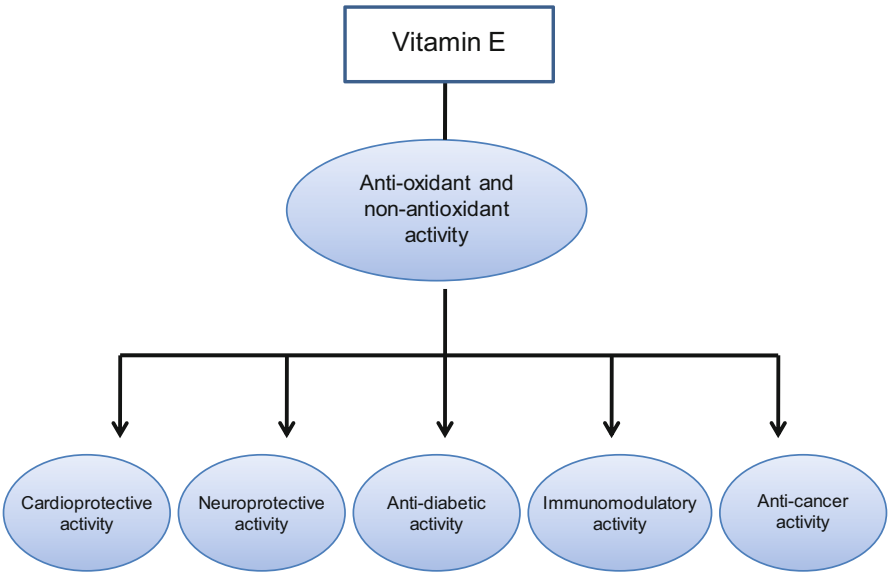

Fig. 1 Summary of vitamin E activities

diseases (Butterfield et al. 2002), and cancer (London et al. 1992) (Fig. 1). Nonetheless, the role of this vitamin in other conditions is still debated (Rossato and Mariotti 2014).

In the recent years, it has become increasingly evident that not all actions of vitamin E are dependent on its antioxidant properties, but may involve non-antioxidant activities, especially those connected with cell signal transduction, protein expression, enzyme activities, and gene expression regulation (Zingg and Azzi 2004).

The purpose of this review is to describe the congenital disorders associated with vitamin E deficiency. In addition, we sought to provide a concise summary of the history, metabolism, and regulatory functions of vitamin E.

History, Metabolism, and Function of Vitamin E: General Overview

Historical Background

Vitamin E was first discovered in 1922 by Evans and Bishop as a dietary factor essential for reproduction in rats. They found that laboratory rats failed to reproduce when lard was their only source of food fat. According to the researchers, there was a compound in both wheat germ and lettuce that corrected the problem and they decided to call it "Factor X" and the "antisterility factor" (Evans and Bishop 1922). Similar results were observed by Sure and he called it "Vitamin E," as by

then vitamins A, B, C, and D were already known (Sure 1924). In 1936, Evans et al. isolated a pure compound from the nonsaponifiable fraction of wheat-germ oil having the properties of vitamin E. This active compound was called alpha-tocopherol (α-tocopherol), from the ancient Greek word *phero* meaning "to bring" and *tocos*, meaning "childbirth" (Evans et al. 1936).

Natural vitamin E now encompasses a family of eight fat-soluble isoforms that exhibit the biological activity of α-tocopherol: the α-, β-, γ-, and δ-tocopherols and the α-, β-, γ-, and δ-tocotrienols, which are synthesized by plants from homogentisic acid (Rimbach et al. 2002).

Tocopherols and tocotrienols both include a chromanol ring and differ in their side chains. Saturated phytyl side chain is involved in the structure of tocopherols, while unsaturated geranylgeranyl side chain with three double bonds is involved in the structure of tocotrienols (Kamal-Eldin and Appelqvist 1996). Tocopherols and tocotrienols are classified as α-, β-, γ-, and δ according to the methyl group on the chromanol ring. Among the eight naturally occurring forms of vitamin E family, α-tocopherol is considered the most common form in human tissues followed by γ-tocopherol, while tocotrienols are usually not detected in tissues (Frank et al. 2012). Tocopherols are exclusively synthesized by photosynthetic organisms, and plant-derived oils are the major sources of vitamin E in the human diet. The most common form of tocopherol in the North American diet is γ-tocopherol, the predominant form of vitamin E in corn oils, while the most common form in European diets is α-tocopherol, found in olive and sunflower oils (Dutta and Dutta 2003). Tocotrienols are found in palm oil, barley, oats, and rice bran, and have higher antioxidant activity than tocopherols.

Metabolism

The vitamin Es present in ingested food, either as a free molecule or esterified, leave the stomach to be hydrolyzed in the duodenal lumen by the pancreatic lipases and subsequently absorbed through the brush border membrane of the enterocytes (Borel et al. 2001; Hacquebard and Carpentier 2005). After their uptake, vitamin E isomers reach the basolateral side of enterocytes to be equally incorporated into chylomicrons together with triacylglycerol, phospholipids, and cholesterol (Hacquebard and Carpentier 2005). The chylomicron-bound vitamin E forms are transported via lymphatic system into the circulation where the triaclyglycerol components of the chylomicrons are hydrolyzed by lipoprotein lipase of the capillary endothelium and adipose tissue forming lipid-depleted chylomicrons components (Hacquebard and Carpentier 2005). The chylomicrons remnants containing most of the absorbed tocopherols and tocotrienols are then taken up by the liver (via the endocytic receptors, such as the low-density lipoprotein (LDL) receptor and heparin sulfate proteoglycans) which sorts out α-tocopherol and preferentially secretes it within very low-density lipoprotein (VLDL) and high-density lipoprotein (HDL) into the bloodstream for distribution in the body (Traber et al. 1993). On the other hand, excess α-tocopherol and the other tocopherols and tocotrienol analogues are either excreted unchanged or metabolized before elimination in urine and bile (Traber

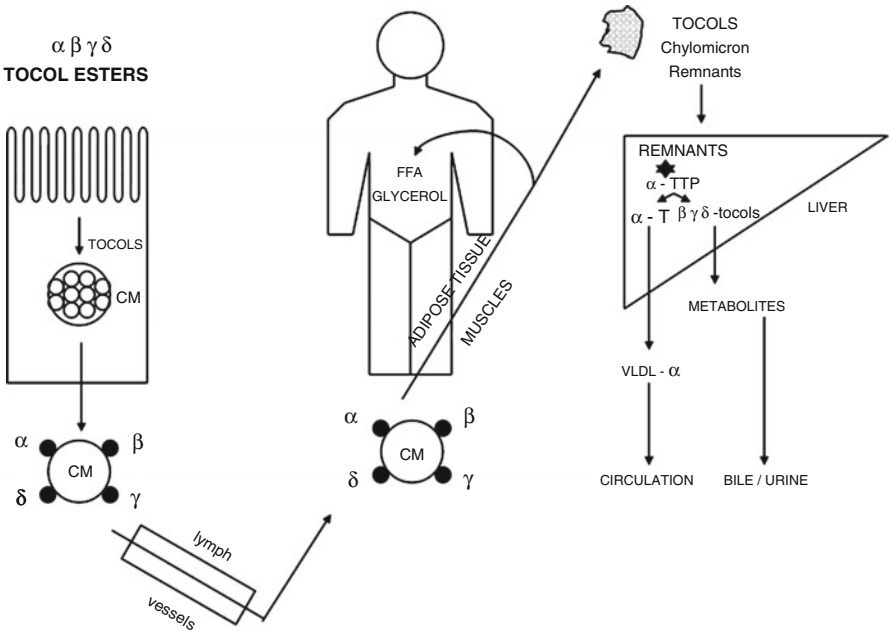

Fig. 2 Absorption, transport, and metabolism of vitamin E. All forms of vitamin E are absorbed equally. Intestinal enzymatic digestion is followed by the distribution to the liver and nonhepatic tissues. Discrimination between the different forms of vitamin E in favor of α-tocopherol occurs mainly in the liver by α-TTP, which protects α-tocopherol from excessive degradation and excretion (With permission from Eggermont 2006)

et al. 1993). This selective accumulation of α-tocopherol is mediated by hepatic cytosolic protein, α-tocopherol-transfer protein (α-TTP) which preferentially binds to α-tocopherol over other vitamers (Eggermont 2006) (Fig. 2). While α-TTP presents high affinity to α-tocopherol (100%), it has much lower affinity toward other vitamin E forms, e.g., 38%, 9%, or 1% affinity to β-, γ-, and δ- tocopherols, respectively (Eggermont 2006). In addition, it was proposed that non-α-tocopherol forms of vitamin E are catabolized in liver into carboxyethyl hydroxychromanol (CEHC) metabolites via cytochrome P450 (CYP4F2) initiated ω-hydroxylation and oxidation followed by β-oxidation of the phytyl chain (Birringer et al. 2002; Sontag and Parker 2007).

Molecular Function of Vitamin E

Antioxidant Activity

Vitamin E is widely accepted as one of the most potent antioxidant. Biochemically, tocopherols and tocotrienols are potent lipophilic antioxidants by scavenging lipid peroxyl radicals via donating hydrogen from the phenolic group on the chromanol ring and thus neutralize free radicals or reactive oxygen species (ROS) (Jiang et al.

2001). At equal molar concentrations in vitro, because of possessing similar phenolic moiety, all vitamin E forms are considered to have potent antioxidant activities. It was reported that the α-tocopherol and γ-tocopherol isoforms and the tocotrienol forms have relatively similar capacity to scavenge ROS during lipid oxidation (Yoshida et al. 2007). In vivo, there is likely more ROS scavenging by α-tocopherol than γ- tocopherol since it is at a 10-fold higher concentration within tissues. In addition to scavenging ROS, γ-tocopherol, in contrast to α-tocopherol, also reacts with nitrogen species such as peroxynitrite forming 5-nitro-γ-tocopherol (Wolf 1997).

Nonantioxidant Activity

As already mentioned in the introduction, it has been reported that vitamin E exhibits some properties that cannot be assigned to its antioxidant capacity. Here below we summarize some of those nonantioxidant functions.

Effects on Enzyme Inhibition

Protein kinase C (PKC) is a serine/threonine kinase that utilizes the cofactors phosphatidylserine, diacylglycerol, and calcium for activation. It is considered one of the major cellular transduction systems triggered by various ligands as hormones, neurotransmitters, and growth factors (Azzi et al. 1992). PKC is one of the pathways used by α-tocopherol (Boscoboinik et al. 1991) where it exerts a specific inhibitory activity compared to β-tocopherol (Tasinato et al. 1995). In monocytes this leads to the inhibition of phosphorylation and translocation of the cytosolic factor p47 (phox) and to an impaired assembly of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and of superoxide production (Cachia et al. 1998). Moreover, α-tocopherol exerted an antiproliferative effect on vascular smooth muscle cell model by inhibiting PKC pathway (Özer et al. 1998). However, these inhibitory effects were not observed by its isomer β-tocopherol or another antioxidant (probucol) (Özer et al. 1998). Additionally, α-tocopherol protects against endothelial damage by regulating the endothelial cell PKC (Abdala-Valencia et al. 2012). Remaining within this context, vitamin E has shown to increase the production of vasodilator prostanoids by suppressing cyclooxygenase (COX) activity in cellular environments and therefore effectively contribute to the anti-inflammatory process (Wu et al. 2005).

Effects on Gene Expression

It has been reported that vitamin E regulates the expression of genes involved in oxidative stress, proliferation, inflammation, and apoptosis. Some of these gene classes modulated by vitamin E include α-TTP, scavenger receptors (CD36, SR-BI, SR-AI/II), P450-Cytochromes, transcriptional factors (NF-κB, AP-1), genes involved in the modulation of extracellular proteins (tropomyosin, collagen-alpha-1, matrix metalloproteinases (MMP-1, -19), and connective tissue growth factor) (Azzi et al. 2004; Brigelius-Flohé 2009).

Moreover, genes connected to adhesion and inflammation (E-selectin, Intercellular Adhesion Molecule 1, integrins, glycoprotein IIb, IL-2, IL-4, IL-1b, and transforming growth factor-beta), lipid metabolism (Apolipoprotein E, peroxisome proliferator-activated receptor gamma, LDL- receptor), and cell

cycle regulation are also affected by α-tocopherol at the transcriptional level (Brigelius-Flohé 2009).

Despite well-documented antioxidant and other beneficial effects as well as negative association between α-tocopherol intake and chronic diseases, supplementation of α-tocopherol has failed to offer consistent benefits to prevention of chronic diseases including cancer and cardiovascular diseases in many large clinical intervention studies (Moya-Camarena and Jiang 2012; Myung et al. 2013; Papaioannou et al. 2011; Dolara et al. 2013). On the other hand, accumulating evidence suggests that metabolites mainly deriving from non-α-tocopherol vitamers have unique properties that are superior to α-tocopherols.

For instance, short-chain carboxychromanols like CEHCs generated by CYP4F2-initiated ω- oxidation of the side chain have been shown to have natriuretic activities (Wechter et al. 1996). In hemodialysis patients, supplementation of γ-tocopherol but not α-tocopherol is associated with a consistent increase of serum CEHC and a decrease in pro-inflammatory IL-6 and C-reactive protein (Himmelfarb et al. 2003). Moreover, long-chain carboxychromanols, including 13′-carboxychromanol, exhibit a potent anti-inflammatory (Jiang et al. 2008) and anticancer (Birringer et al. 2010) effects that may provide new insights into physiological role of less tissue-preserved forms of vitamin E.

Serum Vitamin E Assessment

The adequacy of the mean range of α-tocopherol intake is difficult to define being strongly influenced by concentration of circulating lipids, and does not accurately reflect tissue vitamin levels. Serum α-tocopherol concentrations less than 12 μmol/L were defined by the Institute of Medicine (IOM) to be in the deficient/inadequate range for healthy adults (Institute of Medicine 2000). It is recognized that vitamin E inadequacy is associated with increased erythrocyte fragility. Therefore, results from hydrogen peroxide-induced erythrocyte lysis test were used to define vitamin E status and consequently to assess vitamin E supplementation (Institute of Medicine 2000). As will be discussed in the next section, most commonly low α-tocopherol concentrations are caused by the combination of consumption of diets low in vitamin E, along with inadequate intakes or absorption of fat, protein, and calories. These latter dietary components are necessary for fat absorption and thus for vitamin E absorption and its lipoprotein transport.

Congenital Disorders with Vitamin E Deficiency

Vitamin E deficiency is seldom found in adults but more frequently can be found in children, likely as a result of genetic defects that lead to fat-malabsorption syndromes or a rapid depletion of plasma α-tocopherol. In this section we highlight the pathophysiology, clinical presentation, and management of the main genetic disorders associated with vitamin E deficiency (Table 1).

Table 1 Genetic diseases with vitamin E deficiency (Modified with permission from Eggermont 2006)

Disease	MIM number	Location	Inheritance	Gene defect
Ataxia with vitamin E deficiency	277460	8q13.1	AR	α-TTP
Abetalipoproteinemia	200100	4q22-24	AR	MTP
Familial hypobetalipoproteinemia	107730	3p21.1-22	AD	APOB
Chylomicron retention disease	246700	5q31	AR	SAR1B
Cystic fibrosis	219700	7q31.2	AR	CFTR

Ataxia with Vitamin E Deficiency

Human vitamin E deficiency symptoms began to be reported in the 1960s in various case studies of patients with lipoprotein abnormalities. Since these patients had malabsorption of other nutrients, it was not clear the extent to which various symptoms could be attributed to lack of vitamin E (Kayden et al. 1965). In the early 1980s, studies of humans with vitamin E deficiency symptoms without fat malabsorption began to appear in the literature (Burck et al. 1981). This form of vitamin E deficiency was later called AVED, or familial isolated vitamin E deficiency (Doerflinger et al. 1995). AVED (MIM 277460) is a rare genetic neurodegenerative disease transmitted in an autosomal recessive mode, and caused by mutations in the α-tocopherol transfer protein gene (TTPA) located on chromosome 8q13 (Doerflinger et al. 1995). This defect impairs incorporation of vitamin E into plasma VLDL and thus cannot reach peripheral circulation.

The concomitant presence of specific neurological phenotype and low plasma levels of vitamin E, in the absence of other clinical conditions associated with fat malabsorption, can guide the diagnosis for AVED (Anheim et al. 2010). The onset of neurologic features in AVED is between 4 and 18 years of age with a phenotype that resembles patients with Friedreich's ataxia, a genetic disorder caused by a mutation in the gene for frataxin, a mitochondrial iron-binding protein (Bradley et al. 2000; Yokota et al. 1997).

The signs and symptoms are usually devastating and progressive and mainly including truncal and extremity ataxia, loss of deep tendon reflexes, disturbances in proprioceptive and vibratory sensations, dysarthria, and positive Babinski sign (El Euch-Fayache et al. 2014). Head titubation, retinopathy, and dystonia are more common in patients with AVED while cardiomyopathy, glucose intolerance, scoliosis, and foot deformities in Friedreich ataxia (Cavalier et al. 1998; Benomar et al. 2002).

The molecular mechanism that underlies the neurological damage in patients with AVED is not yet known in detail, but oxidative stress due to reduced delivery of vitamin E to the central nervous system is likely to play a major role (Copp et al. 1999). Neuropathological findings derived from human and animal models with vitamin E deficiency documented the presence of severe dying back-type degeneration of the posterior column and massive accumulation of lipofuscin in neurons including dorsal root ganglion cells (Yokota et al. 2000).

Different mutations have been described in different ethnic groups. In North Africa populations, the most frequent mutation is 744delA on chromosome 8q13 (Cavalier et al. 1998; Gabsi et al. 2001). The mutation 513insTT predominates in AVED families of North European origin, 175 C4T (R59W) on exon 1 and 437delT on exon 3 in the case reported from Netherland as well as G552A on exon 3 in Japan (Yokota et al. 1997). In Mediterranean region, both the 744delA and 513insTT account for approximately 80% of the TTPA mutated alleles in Italian AVED (Doerflinger et al 1998, Cavalier et al. 1998).

Several studies described a correlation between the type of mutation and the function of the α-tocopherol protein and thereby vitamin E serum level and the severity of the neurological signs (Cavalier et al. 1998; Mariotti et al. 2004). Different mutations have been reported, including missense, nonsense, frameshift, and splice site mutations, and may affect the severity of the disease, presumably via residual protein activity with certain mutations (Cavalier et al. 1998).

Today experts agree that there is no specific instrumental approach for establishing the diagnosis of AVED (Harding et al. 1982). However, the presence of sensory neuropathy with normal motor conduction and absent or markedly reduced sensory nerve action potentials (SNAPs) is considered a neurophysiologic hallmark of this disease. Additionally, it has been described a distal motor neuropathy with normal sensory conduction AVED patient (Fusco et al. 2008).

Individuals with AVED are treated with life-long vitamin E supplementation. Remarkably, the administration vitamin E in early stages of the disease seems to prevent the progression of neurological impairment, atherosclerosis, and retinopathy in these patients (Marzouki et al. 2005) and can mildly improve cerebellar ataxia (Gabsi et al 2004). A mouse model has been developed that shows late-onset head tremor, ataxia, and retinal degeneration, the neurological aspects of which resolve with supplementation of vitamin E (Yokota et al. 2001).

Currently, vitamin E is manufactured as the acetate esters of RRR-α-tocopherol by using plant materials and all-racemic-α-tocopherol by chemical synthetic methods. Even though, α-TTP preferentially packages RRR-α-tocopherol into nascent VLDL, however, some AVED individuals lacking α-TTP or with a marked defect in the RRR-α-tocopherol binding site cannot discriminate between α-tocopherol stereoisomers (Traber et al. 1993; Cavalier et al. 1998).

Cavalier et al. suggested that administration of 800 mg RRR α-tocopherol twice daily, with meals that contain fat, results in plasma α-tocopherol levels at or above the normal range (Cavalier et al. 1998).

Abetalipoproteinemia

Abetalipoproteinemia (ABL; MIM 200100) or Bassen-Kornzweig syndrome is a rare autosomal-recessive disease that is characterized by very low plasma concentrations of triglyceride and cholesterol (under 30 mg/dl) and undetectable levels of LDL and apolipoprotein (apo) B (Berriot-Varoqueaux et al. 2000). It is caused by a mutation in microsomal triglyceride transfer protein (MTP) gene on chromosome 4q22-24 (Wang

and Hegele 2000). MTP, physiologically expressed on the luminal side of the endoplasmic reticulum in intestine, is involved in the assembly of chylomicrons in the enterocytes and of VLDL particles in hepatocytes (Wang and Hegele 2000).

Despite the gold standard diagnostic test would be by sequencing the MTP gene, and a clinical diagnosis can be made for ABL based on lipid profile, blood smear, and clinical symptoms. Key clinical features in ABL patients in the first years of life are steatorrhea due to fat malabsorption, and failure to thrive. This is often accompanied by digestive symptoms, such as diarrhea, vomiting, and abdominal distension (Burnett et al. 2012).

Additionally, neurological disorders which may appear later in childhood due to profound vitamin E deficiency often have the greatest impact on quality of life especially if there has been no therapeutic intervention. Absent tendon reflexes is an early clinical sign, followed by deep sensory loss in the lower limbs and then a spinocerebellar syndrome with an ataxic gait, dysmetria, and dysarthria (Berriot-Varoqueaux et al. 2000; Kane and Havel 2001). Ophthalmological findings may include atypical retinitis pigmentosa where the presence of lipofuscin pigment in the retina suggests that vitamin E deficiency plays a central role in this retinopathy (Berriot-Varoqueaux et al. 2000, Kane and Havel 2001).

Among laboratory investigations, a blood smear may show acanthocytosis that results from either vitamin E deficiency or an altered membrane lipid composition (Kane and Havel 2001). As mentioned previously, lipid profile shows decrease of plasma levels of cholesterol and triglycerides and almost undetectable levels of apoB-containing lipoproteins including chylomicrons, VLDL, and LDL (Berriot-Varoqueaux et al. 2000; Wang and Hegele 2000). The current standard treatment consists of strict adherence to specialized diets and oral vitamin E supplementation. Total fat intake should be restricted to less than 30% of the total caloric intake which will eliminate steatorrhea and allow absorption of nutrients essential for growth and development (Berriot-Varoqueaux et al. 2000; Wang and Hegele 2000; Zamel et al. 2008).

High-dose oral vitamin E supplementation (100–300 mg/kg/day) is recommended to halt the progression of the neurological deterioration (Zamel et al. 2008). Combined oral vitamin E and A is usually administered to attenuate the severity of retinal degeneration (Zamel et al. 2008).

Hypobetalipoproteinemia

Hypobetalipoproteinemia (HBL) represents a rare co-dominant condition characterized by low plasma levels of total cholesterol, low-density lipoprotein-cholesterol (LDL-C), and apoB below the 5th percentile of the general population (Tarugi et al. 2007). Familial hypobetalipoproteinemia (FHBL; MIM 107730) is the most frequent monogenic form of HBL. It may be due to loss-of-function mutations in apoB gene (APOB-linked FHBL) or, less frequently, in PCSK9 (PCSK9-linked FHBL) (Tarugi et al. 2007; Schonfeld 2003). However, in many subjects the genetic basis of FHBL remains unexplained (Orphan FHBL) (Tarugi et al. 2007). Most APOB gene mutations lead to the formation of truncated apoB protein of various sizes

(Tarugi et al. 2007, Schonfeld 2003). Missense nontruncating mutations of the APOB gene can also cause FHBL (Burnett et al. 2003).

The best-characterized form of FHBL occurs with a dominant mode of inheritance and it has been linked to heterozygous pathogenic mutations in the APOB gene that are generally asymptomatic but often develop liver steatosis (Tarugi et al. 2007). Patients with the clinical diagnosis of homozygous FHBL are rare and they can be either carriers of homozygous or compound heterozygous mutations in APOB gene (Tarugi et al. 2007). The clinical manifestations of homozygous FHBL show great variability. They may have a similar biochemical and clinical phenotype to patients affected by ABL due to fat malabsorption and fat-soluble vitamin deficiency, particularly vitamins A and E (Lee and Hegele 2014).

Chylomicron Retention Disease

Chylomicron retention disease (CMRD; MIM 246700), also called Anderson disease, is an autosomal recessive disorder condition characterized by the accumulation of lipid droplets within the enterocytes and the selective absence of apoB-48-containing particles from plasma (Boldrini et al. 2001). CMRD is caused by homozigous and compound heterozygous mutations in SAR1B, a gene encoding Sar1b protein, which is involved in chylomicron trafficking from the endoplasmic reticulum to the Golgi apparatus (Jones et al. 2003). Until now, missense mutations have represented the majority of SAR1B mutations.

CMRD presents shortly after birth with malabsorptive diarrhea and failure to thrive, with vomiting and abdominal distension often present (Peretti et al. 2010). Vitamin E is the most affected among the liposoluble vitamins in CMRD, because its transport is highly dependent on apo B-containing lipoproteins (Berriot-Varoqueaux et al. 2000). Hepatomegaly and hepatic steatosis may develop in some patients, but in contrast to ABL and FHBL, liver cirrhosis has not been reported in CMRD (Peretti et al. 2010). The neurological complications are usually an alarm sign of vitamin E deficiency in these subjects and include hyporeflexia and loss of proprioception in adolescents and ataxia, myopathy, and sensory neuropathy in adults (Peretti et al. 2010).

In CMRD, total cholesterol, LDL cholesterol, and HDL cholesterol concentrations are low, but triglyceride levels are generally normal. An increased plasma creatine kinase concentration of up to five times the normal level may be observed from infancy (Peretti et al. 2010).

Regarding the management of CMRD patients, there are no specific recommendations for the follow-up or treatment of CMRD, even if low-fat diet regimens have shown to improve digestive symptoms in these patients (Peretti et al. 2010).

Cystic Fibrosis

Cystic Fibrosis (CF; MIM 219700) is an autosomal recessive disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene,

located on chromosome 7. The CFTR protein is a chloride ion channel and is expressed in many different organs (e.g., the pancreas, airways, lungs, liver, salivary glands, sweat and reproductive tract glands) (Gibson et al. 2003). CFTR protein dysfunction leads to defective ion transport across epithelial surfaces, which causes impaired mucociliary clearance. The most common manifestations in newborns and children include sinopulmonary symptoms, failure to thrive, steatorrhea, and meconium ileus (Gibson et al. 2003). However, intestinal malabsorption mediated by pancreatic insufficiency, deranged bile acid function, and enzyme inactivation by hyperacidity remains the most common feature in CF subjects (Peretti et al. 2005). Vitamin E status in these individuals has shown to be associated with hemolytic anemia (Wilfond et al. 1994), cognitive impairment (Koscik et al. 2005), and increased rate of pulmonary exacerbations (Hakim et al. 2007). Today, it has been recommended to include a dosage of 100 UI to 400 UI per day for all subjects with CF (Sinaasappel et al. 2002).

Conclusion

Vitamin E deficiency is quite rare in adult humans. It is more frequently found in children with an inherited condition that impairs their ability to absorb this vitamin.

It has been shown that vitamin E plays a vital role in various disorders through its oxidative properties, although it has also nonoxidative effects in humans. Therefore, further studies should be addressed beyond the free radical-scavenging properties of vitamin E and its metabolites.

In spite of the promising potential, the experimental analysis of tocotrienols accounts for only a small portion of vitamin E research. Hence, the current state of knowledge deserves further investigation into this lesser known form of vitamin E.

It must be emphasized that early supplementation of vitamin E could halt the disease progress and a fast progression of the disease should be avoided. However, it is well known that this vitamin is transported with lipoproteins, and therefore its plasma concentration is not indicative of whole body and peripheral tissue stores. In this context, additional markers of inadequate vitamin E status especially in newborns are needed. This would also promote the evaluation of vitamin E supplementation outcomes in terms of beneficial effects and safety.

Policies and protocols

In this chapter we described the genetic disorders associated with vitamin E deficiency in humans. Despite vitamin E deficiency is rare, it can occur as a result of genetic abnormalities in α-tocopherol-transfer protein or as a result of fat malabsorption syndromes (abetalipoproteinemia, hypobetalipoproteinemia, chylomicron retention disease, and cystic fibrosis).

- Generally, vitamin E deficiency manifests in early childhood by progressive neurologic damage and serious clinical consequences. Therefore, policies focused on the prompt recognition of individuals with vitamin E deficiency should be considered.
- The physiological role and the health consequences of vitamin E deficiency should get the desired attention in international micronutrient recommendations. In addition, public health authorities should encourage large-scale research studies to determine optimal vitamin E dosage and to evaluate the outcomes.
- As described previously, hydrogen peroxide-induced erythrocyte lysis test is used to define vitamin E status and consequently to assess vitamin E supplementation. However, when interpreting the results it is important to consider serum lipid profile components in these individuals.
- A full clinical investigation is recommended in individuals diagnosed by AVED. In addition to laboratory testing, assessment must include neurologic, ophthalmologic, and cardiac examination. Moreover, it is appropriate to perform a predictive genetic testing in at-risk families for the purpose of early diagnosis and treatment.

Dictionary of Terms

- **Tocopherols and tocotrienols** – Two main families of vitamin E. Each composed of four forms with different chemical structure.
- **Reactive oxygen species** – Highly reactive molecules produced by physiologic and nonphysiologic processes and may cause damage to cell membrane or DNA molecules.
- **Antioxidant** – Is a molecule that protects against harmful chemical reactions inside the organism.
- **Malabsorption** – Impairment in absorption of food nutrients from intestinal tract.
- **Congenital disorder** – When a baby has a disease that is present from birth. It can be inherited from mother and/or father (genetic disorder) or caused by environmental factors.

Summary

- Vitamin E is a fat-soluble vitamin that refers to a group of compounds that include both tocopherols and tocotrienols.
- Vitamin E is a potent antioxidant that acts as a peroxyl radical scavenger and can play a role in regulating cell signaling and modulating gene transcription through a nonantioxidant activity.
- Vitamin E has been shown to play a role in cardiovascular disease, cancer, chronic inflammation, and neurodegeneration.
- In humans severe vitamin E deficiency occurs as a result of genetic defects in the α-tocopherol transfer protein gene or in presence fat-malabsorption syndromes.

- Early supplementation of vitamin E may prevent and reverse clinical complications of vitamin E deficiency.
- Future research should focus on finding biomarkers of inadequate vitamin E status to optimize both diagnostic and therapeutic approaches in individuals with vitamin E deficiency.

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Low Folate Status and Relationship with Betaine and Homocysteine

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Jose M. Colomina and Michelle M. Murphy

Contents

Abstract

Folate and betaine participate in homocysteine metabolism through the methionine synthase (MS) and betaine-homocysteine methyltransferase (BMHT) pathways, respectively. The ubiquitous MS pathway depends on 5-methyltetrahydrofolate and cobalamin as cofactors in the remethylation of homocysteine to methionine. When folate and cobalamin supply are adequate,

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homocysteine remethylation to methionine is predominantly by the MS pathway. The BHMT pathway appears to play an important role in very early embryogenesis and fetal development (in animal studies) but is then limited mainly to the kidney and liver in human adults.

Low or suboptimal folate status still affects non-supplement users and low consumers of voluntarily fortified foods in populations where mandatory fortification with folic acid is absent.

Limited dietary folate and/or cobalamin intake impairs homocysteine remethylation via the MS pathway thus leading to increased blood homocysteine and reduced methionine synthesis. Evidence from in vitro and animal studies shows that the BHMT pathway is upregulated when folate status is low. Preliminary evidence supports the hypothesis that homocysteine remethylation by BHMT is enhanced when the MS pathway is impaired due to low folate status in human adults and during pregnancy. Further research is required to demonstrate that BHMT activity increases in response to low folate status and to determine the extent to which the BHMT pathway can compensate for MS pathway impairment.

Keywords

Folate · Betaine · Cobalamin · Homocysteine · Methionine synthase · Betaine-homocysteine methyltransferase · Methionine

List of Abbreviations

1C	One carbon
1CM	One carbon metabolism
5,10-CH_2THF	5,10-Methylenetetrahydrofolate
5-CH_3THF	5-Methyltetrahydrofolate
BHMT	Betaine-homocysteine methyltransferase
CBS	Cystathionine-beta-synthase
CHDH	Choline dehydrogenase
DMG	Dimethylglycine
DMGDH	Dimethylglycine dehydrogenase
FAD	Flavin adenine dinucleotide
MS	Methionine synthase
MTHFR	Methylenetetrahydrofolate reductase
NTDs	Neural tube defects
PML	Post-methionine load
PMNS	Pune Maternal Nutrition Study
SAM	*S*-adenosyl methionine
SARDH	Sarcosine dehydrogenase
SNPs	Single nucleotide polymorphisms
tHcy	Fasting total plasma homocysteine
THF	Tetrahydrofolate

Introduction

Overview of one carbon metabolism (1CM) and the roles of folate and betaine in homocysteine metabolism

The 1C network, through the intracellular transfer of 1C units to metabolic reactions, regulates purine, pyrimidine, and thymidylate synthesis as well as methionine synthesis from homocysteine. These processes lead to protein, DNA, and RNA synthesis as well as the epigenetic regulation of gene functions. The nutrients that participate in this biological process (amino acids, osmolytes, B vitamins, nitrogenous bases, phospholipids, and methyl donors) are essential for cell proliferation, metabolism, regulation, and differentiation. Amino acids, folates, the universal methyl donor *S*-adenosyl methionine (SAM), and betaine, among others, act as 1C donors in this network. These are directly supplied to the 1C network from the diet or, as in the cases of SAM and betaine, can be generated by the network (Fig. 1). Optimal performance of the network depends on adequate functioning of all relevant enzymes, plus an adequate dietary supply of all the specific micronutrient cofactors, 1C, and methyl group donors involved. Moreover, the network is strongly influenced by lifestyle factors, such as smoking, alcohol consumption, diet, and exercise.

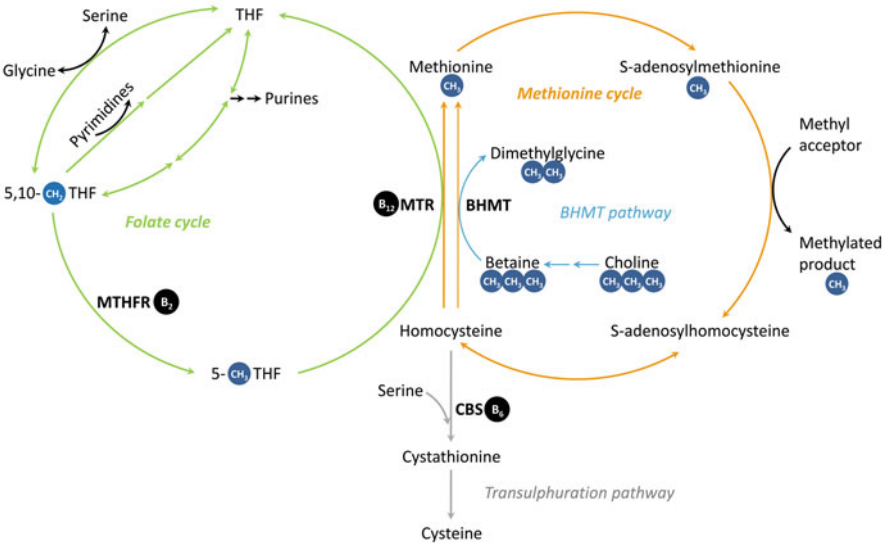

Fig. 1 Folate and methionine cycles, betaine-homocysteine methyltransferase, transulphuration and related pathways. Simplified overview to highlight interrelations between the folate cycle (green arrows), methionine cycle (orange arrows) and betaine-homocysteine methyltransferase pathway (blue arrows), and transulphuration pathway (grey arrows). Enzymes [vitamin cofactor]: BHMT, betaine-homocysteine methyltransferase; CBS, cystathionine-beta-synthase [B_6, pyridoxine]; MTHFR, methylenetetrahydrofolate reductase [B_2, riboflavin]; MS, methionine synthase [B_{12}, cobalamin]. Metabolites: THF, tetrahydrofolate; 5,10-CH_2THF, 5,10-methylenetetrahydrofolate; 5-CH_3THF, 5-methyltetrahydrofolate

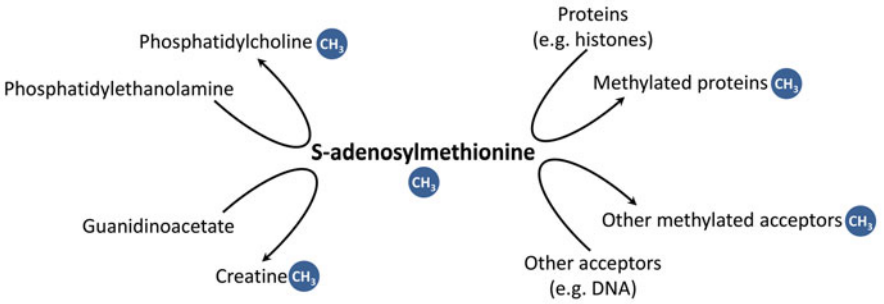

Fig. 2 Main methyl transfer reactions. Schematic representation of methyl transfer reactions with *S*-adenosyl methionine as the methyl donor

Homocysteine is an intermediate in this biological system, and a large body of evidence supports its potential as a biomarker of impaired 1C metabolism. Deficiency in folate, cobalamin, or pyridoxal phosphate, especially, leads to elevated blood homocysteine concentrations. There is also accumulating evidence to support the hypothesis that nutrient-nutrient and nutrient-gene interactions involving any of the nutrients involved in the system may lead to metabolic anomalies affecting this network as well as other metabolic processes.

Folate and betaine participate in the remethylation of homocysteine to methionine at the points of intersection of the respective methionine synthase (MS) and betaine-homocysteine methyltransferase (BHMT) pathways with the methionine cycle. The cobalamin- and folate-dependent MS pathway is ubiquitous, and the betaine-dependent BHMT pathway is limited to the liver and kidney (Finkelstein 1990) and lens (Rao et al. 1998). There are conflicting reports regarding BHMT presence in the brain (Gaull et al. 1973; McKeever et al. 1991). Status in the nutrients and metabolites that regulate the MS and BHMT pathways appears to drive interactions between these.

Methyl groups are transferred from SAM to four important methyl acceptors (Fig. 2) (McBreairty et al. 2013). The methylation of phosphatidylethanolamine and guanidinoacetate produces phosphatidylcholine (phospholipid) and creatine (osmolyte and energy storage form when phosphorylated), respectively.

The Folate Cycle and MS Pathway

Folate acts as an intermediate in the transfer of 1C units for the previously mentioned functions that are crucial for cellular proliferation and genomic stability. It also provides methyl groups for biological methylation reactions such as DNA and protein methylation that are essential epigenetic regulatory processes. Folate and cobalamin play interdependent roles in homocysteine remethylation to methionine via the MS pathway. The enzyme methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. This acts

as a cofactor and as a substrate for MS and provides the methyl group that binds to cobalamin to form methylcobalamin. Methylcobalamin is a cofactor in the MS reaction in which homocysteine is remethylated to form methionine. SAM regulates MTHFR activity by allosteric inhibition. In situations of cobalamin deficiency, intracellular 5-methyltetrahydrofolate accumulates because without cobalamin to bind the methyl group, it cannot be converted to tetrahydrofolate. Folic acid, the oxidised form of folate, is contained in vitamin supplements and fortified foods and has considerably higher bioavailability than the naturally occurring folate forms (Cuskelly et al. 1996). There are concerns that intake of high doses of folic acid, in the presence of cobalamin deficiency, may lead to the normalisation of erythrocyte size and disappearance of the symptomatic megaloblasts of cobalamin deficiency, thus masking cobalamin deficiency.

Betaine and the BHMT Pathway

Betaine is a methylamine which mainly acts as an osmolyte (Yancey et al. 1982) and in protein stabilisation (Sheikh-Hamad et al. 1994). It can also act as a methyl donor in the BHMT catalysed conversion of homocysteine to methionine. Betaine-dependent homocysteine remethylation is as important as folate-dependent homocysteine remethylation in the liver according to rat experiments (Finkelstein and Martin 1984a). Betaine supplementation of patients with homocystinuria, due to inborn errors affecting key enzymes involved in homocysteine metabolism, reduces blood homocysteine and the clinical symptoms of homocystinuria but does not normalise homocysteinemia (Garrow 1996). Therefore, it cannot compensate impairment of other regulatory pathways of homocysteine metabolism.

Dietary betaine intake recommendations have yet to be defined. Apart from dietary sources, betaine is also produced from choline oxidation (Fig. 3). In the BHMT reaction, one methyl group is transferred from betaine to homocysteine to form dimethylglycine and methionine. When dimethylglycine is converted into glycine, it donates two methyl groups to mitochondrial reactions (Wittwer and Wagner 1981). Moreover, glycine and tetrahydrofolate are converted into CO_2, NH_3, and 5,10-methylenetetrahydrofolate by the glycine cleavage system (Yoshida and Kikuchi 1970).

Choline can be obtained from the diet or synthesised endogenously by converting phosphatidylethanolamine to phosphatidylcholine via phosphatidylethanolamine *N*-methyltransferase.

Low Folate Status and the MS Pathway

Plasma folate concentration <6.8 nmol/L (measured by the gold standard *Lactobacillus casei* microbiological assay) is indicative of folate deficiency based on macrocytic anemia as an indicator (WHO 2015). Plasma folate concentration of 10 nmol/L (determined by radioimmunoassay) was proposed as the minimum cutoff concentration to

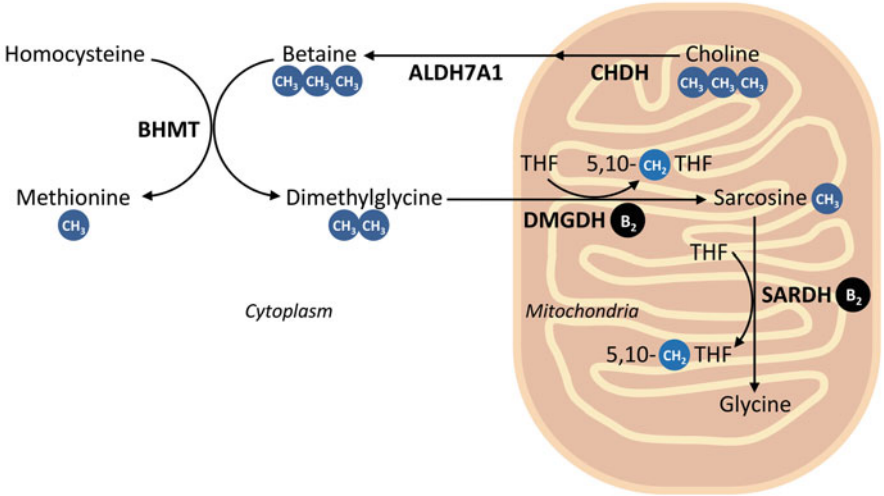

Fig. 3 BHMT pathway and related reactions. Illustration of the distribution between the cytoplasm and mitochondria of choline oxidation, methyl transfer by BHMT, and related reactions. Enzymes [vitamin cofactor]: ALDH7A1, betaine aldehyde dehydrogenase; BHMT, betaine-homocysteine methyltransferase; CHDH, choline dehydrogenase; DMGDH, dimethylglycine dehydrogenase [B_2, riboflavin]; SARDH, sarcosine dehydrogenase [B_2, riboflavin]. Metabolites: THF, tetrahydrofolate; 5,10CH_2THF, 5,10-methylenetetrahydrofolate

prevent moderately elevated fasting plasma total homocysteine (tHcy) (Brouwer et al. 1998), and concentrations below this are accepted as indicative of possible folate deficiency (WHO 2015; Bailey and Hausman 2017). Red blood cell folate concentration >906 nmol/L is optimal for protection against neural tube defect (NTD)-affected pregnancies caused by suboptimal folate status (Daly et al. 1995; WHO 2015).

Insufficient supply of folate or cobalamin to the 1C metabolic network impairs the MS pathway and leads to increased tHcy, as reported in animal and human studies. Thus, moderately elevated tHcy is an indicator of low folate and/or cobalamin status. Both folic acid and cobalamin supplementation reduce tHcy. Homocysteine remethylation via the MS pathway may also be downregulated when folate utilisation is impaired, for example, by single nucleotide polymorphisms (SNPs) associated with impaired intracellular folate metabolism, especially in the absence of mandatory fortification with folic acid or folic acid supplement use (Bueno et al. 2016). The influence of SNPs on folate metabolism is discussed below.

Low Folate Status and the BHMT Pathway

Homocysteine can be remethylated by the BHMT pathway in a reaction that does not require folate or cobalamin. Although limited to the kidneys and the liver, it may play a role in folate and cobalamin sparing, in situations of deficiency. In this case, its

role in homocysteine remethylation may be enhanced in situations of deficiency in either of these vitamins.

Evidence from in vitro, animal, and human studies suggests that 1CM nutrients interact with the BHMT pathway. Evidence from in vitro studies is summarized in Table 1. The effects of different forms of folate on the BHMT pathway have not been studied, but studies have investigated the effects of nutrients/metabolites that are likely to be replete when folate status is high, e.g., methionine and SAM. Methionine was shown to markedly inhibit rat (Finkelstein et al. 1972) and human (Szegedi et al. 2008) hepatic BHMT activity. SAM inhibited BHMT activity in rat liver (Finkelstein et al. 1972; Finkelstein and Martin 1984b) in studies using the semi-purified enzyme but not purified human hepatic BHMT (Szegedi et al. 2008). A dose-dependent

Table 1 Evidence from in vitro and animal studies for an association between folate status (or related metabolites) and the BHMT pathway

	Experiment		Effect
In vitro studies			
Finkelstein et al. 1972	Rat liver extract (semipurified BHMT)	50 μM methionine	93% lower BHMT activity
		50 μM SAM	15% lower BHMT activity
Finkelstein and Martin 1984a		224 μM SAM	32% lower BHMT activity
Castro et al. 2002; Ou et al. 2007	Human hepatoma cells with construct of *BHMT* promoter and luciferase gene; or endogenous *BHMT*	0–5000 μM SAM	SAM dose-dependent decrease in construct/*BHMT* mRNA levels
Szegedi et al. 2008	Purified human hepatic BHMT	200 μM SAM	No effect on BHMT
		200 μM methionine	15% lower BHMT activity
Animal studies			
Halsted et al. 2002	Micropigs	Folate-deficient diet	37% higher hepatic BHMT activity
Chmurzynska and Malinowska 2011	Rat dams	Folic acid-supplemented diet	Lower hepatic *BHMT* mRNA levels in offspring
Christensen et al. 2015	Mice	Excessive folic acid diet	No effect on hepatic *BHMT* mRNA
			Lower hepatic *CHDH* mRNA
Jadavji et al. 2015	Mice	Folate-deficient diet	17% lower hippocampal betaine concentration

BHMT betaine-homocysteine methyltransferase, *CHDH* choline dehydrogenase, *SAM* *S*-adenosyl methionine

reduction in BHMT expression by SAM was observed in human hepatoma cells (Castro et al. 2002; Ou et al. 2007) (Table 1).

Animal experiments suggest that the hepatic BHMT pathway is more active when folate status is low (Table 1). Micropigs on a folate-deficient diet had higher hepatic BHMT activity than pigs fed a folate-replete diet (Halsted et al. 2002). On the other hand, hepatic BHMT expression was lower in the offspring of rats fed with a folic acid-supplemented diet (5 mg/Kg diet) compared to that of rat dams fed on a control diet of 2 mg/Kg of folic acid (Chmurzynska and Malinowska 2011). A diet containing excessive folic acid had no effect on hepatic BHMT expression but was associated with lower hepatic choline dehydrogenase expression in mice (Christensen et al. 2015). In a separate study that did not measure BHMT activity, lower hippocampal betaine concentration was reported in lactating mice on a folate-deficient diet (0.3 mg/Kg diet) compared to those on an adequate folate diet 2 mg/Kg (Jadavji et al. 2015).

Various studies in humans have investigated interactions between folate and betaine status or, indirectly, between the MS and BHMT pathways (Table 2). In a study of coronary angiography patients, plasma betaine was increased by 15% following a methionine load, and there was a strong inverse correlation between post-methionine load (PML) betaine and homocysteine (Holm et al. 2004). In the same study, following random assignment to a 3-month B vitamin supplement regime, the correlation between plasma betaine and PML homocysteine was weakened, except in the placebo group. Supplementation of healthy men and women with 50–800 μg/d of folic acid led to a dose-dependent increase in plasma betaine concentrations (Melse-Boonstra et al. 2005). Furthermore, the proportional decrease in tHcy following folic acid supplementation was associated with the increase in plasma betaine concentration. As we mentioned previously, betaine's principal role does not appear to be as a methyl donor, and the authors interpreted these results as indicative of interaction between the MS and BHMT pathways. They suggested that the BHMT pathway is downregulated when MS pathway activity is optimal and that this is a potential betaine-sparing mechanism. A transversal study investigating the association between dietary betaine intake and tHcy in adult women reported an inverse association when folate status was below 400 μg/d, but there was no evidence for an independent role of dietary betaine in tHcy remethylation when folate intakes were above 400 μg/d (Chiuve et al. 2007). Betaine was a strong determinant of tHcy in adults with the *MTHFR* c.665TT genotype and low folate status (Holm et al. 2007). Before mandatory fortification of flour with folic acid was implemented in the USA, dietary betaine and choline intakes were inversely associated with tHcy in adults with low folate or cobalamin status. Post fortification, such inverse associations between choline and betaine with tHcy were not observed (Lee et al. 2010). The association between plasma betaine and tHcy was measured throughout pregnancy in a longitudinal pregnancy study carried out in the absence of mandatory fortification with folic acid (Fernàndez-Roig et al. 2013). Folic acid supplementation was most prevalent during the first trimester of pregnancy. During early pregnancy, plasma folate was strongly inversely associated with tHcy, but there was no association between plasma betaine concentration and tHcy. As pregnancy

Table 2 Summary of studies in humans that examined the association between betaine and homocysteine according to folate status

Study	Population	Design	Exposure						Outcome
			Intake						
			Mandatory folic acid fortification[a]	*Supplements*	*Dietary folate*	Plasma folate nmol/L	*MTHFR* 677C>T	*BHMT* 716G>A	
Holm et al. 2004	90 coronary angiography patients	Randomized controlled trial		0.8 mg/d folic acid, 0.4 mg/d B_{12}, 40 mg/d B_6 for 3 months					The post-methionine load increase in Hcy was inversely associated with plasma betaine concentration. The association was weakened after supplementation
Melse-Boonstra et al. 2005	308 men and women aged 50–75	Double-blind randomized controlled trial		From 50 to 800 μg/d folic acid for 12 weeks					There was a weak inverse betaine-tHcy association at all folic acid doses There was a dose-dependent increase in plasma betaine
Chiuve et al. 2007	1477 adult women	Transversal, observational							No independent association between betaine intake and tHcy was observed

(*continued*)

Table 2 (continued)

Study	Population	Design	Exposure						Outcome
			Intake						
Holm et al. 2007	10,601 adult men and women	Transversal, observational				<10.1	TT		Betaine was inversely associated with tHcy in participants with the TT genotype and plasma folate in the lowest quartile
Lee et al. 2010	Adult men (1325) and women (1407)	Transversal, observational	Prefortification USA						Choline and betaine intake were inversely associated with tHcy, especially when folate or B_{12} status was low
			Post-fortification						No association between betaine intake and tHcy was observed
Fernàndez-Roig et al. 2013	522 pregnant women	Prospective, observational				≤11.4			Betaine was not associated with tHcy during early pregnancy but was inversely associated with tHcy by mid-

									pregnancy in women with low folate status The reduction in betaine, increase in plasma DMG (pDMG), and inverse association between betaine and tHcy were enhanced as pregnancy progressed in women with low folate status
Colomina et al. 2016	612 pregnant women	Prospective, observational				≤13.4 vs. >13.4		A allele	Carriers of the variant A allele had lower pDMG compared to the GG genotype when folate status was normal-high. pDMG did not vary between genotypes when folate status was low

[a]Mandatory fortification of flour with folic acid in the USA

MTHFR methylenetetrahydrofolate reductase, *BHMT* betaine-homocysteine methyltransferase, B_{12} cobalamin, B_6 pyridoxine, *Hcy* homocysteine, *pDMG* plasma dimethylglycine, *tHcy* fasting total plasma homocysteine

progressed, and as plasma folate status decreased, an inverse association between betaine and tHcy emerged, and by late pregnancy, the strength of the association between betaine and tHcy was similar to that of folate and tHcy, in mothers with plasma folate status below the median. This study provided indirect evidence for shifts in MS and BHMT pathway activities with changing folate status and for a possible upregulation of the BHMT pathway when the MS pathway was impaired.

In summary, evidence from both animal and human studies points toward upregulation of the BHMT pathway when folate status is low. Therefore, the roles of betaine or choline in homocysteine metabolism may be enhanced in situations of folate deficiency.

SNPs Affecting the MS and BHMT Pathways

Two non-synonymous SNPs can be regarded as the main genetic determinants of the two homocysteine remethylation pathways. The *MTHFR* c.665C>T SNP (also known as 677C>T) affects the MS pathway, and the *BHMT* c.716G>A SNP (also known as 742G>A) affects the BHMT pathway. The reported prevalences of *MTHFR* c.665 variant homozygotes range from 5% to 20% (Wilcken et al. 2003) and the prevalence of *BHMT* c.716 variant homozygotes is 10% (Fredriksen et al. 2007; Liang et al. 2014).

The MTHFR enzyme catalyzes the irreversible reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the folate form that acts as the methyl donor in the MS reaction. The variant MTHFR enzyme is thermolabile and has reduced activity (Frosst et al. 1995; Daly et al. 1999), probably due to a higher dissociation rate of the MTHFR homodimer with the subsequent loss of its flavin adenine dinucleotide (FAD) cofactor (Yamada et al. 2001). Higher homocysteine concentrations with increasing number of variant alleles have been reported in a plethora of human studies, and the *MTHFR* c.665C>T polymorphism is the most important genetic determinant of tHcy (van Meurs et al. 2013; Bueno et al. 2016). Folate appears to alleviate the defect of the variant enzyme. In vitro, 5-methyltetrahydrofolate increases the affinity of the enzyme for its FAD cofactor to a greater extent in the variant than normal MTHFR (Yamada et al. 2001). Normalisation of tHcy in the presence of the variant genotype or allele, with increasing folate status, has been reported in numerous human studies (Geisel et al. 2001; Kluijtmans et al. 2003; de Lau et al. 2010).

In the case of the *BHMT* c.716G>A polymorphism, increased affinity for homocysteine and increased (Li et al. 2008) or similar (Weisberg et al. 2003) affinity for betaine in the variant compared to normal enzyme were reported in in vitro studies. However, the effects were observed at supraphysiological homocysteine concentrations (400 or 500 μM) when rat liver concentrations were reported to be 7.6 μM (Svardal et al. 1986) and low betaine concentrations (64 μM) when rat liver betaine concentrations were reported to be 550 μM (Koc et al. 2002). The polymorphism did not affect thermostability (Weisberg et al. 2003) nor did it affect protein expression nor enzymatic activity in two separate studies that tested substrate concentrations of

64 μM of betaine and 400 μM of homocysteine (Li et al. 2008; Feng et al. 2011). No association was observed between the SNP and homocysteine in numerous human studies, but lower plasma or serum dimethylglycine (product of the BHMT pathway) concentrations were reported in the presence of the variant allele compared to the common homozygote genotype. This was observed in a Norwegian study of old adults (Fredriksen et al. 2007) and a Spanish study of pregnant women (Colomina et al. 2016). In the latter study, lower dimethylglycine in heterozygotes compared to the other genotypes was observed during early pregnancy when folate status was high. This suggests that dimethylglycine differences between the different *BHMT* c.716 genotypes may be more evident when folate status is high. Why the apparent downregulation of the BHMT pathway in the presence of the variant genotypes does not appear to affect betaine concentrations might be due to tight control of betaine supply for its principal roles as an osmolyte and in protein stabilization.

Health Consequences of Low Folate Status

The micronutrients that regulate 1C metabolism are essential in maintaining health throughout the life span. Both low folate and low cobalamin status are associated with moderately elevated tHcy. Low folate status and or moderately elevated tHcy has been associated with adverse health outcomes in adults such as chronic diseases including cardiovascular diseases (Boushey et al. 1995), cancer (Blount et al. 1997), dementia (Ravaglia et al. 2005), cognitive decline (Nurk et al. 2005), and bone disease (van Meurs et al. 2004). Low maternal folate status has been associated with increased risk of fetal malformations such as NTDs as well as preeclampsia, preterm birth, and other pregnancy complications (Murphy and Fernandez-Ballart 2011). Importantly, periconception supplementation with folic acid has been shown to protect against folate-sensitive NTD recurrence and occurrence (MRC Vitamin Study Research Group 1991), and this has led to policies worldwide aimed at ensuring adequate maternal folate status to prevent grave fetal developmental anomalies. Moderately elevated maternal tHcy has also been associated with lower weight at birth in the offspring (Murphy et al. 2004; Onalan et al. 2006; Yajnik et al. 2014). This finding was not replicated in some studies, such as a Canadian study in the post-fortification with folic acid era (Dodds et al. 2008). However, a recent meta-analysis confirmed that the inverse association between tHcy and birth weight is consistent across studies and reported an odds ratio for small for gestational age of 1.45 when maternal tHcy before or during pregnancy was above the 90th percentile (Hogeveen et al. 2012). Maternal erythrocyte folate concentration during pregnancy has also been reported to be positively associated with birth weight (Relton et al. 2005).

Some studies have shown that the association between maternal tHcy during pregnancy and health and development in the offspring extends beyond fetal life. Moderately elevated tHcy at preconception was associated with lower cognitive achievement in children at 6 years of age (Murphy et al. 2017) and late pregnancy maternal folate status was positively associated with cognitive performance in 9–10 year old children (Veena et al. 2010). In India, the thin-fat phenotype in which babies have low weight but high

adiposity has been described (Yajnik et al. 2003). The combination of elevated folate and low cobalamin status during pregnancy was associated with increased central obesity and insulin resistance in the offspring during childhood in the Pune Maternal Nutrition Study (PMNS) in India (Yajnik et al. 2008; Yajnik and Deshmukh 2012), and it has been suggested that folate-vitamin B_{12} imbalance in mothers during pregnancy may lead to babies having the thin-fat phenotype. The PMNS investigators also suggested that such imbalance may result from supplementing cobalamin-deficient mothers with folic acid.

Some studies have reported that suboptimal cobalamin status in fertile women or during pregnancy may be more widespread than expected outside of countries such as India where vegetarianism is customary (Ray et al. 2008; Solé-Navais et al. 2018). It is widely recommended that women of fertile age or with the possibility of becoming pregnant take a daily supplement containing 400 μg of folic acid and that this be continued until the end of the first trimester of pregnancy. The recommendation/composition of the prenatal supplement varies between countries. Some recommend including vitamin B_{12} and other micronutrients in the supplement, but the common ingredient in all of them is at least 400 μg/d of folic acid during the first trimester.

Policies and Protocols

In this chapter, we have described the essential role of folate in one carbon metabolism and homocysteine remethylation and some of the health consequences of low folate status. Folate deficiency is especially harmful during embryogenesis and early fetal development and has been associated with grave NTDs such as spina bifida. The adverse health and developmental outcomes associated with this condition, and others derived from grave developmental defects, have huge direct and indirect healthcare costs. Prenatal healthcare protocols around the globe include the recommendation to take 400 μg/d of folic acid during periconception and at least until the end of the first trimester of pregnancy. However, success of this recommendation requires knowledge by the mother to take the supplement (before her first prenatal visit and before knowing she is pregnant). Mandatory fortification of cereal-based products to improve the folate status of women of fertile age and reduce the incidence of NTDs was implemented in the USA in 1998 (US FDA 1996). Numerous countries around the globe subsequently introduced similar policies (see Fig. 4).

The policy led to increased serum and red blood cell folate concentrations in the USA population compared to prefortification, and low serum folate and low red blood cell folate prevalences fell from 24% and 3.5%, prefortification, respectively, to $\leq$1% post-fortification (Pfeiffer et al. 2012). Furthermore, NTD rates in the USA and Canada have fallen by 50% compared to prefortification rates (Mills and Signore 2004).

Overall, mandatory fortification has not been introduced in European countries. In some, it has been postponed due to the widespread intake of voluntarily fortified foods and concern regarding the possible adverse effects of excessive folic acid intake through combined consumption of supplements and fortified foods (Smith et al. 2008).

Fig. 4 Global distribution of mandatory fortification with folic acid. Countries highlighted in blue represent those where mandatory fortification is implemented (Food Fortification Initiative 2016; PhD thesis JM Colomina 2017)

However, in a Spanish study of non-supplement users, and where the intake of voluntarily fortified foods is low, folate deficiency affected 18.8% of adults and 30.2% of men and women of fertile age (Bueno et al. 2016).

As we also indicated in this chapter, folate and cobalamin play interdependent metabolic roles. Low cobalamin status or deficiency largely affects vegetarians (due to a deficit in intake) or old people (due to impaired uptake). We briefly mentioned the risk of masking cobalamin deficiency by high folic acid intake. Folate-cobalamin interactions are outside the scope of this chapter. However, there is some evidence that metabolic anomalies and adverse health outcomes associated with cobalamin deficiency during early and late life may be exacerbated in the presence of high folic acid intake (through combined intake from supplements and fortified foods). Further research is required to confirm these findings. Mandatory fortification is indiscriminate, and future studies should also investigate whether changes in policies, such as reduction in the content of folic acid added to cereal products or the addition of vitamin B_{12}, are required.

Dictionary of Terms

- **One carbon metabolism** – metabolic network fueled by different B vitamins, choline, betaine, and methionine that is responsible for the production and transfer of single C units (e.g., methyl groups) used for processes such as DNA synthesis and repair and methylation reactions
- **Homocysteine remethylation** – binding of methyl group by homocysteine to produce methionine either by the folate-/cobalamin-dependent reaction

(catalyzed by methionine synthase, MS) or the betaine-dependent reaction (catalyzed by betaine-homocysteine methyltransferase, BHMT)
- **Folic acid** – synthetic, oxidized folate form used in supplements and in fortified flour and foods that has greater bioavailability than the naturally occurring folate forms
- **tHcy** – fasting plasma total homocysteine concentration
- **Methylenetetrahydrofolate reductase (MTHFR)** – enzyme catalyzing the unidirectional conversion of 5,10-methylenetetrahydrolate to 5-methyltetrahydrofolate, the methyl donor in folate-/cobalamin-dependent homocysteine remethylation

Summary Points

- Nutrients involved in one carbon metabolism (including amino acids, osmolytes, B vitamins, nitrogenous bases, phospholipids, and methyl donors) are essential for cell proliferation, metabolism, regulation, and differentiation.
- Folate and cobalamin (vitamin B_{12}) play interdependent roles in the methionine synthase pathway, which is the ubiquitous homocysteine remethylation pathway.
- Homocysteine can also be remethylated by the betaine-homocysteine methyltransferase pathway, which is limited to the kidneys and the liver.
- Overall, the role of betaine as a methyl donor is relatively minor compared to folate due to its other essential roles and the limited sites of betaine-homocysteine methyltransferase.
- Folate and/or cobalamin deficiency leads to methionine synthase pathway impairment and to elevated tHcy.
- Evidence from in vitro, animal, and human studies supports the hypothesis that the betaine-homocysteine methyltransferase pathway is upregulated when folate status is low. Betaine or choline may gain importance in homocysteine metabolism in situations of folate deficiency.
- The enzyme coded by the variant allele of *methylenetetrahydrofolate reductase* (*MTHFR*) c.665C>T is thermolabile, has impaired activity, and leads to impaired folate-dependent homocysteine remethylation.
- Human studies reported no differences in fasting plasma total homocysteine concentration between *betaine-homocysteine methyltransferase* (*BHMT*) c.716G>A genotypes. However, lower plasma dimethylglycine concentration in the presence of the variant allele compared to the homozygote common genotype suggests impaired betaine-dependent homocysteine remethylation in its presence.
- Folate status interacts with the *methylenetetrahydrofolate reductase* (*MTHFR*) c.665C>T and *betaine-homocysteine methyltransferase* (*BHMT*) c.716G>A polymorphisms in their effects on the methionine synthase and betaine-homocysteine methyltransferase pathways, respectively.
- Low folate status is associated with grave fetal developmental abnormalities, pregnancy complications, and highly prevalent chronic diseases in adults.

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Folate: Could We Live Without It? A Novel Epigenetic Connection

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Catherine A. Powell, Gabriella Villa, Trevor Holmes, and Mahua Choudhury

Contents

Abstract

Folate is an essential nutrient obtained through diet and supplements. The term folate is used interchangeably with folic acid, its synthetic form. Folate is metabolized in the one-carbon pathway, and its metabolites are used for a number of biological processes. Metabolites of folate are used in nucleotide synthesis and

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methylation. In fact, the one-carbon pathway produces the major methyl donor used in methylation, *S*-adenosylmethionine (SAM). Folate and DNA methylation are, therefore, closely entwined. Folate deficiency is associated with a number of diseases including congenital disabilities. In the last couple of decades, folate or folic acid supplementation is highly promoted in pregnancy because folate deficiency leads to neural tube defects with a wide range of consequences in children. Folate deficiency is also associated with gastric and colorectal cancers, cardiovascular disease, and liver disease. Alterations in global DNA methylation and disease-specific gene methylation patterns are implicated in the development and progression of these diseases. Folate is an important nutrient to understand the epigenetic regulation of disease.

Keywords

Folate · DNA methylation · Epigenetics · Diabetes · Cancer · Folic acid · DNA methyltransferase · DNMT · Cardiovascular disease · Methyl donor

List of Abbreviations

CDC	Centers for Disease Control
CIMP	CpG island methylator phenotype
CpG Island	Cytosine–phosphate–Guanine Island
CVD	Cardiovascular disease
DHFR	Dihydrofolate reductase
DNA	Deoxyribonucleic acid
DNMT	DNA methyltransferase
dTMP	Deoxythymidine monophosphate
dTTP	Deoxythymidine triphosphate
dUMP	Deoxyuridine monophosphate
dUTP	Deoxyuridine triphosphate
5-MTHF	5-Methyltetrahydrofolate
MTHFR	Methylenetetrahydrofolate reductase
PABA	Para-aminobenzoic acid
SAM	*S*-adenosylmethionine
TET	Ten–eleven translocation proteins
THF	Tetrahydrofolate
WHO	World Health Organization

Introduction

Proper nutrition with the correct levels of vitamins and minerals are essential to lead a healthy lifestyle. Nutrient deficiency not only has the potential to make a person feel ill but may also change the epigenome, a regulatory mechanism that can alter gene expression without altering the genetic code (Choi and Friso 2010). Ramifications of changes to the epigenome can affect the individual and, with its heritable trait, subsequent generations of children. In this chapter, we will focus on

folate, an essential nutrient that when deficient leads to a host of complications and alterations in DNA methylation.

Folate

Folate or folic acid is a water-soluble vitamin B (also known as vitamin B_9) with an extensive role in human metabolism (Liew 2016). The chemical structure of folate contains a heterobicyclic pteridine ring, para-aminobenzoic acid (PABA), and glutamic acid (Lindzon and O'Connor 2007) (Fig. 1). Folate is the naturally occurring form first discovered in foliated (green leafy) vegetables. Plants and vegetables such as broccoli, asparagus, celery, lentils, beans, and Brussels sprouts have high levels of folate (NIH 2016) (Fig. 2). On the other hand, folic acid is the synthetic form of folate added to fortified foods (such as cereals, flour, grains, and bread) as of 1998 and found in supplements (Liew 2016; Crider et al. 2011; US Department of Health and Human Services FaDA 1996). The term "folate" is used interchangeably between the natural and synthetic forms.

Folate is essential for the synthesis of nucleic acids, amino acids, and methyl donors necessary for a range of biological processes from DNA synthesis to gene or epigene regulation (Liew 2016; Scaglione and Panzavolta 2014). Folate is metabolized through the one-carbon pathway; this metabolism pathway produces precursors for nucleotide synthesis and methyl donors (Bailey and Gregory 1999). In the one-carbon donor pathway, folate is converted to tetrahydrofolate (THF) and further metabolized to its biologically active form 5-methyltetrahydrofolate (5-MTHF) (Bailey and Gregory 1999). However, synthetic folic acid requires additional enzymatic steps including through dihydrofolate reductase (DHFR), an enzyme that has the potential for drug inhibition that is avoidable with natural sources of folate (Scaglione and Panzavolta 2014). Metabolism of synthetic folic acid can be inhibited by drug interactions with DHFR, potentially leading to downstream metabolite deficiencies (Scaglione and Panzavolta 2014). 5-MTHF is involved in an enzymatic reaction to convert homocysteine to methionine, which is then converted to *S*-adenosylmethionine (SAM), a major methyl-donating molecule (Liew 2016; Bailey and Gregory 1999; Blom and Smulders 2011). SAM, in turn, donates its methyl group via methyltransferase enzymes to DNA, RNA, proteins, and lipids (Shorter et al. 2015) (Figs. 1 and 3).

Folate metabolites are also involved in the production of nucleotides. In DNA synthesis, folate metabolism leads to deoxyuridine monophosphate (dUMP) methylation forming deoxythymidine monophosphate (dTMP) (Liew 2016; Stover 2009). dTMP is then converted to thymidine triphosphate (dTTP), which is one of the four deoxynucleotides needed for DNA synthesis as well as DNA repair (Liew 2016). The low availability of folate can lead to the decreased production of dTMP from dUMP and ultimately a decreased supply of dTTP available for DNA replication and repair. Excess dUMP will then be converted to deoxyuridine triphosphate (dUTP) (Scaglione and Panzavolta 2014; Bailey and Gregory 1999). Due to its inability to distinguish between dTTP and dUTP, DNA polymerases can incorrectly

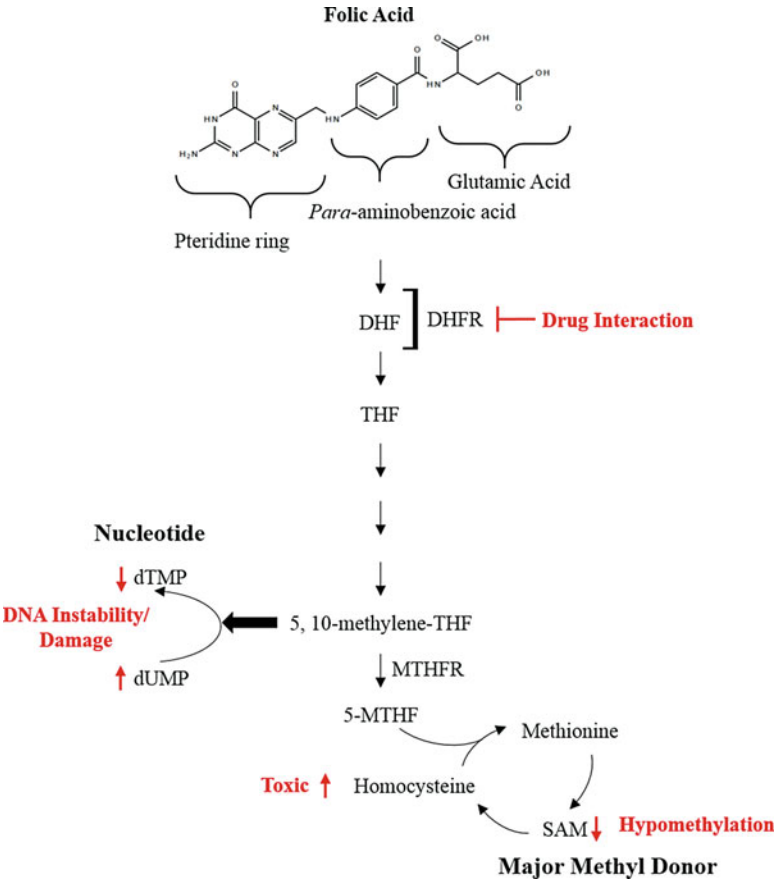

Fig. 1 Folate metabolism to metabolites involved in DNA synthesis and methylation. Folate metabolism via the one-carbon metabolism pathway. Folate is converted to DHF and then tetrahydrofolate (*THF*) by DHF reductase. THF is further metabolized to 5,10-methylene-THF which enters into nucleotide synthesis to convert deoxyuridine monophosphate (*dUMP*) to deoxythymidine monophosphate (*dTMP*) which is then incorporated as a nucleotide into DNA replication. 5,10-Methylene-THF is metabolized to 5-methyltetrahydrofolate (*5-MTFH*) by methyltetrahydrofolate reductase (*MTHFR*). 5-MTHF enters into the pathway to convert homocysteine to methionine, which can then be converted to *S*-adenosylmethionine (*SAM*). SAM is a major methyl donor in DNA methylation and other methylation processes. Figure adapted from Crider et al. (2012) with permissions

incorporate uracil in place of thymidine in the DNA synthesis, which can lead to an overabundance of uracil in the DNA (Blount et al. 1997). Excessive uracil incorporation from folate deficiency can lead to DNA instability, chromosome breaks, and increased risk for certain diseases such as cancer (Liew 2016; Blount et al. 1997; Duthie 1999) (Fig. 1). Folate deficiency is associated with a number of diseases including increased risk of certain cancers, cardiovascular disease, and neurological defects (Blount et al. 1997; Duthie 1999; Mattson et al. 2002; Ward 2001). The

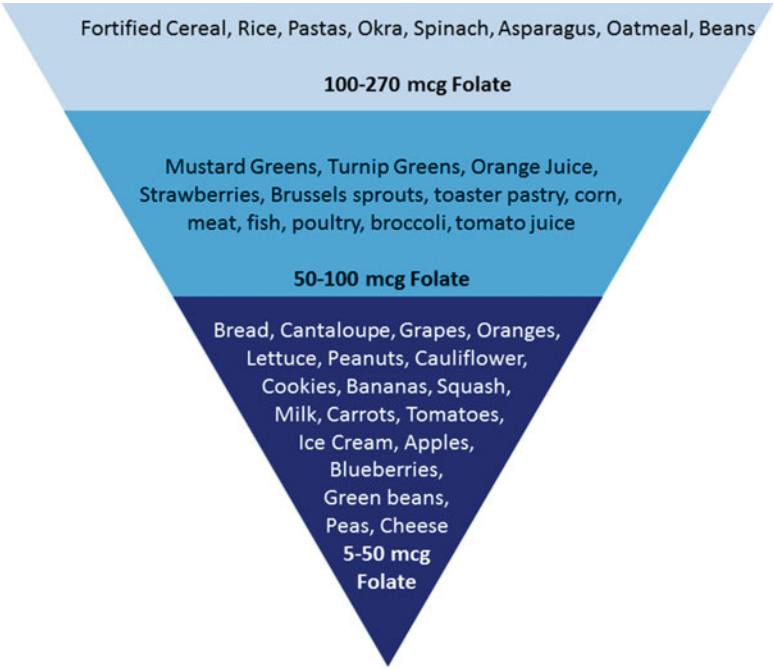

Fig. 2 Folate levels in foods. Common foods are stratified by the amount of folate or folic acid each contains. Foods are displayed by ranking of folate content in micrograms (mcg) from the USDA Food Composition Database (U.S. Department of Agriculture, Agricultural Research Service 2017). The highest folate containing foods are those supplemented with folic acid such as fortified cereals, rice, and pasta as well as some natural occurring high folate containing foods such as asparagus, Brussels sprouts, spinach, broccoli, and beans. Fruits, some vegetables, and dairy contain lower amounts of folate

mechanism underlying folate deficiency and disease risk may provide key diagnostic and therapeutic strategies in the future.

DNA Methylation

DNA methylation is one of the most studied and most well-understood epigenetic marks (Smith and Meissner 2013). It is a repressive mark leading to decreased expression of targeted genes without altering the genetic code (Choi and Friso 2010). During the process of DNA methylation, the addition of a methyl group occurs at the C5 position of the pyrimidine ring of the cytosine nucleotides via DNA methyltransferase (DNMT) enzymes (Kruman and Fowler 2014; Pacchierotti and Spano 2015). Three DNMTs have been identified as DNMT1, DNMT3a, and DNMT3b. DNMT1 is used commonly in maintaining DNA methylation patterns. Whereas DNMT 3a and 3b are involved in *de novo* DNA methylation which is the methylation of DNA during the embryonic stage of development (Qu et al. 2013;

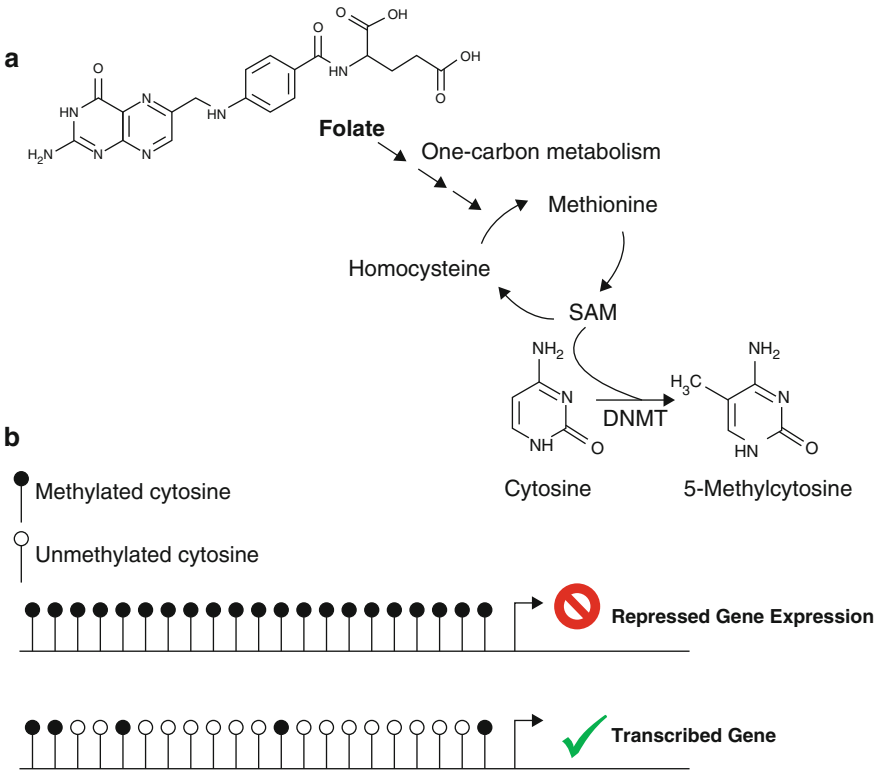

Fig. 3 Overview of DNA methylation by folate metabolite, SAM. (**a**) Folate is metabolized through the one-carbon pathway leading to the generation of SAM, a major methyl donor. DNA methylation occurs when DNMTs (DNMT1, DNMT3a, or DNMT3b) transfer a methyl group from SAM to the 5-position on cytosine generating 5-methylcytosine, a repressive mark. (**b**) Methylated cytosines (solid ball and stick) are repressive marks and prevent gene transcription. Hypomethylated genes or genomic regions where cytosines do not have a methyl group at the 5-position (open ball and stick) can be transcribed, and genes are expressed

Quintero-Ronderos and Montoya-Ortiz 2012). DNA methylation does not occur at random cytosine nucleotides. Instead, methylation occurs at CpG islands (areas of rich CG dinucleotide base pairs) near the promotor region. However, CpG islands found around transcriptional start sites of endogenous and regulatory genes often remain hypomethylated allowing for the binding of transcription factors (Kruman and Fowler 2014). Studies have also shown that methylation occurs at other CpG islands distal to the promoter region which magnifies the role of DNA methylation beyond regulation of gene expression to a more genome-wide function (Kruman and Fowler 2014; Kandi and Vadakedath 2015).

DNA methylation patterns are commonly set during development. As such, DNA methylation is stable in somatic cells, and gene expression is not rapidly altered by DNA methylation as in other epigenetic mechanisms, such as histone modifications

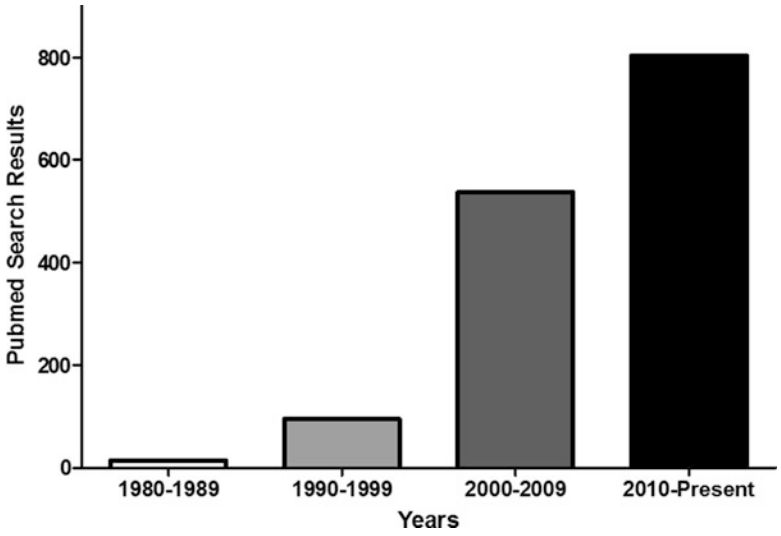

Fig. 4 Publications on folate and DNA methylation by decade since 1980. PubMed search results for the terms "Folate" and "DNA Methylation." Search results are displayed by decade from 1980 to 1989, 1990 to 1999, 2000 to 2009, and 2010 to present (2017)

(Smith and Meissner 2013; Robertson 2005). However, once DNA methylation patterns have been set during embryogenesis, they must be maintained throughout life during DNA replication. Folate is necessary to provide methyl donor pools of its metabolite SAM continually. Folate deficiency, and therefore SAM deficiency, has the potential to lead to a form of passive DNA demethylation where methylation is not maintained on newly synthesized DNA and subsequent cellular generations (Crider et al. 2012) (Fig. 3). Folate deficiency at the onset of development or later in life could have a substantial impact on the future health of the individual by altering DNA methylation patterns (Irwin et al. 2016). Thirty years ago, folate and DNA methylation research was almost nonexistent (Fig. 4). However, as the epigenetics field has emerged, studies into folate and DNA methylation also increased and is still on the rise (Fig. 4).

DNA Demethylation

DNA demethylation is also vital in certain stages of development and programming (Wu and Zhang 2014; Messerschmidt et al. 2014). For instance, DNA methylation marks are cleared from the genome in early stages of the embryogenesis and germline cells, erasing parental marks and priming the cells for pluripotent potential and new genome DNA methylation patterns (Wu and Zhang 2014). This reprogramming step is necessary to create a totipotent state that will allow for sex-determination and germ-line-specific differentiation of the embryo (Messerschmidt et al. 2014).

Although transferring a methyl group involves a covalent bond, DNA methylation is a reversible process (Wu and Zhang 2014). However, not in the same way as other epigenetic modifications such as histone acetylation and deacetylation, which is a coordinated balance between histone acetyltransferases and histone deacetyltransferases. DNA demethylation is mediated through both active and passive mechanisms. Active mechanisms of demethylation are oxidation of the 5-methylcytosine via ten–eleven translocation proteins (TET) and base excision of the methylated cytosine (Wu and Zhang 2014). When DNMT1, responsible for maintaining methylation patterns, does not methylate a strand of replicating DNA, those marks will disappear in subsequent rounds of DNA replication leading to passive demethylation (Wu and Zhang 2014). In conclusion, exploring DNA demethylation mechanisms has the potential to expand the current therapeutic knowledge of diseases.

Folate, DNA Methylation, and Disease

Folate deficiency results from either insufficient dietary intake or genetic defects in the metabolism of folate. This deficiency is prevalent in the population, and the resulting strain on the methyl donor pool may have deleterious effects on overall human health (Niculescu and Zeisel 2002). As for folate deficiency in the one-carbon donor pathway, low levels of folate results in low levels of SAM and may alter global DNA methylation patterns leading to changes in gene expression involved in various diseases including oncogenes (Liew 2016; Niculescu and Zeisel 2002) (Fig. 3).

Pregnancy Complications

Folate supplementation is imperative during pregnancy. The CDC recommends that women who may become pregnant, even before conception, take 400 micrograms of folate daily (CDC 2016). Folate is required for the proper neural tube closure during embryogenesis and development; this process occurs in the first few weeks of pregnancy (Cordero et al. 2015). Therefore, folate deficiencies during pregnancy can lead to a number of pathogeneses resulting from neural tube defects (NTD), including spina bifida (Liew 2016; Irwin et al. 2016; Pitkin 2007; Beaudin and Stover 2007). Up to 70% of neural tube closure defects are thought to be treatable with proper folate supplementation, the reason being that proper supplementation before conception ensures enough folate for neural tube formation (CDC 2016; Cordero et al. 2015). Therefore, it is recommended women who are planning to become or are pregnant take a folate supplement. Another mechanism for folate-associated pregnancy complications is accumulation of homocysteine, the precursor to methionine and SAM in the methyl donor pathway. Homocysteine levels may also affect fetal health, with an accumulation of homocysteine (a possible side effect of folate deficiency) competing for and blocking SAM binding to DNMTs leading to DNA hypomethylation (Iacobazzi et al. 2014). It is suggested that Down syndrome

where mitochondrial DNA is hypomethylated, cleft palates, and congenital heart defects are complications for imbalanced homocysteine and abnormal folate levels and metabolism (Blom and Smulders 2011) (Fig. 5).

Cancer

Alterations in DNA methylation of oncogenes and tumor suppressors can have a profound effect on cancer susceptibility (Clark and Melki 2002; Ehrlich 2002). In a hypermethylation state, tumor suppressor gene expressions are inhibited, and on the other hand during a hypomethylated state, oncogenes are overexpressed (Kandi and Vadakedath 2015). DNA methylation becomes an issue when it occurs abnormally. Anything that leads to this abnormality is of absolute importance in cancer. There

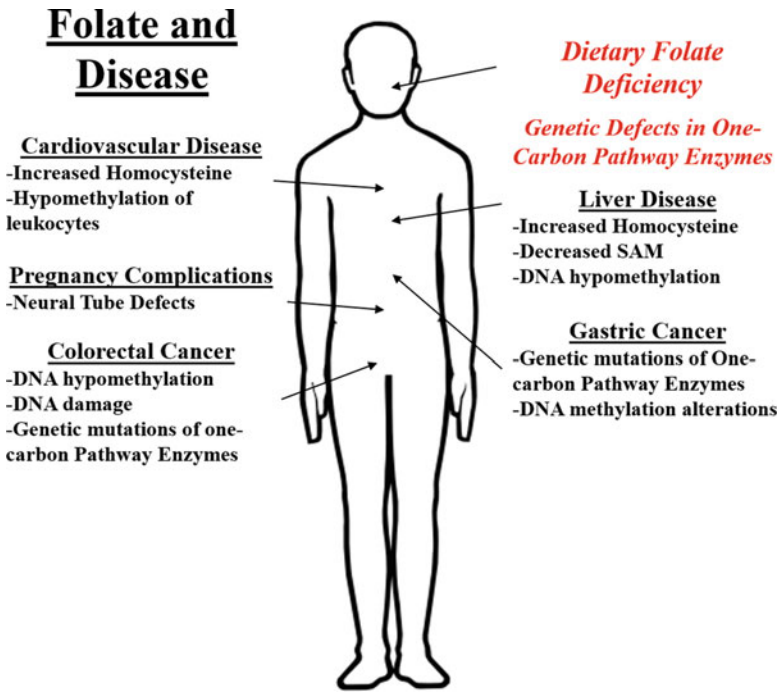

Fig. 5 Overview of folate and disease mechanisms. Folate deficiency or perturbations in folate metabolism have been linked to numerous diseases. Folate deficiency leads to increased homocysteine levels, which is toxic and detrimentally affects the cardiovascular system and liver contributing to a disease state. Folate deficiency produces less SAM, a methyl donor, which in turn results in DNA hypomethylation. DNA hypomethylation may alter oncogene and tumor suppressor gene expressions in cancers, leukocytes in cardiovascular disease, and hepatic genes that regulate liver disease. DNA damage due to DNA hypomethylation as well as nucleotide pool imbalance caused by folate deficiency is a factor in the development of cancer. While a person may take in the correct amount of dietary folate, metabolism of folate may be disrupted by polymorphisms or mutations in key enzymes in the one-carbon metabolism pathway, which contributes to cancer development

has been evidence that chemical pollutants, dietary components, and other factors are involved in changing epigenetic mechanisms such as DNA methylation (Qu et al. 2013). Folate deficiency has been shown to result in hypomethylation due to the low availability of methyl group donors, such as SAM (Qu et al. 2013). Even though methylation states of cancer cells vary, hypomethylation is common and a source of altered gene expression (Duthie 1999) (Fig. 5).

Gastric and Colorectal Cancer

Recent scientific research shows that folate deficiency either by low folate intake or due to a genetic component is associated with increased risk for gastric cancer (Gao et al. 2013; Zhao et al. 2012). Low serum folate levels have been associated with gastric cancer development and invasiveness (Lee et al. 2014). Folate deficiency has also been associated with the susceptibility of colorectal cancer by folate deficiency-induced DNA hypomethylation (Kim 2004; Pufulete et al. 2003). Although the mechanisms between folate and gastric and colorectal cancers are still being elucidated, studies suggest DNA methylation may be playing a role. As with many cancers, methylated promoter sites and aberrant methylation are present in both gastric cancer and colorectal cancer (Qu et al. 2013; Zhao et al. 2012; Bardhan and Liu 2013; Esteller 2002; Wajed et al. 2001). Diagnosis of gastric and colorectal cancers is commonly based on gastrointestinal endoscopy, colonoscopy, and fluoroscopy, which are invasive and not comfortable for the patient (Winawer et al. 2003; Pasechnikov et al. 2014). Thus, using DNA methylation as a possible diagnostic screening tool to detect cancer is becoming important. DNA methylation as a screening tool uses gastric fluids (serum or gastric washes) or biopsy specimens, and it identifies the prevalence of methylation in DNA within the specimen (Nakamura et al. 2014; Ye et al. 2010; Kalnina et al. 2015). Such DNA is the secreted product from gastric cancer cells; thus, the methylation patterns observed in the specimen are thought to be correlated with those in cancer cells (Nakamura et al. 2014; Ye et al. 2010; Kalnina et al. 2015). Numerous genes have been identified as having altered DNA methylation patterns in cancers versus normal tissue. In colorectal cancer, CpG island methylator phenotype (CIMP) is used to classify colorectal cancers (Bardhan and Liu 2013). These genes, which are hypermethylated in colorectal cancer, include MLH1, p16, MINT1, MINT2, and MINT31, common genes in DNA mismatch repair mechanisms and tumor suppressors (Bardhan and Liu 2013). CIMP panels can further be expanded to include a number of other genes, including RUNX3, IGF2, and WRN (Bardhan and Liu 2013). Furthermore, DNA methylation of serum RUNX3 and serum RASSF1A used to diagnose gastric cancer with the latter having high specificity, but low sensitivity (Qu et al. 2013). The promoters of RUNX3 and RASSF1A are hypermethylated in gastric cancer tissue compared to normal tissue (Qu et al. 2013; Chen et al. 2010; Kim et al. 2004; Waki et al. 2003; Wang et al. 2008; Ye et al. 2007) (Table 1). These hypermethylation marks can be used as biomarkers of disease risk and state. A significant difference between cancer and normal DNA is the methylation of the promoter site for gene p16, so it has become of

Table 1 Alterations in genes leading to colorectal and gastric cancer susceptibility. Changes in gene methylation or gene polymorphisms involved in colorectal or gastric cancers. Changes in DNA methylation or polymorphism increase the risk for colorectal and gastric cancers. Certain markers are used as biomarkers in disease to sub-classify cancers

Cancer	Property	Outcome or marker	References
Gastric	RUNX3 hypermethylation	Increased risk, biomarker	Qu et al. (2013), Chen et al. (2010), Kim et al. (2004), and Waki et al. (2003)
	RASSF1A hypermethylation	Increased risk, biomarker	Qu et al. (2013), Wang et al. (2008), and Ye et al. (2007)
	p16 hypermethylation	Increased risk, biomarker	Qu et al. (2013)
	MTHFR polymorphism	Increased risk	Gao et al. (2013)
Colorectal	DNA hypomethylation	Increased susceptibility	Kim (2004) and Pufulete et al. (2003)
	CIMP hypermethylation	Used to subtype	Bardhan and Liu (2013)

special interest to use this as a biomarker for gastric cancer (Qu et al. 2013) (Table 1). As for potential treatment, the idea of demethylation of tumor suppressor genes has gained much appeal since demethylation of these genes leads to cell apoptosis (cell death) (Qu et al. 2013). Folate deficiency or disruptions in the folate metabolism pathway may lead to alterations in DNA methylation patterns, and impact the development of these cancers. However, further studies into the connection between folate status, DNA methylation of these genes, and gastric and colorectal cancer is needed. Elucidating a connection between folate and DNA methylation patterns will hold diagnostic value in gastric and colorectal cancers.

Defects in the folate metabolism pathway may also markedly impact cancer development. Polymorphisms in an essential enzyme in folate metabolism, methylenetetrahydrofolate reductase (MTHFR), correlated with increased risk for gastric cancer and when associated with folate intake, decreased folate intake and further increased the risk of gastric cancer above the MTHFR polymorphism and folate deficiency alone (Gao et al. 2013) (Table 1 and Fig. 5). Deficient folate intake and the resulting DNA hypomethylation in colorectal cancer leads to damage- and mutation-prone DNA and insufficient repair mechanisms, some of which is reversible with proper folate supplementation (Kim 2004; Pufulete et al. 2003). In addition, in several cases, colorectal cancers have polymorphisms in the enzymes in the one-carbon pathway where regardless of folate intake, metabolism to methyl donors is affected due to genetics rather than environment (Zhao et al. 2012; Pufulete et al. 2003). It is thought that the reactivation of these enzymes may be possible, and whose function may trigger tumor suppression (Zhao et al. 2012) (Table 1 and Fig. 5).

Cardiovascular Disease

The evidence demonstrated a connection between DNA methylation in cardiovascular diseases (CVD) such as atherosclerosis, heart failure, myocardial infarction, and cardiac hypertrophy (Blom and Smulders 2011; Ward 2001; Li et al. 2016). Although evidence for DNA methylation and folate in CVD are at its initial stages, researchers are actively exploring the field in hopes to use epigenetics in the treatment of patients with CVD (Voelter-Mahlknecht 2016). Folate deficiency contributes to CVD in that it causes high levels of homocysteine in the body, which is damaging to the cardiovascular system (Mattson et al. 2002; Winder 1998). High levels of homocysteine are due to folate deficiency in which levels of folate are inadequate to methylate homocysteine to methionine (Mattson et al. 2002; Winder 1998; Castro et al. 2003; Rosenquist 2013). Folate supplementation lowers homocysteine levels, but its beneficial effects vary in disease states; folate supplementation has shown benefits in stroke prevention while it has shown no benefit in CVD (Voelter-Mahlknecht 2016). Although the mechanism is unknown, leukocytes in the serum of patients with vascular disease are globally hypomethylated compared to healthy controls (Kandi and Vadakedath 2015; Castro et al. 2003). This hypomethylation correlates with increased homocysteine levels in the serum; this pathway is consistent with characteristics of folate deficiency and one-carbon pathway alterations (Castro et al. 2003). Scientists speculate that the folate supplementation would help bring homocysteine levels back to safe levels, a major contributor to the development of various heart-related diseases (Li et al. 2016) (Fig. 5).

Liver Disease

Medici and Halsted demonstrated that folate deficiency is associated with the development of alcoholic liver disease through numerous mechanisms (Medici and Halsted 2013). The group showed that low levels of folate, thiamine, and vitamin B6 play a role in the development of alcoholic liver disease. It is thought that the low availability of folate and similar compounds in the human body will lead to hypomethylation of DNA and DNA instability in the liver and will cause expression of harmful liver enzymes that result in liver damage (Medici and Halsted 2013). Furthermore, folate feeds into metabolism of methionine and affects homocysteine levels, antioxidant effects, and lipid mobilization (Medici and Halsted 2013). Chronic alcohol intake disturbs the one-carbon metabolism; its hallmark effect is elevated levels of homocysteine (Kruman and Fowler 2014). Since the one-carbon metabolism is disturbed by chronic alcohol intake, it is safe to say that the effects also lead to changes in DNA methylation and subsequent gene expression, not only at the liver but multiple organs as well (Kruman and Fowler 2014). The mechanism of how chronic alcohol intake affects is not entirely understood, but it may be a result from two possible pathways: the direct interference of enzyme function from ethanol inhibition, or the decreased levels of folate due to the poor diet as seen in alcoholics (Kruman and Fowler 2014). Either way, one-carbon metabolism dysfunction leads to low levels of SAM which leads to hypomethylation of DNA (Kruman and Fowler 2014). Additionally,

increased alcohol intake strongly associates with low folate intake and colorectal cancer via mechanisms of promoter hypomethylation and elevated homocysteine levels (van Engeland et al. 2003; Giovannucci et al. 1995; Kato et al. 1999) (Fig. 5).

Conclusions

In summary, folate is an important dietary requirement, especially during pregnancy. Folate deficiency during pregnancy leads to detrimental neural tube defects, but more, folate deficiency correlates with gastric and colorectal cancer, as well as, cardiovascular and liver disease later in life. Its metabolism pathway, the one-carbon pathway, generates the methyl donor SAM for DNA and other methylation processes. Folate deficiency may be leading to unstable DNA by disrupting nucleotide synthesis and increasing susceptibility to disease. Further, folate deficiency may deplete methyl donor pools affecting DNA methylation and altering expression of genes associated with disease. Epigenetics and epigenetic regulation of disease is an emerging and fast developing field and folate's role in epigenetic regulation of disease is still unfolding. Therefore, more attention needs to be focused on the epigenetic impact of folate in human health and disease. Folate and its metabolites have potential to be used in preventative, diagnostic, and therapeutic measures in a number of diseases. Determining folate status and folate supplementation may be beneficial in treating diseases associated with elevated homocysteine levels in the blood or tissue, as in CVD and liver disease. Further, evaluating folate levels and DNA methylation in a disease context, globally or disease gene-specific, may provide valuable information on the underlying mechanism of disease, and the potential for folate supplementation in restoring proper DNA methylation. Therefore, maintaining proper folate supplementation throughout life, not just at critical stages such as pregnancy, should be evaluated in reducing the risks of developing diseases such as cancer, CVD, and liver disease.

Policies and Protocols

The World Health Organization (WHO) issued guidelines in 2015 reflecting the appropriate folate levels in red blood cells in women of reproductive age. Folate levels must exceed 400 ng/mL (906 nmol/L) in RBCs in women of reproductive age to reduce the chances of fetuses being affected by neural tube defects during development (Cordero et al. 2015). The CDC recommends women of reproductive age to consume 400 mcg of folate daily (CDC 2016).

Dictionary of Terms

- **Epigenetics** – The study of how environmental pressures, such as nutrient deficiency, alter the expression of genes without altering the genetic code.

- **DNA methylation** – An epigenetic mechanism where a methyl group is added to the 5-carbon of cysteine in CpG islands and canonically represses gene transcription.
- **DNA methyltransferase** – An epigenetic enzyme family consisting of DNMT1, DNMT3a, and DNMT3b that adds a methyl group to the C5 position of cytosine by de novo either methylation (DNMT3a and DNMT3b) or maintenance of already methylated DNA (DNMT1).
- **Folate** – An essential dietary nutrient commonly found in broccoli, celery, asparagus, beans, and Brussels sprouts and its synthetic form, folic acid, that is added to fortified foods since 1998 and found in supplements.
- **One-carbon metabolism** – Folate participates in this pathway where it is metabolized into tetrahydrofolate and undergoes further metabolism to produce nucleotides or 5-methyltetrahydrofolate, which is further metabolized into the primary methyl donor in methylation processes, *S*-adenosylmethionine.

Summary Points

- This chapter discusses the dietary nutrient, folate, and its connection to epigenetics.
- Folate is metabolized through the one-carbon metabolism pathway, and its metabolites are used in DNA synthesis and methylation processes.
- One such metabolite is *S*-adenosylmethionine, the major methyl donor in methylation processes.
- Folate deficiency leads to pregnancy complications leading to neural tube defects in children and disease such as cancer, cardiovascular diseases, and cancer in mothers and children.
- Folate deficiency may lead to insufficient methyl donor pools to maintain proper methylation of the DNA.
- Hypomethylation, or decreased methylation, of the DNA, can lead to DNA instability leading to DNA damage.
- In addition, folate deficiency and resulting hypomethylation can lead to the expression of oncogenes leading to the development of cancers such as colorectal and gastric cancers.
- Folate deficiency can also alter the balance in one-carbon metabolism to an accumulation of homocysteine, which causes damage to the cardiovascular system associated with a number of cardiovascular diseases.
- Proper supplementation of folate (400 mcg/day) has shown to prevent pregnancy complications.
- Folate levels and methylation have diagnostic and therapeutic potential; in fact, supplementation of folate has shown improvement in certain diseases.

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Vitamin K Status in Nutritionally Compromised Circumstances

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Mina Yamazaki Price and Victor R. Preedy

Contents

Abstract

Vitamin K deficiency is very rare except in neonatal populations. This is due to dietary sources, particularly plant-derived phylloquinones (vitamin K1) being abundantly distributed in nature and ubiquitously available in common foods. However, there is very little information on the bioavailability of vitamin K from foods. Furthermore, despite the increased understanding of vitamin K's biological

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roles, there are difficulties in establishing a causal link between plausible biomarkers of vitamin K deficiency and reproducible health outcome measures. Additionally, with vitamin K there is the added complication that this vitamin is also synthesized in the gastrointestinal tract by gut microflora. As a result, the exact dietary requirements for vitamin K in numerical terms have not been fully established. Clinically significant vitamin K deficiency is almost nonexistence in healthy populations. However, there are states in which it is compromised in some population cohorts other than neonatal populations. This review illustrates some examples of vitamin K insufficiency states, which include eating disorders, undernourished children, inflammatory bowel disease, and chronic kidney disease. It also describes some biomarkers of vitamin K status used in recent studies.

Keywords

Vitamin K · Phylloquinones · Menaquinones · Natto · Carboxylation · Uncarboxylated · Glutamate residues

List of Abbreviations

BMI	Body mass index
ESRF	End-stage renal failure
IBD	Inflammatory bowel disease
PIVKA-II	Protein induced by vitamin K absence-II
ucMGP	Undercarboxylated matrix Gla protein
ucOC	Undercarboxylated osteocalcin

Introduction

Vitamin K is a one of four fat-soluble vitamins (the other three being vitamin A, D, and E). Vitamin K is categorized into two major forms. They are the plant-derived phylloquinones (vitamin K1) and the bacterial-derived menaquinones (vitamin K2) (Price and Preedy 2015). There are number of vitamers within each form. Some texts describe a third class, namely, menadione (sometimes denoted as vitamin K3), but it does not occur naturally (it is synthesized) and is used in animal feeds (Coombs and McClung 2017). Structurally, they have a common 2-methyl-1, 4-napthoquione ring, and the differences at the three position of the side chain separate the two major forms (phytyl side chain and prenyl side chains, phylloquinones and menaquinones, respectively) (Fig. 1).

The nomenclature of vitamin K can be somewhat challenging as they are also denoted by the number of isoprenoid units. For example, phylloquinone-1 is denoted by K-1 and menaquinone-7 is denoted as MK-7 etc. Natto, for example, (Table 1) is particularly high in MK-7. There are a number of phylloquinones and menaquinones, with different biological activities. Coombs and McClung (2017) list six phylloquinones (K-1 - K-6) and six menaquinones (MK-2 - MK-7) in terms of their bioactivity (potency). For example, K-4 is 20 times more potent than K-1. MK-5 is eight times more potent than MK-2 (Coombs and McClung 2017). There

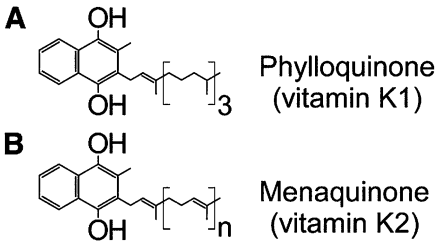

Fig. 1 Chemical structure phylloquinone (vitamin K1) and menaquinones (vitamin K2). Fig. A shows phylloquinones (vitamin K1) and B shows menaquinones (vitamin K2). (Source: Rishavy and Berkner 2012)

are probably about 20 different vitamin K isomers (both phylloquinones and menaquinones) that have been investigated or characterized in depth (see Coombs and McClung 2017).

Dietary Sources

The main dietary sources of plant-derived phylloquinone are leafy green vegetables, pulses, and certain plant oils. The phylloquinones are synthesized by chloroplasts. Phylloquinone is abundantly distributed in nature and is the primary form in the Western diet (Hayes et al. 2016). On the other hand, dietary sources of bacterial-derived menaquinones are primarily found in animal sources including dairy products and meats including processed meat products. Additionally, menaquinones are synthesized by the gut microflora in the large intestine in humans and then subsequently enter the circulation. Bacterially fermented products such as natto are a rich source of menaquinones. Furthermore, although the biological pathway is unclear, phylloquinone is converted to one form of menaquinone, i.e., menaquinone-4, in vivo which is independent of gut microflora activity (Price and Preedy 2015). Table 1 shows the vitamin K concentrations in some of the common foods consumed in the Western diet. It highlights the ubiquitous distribution of phylloquinones compared to the less common distribution of menaquinones (Schurgers and Vermeer 2000).

Biological Role

The primary or traditionally accepted role of vitamin K, established since the 1970s, is its participation in hemostasis (antihemorrhagic). It acts as a cofactor to facilitate the post-translational carboxylation of specific peptide-bound glutamate residues to γ-carboxyglutamate (in some text the Greek symbol is replaced with the term "gamma") residues in plasma-clotting proteins, namely, factors II (prothrombin), VII, IX, and X to initiate the coagulation cascade (Price and Preedy 2015).

Proteins which require vitamin K as a cofactor for the posttranslational γ-carboxylation of glutamate residues for its activation are termed as vitamin K-dependent proteins. Further discovery of a number of vitamin K-dependent proteins (a total of 16 proteins including the aforementioned 4) leads to the general consensus that the role of vitamin K extends beyond hemostasis (Price and Preedy 2015).

Table 1 Vitamin K concentrations in some of common foods consumed in the Western diet. The below values show mean values of at least three to six different samples or brands. Natto (fermented soy beans) is not commonly consumed in the Western diet but commonly consumed in Japan. It is listed as an example of extremely high content of menaquinones (Vitamin K2) in fermented foods (Source: Schurgers and Vermeer 2000)

Food group	Food	Phylloquinone (vitamin K1) concentration μg per 100 g	Menaquinone (vitamin K2) concentration μg per 100 g
Vegetables	Kale	817.0	Not detectable
	Spinach	387.0	Not detectable
	Broccoli	156.0	Not detectable
	Sauerkraut	25.1	4.8
	Natto (fermented soy beans)	34.7	1088.4
Fruits	Apple	3.0	Not detectable
	Banana	0.3	Not detectable
	Orange	0.1	Not detectable
Fat and oils	Olive oil	53.7	Not detectable
	Margarine	93.2	Not detectable
	Sunflower oil	5.7	Not detectable
	Butter	14.9	15.0
Dairy	Whole milk	0.5	0.9
	Buttermilk	Not detectable	2.5
	Whole yoghurt	0.4	0.9
	Hard cheese	10.4	76.3
	Soft cheese	2.6	56.5
Meat and egg	Beef	0.6	1.1
	Chicken breast	Not detectable	8.9
	Pork steak	0.3	2.1
	Pork liver	0.2	0.3
	Salami	2.3	9.0
	Goose liver paste	10.9	369.0
	Egg yolk	2.1	31.4
Fish and shell fish	Mackerel	2.2	0.4
	Herring	0.1	Not detectable
	Salmon	0.1	0.5
	Prawn	0.1	Not detectable
Bread	Wheaten bread	1.1	Not detectable
	Sourdough bread	1.0	Not detectable

For example, vitamin K is involved in vascular and bone health by protecting against vascular calcification and bone loses, respectively. Table 2 shows a list of vitamin K- dependent proteins and their physiological roles (Berkner and Runge 2004; Booth 2009; Price and Preedy 2015). There is also increasing evidence that

Table 2 Vitamin K-dependent proteins and their biological roles. The table shows 16 vitamin K-dependent proteins. Many of biological pathways of roles listed in this table are unclear (Berkner and Runge 2004; Booth 2009)

Name of proteins	Biological role
Prothrombin (factor II)	Hemostasis/coagulation
Factor VII	Hemostasis/coagulation
Factor IX	Hemostasis/coagulation
Factor X	Hemostasis/coagulation
Protein Z	Hemostasis/coagulation
Protein S	Hemostasis/coagulation/anti-inflammatory
Protein C	Hemostasis/coagulation/anti-inflammatory
Osteocalcin	Bone health/regulates bone mineral maturation/possible role in glucose homeostasis
Gla-rich protein	Bone health/regulates extracellular calcium
Periostin	Bone health/involves extracellular matrix mineralization
Matrix Gla protein	Vascular health/inhibits vascular calcification
Gas-6	Vascular health/involves vascular smooth muscle cell apoptosis and movement
Transmembrane Gla 1	unclear/possible role in signal transduction
Transmembrane Gla 2	unclear/possible role in signal transduction
Transmembrane Gla 3	unclear/possible role in signal transduction
Transmembrane Gla 4	unclear/possible role in signal transduction

vitamin K status has a bearing on bone and cardiovascular health and well-being. Indeed, there is some evidence that supplemental vitamin K may confer a biological advantage in terms of bone health.

Dietary Requirements and Recommendations

Despite the increased understanding of vitamin K's biological roles, its exact dietary requirements in numerical terms have not been fully established. This uncertainty may be due to the difficulties in inducing vitamin K deficiency through dietary deprivation alone or finding individuals who are vitamin K deficient via classical undernutrition studies (DH 1991). Furthermore, there are difficulties in establishing a causal link between plausible biomarkers of vitamin K deficiency and reproducible health outcome measures. This is compounded by the fact that there is very little information on the bioavailability of vitamin K from foods. As a consequence, it is somewhat problematical to derive specific dietary recommendations or reference values for vitamin K (Shearer et al. 2012).

For the UK, the Dietary Reference Values consist of 3 main components as follows:

Estimated average requirements (EAR). On the population level, half will require more than the EAR and half less.

Lower Reference Nutrient Intake (LRNI). Intakes at the LRNI will only satisfy the few people in a group who have low needs (less than 3% of the population).
Reference Nutrient Intake (RNI). Intakes at the RNI will satisfy the needs of the majority of the people in a group (about 97% of the population). The risk of deficiency in the group is very small if intakes Are at the RNI or above.

Occasionally, there is not sufficient information to provide either an EAR, LRNI, or RNI. In these circumstances safe intakes have been derived for UK-based reference values. Safe Intakes are defined as "a term used to indicate intake or range of intakes of a nutrient for which there is not enough information to estimate RNI, EAR or LRNI. It is an amount that is enough for almost everyone but not so large as to cause undesirable effects" (DH 1991).

For a full explanation of these terms, their derivation, and implications for health and disease prevention, one is referred to the original report on the Dietary Reference Values (DH 1991).

In the UK, safe intakes have been derived for vitamin K dietary reference values for adults and infants (in other words and to reiterate a point, there are no EAR, LRNI, nor RNI for vitamin K). In the UK, safe intake for adults for vitamin K is 1 μg per body weight (kg) per day (DH 1991). This equates to about 69–72 and 59–60 μg/day adults, for male and female, respectively (values are calculated based on reference weight for age range between 19 and 54 years) (SACN 2015, Table 3). Safe intake for vitamin K for infants is 10 μg/day in the UK (DH 1991). These values are similar for the USA and Japan (Table 3). Both countries have only values for adequate intake, which is considered to be the UK equivalent of safe intake. In the USA and Japan, for adults, reference intakes are higher, i.e., 150 μg for both genders for the USA and 120 and 90 μg/day in Japanese men and women, respectively. For infants, they are 2.0–2.5 μg/day for the USA and 4–7 μg/day for Japan (NASEM 2016, MHLW 2015). For the definition of infants, it has to be mentioned that there is no age specification for the UK. However, they are defined as 0–12 months, for the USA, and 6–11 months for Japan, respectively (NASEM 2016, MLHW 2004).

Table 3 shows dietary reference values and definitions of the terminology for vitamin K used in the UK, USA, and Japan.

As mentioned earlier, vitamin K is widely distributed in a variety of foods. Therefore the clinical deficiency caused by dietary deficiency is extremely rare. However, there are states in which it is compromised. In the following text, we describe these compromised states pertaining to vitamin K insufficiency.

Different biomarkers have been used to define reduced vitamin K status. Table 4 summarizes the biomarkers used in studies described in the text below.

Vitamin K and its Deficiency States

Clinically significant vitamin K deficiency is almost nonexistence in healthy populations except for neonatal populations (Lee et al. 2016). Neonatal populations are particularly vulnerable to vitamin K deficiency. Underlying causes include their

Table 3 Reference values of vitamin K, definition of the terminology and current recommendation for adults used in the UK, USA, and Japan

Country	Reference values	Definition	Current recommendation or reference intakes (μg/day)
UK	Safe intake	A term used to indicate intake or range of intakes of a nutrient for which there is not enough information to estimate reference nutrient intake, estimated average requirements or lower reference nutrient intake. It is an amount that is enough for almost everyone but not so large as to cause undesirable effects	68.8–71.5 adult male 59.0–59.9 adult female 10.0 infants
USA	Adequate intakes	A recommended average daily nutrient intake level based on observed or experimentally determined approximations or estimates of mean nutrient intake by a group (or groups) of apparently healthy people. An adequate intakes is used when the recommended dietary allowance cannot be determined	120.0 adult male 90.0 adult female 2.0–2.5 infants
Japan	Adequate intake	A less well-defined value, generally the median of the population without evidence of deficiency	150.0 adult male 150.0 adult female 7.0 infants

The current recommendations or reference intakes for adults shows values 19–50 years in the UK and USA and 19–49 years in Japan. For the UK, figures are calculated based on μg/body weight (kg)/day (DH 1991) using weight for males 19–24 years, 71.5 kg; 25–34 years, 71.0 kg; 35–44 years, 69.7 kg; 45–50 years, 68.8 kg. For females, 19–24 years, 59.9 kg; 25–34 years, 59.7 kg; 45–50 years, 59.0 kg (SACN 2011). For infants, there is no age definition for the UK, for the USA, it is defined as 0–12 month old (4 μg/day for 0–6 month, 2.5 μg/day for 6–12 month); for Japan, it is defined as 6–11 months old. Sources: for the UK (DH 1991); for the USA (NASEM 2016); for Japan (MHLW 2015)

low hepatic reserve and plasma concentration of vitamin K at birth due to poor placental transport of vitamin K and low levels of vitamin K in breast milk (DH 1991; Mihatsch et al. 2016). Additionally, insufficient gut microflora colonization in the large intestine also likely contributes to endogenous deficiency (Lippi and Franchini 2011). However, some other population cohorts may also be vulnerable to vitamin K deficiency in feeding disorders, starvation, and fat malabsorption, as described below.

Eating Disorders of Anorexia Nervosa and Bulimia Nervosa

Compromised bone health (i.e., osteopenia and osteoporosis) is one of the well-recognized clinical complications of deficiency-related eating disorders and their subtype, such as anorexia nervosa and bulimia nervosa (Howgate et al. 2013). This is because of the essential role that vitamin K plays in bone metabolism

Table 4 Vitamin K biomarkers

Biomarker	Rationale
Undercarboxylated osteocalcin	Osteocalcin, a bone matrix protein is exclusively produced by osteoblasts, bone forming cells. Osteocalcin is a bone matrix protein that undergoes a post-translational carboxlylation of protein-bound glutamate residues into gamma-carboxyglutamate which requires vitamin K as a cofactor. Undercarboxylated osteocalcin has no role in bone metabolism
Undercarboxlylated prothrombin	Also called protein induced by vitamin K absence or antagonist -II (PIVA-II). Prothrombin (factor II) is one of four plasma-clotting proteins (other 3 are factor VII, IX, X). Prothrombin undergoes post-translational carboxylation of glutamate residues into gamma-carboxyglutamate which requires vitamin K as a cofactor. Undercarboxylated prothrombin is inactive/defective in blood coagulation
Undercarboxylated matrix Gla protein	Matrix Gla protein is a calcification inhibitor secreted primarily by vascular smooth muscle cells. Gamma-carboxylation of its five glutamate residues requires vitamin K as a cofactor. Undercarboxylated matrix Gla protein does not inhibit the process of vascular calcification

The above biomarkers increase in the circulation in the case of reduced vitamin K availability in vivo (Urano et al. 2015; Nakajima et al. 2011; Lee et al. 2016; Wyskida et al. 2016). These have been selected as they are mentioned in this review in the context of specific studies

(i.e., involvement by the vitamin K-dependent proteins, namely, osteocalcin, Gla-rich protein, and periostin). Urano et al. (2015) investigated vitamin K status of 54 females with eating disorders and 15 age-matched healthy controls (mean age of 28 years).

In their aforementioned study, serum undercarboxylated osteocalcin (ucOC) levels of 4.5 ng/ml or higher were defined as vitamin K deficiency. Osteocalcin is a bone matrix protein which is produced by the osteoblasts, the bone forming cells. Vitamin K is a necessary cofactor for the posttransitional carboxylation of osteocalcin. When the supply of vitamin K is insufficient or abnormal, ucOC, which has no biochemical role in bone metabolism, is released into the blood stream. UcOC is considered as a sensitive biomarker for vitamin K status (Urano et al. 2015).

The 54 subjects included 29 with anorexia nervosa and 25 with bulimia nervosa (Urano et al. 2015). The mean body mass index (BMI) of eating disorder subjects was 14.8 kg/m^2 (95% confidence interval 14.1–15.5 kg/m^2). BMI of this magnitude indicates severe thinness (BMI less than 16.0 kg/m^2) (WHO 2016). The mean BMI of the healthy subjects was 20.1 kg/m^2 (95% confidence interval 18.7–21.4 kg/m^2). All eating disorder subjects were diagnosed with osteopenia or osteoporosis, and a total of 28% of the eating disorder subjects (anorexia nervosa n = *5*, bulimia nervosa $n = 10$) were found to be vitamin K deficient. The prevalence of vitamin K deficiency within healthy subjects was not shown. However, serum levels of ucOC of bulimia nervosa subjects were statistically significantly higher than healthy subjects ($p < 0.05$), whereas differences between anorexia nervosa and healthy subjects did not reach statistical significance (Urano et al. 2015).

Interestingly, this study showed significant negative correlation between serum level of ucOC (a surrogate marker of vitamin K deficiency) and dietary vitamin K intake for both types of eating disorder ($p < 0.01$). The authors argued that the cause of vitamin K deficiency in this cohort was likely multifactorial (Urano et al. 2015). For example, complex behaviors associated with eating disorders such as severe restriction of dietary intake and vomiting/purging behaviors could cause reduction in overall vitamin K status. Regular laxatives misuse could also lead to malabsorption of dietary vitamin K. Furthermore, laxative abuse may cause alterations in the gut microflora populations which could negatively affect in vivo vitamin K synthesis (Urano et al. 2015).

The importance of mentioning the effects of anorexia nervosa and bulimia nervosa is their potential appreciation in understanding the consequence of undernutrition/starvation. In conceptual terms, these two conditions should provide us with information as to the effects of undernutrition/starvation on vitamin K status. However, as the previous text alludes, with vitamin K there is the added complication that this vitamin is also synthesized in the gastrointestinal tract.

Children and Undernutrition

There have been some studies showing positive associations between vitamin K status and childhood bone health, such as total body bone mineral content (van Summeren et al. 2008). However, in children, results are inconclusive in terms of relationships between dietary vitamin K intake and vitamin K status. This could be due to several potential reasons including the large variation in day to day vitamin K intake and difficulties in assessing bioavailability of dietary vitamin K and synthesis by gut microbial population (Cashman 2005).

Recently, Lee et al. (2016) studied 500 children of 6–8 years of age in the southern rural part of Nepal. The study found 100 children (mean age 7.3 years) to be vitamin K deficient. In this study, vitamin K deficiency was defined by plasma concentration of undercarboxylated prothrombin, also known as protein induced by vitamin K absence-II (PIVKA-II) to be more than 2 μg/L. Prothrombin (blood coagulant factor II) is a hepatic vitamin K-dependent protein and abundant in the circulation. PIVKA-II is secreted by the liver when the liver storage of vitamin K is depleted (Lee et al. 2016).

Among the 100 children identified with vitamin K deficiency, 37% and 44% were classified as stunted and underweight, respectively. However, when vitamin K-deficient ($n = 100$) and vitamin K-sufficient ($n = 400$) children were compared, the study showed no significant differences in anthropometric measurements (i.e., weight, height, BMI, and mid-upper arm circumference), prevalence of stunting and underweight. The dietary intake of certain foods (i.e., intake of milk, eggs, meat, fish, dark leafy vegetables in the past week) and most of other profiles (e.g., literacy and economics of household) were also not different between vitamin K-deficient ($n = 100$) and vitamin K-sufficient ($n = 400$) children. For example, the prevalence of underweight was 44% and 50%, in vitamin K-deficient and vitamin K-sufficient

groups, respectively. The authors hypothesized there may be diverse homeostatic responses to subclinical vitamin K status in a generally undernourished young children population (Lee et al. 2016).

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is the collective term for ulcerative colitis and Crohn's disease. IBD is characterized as a chronic relapsing and remitting inflammation of the digestive tract (Nakajima et al. 2011). Micronutrient deficiency including vitamin K as well as protein energy malnutrition is known to be commonly prevalent in patients with IBD. The prevalence of malnutrition could be as much as 85% of patients regardless of their disease status (i.e., active or remission) (Weisshof and Chermesh 2015). The degree of malnutrition depends on the disease severity including inflammatory processes and, in the case of Crohn's disease, the area of the gut affected by the disease. Therefore, the etiology of malnutrition in IBD is multifactorial. Common causes of malnutrition include failure to maintain a sufficient dietary intake (poor appetite and imposed dietary restrictions), impaired intestinal absorption (depending on the location and severity of inflammation), and nutrient loss from the inflamed and ulcerated gut (Altomare et al. 2015). Furthermore, nutritional requirements in general increase particularly in the active stages of IBD due to physiological response to inflammation. Moreover, the increased nutritional requirements during the active stages of inflammatory disease may not be coupled with increased nutritional intake for many experiencing physiological symptoms such as abdominal pain, nausea, and diarrhea (Weisshof and Chermesh 2015).

Nakajima et al. (2011) investigated vitamin K status in IBD patients. In their study, vitamin K insufficiency was defined by the comparison of serum concentration of undercarboxylated osteocalcin (ucOC) between IBD patients and healthy subjects. The cutoff value for serum concentration of ucOC to define vitamin K insufficiency was not specified (Nakajima et al. 2011).

Forty seven Crohn's disease patients, 40 ulcerative colitis patients, and 41 age- and gender-matched healthy subjects were compared (all were Japanese) (Nakajima et al. 2011). Serum ucOC level was statistically significantly higher ($p < 0.05$) in Crohn's disease patients when compared to ulcerative colitis patients and healthy subjects. However, when compared ulcerative colitis patients and healthy subjects, serum ucOC level was higher in ulcerative colitis patients compared to healthy subjects, but the differences did not reach of statistical significance.

Although this study did not examine vitamin K dietary intake, the authors argued that insufficient vitamin K intake may not be the main reason to explain the results. This is because, previously, Kuwabara et al. (2009) found vitamin K dietary intake in IBD patients exceeded the daily adequate intake level at the time the study was performed (Kuwabara et al. 2009). The mean vitamin K intake in IBD patients was 131.1 μg in this study (Kuwabara et al. 2009). At the time the adequate intake for vitamin K was 75 μg for adult male and 65 μg for adult female in Japan (MWLH 2004). The adequate intake for vitamin K in Japan was then revised to 150 μg for

adult male and female in 2015 (MHLW 2015). Rather than insufficient vitamin K dietary intake, the authors suggested that the statistically higher serum concentration of ucOC found in Crohn's disease patients compared to the healthy subjects is attributed to reduced dietary fat intake by Crohn's disease patients. In Japan, Crohn's disease patients are commonly treated with a fat-reduced diet. The absorption of fat-soluble vitamin K may be compromised in Crohn's disease patients. Finally, endogenous synthesis of vitamin K by intestinal bacteria may differ between these cohorts, as the bacterial flora composition is significantly altered, particularly in Crohn's patients, due to inflammation of the gut (Nakajima et al. 2011).

Here, we can suggest that there is a common theme in the aforementioned studies related to children with malnutrition, subjects with anorexia, and patients with IBD. Namely, the microbiological profile of the intestinal tract may complicate the understanding of the relationship between dietary intake of vitamin K and the status of vitamin K sufficiency or deficiency. However, there is little information on the exact amount of vitamin K (menaquinones) produced by intestinal bacteria. Furthermore, some have suggested de novo synthesis of vitamin K (menaquinones) may not be fully dependent on intestinal bacteria as animals lacking a gut microflora still synthesize vitamin K (menaquinones) (LeBlanc et al. 2013). Such theory adds further complication to our understanding of vitamin K homeostasis.

Chronic Kidney Disease

It is widely recognized that cardiovascular diseases are the leading cause of the mortality, morbidity, and hospitalization in end-stage renal failure (ESRF). Coronary artery calcification, a risk factor of cardiovascular disease, is commonly prevalent in ESRF patients compared to population without kidney disease (Chen et al. 2017). The link between vascular calcification, vitamin K status, vitamin K-dependent proteins, and the negative effects of commonly used medications in this cohort (i.e., vitamin K inhibitory action of warfarin) has received attention (Gallieni and Fusaro 2014).

The compromised vitamin K status of this cohort could be due to therapeutic dietary restrictions such as limiting intake of potassium and phosphorous. Commonly restricted dietary components including dairy products, legumes, fruits, and vegetables. Many of these foods contain high concentration of vitamin K as discussed previously (see also Table 1).

Wyskida et al. (2016) investigated the level of functional vitamin K deficiency and its relation to vitamin K1 (phylloquinones) intake in 153 Polish ESRF patients on hemodialysis. In their study, vitamin K status was measured using two surrogate markers, namely, plasma concentration of protein induced by vitamin K absence-II (PIVKA-II) and undercarboxylated matrix Gla protein (ucMGP). The 95% confidence interval around the mean in 20 healthy adult subjects (similar ages to ESRF patients with normal kidney function) were established as a normal, 0.37–0.66 ng/mL and 5.1–9.2 mg/mL, PIVKA-II and ucMGP, respectively. The values in ESRF patients were compared with normal ranges. ESRF patients which had surrogate

markers below normal ranges were defined as vitamin K deficient. Dietary vitamin K1 (phylloquinones) intake was assessed for the past year, using a food frequency questionnaire.

The results showed increased plasma concentration of PIVKA-II (>0.66 ng/mL) in 27.5% of ESRF patients. However, the mean plasma concentration of PIVKA-II was not significantly different between ESRF patients and healthy subjects (0.59 ng/mL and 0.51 ng/mL, respectively). The results also found significantly higher mean plasma concentration of ucMGP (17.9 mg/mL) in ESRF patients compared to healthy subjects (7.1 mg/mL, $p < 0.001$). Increased ucMGP level (>9.2 mg/mL) was found in 77% of ESRF patients (Wyskida et al. 2016).

Median vitamin K1 intake was 103 μg/day in ESRF patients. There were no significant differences between men and women. Of these109 ESRF patients, 34% subjects found to have less than the recommended values for the Polish population (at least 65 μg and 55 μg/day for adult men and adult women, respectively; (Wyskida et al. 2016).

Forty five percent of ESRF patients who showed increased plasma concentration of PIVKA-II (>0.66 ng/mL) had daily intake less than the amount recommended for the Polish population. Authors conducted further analysis of the subgroup of ESRF patients with increased PIVKA-II level > 0.66 ng/mL with the receiver operator curve analysis. ESRF patients with increased plasma concentration of PIVKA-II level > 0.66 ng/mL had lower daily vitamin K1 intake less than 40.2 μg/day, which is below aforementioned Polish daily recommendation. However, for the level of ucMGP, there was no significant difference between patient group whose vitamin K1 intake was greater than the recommendation for the Polish population and the group whose vitamin K intake was below the recommended value.

There was no correlation between plasma concentration of ucMGP and PIVKA-II and between ucMGP and daily vitamin K1 intake. The study demonstrated a correlation between plasma PIVKA-II level and dietary vitamin K intake in ESRF patients. The authors indicated PIVKA-II is superior as a surrogate marker for vitamin K deficiency compared to ucMGP.

Possible factors other than vitamin K dietary intake which could have influenced negatively in vitamin K status of ESRF patients include gut microflora composition, in vivo synthesis of vitamin K2 (menaquinones), impaired vitamin K absorption, or disturbed vitamin K metabolism (Wyskida et al. 2016).

Policies and Protocols

Readers are reminded that to ensure adequate vitamin K intake, one must have a balanced diet. However, one needs to take into account the fact that intake of vitamin K1 or K2 will depend on dietary variations. For example, vitamin K2 intake in the Japanese population is higher than that of the UK population. This is because in Japan fermented foods are an important source of vitamin K2. In the UK the majority of vitamin K comes in the form of K1 from vegetables. One also needs to consider compositional tables in context of portion size, frequency, and also concentrations

within foods. For example, natto has a vitamin K concentration of approximately 1 mg per 100 g. It is rarely consumed in the UK. So it is erroneous to say that natto is an important source of vitamin K unless the country (hence the dietary profile of that country) is specified.

In examining reference intakes, one needs to emphasize that we have only discussed values for the UK, USA, and Japan. However, other countries or bodies have different reference intakes. For example, the World Health Organization suggests 55 and 65 micrograms per day for adult women and men, respectably.

Dictionary of Terms

- **Anorexia nervosa** – A major form of eating disorders in which the patients starve themselves to induce weight loss. It is a primary psychological illness which can cause significant acute and chronic medical complications. It is not unknown for patients with anorexia nervosa to starve themselves to death.
- **Bioavailability** – The proportion of a nutrient or other substance such as a drug that enters the circulation or body system when compared with the amount ingested. A variety of processes can impact on bioavailability, such as the nature of the food matrix, transport processes in the intestine, degradation or metabolism upon entering the gastrointestinal tract, and so on.
- **Bulimia nervosa** – A major form of eating disorders. It is a psychological disorder characterized by the binge eating and purging behaviors. It is associated with a numbers of adverse health consequences including increasing suicide risk.
- **Chronic kidney disease** – A long-term condition where the functions of the kidneys are gradually impaired. The degree of impairments separates stages of diseases.
- **Crohn's disease** – A condition in which segments of the digestive system become inflamed. Unlike ulcerative colitis, it can affect any part of the gastrointestinal tract. However, most commonly Crohn's disease affects the terminal part of the ileum or the colon.
- **End-stage renal failure** – The final stage of chronic kidney disease where the kidneys are hardly functioning or not functioning at all. The patients can be treated with dialysis or kidney transplant.
- **Fat-soluble vitamins** – Fat-soluble vitamins are absorbed and transported with fats in the diet. Fat-soluble vitamins include vitamins A, E, D, and K.
  They have predominantly aromatic and aliphatic characteristics and are soluble in nonpolar solvents. They are stored in the liver and adipose tissue. The term "fat soluble" is used in contrast to "water-soluble vitamins" (such as the B vitamins, folate and vitamin C).
- **Gut microflora** – The term is often used interchangeably with gut microbiota. It is the complex communities of microorganisms (e.g., bacteria, fungi and viruses) that inhabit in the digestive tracts of all mammals. In humans, the composition, structure, diversity, and functional capacity of gut microflora are thought to be

influenced by various factors. These factors include heredity influences, diet, and medical interventions (the latter, e.g., may be the effects of antibiotics).

- **Hemostasis** – The arrest or cessation of bleeding. This involves the physiological process of blood coagulation and constriction of damaged blood vessels. The role of vitamin K within blood coagulation is the most recognized biological role of vitamin K.
- **Natto** – A fermented soybeans dish which has high concentrations of vitamin K2.
- **Ulcerative colitis** – A condition where varying amounts of the colon and almost always the rectum become inflamed. The most common symptoms include frequent diarrhea (sometime with blood and mucus) and abdominal pain.
- **Vitamin K** – Vitamin K is a fat-soluble vitamin, which has two major forms. They are the plant-derived phylloquinones (vitamin K1) and the bacterial-derived menaquinones (vitamin K2). They are abundant in nature and widely distributed in a variety of foods. Phylloquinones are commonly found in green leafy vegetables, pulses, and plant oils. Menaquinones are primarily found in animal sources such as dairy products and meats. Menaquinones are also produced by bacteria. As a consequence relatively high concentrations of menaquinones are found in fermented foods such as natto.

Summary Points

- Vitamin K is categorized into two major forms. They are plant-derived phylloquinones (vitamin K1) and bacterial-derived menaquinones (vitamin K2). There are many vitamers within each form.
- Dietary sources of vitamin K are widely distributed. The contributions of different foods will depend on the cultural significance (common or uncommon foods), portion size, and frequency.
- Phylloquinones (vitamin K1) are commonly found in leafy green vegetables. Menaquinones (vitamin K2) are primarily found in animal sources (e.g., dairy products and meat) and bacterially fermented foods and synthesized by the gut microflora in the large intestine in humans.
- The exact dietary requirements of vitamin K in numerical terms have not been fully established due to various reasons including difficulties in determining a causal link between plausible biomarkers of vitamin K deficiency and reproducible health outcome measures.
- Clinically significant vitamin K deficiency is almost nonexistence in healthy populations except for neonatal populations. However, some other population cohort may also be vulnerable. This includes undernourished children, patients with eating disorders, those with inflammatory bowel disease, and patients with chronic kidney disease.
- Compromised vitamin K deficiency status is multifactorial. These include reduced vitamin K intake, perturbations in absorption, altered de novo synthesis, and interaction with medications.

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The Biological and Health Outcomes of Copper Inadequacy: A Public Health Perspective

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Abstract

Copper is involved in a wide range of physiological functions. In humans, copper deficiency impairs growth and reproduction and biochemical alterations and health outcomes due to copper deficiency have been reported. Biochemical or clinical copper deficiency is prevalent in infants recovering from malnutrition, children with chronic diarrhea, in some cases of malabsorption, and in some regions depending on food availability, soil concentrations, or behaviors. The effects of food choices on markers of micronutrients are still insufficiently investigated. The competitive absorption of zinc and copper is considered. The copper intake in representative samples of healthy populations has been assessed in several national dietary surveys sometimes with no concordance to copper serum concentrations. The wordwide multiplicity of dietary reference values illustrates the lack of consensus regarding requirements. The number of large-scale, high-quality studies examining the relationship between copper intakes, biomarkers of copper status, and health outcomes is still limited. None of several putative biomarkers of copper status have yet unequivocally proved to reflect changes in copper intake, in nutritional ranges. Monitoring copper status should rely on intake assessments for which several issues are reviewed here. Due to a lack of sensitive biomarkers of copper status, the question under-recognition deficiency is raised. Based on current knowledge the question of copper deficiency-related health outcomes is addressed. Policies options are considered.

Keywords

Copper · Metabolism · Cuproenzyme · Superoxide dismutase · Cytochrome c oxidase · Lysyl oxidase · Redox reaction · Oxidation · Requirement · Biomarkers · Fortification · Upper limit · Risk-benefit assessment · Intakes

List of all Abbreviations

AI	Adequate intake
ANSES	Agence Nationale de Sécurité Sanitaire (French Agency for Food, Environmental, and Occupational Health and Safety)
bw	body weight
D-A-CH	States of the German language Sprachraum (Germany, Austria, Switzerland)
DRV	Dietary reference value
EFSA	European Food Safety Authority

EURRECA	European micronutrient RECommendations Aligned
FFQ	Food frequency questionnaire
IOM	Institute of Medicine
KC	Keratoconus
LDL	Low-density lipoprotein
MT	Metallothioneins
NCM	Nordic Council of Ministers
NHMRC	(Australian) National Health and Medical Research Council
NNR	Nordic Nutrition Recommendations
RCT	Randomized control study
RDA	Recommended dietary allowance
ROS	Reactive oxygen species
SOD	Superoxide dismutase
UL	Tolerable upper intake level
VLDL	Very-low-density lipoprotein
WHO	World Health Organization

Introduction

Copper (Cu) was established as an essential trace element in the 1920s. In the Western diet, decreased copper in food and diet over the last several decades has been suggested to contribute to copper inadequacy (Klevay 2011); in developing countries, malnutrition, protein-energy malnutrition, and micronutrient deficiencies continue to be major health burdens (FAO 2004). Being a transition metal, copper is a cofactor for many redox enzymes involved in several biochemical processes and physiological functions. The wide range of clinical features resulting from disturbances in the activities of cuproenzymes means that although severe copper deficiency is relatively straightforward to diagnose, identifying marginal deficiency is more difficult (Danzeisen et al. 2007). Therefore, monitoring copper status in the general population should rely on intake assessments for which several issues are reviewed here. Based on current knowledge regarding copper intake, biomarkers, and the relationship between dietary copper and health, the question of copper deficiency-related health outcomes are addressed. Policies options, including fortification, will be considered.

Copper Functions, Metabolism, and Balance

Copper Metabolism and Balance

Needed in trace amounts, copper is present in the order of approximately 110 mg Cu in the body of a healthy 70 kg human adult (10 mg in the liver, 8.8 mg in the brain, 6 mg in blood, 3 mg in the kidney, 46 mg in the skeleton, including bone marrow,

and 26 mg in skeleton muscle). Its absorption occurs mainly in the proximal part of the small intestine, where it is transported to the liver via the portal vein. The absorption rate varies between 12% and 71% depending on age, gender, food matrix, amount of dietary copper, and the use of oral contraceptives (van den Berghe and Klomp 2009). Copper absorption is consistently 10% higher in women than men, without being affected by hormone use in women and without any effect of age on fractional absorption (Johnson et al. 1992). In fully or partially breast-fed infants, limited evidence from monitoring fecal 65Cu elimination after an oral dose suggests that intestinal absorption of copper is close to 80% (Olivares et al. 2002). This estimation was not corrected for intestinal copper reexcretion; therefore, we assume that copper uptake from breast milk is almost total.

Following intestinal absorption, 75% of portal copper is taken up by the liver (Harvey et al. 2005) and the remainder flows into the peripheral circulation, mainly bound to albumin. In the liver, 20% is reexcreted back into the gastrointestinal tract and 80% is exported to the periphery, bound to ceruloplasmin (Harvey et al. 2005). An excessive intake of zinc predispose an individual to secondary copper malabsorption (Beshgetoor and Hambidge 1998).

Based on concentration in gastrointestinal fluids, an estimated 2.5 mg of copper is excreted daily in the biliary flow, and as much through other gastrointestinal secretions (including saliva, gastric juice, pancreatic and intestinal fluid secretions) (Linder et al. 1998). Most of the endogenous copper secreted into the gastrointestinal lumen is reabsorbed across the intestinal epithelium (Harvey et al. 2003; Turnlund et al. 1989, 1998, 2005). The gastrointestinal secretion of copper plays a major role in the control of copper homeostasis, with endogenous losses increasing from 0.45 to 2.46 mg per day (mg/d) for intakes increasing from 0.7 to 6 mg/d (Harvey et al. 2003).

Urinary excretion ranges from 10 to 25 μg/d (Turnlund et al. 1990, 2005, 1997) and does not seem to play a significant role in the control of copper homeostasis in response to changes in copper intake.

Sweat and integumentary losses average 42 μg/d (Milne et al. 1991), and sweat losses are believed to generally have a small overall effect on the estimated fractional losses (Snyder et al. 1975). Due to similar physiochemical properties between copper and zinc, a high intake of zinc or high molar ratio of zinc to copper has long been recognized to interfere with copper metabolism (Beshgetoor and Hambidge 1998).

Information regarding Cu flux and organ pools is summarized in Fig. 1.

Biochemical Functions

Copper is required for the function of over 30 proteins (Arredondo and Nunez 2005), involved in redox reactions in a myriad of biological processes, such as cellular respiration (cytochrome C oxidase), free radical eradication (superoxide dismutase, SOD1), connective tissue biosynthesis (lysyl oxidase), neurological development (dopamine beta-hydroxylase), iron homeostasis (ceruloplasmin), antioxidant defense, neuropeptide synthesis, regulation of gene expression, and immune

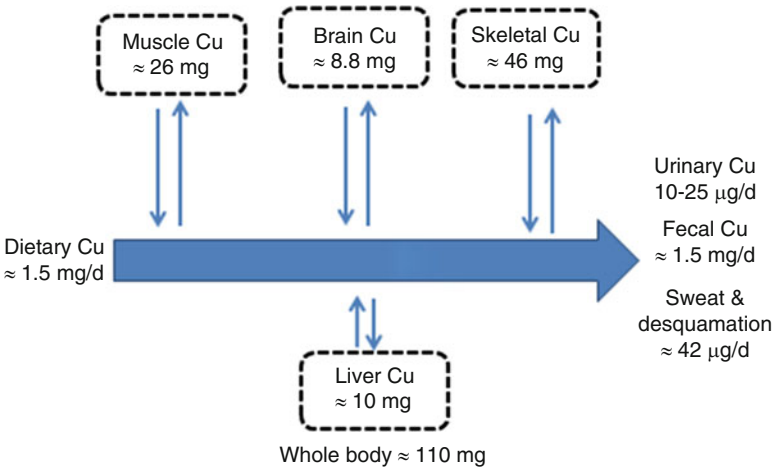

Fig. 1 Whole-body pools and flux

function (Prohaska and Gybina 2004). A deficiency of copper suppresses SOD1 activity, weakens free radical defense, and may also cause membrane damage. In SOD1, copper, as cofactor, works together with zinc, and it is the copper-to-zinc ratio, rather than the absolute amount of either metal alone, that helps the enzyme function properly. The competitive absorption of zinc and copper has to be considered. However, copper has an ambivalent role and, in its free form (as a transition metal), it can be a source of reactive oxygen species (ROS). Copper can therefore be toxic at high levels. An overload of this metal leads readily to Fenton-type redox reactions, resulting in oxidative cell damage and cell death. However, copper toxicity as a result of dietary excess is generally not considered a widespread health concern, probably as a result of the homeostatic mechanisms controlling copper absorption and excretion (Turnlund et al. 2005).

Copper Deficiencies and Health Outcomes

Biochemical alterations due to copper deficiency have been reported such as alterations in copper levels, lower hemoglobin, reduced melanin, and elevated hepatic iron content. Some of the most salient changes to organs include alterations in the hematological, cardiovascular, nervous, and immune systems. Anemia, cardiac enlargement, neuronal degeneration, and thymic hypoplasia have been observed in a number of species (Prohaska 1990). In humans, copper deficiency impairs growth and reproduction. Prolonged marginal deficiency has also been associated with alterations in cholesterol metabolism (Klevay et al. 1984; Reiser et al. 1987).

During pregnancy, deficiencies in some trace elements including copper have been described as possibly associated with complications in childbirth or fetal development (Darnton-Hill and Mkparu 2015). In utero, copper deficiency may

result in impaired development of the cardiovascular system, bone malformation and ongoing neurologic and immunologic abnormalities into infancy and beyond (Gambling and McArdle 2004; Georgieff 2007).

Lipoprotein Profile

Two cross-sectional studies (Bo et al. 2008; Ghayour-Mobarhan et al. 2005) suggest that high copper intake and status is associated with a better metabolic profile.

In a randomized control study (RCT) (Davis 2003), total and low-density lipoprotein (LDL) cholesterol did not differ between the low (0.59 mg Cu/d) and adequate (2.59 mg Cu/d) copper periods (6 weeks each). Supplementation with 2 mg Cu/d for 6 weeks (Medeiros et al. 1991) triggered an increase in total serum LDL cholesterol at 4 weeks whereas very-low-density lipoprotein (VLDL) cholesterol declined in the placebo group. The percentage of LDL increased after 6 weeks of supplementation compared with the initial baseline value, while the percentage of cholesterol as VLDL decreased compared with the baseline value in the supplemented group.

Limited results from cross-sectional studies tend to suggest that dietary copper is associated with a better lipoprotein profile. This observation is not fully supported by the results of RCTs, but the short duration of these studies may preclude the occurrence of such an effect.

Cardiac Arrhythmia

A significant increase in the number of ventricular premature discharges was observed in 3 women out of 13 after 3–12 weeks on a low copper diet (0.57 mg Cu/d for 105 days) (Milne and Nielsen 1996). In another study, 3 women out of 12 on a low copper diet (1 mg Cu/d during a 90 day controlled period) exhibited ventricular premature discharge. Although three women received supplements before the end of the study, two still exhibited an increased number of abnormal ventricular discharges. Limited evidence from the RCTs also suggests that marginal intake of copper may lead to cardiac arrhythmia but no conclusion can be drawn regarding the relationship between dietary copper and cardiac arrhythmia (Milne et al. 2001).

Neurologic Alterations

The hypothesis that copper intake is linked to cognitive decline is based on the long-recognized age-related accumulation of metal-transporting proteins and compounds in key sites of the attentional circuits (Zatta et al. 2008).

Copper concentrations have been found to be inversely associated with cognitive performance in a large cohort of elderly healthy women but not in men. Long-term and short-term recall scores are significantly lower in women with serum copper over 2.15 mg/L than women with serum copper below 0.9 mg/L. (Lam et al. 2008).

A potential role of copper in depression has been hypothesized. No significant differences have been found in serum copper concentrations between patients in a current depressive episode or in remission and healthy volunteers. Moreover, copper levels in the active stage of the disease are not correlated with clinical features of the population (Styczen et al. 2016). This study seems to be the first large-scale investigation on this topic.

An association between serum copper concentrations and sleep duration in aging men has been suggested (Luojus et al. 2015). Authors hypothesized that copper contributes to sleep regulation through pro-oxidative processes and copper-dependant N-methyl-d-aspartate receptor activity. Mechanisms underlying the relationship require further investigation.

Cancers

Two cohort studies have examined the link between copper intake and lung cancer and lymphoma.

A large cohort study (Mahabir et al. 2010) showed no relationship between total copper intake and the risk of lung cancer. In the Iowa Women's Health Study (Thompson et al. 2010), there was no link between total or dietary copper and the risk of non-Hodgkin's lymphoma, diffuse large B-cell lymphoma or follicular lymphoma. In a third cohort study in subjects aged 65 years and over (Bates et al. 2011), dietary copper intake was not predictive of cancer mortality over 14 years.

The link between copper intake and breast cancer was not demonstrated in one cross-sectional study (Dabek et al. 1994) and in one case-control study (Cavallo et al. 1991).

For other cancers, no relevant studies assessing copper intake were identified. To date, no conclusion can be drawn regarding copper intake and cancers.

Arthritis

One cohort study in women (mean age, 61.4 years) (Cerhan et al. 2003) found no link between dietary copper intake – with or without supplementation – and risk of rheumatoid arthritis. There was a weak, but significant inverse relationship between the use of copper supplements and the risk of rheumatoid arthritis, which did not persist after further adjustment for confounding factors (age and energy intake, other risk factors). Thus, no conclusion can be drawn regarding copper intake and rheumatoid arthritis.

Immunosuppression

In healthy humans, diets providing 0.38 mg Cu/d for 42 days reduces peripheral blood mononuclear cell proliferation and secretion of the interleukin-2 receptor in culture media. Neither parameter returned to baseline levels at the end of the 24 day

repletion period. Peripheral blood number of leucocytes, monocytes, neutrophils, lymphocytes or natural killer cells were unaffected by diets of 0.66, 0.38, and 2.49 mg/d Cu during 24, 42, and 90 days, respectively (Kelley et al. 1995).

A significant impact on the immune function with both leucopenia and neutropenia has been reported in specific situations where Cu malabsorption combined with low Cu intakes resulted in impaired Cu status, such as post-bariatric surgery patients (Saltzman and Karl 2013).

Together with zinc and iron, copper plays a pivotal role either in maintaining and reinforcing the immune and antioxidant performances or in affecting the complex network. The relevance of dietary copper in aging because affecting some genes related to inflammatory/immune response is suggested despite sensitive biomarkers are missing to assess marginal or moderate copper deficiency in aging of genes involved in encoding proteins for a correct inflammatory/immune status (Mocchegiani et al. 2012).

Keratoconus

Copper has been called into question in relation to keratoconus (KC). A hypothesis linking the entire putative pathway of KC development suggests that copper imbalance in corneal tissue may be an independent risk factor for the disease. Current evidence suggests that, in the vast majority of patients, the development of KC depends on the interplay between genetic and environmental factors. Copper deficiency may be an unrecognized factor increasing susceptibility to the disease (Dudakova et al. 2015). It has been hypothesized that lower plasma levels of copper in males compared with females could explain a higher prevalence of KC (Johnson et al. 1992). The assessment of copper levels and its distribution in keratoconic corneas warrants further investigation (Dudakova et al. 2015).

Copper Requirements and Dietary Reference Values

Requirements for Adults

To set nutritional requirements in adults, calculation of nutrient balances requires a thorough assessment of nutrient losses. For copper, four balance studies are available (Harvey et al. 2003; Milne et al. 1990; Turnlund et al. 1998, 2005). Copper balance values are reported to be positive for intakes greater than 2.4 mg Cu/d and negative for intakes less than 0.8 mg Cu/d (Harvey et al. 2003; Turnlund et al. 1998).

Fractional copper absorption has been measured using extrinsic meal labelling with 65Cu and fecal copper monitoring for several days to accurately quantify the amount of absorbed copper reexcreted in fecal matter (Harvey et al. 2005; Harvey et al. 2002, 2003). The results allow considering the true fractional absorption of copper as being close to 50%, and constant for copper intakes ranging from 0.7 mg/d to 6.0 mg/d.

Table 1 Copper requirements and dietary reference values (DRV) (mg Cu/d) according to several public health authorities

	ANSES (2017)	D-A-CH (2013)	EFSA (2015)	IOM (2001)	NHMRC (2006)	NCM (2012)
Men						
EAR	1	–	–	0.7[a]	ND	0.7
DRV	2.0 1.5 (51–65 year)	1.0–1.5	*1.6*	0.9	1.7	0.9
Women						
EAR	0.8	–	–	0.7	ND	0.7
DRV	1.5	1.0–1.5	*1.3*	0.9	1.2	0.9

[a]Based on variations in copper status estimated from copper in serum, ceruloplasmin, SOD in erythrocytes in depletion/repletion studies

These data suggest that the minimal amount of dietary copper required to achieve a null balance lies somewhere between 0.8 and 2.4 Cu mg/d. Consequently, there is no consensual requirement value and, depending on the public health authority, this requirement has been set based on balance studies available or other types of data. Some of these values are summarized in Table 1.

Requirements for Children

In infants, copper requirements can reach 40–80 μg Cu/kg body weight (bw)/d. Copper stored in the liver of the fetus and delivered by the mother during the last trimester of pregnancy is essential to cover this requirement (Afssa 2001). Therefore in pregnant women, copper intake should be increased of 0.2–0.5 mg/d.

Human breast milk usually contains 300–400 μg/L of copper (Dorea 2000) and, typically, initially provides 50 μg/kg bw/d, declining to approximatively 13 μg/kg bw/d after 6 months of lactation (Casey et al. 1989). Copper balance has been reported in infants receiving 27 μg/kg/d of copper from breast milk (Aggett et al. 1983).

Therefore, the World Health Organization (WHO) has proposed that all infants (0–6 months of age) not given breast milk should receive cow's milk-based or other formula diet providing at least 50 μg/kg bw/d of copper. This intake may have to be increased in formulae where the bioavailbility of copper is likely to be reduced (e.g., soy-based formulae).

The UK Committee on Medical Aspects of Food Policy (DH 1991) set a requirement for infants based on a factorial approach. In the UK, requirements of 47, 39, and 36 μg/kg bw/d were set for successive 3-month periods of infancy.

In the Nordic Nutrition Recommendations (NNR 2012), for infants aged 6–11 months and children, copper requirements were extrapolated from adult requirements, taking into account an allowance for growth, in line with the Institute of Medicine (IOM 2001).

Clinical and biochemical studies monitoring copper intakes in children are very rare. Data on copper intakes required to establish equilibrium between input and

output differ widely, ranging from 35 to 2000 μg/kg bw/d (Alexander et al. 1974). Although most suggest that copper balance can be reached at intakes in the range of 50–100 μg/kg/d, most datasets also reflect the difficulty of interpreting copper balance studies when the copper status of experimental subjects has not been previously defined.

The IOM (2001) extrapolated the copper requirement for children from the requirement for adults using allometric scaling (body weight to the power of 0.75), owing to the absence of data for children. The choice of this scaling approach was justified by the structural and functional role of copper in many enzymes and because it resulted in higher values than extrapolation based on isometric scaling (i.e., linear with body weight).

Dietary Reference Values (DRVs)

DRVs are established for each nutrient and target population based on scientific data of various types (biochemistry, epidemiology, etc.). The methods of "construction" to establish these values vary depending on the nature of nutrients and the data available. For copper, due to the lack of appropriate balance data, a few institutions (Anses 2016; IOM 2001) have derived a recommended dietary allowance (RDA) based on requirement estimations. Other authorities (e.g., NHMRC, EFSA, DASH) therefore proposed recommended values based on observed intake (adequate intakes, AI). Some of the proposed values are summarized in Table 1.

To date, at the international level, the multiplicity of values illustrates the lack of consensus regarding nutritional requirements and reference values for copper.

Copper Intake and Assessment of Copper Status

The calculation of the prevalence of inadequacy must take into account both copper content in food, intake frequencies, and food composition data to calculate copper intakes compared with DRVs.

Dietary Sources of Copper

Copper content in food varies with regional specificities, which explain some of the differences between composition tables among regions or countries. Naturally low soil copper concentrations are responsible for low contents in food and drinking water. Use of copper compounds as bactericides or fungicides on many crops also affects its content in cereals, fruits, and vegetables and, to a lesser extent, meat and animal products (Olivares et al. 2004).

The most bioavailable source of copper is meat and the major storage is in the liver, primarily bound to metallothioneins (MT) (Hartmann et al. 1993). Food groups such as offal (7–20 mg/100 g), nuts (1–2 mg/100 g), shellfish (1–3 mg/100 g), and, to

a lesser extent, cereals and fruits (0.5–2 mg/100 g) can be regarded as good sources of copper, while milk and dairy products contain low amounts (<1 mg/100 g) (Ciqual, ANSES 2016). The largest contributors to intake in the French adult population are bread and bread products (14.1%), vegetables (7.8%), offal (7.4%), fruits (6.1%), and potatoes (5.5%).

Copper density in a vegan diet has been shown to be twice that of an omnivorous diet (2.0 ± 0.34 mg/1000 kcal vs 0.7 ± 0.29 respectively) (Abdulla et al. 1981). Daily copper intake has been reported to be 27% higher in vegetarians than in omnivorous adolescent females (Donovan and Gibson 1996). The higher copper content of a plant-based diet has been consistently shown to compensate for its slightly reduced bioavailability resulting from the presence of phytates and fibers, suggesting that diets low in animal does not cause copper deficiency (Hunt and Vanderpool 2001; Turnlund 1983, 1985).

Dietary Intakes and Nutritional Status

The copper intake in representative samples of healthy populations has been assessed in several national dietary surveys (Table 2).

Similar levels of copper intake have been reported in European countries for adults and the elderly (Table 2), according to recent reviews of representative national dietary surveys and total diet studies (Sadhra et al. 2007; Vinas et al. 2012) and in the US adult population (US Department of Agriculture 2012).

Several methods are used for calculating the prevalence of inadequacy which require knowledge on intake and DRVs values. Nutritional status is mostly assessed

Table 2 Mean copper intake in adults and the elderly in various European countries according to representative national dietary surveys.

Country	Study – years	Method	Men		Women	
			N	Mean ± SD	N	Mean ± SD
Adults						
Finland	FINDIET 2007	Adj 48 h record	730	1.6 ± 0.7	846	1.3 ± 0.5
Ireland	SLAN 2007	FFQ[a]	662	1.5 ± 0.8	717	1.2 ± 0.7
Italy	INN-CA 1994–1996	7 day record	660	1.6 ± 0.7	801	1.3 ± 0.5
UK	HSE 2000–2001	7 day record	219	1.4 ± 0.7	210	1.0 ± 0.4
France	INCA2 2006–2007	7 day record		1.6 ± 0.08		1.3 ± 0.06
USA	NAHNES 2009–2010			1.5 ± 0.02		1.2 ± 0.02
Elderly (>64 year)						
Finland	FINDIET 2007	Adj 48 h record	229	1.4 ± 0.7	234	1.2 ± 0.5
Ireland	SLAN 2007	FFQ	580	1.4 ± 0.9	742	1.3 ± 0.7

[a]*FFQ* food frequency questionnaire

using the estimated average requirement (EAR) cut-point method. Because biological status is rarely measured due to technical or methodological reasons, the interpretation of prevalence of inadequacy must be made with caution.

A Greek cross-sectional epidemiological study showed wide variation in copper intakes across age. The prevalence of inadequacy assessed using the EAR cut-point method showed inadequacies ranging from 1.9% to 8.4% in children from 9 to 13 years old, 18.3% and 37.5% in 40–60 years old, respectively, males and females (Manios et al. 2014). For the authors, the significant age-related increases are consistent with previous studies conducted in Greece as well as in other developed countries.

In Chile, copper intake inadequacy assessed using the EAR cut-point method was observed to be most common in adult males (33.3%) and in women (21.1%).

In Nordic countries, referring to an EAR of 0.7 mg/d, the percentage of the population over 65 years at risk for inadequate dietary intakes of copper from food alone was 14% for men and 18% for women (ter Borg et al. 2015).

Data from the literature have shown that biochemical or clinical copper deficiency is prevalent in infants recovering from malnutrition or children with chronic diarrhea (Araya et al. 2003). The early introduction of cow's milk as a primary nutrient source leads to the development of copper deficiency, particularly in low-birth-weight and premature infants (Naveh et al. 1981). Case studies (Levy et al. 1985) also describe signs of deficiency in two 6-month-old male infants who were neither premature nor seemingly malnourished but were fed cow's milk since birth. These studies suggest that deficiency during the first year of life in infants fed with a cow's-milk-based diet may not be that uncommon and, unfortunately, may often go undiagnosed.

In some cases, sufficient intake can be even associated with low copper serum concentrations as observed in Mexico, where serum concentration below 65 μg/dl has been reported for 30.6% in children from 1 to 11 years old and 14.1% in adolescents (Morales-Ruan Mdel et al. 2012). The meaning is not quite understood and authors speculate that copper absorption is inhibited by a high intake of absorption antagonists, abundant in the Mexican diet. This study illustrates the limits of basing conclusions on the relationship between intake and a marker of biological status.

Copper deficiency is also commonly observed in formerly obese patients after gastric bypass surgery, with prevalence ranging from 10% to 15% in cross-sectional studies and 4–18% in longitudinal studies (Jaiser and Winston 2010; Gletsu-Miller et al. 2012). Its prevalence is higher after Roux-en-Y gastric bypass and Biliopancreatic diversion with duodenal switch compared to other types of bariatric surgery and is a consequence of both reduced copper intake and malabsorption resulting from decreased hydrochloric secretion in the remnant stomach and duodenal bypass (Gletsu-Miller et al. 2012).

Overall, data on intakes alone are not sufficient to determine prevalence of nutrient inadequacy. Dietary inhibitors and enhancers of absorption influence copper bioavailability and, hence, determines absorption efficiency (IOM 2001). Further, fractional absorption and excretion depends on its availability in the diet and subjects can adapt to intake below levels set by requirements. Therefore, insufficiencies identified by dietary assessment need to be confirmed by complementary parameters

that are seldom included in protocols (clinical, biochemical, and functional). The effects of dietary behavior on markers of micronutrients are still insufficiently investigated. Nevertheless, when trace element levels are inconsistent with medical evaluations, a test for activity of the suspected enzyme(s) may lend support to the differential diagnosis.

Biomarkers of Copper Exposure

Although serum and plasma copper are often used as markers of copper status (Harvey et al. 2009), they show no variation for copper intake values ranging from 0.57 to 6 mg/d in controlled studies. For other putative biomarkers, either their specificity and sensitivity is too low or the quality and number of studies examining their link with copper intake is insufficient to support their use in the evaluation of copper exposure.

Even being available, erythrocyte and extracellular SOD, leucocyte copper, platelet cytochrome c oxidase, or serum lysyl oxidase (Klevay 2011) is less frequently used. The protein-metal speciation analysis has also been proposed as an added value for a precise diagnosis of nutrient deficiency or overload, and subsequently provides a more complete picture for defining correct individual supplementation (Mocchegiani et al. 2012).

Additionally, using only biochemical parameters may be misleading because of a nonspecific increase in serum concentration of ceruloplasmin during acute-phase response against various infections and inflammatory conditions (Muataz Elsiddig and Elmuataz 2008). A Cu:Zn ratio exceeding 2 indicates a severe infection (Hua-Dong et al. 1999).

The lack of a suitable biomarker for copper intake and status highlights the need for high quality RCTs in healthy subjects evaluating the responsiveness of putative new biomarkers of copper status over wide copper intake ranges (Bost et al. 2016). Generally speaking, more informative biomarkers are needed to assess copper status at the population level. To date, information on circulating levels in the general population and children is still scarce. Therefore, data are lacking to accurately estimate the copper status in the populations.

Discussion

The number of large-scale, high-quality studies examining the relationship between copper intakes, biomarkers of copper status, and health outcomes is still limited. It remains difficult to assess the links between copper intake and health and copper requirements. To date, none of several putative biomarkers of copper status have yet unequivocally proved to reflect changes in copper intake within the range of nutritional intakes. The question could be raised of whether deficiency is under-recognized due to a lack of sensitive biomarkers of copper status.

The weakness of food composition tables in providing a clear nutrient composition of all individual food items is also among methodological limitations in assessing the actual dietary intake in studies.

Additionally, the prevalence of inadequacy depends on the chosen EAR value, which varies among countries. International projects, such as the European micronutrient RECommendations Aligned (EURRECA) project have the potential to lead to a standardized way of estimating the adequacy of micronutrient intake in populations, including copper which is considered as one of the top 10 priorities (Cavelaars et al. 2010).

The unresolved issues of copper intake and inadequacy assessment raise the issue of the relevance of copper fortification.

In Europe, few data are available on the contribution of dietary supplements to daily copper intake (Flynn et al. 2009). In the USA, dietary supplements contribute significantly to daily copper intake (50% in male supplement users and 60% in female users) (Bailey et al. 2011). Although the use of dietary supplements results in a small but significant reduction in the prevalence of copper inadequacy in the adult population, it also leads to an increasing risk of intake above the upper intake levels (UL) in children 2–8 years old (7.7% vs. 39% for nonusers and users, respectively) (Bailey et al. 2012). Because fortification with micronutrients should be safe for the whole population, unacceptably large intakes of micronutrients from all sources should be prevented. As voluntary food fortification expands, careful scrutiny and monitoring of this practice is needed (Sacco et al. 2013) to avoid excessive intakes. Among trace elements, copper intake is not frequently assessed in Europe (Novakovic et al. 2013), and the need for an increase in copper intake has not been clearly demonstrated.

High interindividual genetic variability, interactions between dietary habits and genetic factors and the unreliability of specific biomarkers for testing zinc, copper, iron status together contribute to the poor success of supplementations (Klevay 2011). Regarding the heterogeneity of intake data, and considering the risks related to both deficiency and excess, it is judicious to consider restricting the addition of micronutrients to cases in which health benefits are demonstrable. This consideration includes zinc fortification, because the well-known competitive absorption between copper and zinc which can alter copper status under zinc fortification.

Copper has an ambivalent role and may demonstrate U-shaped exposure-response relationships due to toxic responses occurring as a result of both excess and deficiency (Milton et al. 2016). Rather than focus entirely on a single optimal intake level, it may be more appropriate to consider an acceptable range of copper intake, allowing for uncertainties in the data and interindividual variability in the response.

Conclusions

Fortification policies aiming at reducing inadequate nutrient intakes in the general population need to be weighed against the risks of intakes above the UL in other parts of the population. The scientific evidence needed to support fortification policies requires a dedicated risk/benefit assessment.

In the case of copper, uncertainties regarding concentration in foods and the lack of accurate biomarkers of copper status may lead to misclassification in terms of copper exposure and confuse the link between copper intake and health. To understand the public health significance of copper malnutrition and identify the most appropriate measures to prevent it, the number of unresolved issues and uncertainties calls for studies with a more complete and precise assessment of copper exposure based on reliable biomarkers of copper status. The physiological role and the health consequences of copper-deficient diets have to be better understood to establish criteria for defining the degree of malnutrition, and to develop prevention and control strategies.

Policies and Protocols

Policies

Dietary Copper Intakes and Nutritional Status Display Heterogeneous Scenarios that Need Differentiated Approaches Regarding Regions and Populations

- Governments have to encourage research to better understand the physiological role and the health consequences of copper-deficient diets
- The estimation of copper intake in populations has to be standardized at an international level
- The right answer to copper deficiency when occurring in the population has to target the affected population
- Criteria for defining the degree of malnutrition, and to develop prevention and control strategies has to be established by the public health authority
- If considered, fortification policies need to be weighed against the risks of excessive intakes in the nonaffected parts of the population
- Fortification policies require a dedicated risk/benefit assessment in the whole population

Protocols

Assessment of the Responsiveness of Putative new Biomarkers of Copper Status

Uncertainties regarding concentration in foods and the lack of accurate biomarkers of copper status may lead to misclassification in terms of copper exposure and confuse the link between copper intake and health. Intake is insufficient to determine prevalence of nutrient inadequacy and need to be confirmed by complementary information to be included in clinical, biochemical, and functional protocols. This review highlights the need for a more complete and precise assessment of copper exposure based on reliable biomarkers of copper status at the population level and in healthy subjects. The link between biomarkers and

copper intake are insufficiently established to support their use in the evaluation of copper exposure. Specific and sensitive markers have to be identified or developed – using high quality RCTs – to be specific and sensitive over wide copper intake ranges.

Dictionary of Terms

- **Cuproenzyme** – enzyme in which copper is bound in an active site and used as a cofactor.
- **Biomarker** – substance contained in tissues (mainly blood serum or hair) that can be accurately and reproducibly measured. It is supposed to objectively reflect a physiological state.
- **Estimated average requirement (EAR)** – average daily need within the population, as estimated from individual intake data in relation to a criterion of nutritional adequacy in experimental studies
- **Adequate intake (AI)** – average daily intake of a population or subgroup whose nutritional status is considered adequate.
- **Dietary reference value (DRV)** – daily intake that covers the requirement of almost the entire population considered, as estimated from experimental data. The DRV is usually derived from the EAR or an AI by default.
- **Nutritional adequacy** – comparison between nutritional intakes and requirements in order to assess population risk of inadequate intakes. The prevalence of nutrient inadequacy can be assessed by the probability approach or using the estimated average requirement (EAR) cut-point method.
- **EAR cut-point method** – defines the prevalence of inadequate intakes as the proportion of the population with intakes below the median EAR.
- **Fortification** – addition of vitamins, minerals, or other substances that has a nutritional of physiological effect in common foods. Fortification can be voluntary, at the initiative of the industrial, or mandatory, as a public health intervention.

Summary Points

- This chapter focuses on dietary copper which is an essential trace element naturally present in soils and water.
- Copper is involved in many biological functions, thus low copper intake may have various health effects, such as alterations in the hematological, cardiovascular, nervous, or immune systems.
- Due to the lack of putative biomarkers, monitoring copper status mainly relies on dietary intake data that may confuse exposure measure and the assessment of the link between intakes and health.

- The lack of high quality studies examining the relationship between copper intakes, biomarkers of copper status, and health outcomes leads to the absence of consensual requirement values.
- The unresolved issues concerning copper intake and health makes difficult to suggest appropriate policies and to assess their efficiency.

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Importance of Chromium in the Diet

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Contents

Abstract

Chromium is a micronutrient found in several oxidation states, being trivalent chromium and hexavalent chromium the most prevalent. Although it is present in several foods in small quantities, there is still no recommended average requirement. Studies show that during the various life stages, there are

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different needs of ingestion of this mineral. Despite the low molecular weight, there is a small absorption capacity of chromium and its absorption occurs in the intestine by passive transport. Along with other metallic ions, its transport is related to the performance of transferrin, and there may be competition for sites that bind to iron and other minerals. Chromium is related to changes that encompass carbohydrate and lipid metabolism. Therefore, some studies indicate that chromium-deficient diets may favor insulin resistance, with consequent development of type 2 diabetes. This mineral is also present in nutritional supplements featuring various structures such as chromium picolinate, chromium histidinate, chromium chloride, and chromium nicotinate. Trivalent chromium demonstrated an important role in gene expression, mainly in hepatocytes, insulin activity, and adiposity. Studies have investigated the effects of chromium supplementation on diabetes, obesity, and dyslipidemia, but the results are still incipient for the development of guidelines recommending supplementation in risk groups.

Keywords

Chromium · Biological availability · Nutritional requirements · Glucose · Insulin resistance · Insulin · Type 2 diabetes mellitus · Cholesterol · Dyslipidemias · Deficiency · Micronutrients · Toxicity

List of Abbreviations

AI	Adequate intake
CASQ 1	Calsequestin 1
CIDEA	Cell-death-induced DNA fragmentation factor
Cr^{3+}	Trivalent chromium
Cr^{6+}	Hexavalent chromium
CrCl3	Chromium chloride
CrPic	Chromium picolinate
CrSP	Complex of chelated chromium with small peptides
DGAT 2	Decylglycol transferase
EAR	Estimated average requirement
ENO 3	Enolase 3
Glut 4	Glucose transporter in muscle and adipose tissue dependent on insulin
GPI 1	Glucose phosphate isomerase1
IGF-1	Insulin-like growth factor 1
IRS-1	Insulin receptor substrate 1
IRS-2	Insulin receptor substrate 2
Kg	Kilograms
LDL-c	Low-density cholesterol
mRNA	Messenger ribonucleic acid
NBC	Chromium nicotinate
Ph	Hydrogenation potential
PI3k	Phosphatidylinositol-3-kinase
RDA	Recommended dietary allowance

TPM 1	Tropomyosin-1
TTP	Tocopherol transfer protein
UCP 1	Uncoupled protein 1
UL	Tolerable upper intake levels
VLDL	Very low-density lipoproteins
WHO	World Health Organization
μg	micrograms

Introduction

Since its discovery in the eighteenth century, chromium has had several uses as dyes in the textile industry, manufacture of refractories, and metal alloys due to its corrosion capacity (Ensminger et al. 1990; Zelicoff and Thomas 1998). In the twentieth century, the scientific community obtained important findings about the role of chromium in carbohydrate metabolism through the potentiation of insulin signaling (Jeejeebhoy et al. 1977; Vincent 1999). Due to the importance of this micronutrient in the diet, this chapter aims to present the nutritional needs and the main dietary sources of this element, the processes involved in digestion, absorption, transport, and mechanisms of action of chromium in metabolism and its relation with the modulation of gene expression. Because of the increasing use of chromium as a nutritional supplement, this chapter further discusses its effects on type 2 diabetes mellitus and obesity, as well as adverse reactions and toxicity.

Studies indicate a relationship between chromium, health, and disease. But it is still necessary to better understand this micronutrient, such as food, nutritional, biochemical, and public health aspects in order to enable the findings to determine nutritional recommendations for healthy individuals and support guidelines for chromium supplementation in vulnerable groups.

Nutritional Requirements and Food Source

Chromium is a micronutrient present in nature in various valence states, being trivalent (Cr^{3+}) and hexavalent (Cr^{6+}) the most common. Cr^{3+} is the most stable oxidation state and, possibly, the most common form present in the diet, due to the reducing action of food substances (IOM 2001).

In foods, Cr^{3+}can be found, originally identified in brewer's yeast (Schwarz and Mertz 1959). Despite being found in various foods, its amount in most of them does not exceed 2 μg per serving (Roussel et al. 2007) (Table 1).

Cr^{3+} is an important nutrient in the diet, but in the absence of scientific evidence, the Estimated Average Requirement (EAR) of its daily intake has not been established yet. Adequate Intake (AI) (Table 2) is the only establishment to date. The Tolerable Upper Intake Level (UL) has also not been defined, since only a few serious adverse effects were found as a result of its high intake (IOM 2001).

Table 1 Chromium content of foods

Foods	Cr content (μg/serving)
Radish	2.61
Beef steak	2.88
Grapefruit juice	2.94
Grilled chicken	3.83
Pork rib	4.20
Broccoli gratin	4.40
Leek	5.18
Kiwi	5.09
Orange	6.22
Apple	6.80
Boiled potatoes	10.20
Dark chocolate	14.28

Roussel et al. (2007)

Table 2 Daily adequate intake (AI) of chromium (μg)

		Female		
Age	Male		Pregnancy	Lactation
0–6 months	0.2	0.2		
7–12 months	5.5	5.5		
1–3 years	11	11		
4–8 years	15	15		
9–13 years	25	21		
14–18 years	35	24	29	44
19–30 years	35	25	30	45
31–50 years	35	25	30	45
51–70 years	30	20		
>70 years	30	20		

IOM (2001)

Considering life stages, healthy individuals do not represent risk groups for chromium deficiency. Thus, the AI of chromium was based on the average of food consumption. In this case, for children from 0 to 6 months of exclusive breastfeeding, the nutritional recommendation was based on the content of chromium in breast milk, and for children from 7 to 12 months, the quantity of this mineral present in breast milk and in healthy complementary foods was considered (IOM 2001).

For people from 1 to 50 years, there appears to be no increased nutritional requirements of chromium; therefore, the AI of this mineral was based on the average of chromium present in an adequate diet (IOM 2001). For individuals above 51 years, the AI of chromium was also determined from the average amount of this nutrient found in a balanced diet, within the energy requirement for this age group (IOM 2001). There is not sufficient scientific evidence to prove a greater

requirement for chromium in this age group for the prevention of diseases, such as diabetes mellitus type 2 (Mccormick 2012).

In the case of pregnant women, there are no studies that confirm the requirement for chromium supplementation during pregnancy (IOM 1990). Therefore, to obtain the AI of chromium, the recommendation for a nonpregnant woman, of the same age group, added to the requirement for this mineral to fetal supply was considered (IOM 2001). For lactation, the AI of chromium was based on the AI for women of the same age group added to the amount of chromium required for replacement of chromium found in breast milk (IOM 2001).

For individuals with chronic diseases such as diabetes, several studies used additional doses in order to verify the effects of chromium in humans and generate evidence for supplementation recommendations. However, the current evidence does not support a positive effect to the supplementation with chromium in the treatment of diabetes (Abdollahi et al. 2013).

Digestion, Absorption, and Transportation

Although Cr^{3+} is the most stable oxidation state, its absorption is low because it presents difficulty in crossing the plasma membrane (Mertz 1992). As for Cr^{6+}, it has strong oxidative capacity, mainly in acid media, and is linked to oxygen in the form of chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$), which are the easiest compounds to absorb by the plasma membrane. During transport through the membrane, Cr^{+6} is detoxified to Cr^{+3} and reacts with protein components and nucleic acids inside the cell (Pechova and Pavlata 2007).

Specifically during ingestion, the Cr^{+6} dichromate is mixed with saliva and in the stomach it is reduced in Cr^{+3} by hydrochloric acid and thermosensitive-reducing agents present in the gastric juice (Kirman et al. 2013). An enzyme of great importance in the process of chromium reduction is pent gastrin, which stimulates gastric secretion, and Cr^{6+} is reduced into Cr^{3+} by hydrochloric acid, especially in individuals who remain in prolonged fasting (Stollenwerk and Grove 1985).

An in vitro study also demonstrated the conversion of Cr^{6+} to Cr^{+3} by the enzyme glutathione (GSH). By means of the spectrophotometric analysis, it was observed that the excess of the GSH enzyme accelerated the conversion reaction of Cr^{6+} to Cr^{+3}. It has also been shown that this reaction is strongly pH-dependent, being slower in pH 7.4 solutions than at pH values below 5.0 (Wiegand et al. 1984).

The absorption of chromium occurs in the intestine by passive transport, along with other metal ions, mainly in the portion of the jejunum and, to a lesser extent, in the ileum and duodenum, as demonstrated in an experiment with rats (Chen et al. 1973). In humans, there is also evidence that the absorption of chromium begins in the jejunum (Ducros 1992). Chromium in foods has increased absorption by the presence of amino acids, ascorbic acid, high levels of carbohydrates, oxalate, and aspirin in the diet, while phytates and antacids (sodium hydrogen carbonate, magnesium hydroxide) reduce Cr concentrations in the blood and in others tissues (Stoecker 1999).

Inorganic chromium compounds exhibit low absorption, less than 3%, regardless of the dose or chromium status. On the other hand, chromium complexes in the diet are more available than simple chromium salts (Fairweather-tait 1992), that is, the organic sources of Cr^{+3} are better absorbed (Ohh and Lee 2005).

In nutritional supplements, studies on the bioavailability of chromium are controversial; some show that chromium chloride has less bioavailability (0.1–0.4%) than chromium picolinate (2.8%) (Commission 2002). However, due to the toxic effect of picolinate in inducing renal insufficiency, anemia, and hemolysis, the nicotinate compound of chromium has shown greater bioavailability and lower toxicity (Bagchi et al. 2002). More recently, the soluble and ionic forms of chromium phenylalaninate [Cr (D-Phen3), Cr (L-Phen3)] and chromium hydrochloride (CrCl3) have been found to be better absorbed than the organic chromium trispicolinate (CrPic3), chromium nicotinate (CrNic2), and chromium propionate (CrProp) compounds (Laschinsky et al. 2012).

After absorption, chromium is transported in the blood by transferrin and can compete with binding to iron and other minerals (Quarles et al. 2011). The transferrins are proteins (molar mass ~ 80 kDa) that bind reversibly to metal ions, exhibiting greater selectivity for Fe^{+3}. However, the binding of Cr^{+3} to the Fe^{+3} sites of the carrier protein may be related to the detoxification process, more than the transport of an essential trace element (Levina et al. 2016).

The affinity of transferrin to metal ions varies according to environmental conditions, especially pH (Brock 1985). The binding of transferrin to chromium occurs similarly to its attachment to iron. The chromium binds to transferrin's two sites. When each chromic ion binds to the tyrosine residues of transferrin, changes occur in the ultraviolet spectrum of the protein, which was detected by means of Raman resonance (Aisen et al. 1969; Ainscough et al. 1980).

Chromium in Glucose and Lipid Metabolism

Chromium is postulated with functions that mainly cover carbohydrate metabolism. Increased plasma glucose levels stimulate insulin secretion, which binds to the α subunit of its trans membrane receptor and favors the transport of Cr^{+3} through the chromo-transferrin (Cr-Tf) complex. In the intracellular medium, four chromium atoms bind to a protein called apo-chromodulin which becomes active in the form of holo-chromodulin (Fig. 1). Holochromodulin binds to the β subunit of the insulin receptor, amplifies the signal, and activates the kinase activity of the receptor (Vincent 2000). However, the European Food Safety Authority (EFSA) by the Scientific Opinion on Dietary Reference Values for Chromium suggests there is insufficient evidence on chromium's action mechanism that supports its essentiality on glucose metabolism (EFSA 2014).

Intracellular signaling of insulin begins with its binding to the α subunit, which promotes conformational change and β subunit auto-phosphorylation, with a consequent increase in receptor kinase activity. Activation of the insulin

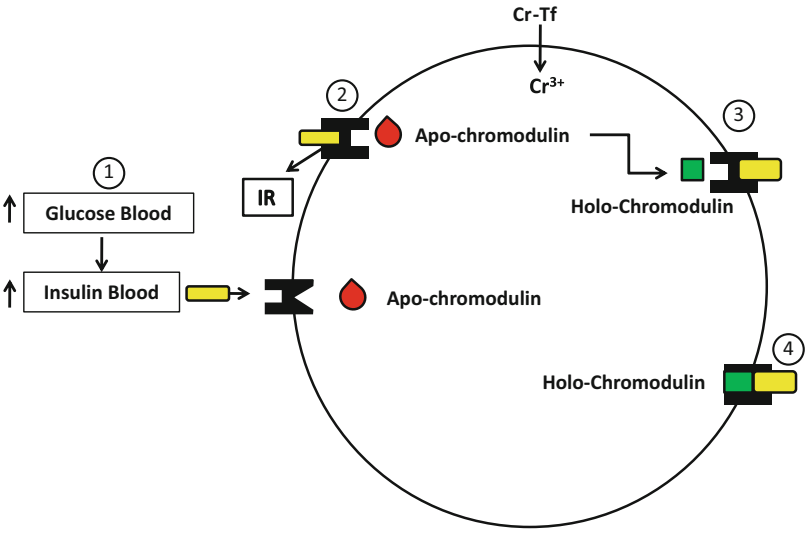

Fig. 1 Action of chromium in glucose metabolism. ◯: Increased glucose and insulin secretion that bind to the α subunit of its receptor, ➔ ▬, ◯: The insulin receptor (*IR*) becomes active through insulin binding and promotes conformational change in the β subunit (▬) and favors the entry of Cr^{+3} into the cell through the chromo transferrin complex (*Cr-Tf*), ◯ to ◯: conversion of the inactive form apo-chromodulin (●) to the active form holochromodulin (■), ◯: Final active form holo-chromodulin attaches to the site at the insulin receptor (▬) (Adapted from Vincent 2000)

receptor kinase triggers a cascade of intracellular phosphorylation. Initially, phosphorylation of its intracellular protein substrates (IRS-1 and IRS-2, respectively) phosphorylates the p85 regulatory subunit and activates the p110 subunit of phosphatidylinositol-3-kinase (PI3k), favoring the conversion of phosphatidylinositol-4,5 -phosphate (PI3k -inactive) in phosphatidylinositol-3,4,5-triphosphate (PI3k-active) (White and Kahn 1994).

Active PI 3-kinase is important in regulating mitogenesis, cell differentiation, and insulin-stimulated glucose transport. It promotes phosphorylation of protein kinase B (AKT) and other phosphoinositois kinase-dependent (PDKs) (White and Kahn 1994; Myers and White 1993).

The phosphorylation mechanism of the p110 subunit of PI3K is stimulated by the chromium present in the cytosol of the cell. Chromium also activates AKT, which stimulates the translocation of the glucose transporter (GLUT4) to the membrane, which is important for the glucose uptake process (Whiteman et al. 2002). The activated AKT phosphorylates other pathways assisting in the conduction of glucose transport (Wang et al. 2006; Dong et al. 2008). Chromium still inactivates PTP-1B which is a protein phosphatase considered to be a negative regulator of insulin signaling (path not shown in the figure) (Goldstein 2002; Sreejayan et al. 2002).

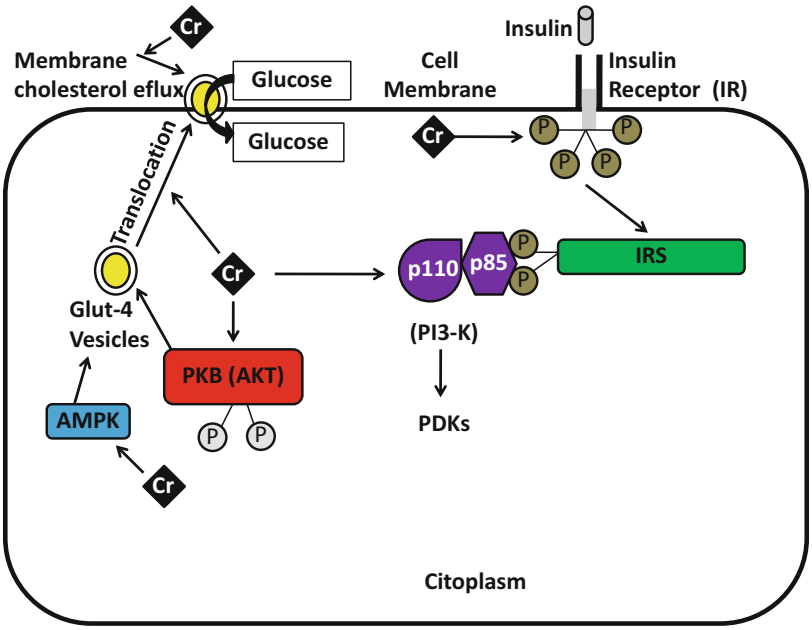

Fig. 2 Putative mechanisms by which chromium augments cellular glucose uptake. Chromium (*Cr*) showed increased kinase activity via phosphorylation of the insulin receptor β (*IR-β*), which was associated with the downstream insulin receptor (*IRS-1, IRS-2* respectively), which was phosphorylated thereby the p85 regulatory subunit and p110 subunit of the phosphatidylinositol 3-kinase (*PI3K*) protein and phosphoinositde-dependent kinases (*PDKs*). Chromium also assists in the signaling and phosphorylation of protein kinase B (*AKT*) leading to translocation of vesicles of glucose 4 (*Glut4*). The transient upregulation of cyclic adenosine monophosphate-activated protein kinase (*AMPK*) leads to increased uptake of glucose. Chromium mediates cholesterol efflux from membranes causing Glut4 translocation and glucose uptake (Adapted from Hua et al. 2012)

The transient positive regulation of AMPK by chromium leads to a higher uptake of glucose, that is, chromium favors the cholesterol efflux of membranes, which causes GLUT4 translocation and consequently glucose uptake (Fig. 2) (Chen et al. 2006).

Although chromium is involved in various mechanisms of glucose metabolism, it was found that chromium nicotinate supplementation did not promote increased insulin sensitivity and reduce blood glucose in diabetic subjects (Guimarães et al. 2013; Abdollahi et al. 2013).

There are also studies that report the relationship of chromium with lipid metabolism. Elevated blood cholesterol levels and aortic plaque formation were observed in rats fed a chromium-deficient diet, but not in animals supplemented with chromium chloride (Schroeder 1969). Later, a reduction in atherosclerotic plaque was observed in rabbits when they received potassium chromate injection (Abraham et al. 1980).

In humans, evidence on the importance of chromium in lipid metabolism has occurred from analyses of aortas of people who died of cardiovascular disease. They had less chromium than aorta from healthy people who were victims of an accident (Schroeder et al. 1970). Later, it was found that people with cardiovascular disease

had lower serum chromium concentrations when compared with healthy people (Newman et al. 1978; Simonoff et al. 1984).

Studies on the effect of chromium supplementation on improving lipid disorders are controversial. Press et al. (1990) have shown the potential of chromium picolinate in improving the lipid profile of people aged 25–80 years; however, Amato et al. (2000) found no promising effect of chromium picolinate on dyslipidemia in the elderly. In a study performed with diabetic subjects who received chromium supplementation by means of brewer's consumption, it showed a decrease in the levels of triglycerides and low-density lipoprotein (LDL-c) (Sharma et al. 2011). However, another study with diabetic subjects showed no change in total cholesterol, LDL-c, high-density lipoprotein (HDL-c), and triglycerides after 90 days of supplementation with chromium nicotinate (Guimarães et al. 2013).

Status, Toxicity and Adverse Chromium Effects

As previously discussed, chromium is a potentiating agent for insulin signaling (Vincent 1999; Chen et al. 2011). Thus, it is assumed that their dietary deficiency may contribute to the development of type 2 diabetes mellitus (IOM 2001). Most of the patients with diabetes mellitus have low concentrations of serum chromium (Guimarães et al. 2013), which makes clear the inverse relationship between HbA1c and serum levels of chromium (Rajendran et al. 2015). Thus, hyperglycemia may lead to a decrease in serum chromium concentrations and increase its urinary excretion, worsening diabetes (Gaméz et al. 2002). In healthy subjects, urinary excretion of chromium did not differ after high glycemic index diets compared to low glycemic index diets (Hajifaraji and Leeds 2008). Regarding age, there is a decrease in serum chromium levels in healthy individuals (Rocha et al. 2016). Table 3 shows studies on chromium status in health and disease.

Regarding obesity, serum chromium levels among obese and eutrophic children did not differ (Azab et al. 2014). The relationship between serum chromium and obesity was also not observed in obese women (Yerlikaya et al. 2013). Despite this, the relationship between obesity and insulin resistance is well established (Zhang et al. 2015). In this sense, chromium status in adults with visceral obesity plays an important role in insulin resistance, due to the inverse relationship between capillary chromium level and HOMA-IR (Kim and Song 2014). The chromium status of the toenails was also inversely associated with the incidence of metabolic syndrome, as a function of its relationship with blood lipids (Bai et al. 2015).

Regarding the deficiency diseases, the majority of anemic children due to iron deficiency presented chromium deficiency (Angelova et al. 2014). Despite the antagonistic effect of chromium on iron, by competing for the binding to apotransferrin (Quarles et al. 2011), insufficient intake of iron from the diet can be accompanied in many cases by borderline or insufficient intake of other micronutrients. On the other hand, in vitamin A deficiency, children with nocturnal blindness presented high levels of chromium in biological samples (blood, scalp, and urine (Afridi et al. 2011).

Table 3 Chromium status in health and disease

Reference	Study design	Sample characteristics	Sample size	Results
Healthy individuals				
Rocha et al. 2016	Cross-sectional study	Healthy individuals 18 to 74 years old	240	There was no difference in serum and urinary levels of chromium between the sexes. Serum chromium levels decreased with age.
Metabolic syndrome				
Bai et al. 2015	Cohort	American adults, aged 20–32 years	3648	Higher toenail chromium levels in young adulthood were associated with lower incidence of metabolic syndrome.
Prediabetic individuals				
Rafiei et al. 2014	Cross-sectional study	Prediabetic patients	132	In the group with a normal level of Cr, serum chromium levels decreased with age.
Type 2 diabetes				
Rajendran et al. 2015	Cross-sectional comparative study	Newly diagnosed type 2 diabetes mellitus patients without any pre-existing complications	42	Mean serum chromium concentration was significantly lower in uncontrolled type 2 diabetic patients. There is a decrease in serum chromium levels with age. The decrease in serum levels of chromium is greater after 40 years old.
Harani et al. 2012	Cohort	Adults aged 40 to 60 years	278	Serum chromium levels vary according to glycemic control in subjects with type 2 diabetes. Individuals with poor glycemic control had chromium levels 33% lower than healthy individuals. Serum chromium levels correlated strongly with insulinemia and HOMA-IR.
Guimarães et al. 2013	Randomized double-blind clinical trial	Adults with type 2 diabetes	42	Serum chromium deficiency was observed in 72% of individuals with type 2 diabetes.

(*continued*)

Table 3 (continued)

Reference	Study design	Sample characteristics	Sample size	Results
Enteral nutrition				
Santos et al. 2017	Prospective observational study	Patients aged 26–95 years that underwent percutaneous endoscopic gastrostomy	129	The majority (94%) of individuals with long-term dysphagia due to head and neck cancer or neurological dysphagia had normal serum levels of chromium. None of the patients who had low levels of serum chromium had diabetes or glucose intolerance. Low levels of serum chromium were not related to gender, glycemia, body mass index, serum albumin or transferrin.
Parenteral nutrition				
Capone et al. 2017	Cross-sectional comparative study	Preterm infants receiving parenteral nutrition therapy	706	Chromium supplementation via parenteral nutrition promoted better glucose tolerance and calorie delivery during the first week of life, especially in very low birth weight infants.
Anemia				
Angelova et al. 2014	Cross-sectional study	Children younger than 3 years with iron deficiency anemia	30	Low serum chromium in 73% of children.

High levels of chromium are generally observed in individuals who are submitted to occupational exposure (Scheepers et al. 2008). Inhalation of dust, mist, or vapor and dermal contact of products containing Cr^{+6} are the major routes of occupational exposure to chromium. In humans, there is sufficient evidence for the carcinogenicity of chromium VI compounds. Cr^{+3} compounds, found in foods, were not classifiable as to their carcinogenicity (IARC 2012).

Chromium is also present in nutritional supplements such as chromium picolinate, chromium histidinate, chromium chloride, and chromium nicotinate. The toxicity of supplements containing Cr^{+3} compounds depends on the binder. Study showed chromium picolinate as a mutagenic (Stearns et al. 2002), but the National Toxicology Program's technical report found no evidence of carcinogenic activity of chromium picolinate monohydrate (NTP 2010). Chromium picolinate,

chromium histidinate, and chromium chloride in high concentrations did not result in oxidative damage to DNA, in situations of oxidative stress induced by hydrogen peroxide (Hininger et al. 2007). However, when compared to chromium bound to niacin, chromium picolinate showed higher production of harmful superoxide anion and increased DNA fragmentation. Despite this, Cr^{+3} compounds are relatively nontoxic and exhibit less oxidative stress and DNA damage when compared to Cr^{+6} (Bagchi et al. 2002). The genotoxic effects of Cr^{+3} are unlikely to occur in humans or animals when exposed to the physiological or moderately elevated level of ingestion (Eastmond et al. 2008).

In addition to genotoxicity, adverse effects were reported during studies with chromium supplementation, such as dizziness, headache, nausea, constipation, flatulence, watery stools (Suksomboon et al. 2014), and itching in the palm of the hands (Guimarães et al. 2013). However, short-term chromium supplementation at usual doses of 150–1000 mcg does not increase the risk of adverse effects when compared with placebo. Even so, the safety of long-term supplementation is not established (Suksomboon et al. 2014).

The Role of Chromium in Modulating Gene Expression

Cr^{+3} has been presenting important effects on gene expression. Changes in hepatic cells, insulin activity, and obesity have already been observed (Peng et al. 2010; Rink et al. 2006; Wang et al. 2016).

Regarding hepatic cells, an in vitro study with Cr^{+3} noted improvement in oleic acid-induced steatosis, as it led to a reduction in the accumulation of lipids, fatty acid content, and the amount of triglycerides. This occurred because chromium blocked the transport of oleic acid excess inside the cells to suppress the mRNA and proteins expressed by the gene cluster of differentiation 36 (CD36); and also to downregulate the expression of diacylglycerol acyltransferase 2 (DGAT2) (Wang et al. 2016). CD36 genes express membrane glycoproteins that help in the capture of chylomicrons, VLDL, and long chain free fatty acids by cells (Iqbal and Hussain 2009) as well as stock and secretion of triglycerides in the liver (Kennedy et al. 2011). In turn, the DGAT2 genes express proteins that help to modulate the synthesis of triglycerides, and their excess can lead to the accumulation of this substance in the liver, and, consequently, liver steatosis (Yen et al. 2008).

As for the effect of chromium on the activity of insulin, an in vitro study demonstrated that the use of chromium picolinate (CrPic), complex of chromium chelated with small peptides (CrSP) and chromic chloride (CrCl3), potentiates the insulin action. In the presence of chromium, insulin increased the expression of insulin-like growth factor 1 (IGF-1) gene, responsible for protein synthesis, and reduced levels of ubiquitin mRNA, responsible for protein degradation (Peng et al. 2010).

Finally, the effect of chromium on obesity was observed with supplementation of niacin-bound chromium (NBC), which acted on adipose tissue by *upregulating* the expression of calsequestrin 1 (CASQ1), tropomyosin-1 (TPM1), enolase 3 (ENO3),

and glucose phosphate isomerase1 (GPI1) genes and downregulating the expression of Cell-death-induced DNA fragmentation factor (CIDEA), thermogenic uncoupled protein 1 (UCP1), and tocopherol transfer protein (TPP) genes (Rink et al. 2006). CASQ1 expressed proteins related to stocks of calcium in the sarcoplasmic reticulum of cells, which may reduce the levels of free calcium inside the cells, thus promoting better insulin signaling in fat cells (Beard et al. 2004; Lau et al. 2008). As for the protein tropomyosin, expressed by TPM1 gene, when their levels are reduced, there is an increasing differentiation of preadipocytes into adipocytes; therefore, the presence of this protein reduces the amount of fat in adipocytes (Lau et al. 2008). ENO3 and GPI 1 genes express key enzymes for glycolysis (Lau et al. 2008; Rink et al. 2006). However, CIDEA and UPC1 genes act on the increase in brown adipose tissue; in addition, a study reported that mice deficient in CIDEA are slim and have better resistance to the development of obesity and diet-induced diabetes, because it presented a higher metabolic rate and lipolysis (Lau et al. 2008; Zhou et al. 2003). TPP gene is involved in the transport of α-tocopherol that will be incorporated into LDL-c; therefore, the reduction in the expression of this gene can reduce levels of this lipoprotein. The increased expression of TPP can also increase the antioxidant defense of adipocytes, making breakage of adipose tissue (Lau et al. 2008; Rink et al. 2006).

Thus, chromium supplementation appears to have beneficial effects regarding the modulation of gene expression and liver health, obesity, and diabetes. Despite these results, further studies are needed to generate scientific evidence, since the studies already carried out were in vitro and in animals.

Policies and Protocol

According to the World Health Organization (WHO), the formulation of a guideline follows the steps to identify the issues and priority outcomes for public health, to observe and evaluate the evidence, so that recommendations and implementation for the prioritized issue solution can be formulated (WHO 2010). The WHO considers some micronutrients deficiency in specific population groups to the proposition of guidelines such as the fortification of multiple micronutrients (sachet containing iron, vitamin A, and zinc and other vitamins and minerals that the country regards necessary) to children aged between 6 and 23 months (WHO 2011) and the daily supplementation of iron and folic acid for pregnant women (WHO 2012). Thus, it appears that the health authorities recommend micronutrient supplementation, whose deficiency in risk groups is well consolidated by the scientific literature.

In the case of chromium, so far, no policies, programs, and guidelines recommend this mineral supplementation for risk groups. The evidence is insufficient to support the use of chromium to improve glycemic control in diabetic subjects. The results of the studies with chromium supplementation are conflicting and confused, due to differences in dosage, micronutrient levels achieved with the initial status of chromium supplementation, and methodologies used (Evert et al. 2013). Thus, although clinical trials are already suggesting that chromium supplementation helps in

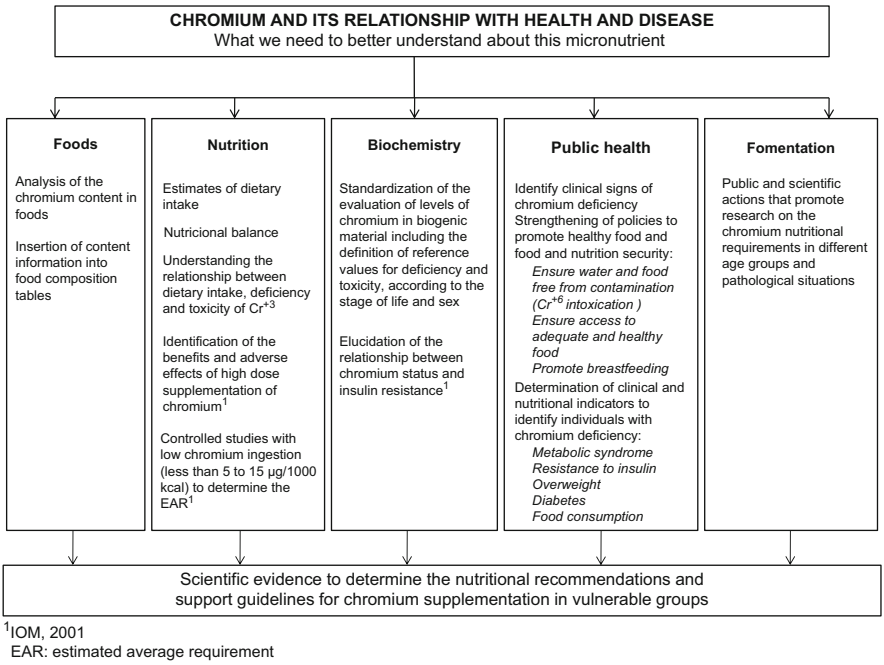

Fig. 3 Research protocol on chromium, health, and disease

controlling diabetes, obesity, and dyslipidemia, the results are still incipient (Onakpoya et al. 2013).

Therefore, chromium still does not meet the requirement of consolidated scientific evidence to develop a guideline recommending supplementation in high-risk groups. More studies are needed on the use of this mineral (Fig. 3), which evaluate the benefits and safety of use in the short, medium, and long term and showing more consistent results, so that it forms part of a policy or program of supplementation for the prevention and/or treatment of both deficiency diseases as chronic. The policies for the promotion of healthy eating and food and nutritional security can be effective initiatives for the prevention of micronutrient deficiencies, including chromium, and for coping with chronic diseases.

Dictionary of Terms

- **Nutritional requirements** – sufficient intake levels to get nutrient requirements in healthy individuals.
- **Adequate intake (AI)** – reference value used when there is insufficient information to determine Recommended Dietary Allowance (RDA).
- **Upper intake levels (UL)** – daily intake limit of the nutrient with no adverse health effects for the majority of the population.

- **Estimated Average Requirements (EAR)** – estimate of daily intake to get needs, indicator, or criteria in half of healthy individuals of a given sex or stage of the life cycle.
- **Recommended Dietary Allowance (RDA)** – daily consumption level that gets the nutrient requirement for almost all healthy individuals at a given stage of life and gender.
- **Bioavailability** – ratio of drug or nutrient concentration to concentration of drug or nutrient at the locus of action.
- **Chromodulin** – low-molecular-weight chromium-binding substance. A 1,5 kDa peptide, composed of four glycine, cysteine, glutamate, and aspartate residues, presenting a tetranuclear characteristic linked to four trivalent chromium ions, being important for insulin signaling.
- **Insulin resistance** – condition where the physiological response induced by insulin is lower than expected considering insulin concentration.
- **Modulation of gene expression** – mechanisms that regulate the synthesis of proteins or other biomolecules by controlling the transcription of genes responsible for the production of these substances.

Summary Points

- Chromium is an important nutrient in food but, because of the lack of scientific evidence, the estimated average requirement (EAR) of its daily intake and the tolerable upper intake levels (UL) have not yet been established.
- Chromium in foods has increased absorption by the presence of amino acids, ascorbic acid, high levels of carbohydrates, oxalate, and aspirin in the diet, while phytates and antacids (sodium hydrogen carbonate, magnesium hydroxide) reduce the absorption of chromium.
- Chromodulin, a low-molecular-weight chromium-binding substance, participates in the mechanism of amplification of insulin-cell signaling.
- Chromium is present in nature in different states of oxidation, with trivalent chromium and hexavalent chromium being the most common forms.
- Chromium is absorbed in the small intestine by passive transport. Due to difficulties in traversing the plasma membrane, the absorption of trivalent chromium is low.
- Hexavalent chromium has strong oxidative capacity and its absorption occurs more easily through the plasma membrane.
- Chromium is transported by ferritin and there may be competition with other metals, such as iron.
- Chromium is present in nutritional supplements in various forms such as chromium picolinate, chromium histidinate, chromium chloride, chromium nicotinate.
- Serum chromium levels vary according to glycemic control in subjects with type 2 diabetes. Serum chromium deficiency appears to be greater in uncontrolled diabetic subjects.

- Serum chromium levels decreased with age in healthy subjects.
- Although chromium is involved in several mechanisms of glucose metabolism, the evidence is not sufficient for long-term therapy in diabetic subjects.
- High levels of chromium are generally observed in individuals who are submitted to occupational exposure.
- In humans, there is sufficient evidence for the carcinogenicity of Cr^{6+} compounds. Cr^{3+} compounds, as found in foods, were not classifiable as to their carcinogenicity.
- Policies to promote healthy eating and food security and nutrition initiatives can be effective for the prevention of micronutrient deficiencies, including chromium, and for coping with chronic diseases.

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Screening for Iodine Deficiency

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Nilgün Çaylan and Gonca Yılmaz

Contents

Abstract

Iodine is an essential micronutrient for normal growth and development and essential component of the thyroid hormones synthesized in the thyroid gland. Iodine deficiency (ID) is one of the most common nutritional disorders. The global efforts to control ID have been very successful, largely because of universal salt iodization

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(USI) programs and the number of countries where ID is a public health problem has decreased over the last two decades. Initial assessment and regular monitoring of the iodine status of a population is crucial to develop optimum public health policies and to monitor the outcomes of ID control programs. For this purpose, it has been recommended that the iodine status of a population should be assessed every 3–5 years in surveys that use valid and well-described methods. Generally, four major methods are recommended for the assessment of a population's iodine status: urinary iodine concentration (UIC), total goiter rate (TGR) (assessed by palpation or ultrasound), thyroid-stimulating hormone (TSH) levels in neonates, and serum or dried blood spot (DBS) thyroglobulin (Tg). Currently, the median UIC in spot urine specimens from a representative sample is the most common measure used to assess a population's iodine status. In conclusion, UIC is the universal, well-validated, readily applicable, cost-effective indicator of population iodine status.

Keywords

Iodine · Iodine Deficiency · Iodine Deficiency Disorders · Iodine Status · Universal Salt Iodization · Screening · Indicator · Monitoring · Urinary Iodine Concentration · Total Goiter Rate · Thyroid- Stimulating Hormone · Thyroglobulin

List of Abbreviations

CH	Congenital hypothyroidism
DBS	Dried blood spot
EFSA	European Food Safety Authority
ICCIDD	International Council for Control of Iodine Deficiency Disorders
ID	Iodine deficiency
IDD	Iodine deficiency disorders
SAC	School-age children
T3	Tri-iodothyronine
T4	Thyroxine
Tg	Thyroglobulin
TGR	Total goiter rate
TSH	Thyroid-stimulating hormone
USI	Universal salt iodization
UIC	Urinary iodine concentration
UNICEF	United Nations Children's Emergency Fund
WHO	World Health Organization

Introduction

Iodine deficiency (ID) is one of the most common nutritional disorders and, yet, it is an easily preventable cause of mental impairment worldwide (Zimmermann 2008). This mental impairment has an immediate effect on children's learning capacity, women's health, the quality of life of communities, and economic productivity

(World Health Organization (WHO) et al. 2007). Pregnant women, newborns, infants, and young children are sensitive to the effects of ID. Health consequences of ID including endemic goiter, cretinism, intellectual impairments, growth retardation, neonatal hypothyroidism, increased pregnancy loss, and infant mortality are collectively termed as iodine deficiency disorders (IDD) (WHO et al. 2007; Zimmermann 2009).

It is estimated that approximately one-third of the world's population lives in iodine-deficient areas (Andersson et al. 2012). ID is particularly common in countries in the Eastern Mediterranean region, Asia, Africa, and large parts of Eastern Europe (Ramakrishnan 2002). Although the populations in inland areas, especially mountainous areas such as the Alps, the Himalayas, and the Andes, are particularly vulnerable to having high levels of ID, ID can also occur in coastal areas and on islands (Zimmermann 2009).

In 1991, the World Health Assembly adopted the goal of eliminating ID worldwide as a public health problem. In order to prevent and treat IDD globally, in 1993, universal salt iodization (USI) was recommended by the United Nations Children's Emergency Fund (UNICEF) and the WHO (UNICEF-WHO 1994). This strategy has been approved and implemented in most countries where ID is a public health problem, and elimination of IDD has been an essential part of many nutritional intervention programs (Zimmermann 2008). The global efforts to control ID have been very successful, largely because of USI programs, in line with the recommendations by the WHO and the International Council for Control of Iodine Deficiency Disorders (ICCIDD) (WHO et al. 2007) (Fig. 1). The number of countries where ID is a public health problem has decreased over the last two decades, making the control of ID a major public health success. Despite the changes in data coverage,

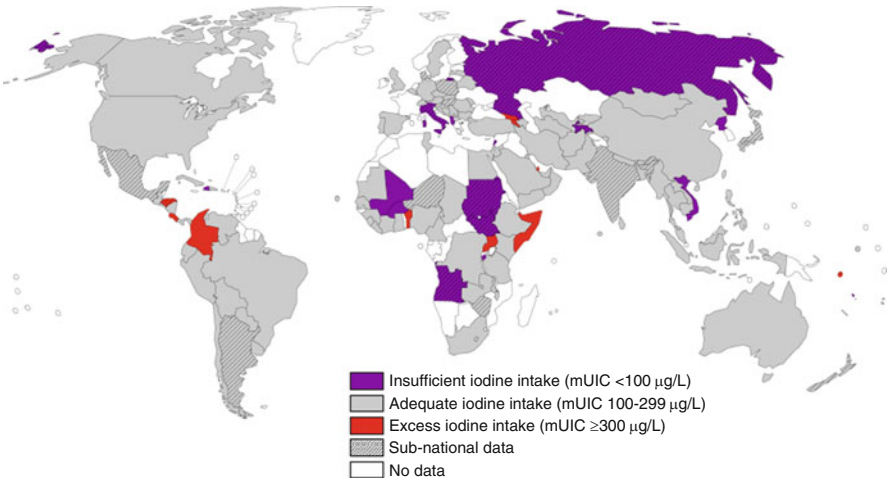

Fig. 1 Global iodine nutrition in 2016 based on median urinary iodine concentrations in school-age children. *mUIC* median urinary iodine concentration (Source: http://www.ign.org/cm_data/2016_SAC.pdf, Reproduced with permission)

Table 1 Changes in number of countries in iodine status from 1993 to 2016

Iodine intake	1993	2003	2007	2011	2016
Insufficient intake	113	54	47	32	15
Sufficient intake	8	67	76	105	102
Excessive intake	–	5	7	11	10
Countries with data	121	126	130	148	127
No data	62	66	63	45	67

Sources: Andersson et al. 2005, 2012; de Benoist et al. 2008; Gizak et al. 2017; http://www.ign.org/scorecard.htm

from 1993 to 2016, the number of iodine-deficient countries decreased from 103 to 15, and the number of iodine-sufficient countries increased from 8 to 102 (Andersson et al. 2005; de Benoist et al. 2008; Andersson et al. 2012; Gizak et al. 2017) (Table 1). However, these achievements are fragile and sustainability is absolutely critical for ID control programs. As ID is a nutritional deficiency that is primarily the result of permanent ID in soil and water, when control programs are interrupted, ID can return at any time after their elimination (Li et al. 2006).

Iodine Cycle and Dietary Considerations

Iodine is an essential micronutrient and it is obtained solely via exogenous dietary sources. As distinct from other essential dietary nutrients, iodine status is not linked to socioeconomic development but rather to geographical location (Rohner et al. 2014). Iodine (as iodide) is widely but unequally distributed across the earth's environment. Most of the iodide is found in the oceans. Iodide ions in seawater are converted into elemental iodine, a volatile form that is evaporated into the atmosphere, and the iodine is returned to the soil by precipitation. However, in many regions, the iodine cycle is slow and incomplete. Additionally, many other factors including geological formation, glaciation, flooding, and soil erosion impact the natural iodine content of the soil. As a result, the iodine in soil and groundwater becomes insufficient (WHO et al. 2001). The iodine content of local foods is dependent on the soil and water iodine content, and therefore, low iodine concentrations in the soil and water result in an iodine-deficient biological environment (Rohner et al. 2014). ID in populations residing in these areas will persist until iodine enters the food chain via foods produced in iodine-sufficient regions or until the addition of iodine to foods (the methods of proven value for mass use are iodized salt and iodine supplementation) (Zimmermann 2009). The natural iodine content of most foods and beverages is low. In general, commonly consumed foods provide 3–80 μg per serving (Haldimann et al. 2005). Foods of marine origin have higher iodine content because marine plants and animals concentrate iodine from seawater. The main sources of dietary iodine are iodized salt, saltwater fish, seaweed, and grains (Zimmermann 2009).

Table 2 Recommendations for daily iodine intake by age and population group

Age/population group	Iodine intake (μg per day)
Children aged 0–59 months	90
Children aged 6–12 years	120
Adolescents (above 12 years)	150
Adults	150
Pregnancy	250
Lactation	250

Source: Reproduced from reference WHO et al. 2007 with permission

Table 2 shows the UNICEF, ICCIDD, and WHO recommendations regarding iodine requirements by age group and for pregnant and lactating women (WHO et al. 2007). As shown in Table 2, the daily iodine requirement during pregnancy increases from 150 μg to 250 μg due to (1) the increase in maternal thyroid hormone production to maintain maternal euthyroidism, (2) transfer of thyroid hormones and iodine to the fetus to cover its needs, and (3) the increase in renal iodine clearance (Glinoer 1997, 2006).

ID is the main cause of endemic goiter, but other substances termed "goitrogens" can interfere with thyroid metabolism and potentiate the effect of ID (Vanderpas 2006). Most goitrogens can cause a major clinical effect when there is coexisting ID (Zimmermann 2008). Iodine absorption can be reduced by the presence of goitrogens in certain foods (including cabbage, broccoli, cassava, lima beans, sweet potato, soy, and millet) and by deficiency in other micronutrients (such as selenium and iron) (Köhrle 1999; Zimmermann 2010). Industrial pollutants, such as perchlorate and disulfides from coal processes, are also important goitrogens. In infants, particularly during periods of exclusive breastfeeding, thyroid function depends solely on iodine in the maternal milk. Maternal smoking during breastfeeding is associated with reduced iodine concentrations in breastmilk, and it dose-dependently reduces breastmilk iodine content up to about half of the normal content and, consequently, may expose the infant to an increased risk of ID (Lauberg et al. 2004).

Iodine Biology

Iodine is an essential component of the thyroid hormones tri-iodothyronine (T3) and thyroxine (T4) synthesized in the thyroid gland. Thyroid hormones, and therefore iodine, are crucial for the regulation of metabolism and for normal physical and neurological development (Zimmermann 2009). The human body contains ~15–20 mg of iodine, of which 70–80% is concentrated in the thyroid gland (WHO 2004). To maintain the normal concentration of iodine in the body, humans require exogenous sources in any of the following chemical forms:

- Iodide, which is rapidly and almost completely (>90%) absorbed in the intestine
- Iodate, which is widely used in salt iodization and is reduced in the gut and absorbed as iodide
- Organically bound iodine (Alexander et al. 1967)

Like iodate, dietary iodine is converted into iodide in the gut lumen, and, as mentioned above, >90% is rapidly absorbed in the stomach and upper small intestine. Fifteen percent of ingested iodine is retained by the thyroid gland (European Food Safety Authority (EFSA) 2006). A sodium/iodide symporter transfers iodide into the thyroid at a concentration gradient of 20–50 times the concentration in the plasma (Eskandari et al. 1997). In individuals with chronic ID, the fraction of iodine absorbed by the thyroid can exceed 80% (Wayne et al. 1964). The half-life of plasma iodine is about 10 h, but this time is reduced in individuals with ID.

The thyroid gland uses iodine for the synthesis of the hormones T4 and T3. T3, the active hormone, is essential for the maintenance of the body's metabolic rate (as it helps to control energy production and oxygen consumption by cells), normal growth, and neural and sexual development (Larsen et al. 1981). T4 is converted to T3 in peripheral tissues by deiodinases (by removing an outer-ring iodine from the T4 molecule). More than 99% of the T3 and T4 circulating in the blood is bound to proteins, and it is only the small amount of free (unbound) thyroid hormone that is bioactive (Rohner et al. 2014). The iodine released when T4 is converted to T3 enters the plasma iodine pool and can it be taken up again by the thyroid or excreted by the kidney. Approximately 90% of ingested iodine is ultimately excreted in the urine (Zimmermann 2008). Thyroglobulin (Tg) is a key precursor in the production of thyroid hormones. The production and release of thyroid hormones are regulated by thyroid-stimulating hormone (TSH), which is released from the pituitary gland into the circulation. This regulation is maintained by feedback inhibition (Ristic-Medic et al. 2009).

Elimination of ID and the Biomarkers to Assess Population Iodine Status

In nearly all countries, the best strategy to control ID is iodization of table salt because salt is widely available and consumed in regular amounts throughout the year, and the cost of salt iodization is quite affordable (WHO et al. 2007; WHO 2014a). When access to iodized salt is inadequate or absent in iodine-deficient areas, temporary iodine supplementation should be considered to ensure optimal iodine intake among vulnerable groups (WHO Secretariat et al. 2007). Giving priority (regarding iodine supplementation) to pregnant and lactating women should be considered until the salt iodization program is scaled up. Additionally, for infants aged 7–24 months, either iodine supplementation or the use of iodine-fortified complementary foods could be considered as temporary public health measures

(WHO et al. 2007). However, in countries or regions in which USI program covers >90% of households and has been sustained for =>2 years, and the monitoring studies indicate iodine sufficiency in the population (within last five years), pregnant and lactating women do not require iodine supplementation (WHO Secretariat et al. 2007; WHO et al. 2007; Zimmermann 2010).

There are three major components of a sustainable program to eliminate IDD:

- Political will and support
- Administrative and legislative arrangements
- Baseline assessment and monitoring systems (WHO et al. 2001, 2007)

Initial assessment and regular monitoring of the iodine status of a population is crucial to develop optimum public health policies and to monitor the outcomes of ID control programs (WHO et al. 2007). For this purpose, it has been recommended that the iodine status of a population should be assessed every 3–5 years in surveys that use valid and well-described methods (WHO Secretariat et al. 2007; WHO et al. 2007).

The most frequently assessed population group that is used to indicate the iodine status of a population is school-age children (SAC), as this group is easy to survey and the iodine status of SAC usually reflects the iodine status of the general population. When SAC are not available for surveys, preschool children and other populations may also be used for population surveys (WHO et al. 2007).

Generally, four major methods are recommended for the assessment of a population's iodine status: urinary iodine concentration (UIC), total goiter rate (TGR) (assessed by palpation or ultrasound), TSH levels in neonates, and serum or dried blood spot (DBS) Tg (WHO et al. 2007) (Table 3). These indicators are complementary because changes in the TGR reflect long-term iodine nutrition (months to years), UIC is a sensitive indicator of recent iodine intake (days), and Tg is associated with an intermediate response to iodine intake (weeks to months) (Zimmermann 2009, 2010).

Urinary Iodine Concentration

UIC is a sensitive, widely accepted, readily applicable, and cost-effective indicator of a population's iodine status. As >90% of ingested iodine is excreted in the urine, UIC is considered a sensitive marker of recent dietary iodine intake, and it is often used for monitoring iodine interventions (Gibson 2005; Nicola et al. 2009). Although UIC does not provide direct information on thyroid function, a low value indicates that a population is at higher risk of developing thyroid disorders (Zimmermann 2010). UIC can be expressed as a concentration (μg/L), in relation to creatinine excretion (μg iodine/g creatinine), or in terms of a 24-h excretion rate (μg/day). An individual's UIC can vary diurnally and day-to-day. Therefore, UIC is not recommended for the diagnosis and monitoring of individuals. However, the variation tends to even out across populations, so UIC can provide a useful measure of the

Table 3 Summary of indicators of iodine status by age and population group

Biomarker	Age group	Advantages	Disadvantages	Rationale/recommendation	Assessment
Median urinary iodine concentration	School-age children (aged 6–12 years) Pregnant women	Spot urine samples are easy to collect Low cost Cut-off points for classifying population iodine status are available Iodine status categories are well established External quality-control program in place	Assesses iodine intake only over the past few days Meticulous laboratory practice is required to avoid contamination with iodine A sufficiently large number of samples must be collected to allow for varying degrees of subject hydration and other biological variation among individuals Not useful for individual assessment	As >90% of ingested iodine appears in the urine, UIC is considered a sensitive marker of recent dietary iodine intake The median UIC from a representative sample of spot urine collections from different population groups is the most common way to classify a population's iodine status	Table 4
Goiter rate assessed by palpation	School-age children	Simple and rapid screening test Requires no specialized equipment	Responds slowly to changes in iodine repletion (months to years) High interobserver variation Specificity and sensitivity are low	Goiter can be used as a baseline assessment of iodine status and as a long-term indicator of the success of an ID elimination program	Table 5 Table 6
Goiter rate assessed by ultrasound	School-age children	More quantitative measurement than palpation Safe and non-invasive International reference values for thyroid volume in schoolchildren are available	Expensive equipment and a source of electricity is needed A specially trained operator is necessary Responds slowly to changes in iodine repletion (months to years)	Goiter can be used as a baseline assessment of iodine status and as a long-term indicator of the success of an ID elimination program	Table 6

TSH	Newborns	Measures thyroid function at a vulnerable age Low costs if a newborn screening program is in force Sample collection and storage on filter paper is simple Blood spots can be stored for several weeks in cool, dry conditions at room temperature	Requires a standardized, sensitive assay Not useful if iodine antiseptics are used during delivery Sampling time should be at least 48 h after birth	TSH is a sensitive indicator of iodine status in newborns For countries and regions that already have a system of neonatal screening in place, the data can be analyzed	A frequency in a population of <3% of individuals with TSH >5 mIU/L Indicates iodine sufficiency
Thyroglobulin	School-age children	Sample collection and storage on filter paper is simple Blood spots can be stored for several weeks in cool, dry conditions at room temperature Can measure improvement of thyroid function within weeks to months after iodine repletion An international reference range has been established	Expensive immunoassay Requires laboratory infrastructure Standard reference material is available, but there is wide interassay variation	Measurement of DBS Tg in school-age children is a sensitive indicator of iodine status in a population and can be used to monitor improving thyroid function after iodine repletion	The Tg reference interval for iodine-sufficient school-age children is 4–40 μg/L

Source: WHO et al. 2007; Reproduced with permission
DBS dried blood spot, *ID* iodine deficiency, *TSH* thyroid-stimulating hormone, *Tg* Thyroglobulin, *UIC* urinary iodine concentration

iodine status of a population. As UICs tend not to be normally distributed, the median is the preferred measure of central tendency (WHO et al. 2007). As it is impractical to collect 24-h samples in population studies, UIC can be measured in spot urine specimens from a representative sample of the target group, and expressed as the median, in μg/L (WHO et al. 2007).

In 1990, UIC was recommended as an effective biochemical indicator of a population's iodine status, and a median UIC of ≤50 μg/L was established as the cut-off value for ID (WHO et al. 1990). Thereafter, in a consultation held by the WHO in 1992, the cut-off value for ID was raised to a median UIC of <100 μg/L, and cut-off values were defined for classifying ID as mild, moderate, or severe (WHO et al. 1994). Importantly, all these cut-off values are based on the median UIC among SAC (WHO 2013).

Table 4 Epidemiological criteria for assessing iodine nutrition based on median urinary iodine concentrations by age group

Age/population group Median urinary iodine concentration (μg/L)	Iodine intake	Iodine status
School-age children		
< 20	Insufficient	Severe ID
20–49	Insufficient	Moderate ID
50–99	Insufficient	Mild ID
100–199	Adequate	Adequate iodine nutrition
200–299	Above requirements	May pose a slight risk
≥300	Excessive	Risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid disease)
Pregnant women		
<150	Insufficient	
150–249	Adequate	
250–499	Above requirements	
>500	Excessive	
Lactating women[a]		
<100	Insufficient	
≥100	Adequate	
Children aged < 2 years		
<100	Insufficient	
≥100	Adequate	

Source: WHO et al. 2007; WHO Secretariat et al. 2007; Zimmermann et al. 2008. Reproduced with permission.
ID iodine deficiency
[a]For lactating women, the figures for the median urinary iodine concentration are lower than the iodine requirements because of the iodine excreted in breastmilk

Iodine requirements are greatly increased during pregnancy and lactation due to metabolic changes. During pregnancy, the transfer of T4 and iodine from the mother to the fetus is essential for fetal brain development and thyroid function (Glinoer 1997). During lactation, the rate of production of thyroid hormones and urinary iodine excretion returns to normal. However, iodine requirements remain elevated, as the iodine is concentrated in the mammary glands for excretion in breastmilk (WHO Secretariat et al. 2007). Additionally, children <2 years of age are also at a high risk of ID because of their continuing high iodine demands, as high levels of iodine are necessary to support brain and thyroid development (UNICEF 2008). Therefore, the cut-off values for assessing iodine nutrition based on the median UIC in vulnerable populations such as pregnant and lactating women and children <2 years of age are different from those of the general population (WHO et al. 2007; WHO Secretariat et al. 2007). Despite this, UIC cut-offs for vulnerable groups are still in need of validation using functional biomarkers in field studies (Rohner et al. 2014). Epidemiological criteria for assessing iodine nutrition based on the median UIC in different age groups and pregnant and lactating women are presented in Table 4.

Thyroid Size and Total Goiter Rate

The thyroid gland is not visible when the neck is in a normal position, and the lateral lobes are often barely palpable on both sides of the trachea. The term "goiter" refers to a thyroid gland that is enlarged (Perez et al. 1958). Goiter is an adaptive process that occurs in response to low iodine intake, and chronic ID is the primary cause. However, goiter is sometimes the result of a combination of low iodine intake along with additional goitrogenic factors. When dietary iodine intake is insufficient for thyroid hormone synthesis, serum T4 decreases. This stimulates the pituitary gland, which releases more TSH. As a result, TSH induces the growth and metabolic activity of the thyroid follicular cells, leading to the enlargement of the thyroid gland (Zimmermann and Andersson 2012).

Goiter is often a reflection of chronic ID, and the level of goiter in a population can be used as a baseline assessment of a region's iodine status and as a sensitive long-term indicator of the success of an ID elimination program. However, changes in goiter prevalence lag behind changes in iodine status and, therefore, goiter cannot be relied upon to accurately reflect current iodine intake (WHO et al. 2007).

Two methods are available for assessing goiter: neck inspection with palpation and thyroid ultrasonography. Assessment of thyroid size by inspection and palpation is the time-honored method of assessing the prevalence of IDD. Ultrasonography provides a more precise and objective method, for grading goiter (WHO et al. 2007). Although the highest prevalence of goiter occurs during puberty and childbearing age, SAC is the preferred group for goiter surveys, as it is easily accessible. Goiter is also common and responds well to the iodine replacement in this age group (WHO et al. 2007; Zimmermann and Andersson 2012).

Table 5 Classification of goiter by palpation

Grade	Examination findings
0	No palpable or visible goiter
1	A goiter that is palpable but not visible when the neck is in a normal position. Thyroid nodules in a thyroid that is otherwise not enlarged fall into this category
2	A swelling in the neck that is clearly visible when the neck is in a normal position and which is consistent with an enlarged thyroid when the neck is palpated

Source: WHO et al. 2001. Reproduced with permission

Table 6 Epidemiological criteria for evaluating the severity of ID based on the prevalence of goiter in SAC

	Degree of ID, expressed as a percentage of the total number of children surveyed			
Indicator	None	Mild	Moderate	Severe
TGR by palpation	0.0–4.9	5.0–19.9	20.0–29.9	≥30
Thyroid volume > 97th Percentile (as assessed by ultrasonography)	0.0–4.9	5.0–19.9	20.0–29.9	≥30

Source: WHO et al. 2007; WHO 2014b. Reproduced with permission
ID iodine deficiency, *SAC* school-age children, *TGR* total goiter rate

Assessing Thyroid Size by Palpation

When using the palpation method, a goiter is diagnosed when "each of the lateral lobes of the thyroid gland has a volume greater than the terminal phalanx of the thumb of the person being examined" (Perez et al. 1958). The palpation method is quite simple and, since 1960, it has been used in most epidemiological studies of endemic goiter (WHO et al. 2007). However, the palpation method has several limitations including high interobserver variation and reduced sensitivity and specificity, so the use of ultrasound is necessary if increased diagnostic precision is required (Delange et al. 2001; Zimmermann et al. 2003).

Goiter assessed by inspection and palpation is graded according to the classification system presented in Table 5. The prevalence of goiter can be quantified using the TGR, which is equivalent to the number of goiters of grades 1 and 2 detected in a population divided by the total number of individuals examined (WHO et al. 2007). A TGR >5% among SAC is recommended by the WHO as an indicator of a public health problem (WHO et al. 2007). Table 6 shows the epidemiological criteria for assessing ID severity based on the prevalence of goiter among SAC.

Assessing Thyroid Size by Ultrasonography

The introduction of the use of ultrasonography to visualize the thyroid gland has been an important development. Ultrasonography is a safe, noninvasive, and specialized technique that can be used to measure thyroid volume more precisely and

objectively than palpation (WHO et al. 1994, 2007). It does not take much time (2–3 min per subject) and is feasible (using portable equipment) even in remote areas (WHO et al. 2007).

The major disadvantages of this method are its high cost, the need for well-trained/experienced technicians, and the inter-rater variability in its application and interpretation (Zimmermann and Andersson 2012). The thyroid is visualized transversely and longitudinally in order to determine the gland's dimensions, and these measurements are used to calculate the thyroid volume (WHO et al. 2007). International thyroid volume reference values for goiter screening have been established (as a function of age, sex, and body surface area) among SAC (Zimmermann et al. 2004). Table 6 shows the epidemiological criteria for assessing ID severity based on the TGR in terms of a thyroid volume > 97th percentile (as assessed using ultrasonography) among SAC.

Thyroid Stimulating Hormone

TSH is secreted by the anterior pituitary gland, and it regulates thyroid hormone synthesis and secretion. Testing for serum TSH is the principal screening test for thyroid dysfunction. Serum TSH concentrations increase when thyroid hormone concentrations are low (hypothyroidism) and decrease when thyroid hormone concentrations are high (hyperthyroidism) (Rohner et al. 2014).

In newborns, the thyroid has smaller iodine stores and a higher iodine turnover than in adults. This high turnover, which is increased in newborns with ID and in fetuses with mild ID, can compromise the neonatal thyroidal secretion of T4, causing increased TSH secretion by the pituitary gland. Hence, in iodine-deficient populations, TSH levels are increased among newborns in the first few weeks of life, a condition known as transient hyperthyrotropinemia (Zimmermann 2010). This fact underpins the use of newborn TSH screening for indicating a population's iodine status. The neonatal period is the only setting in which TSH can provide a useful index of population iodine status (Delange 1997; Zimmermann 2010). Moreover, newborn TSH screening is an important test because it reflects iodine status during a period when the developing brain is particularly sensitive to ID. In older children and adults, although serum TSH may be slightly increased by ID, values are usually in normal range. Therefore, TSH is an insensitive marker for detection of ID in older children and adults, and its routine use in school-based surveys is not recommended (WHO et al. 2007).

When a sensitive TSH assay is used on samples collected 3–4 days after birth, the prevalence of neonatal TSH levels >5.0 mIU/L should be <3% for a region to be classed as an iodine-sufficient region (Zimmermann et al. 2005). In contrast, the TSH levels among other age groups cannot be used to discriminate between iodine-sufficient and iodine-deficient populations (Rohner et al. 2014).

In most developed countries, routine neonatal TSH screening is carried out to detect congenital hypothyroidism (CH) (Pearce and Caldwell 2016). After the pioneering studies were conducted, in addition to its role in diagnosing individual

neonates with CH, neonatal TSH screening was recommended for monitoring population iodine status (Thilly et al. 1978; Kochupillai et al. 1986; Delange 1998, 1999). Subsequently, neonatal TSH screening started to be used as a tool to assess the severity of ID and monitor the outcomes of iodine prophylaxis programs in countries and subnational regions. Although conflicting results have been reported by some studies, especially regarding appropriate cut-off values (Rajatanavin 2007; Burns et al. 2008), many countries have developed successful models (Tylek-Leman'ska et al. 2003; Zimmermann et al. 2005; Gyurjyan et al. 2006; Charoensiriwatana et al. 2008). In Thailand, the use of a geographic information system in the neonatal TSH screening program has enabled the identification of ID at the subdistrict level (Charoensiriwatana et al. 2008). A study conducted in Poland indicated that with the reintroduction of iodized salt in 1992, the prevalence of neonatal TSH levels >5 mIU/L dropped from above 20% in 1991 to just above 5% between 1995 and 2000 (Tylek-Leman'ska et al. 2003). Another study conducted in Switzerland demonstrated that a 25% increase in the concentration of iodine in iodized salt resulted in a reduction of the prevalence of neonatal TSH levels >5 mIU/L from 2.9% to 1.7%, and the iodine status among children and pregnant women has increased from "borderline" to "sufficient" (Zimmermann et al. 2005).

However, the use of neonatal TSH as a biomarker has several limitations. Factors other than ID (including prematurity, birth weight, mode of delivery, maternal or newborn exposure to iodine-containing antiseptics, type of collection paper used for the blood spot, and TSH assay methodology) can affect newborn TSH levels and lead to misinterpretation of the results (Li and Eastman 2010). Evidence shows that the mean serum TSH level sampled from neonates less than 24 h after birth is significantly higher than that sampled from neonates after the first 24 h (Lott et al. 2004). Thus, as a result of the physiological surge in serum TSH in the first 24 h after birth, the appropriate sampling time point for CH screening is between 2 and 4 days after birth (American Academy of Pediatrics et al. 2006).

In conclusion, when a neonatal TSH screening program is in force, the data can be used to assess iodine status at the population level (WHO et al. 2007). However, the implementation of a neonatal TSH screening program requires technical experience and financial resources. Therefore, using this type of program solely to assess community iodine status is not cost-effective and therefore not recommended (Delange 1999; WHO et al. 2001; Ristic-Medic et al. 2009).

Thyroid Hormone (T4 and T3) Concentrations

Elevated serum TSH combined with normal serum T4 and T3 is termed subclinical hypothyroidism, while unchanged or increased serum T3 and TSH combined with decreased serum T4 is termed overt hypothyroidism, which tends to occur in iodine-deficient populations (Delange et al. 1972). An increased T3 concentration is an adaptive response of the thyroid to ID (while, in contrast, fasting and malnutrition are associated with low T3 concentrations). Among individuals with

hypothyroidism, T3 concentrations may not decrease until the disease has progressed to a great extent, because increased TSH concentrations stimulate T3 release from the thyroid (Rohner et al. 2014). Usually, these changes are within the normal range, and the overlap between thyroid hormone concentrations in iodine-deficient and iodine-sufficient populations is large enough to make thyroid hormone concentrations poor indicators of iodine status (WHO et al. 2001, 2007; Zimmermann 2009). Serum concentrations of the thyroid hormones are not recommended as an indicator of population iodine status due to impracticability, high cost, and less sensitivity (WHO et al. 2007).

Thyroglobulin Concentration

Tg is a highly abundant intrathyroidal protein comprising two 330-kDa protein chains, and it is synthesized only in the thyroid gland (van de Graaf et al. 2001). When iodine intake is sufficient, small amounts of Tg are secreted into the circulation, and serum Tg is normally <10 μg/L (Spencer and Wang 1995). The thyroid hyperplasia and goiter that are characteristic of ID both increase the amount of Tg that is released into the blood (in response to TSH stimulation) (WHO et al. 2007; van de Graaf et al. 2001).

Tg can be measured in serum or dried blood spots (DBSs). The implementation of DBS technology is a significant development, as it makes sampling practical even in remote and resource-poor regions (Zimmermann et al. 2006). The measurement of DBS Tg among SAC is recommended as a sensitive indicator of the iodine status of a population, and DBS Tg can be used to monitor improvements in thyroid function after iodine repletion (WHO et al. 2007).

An international reference range for DBS Tg was established in a multicenter study of healthy 5- to 14-year-old children that can be used for monitoring iodine status (Zimmermann et al. 2006). According to this study, the DBS Tg reference range for iodine-sufficient SAC is 4–40 μg/L (Zimmermann et al. 2006; WHO et al. 2007). This is similar to the reference range for adults, which is reported to be 3–40 μg/L (Baloch et al. 2003). In 1994, the WHO, ICCIDD, and UNICEF proposed that a median Tg concentration < 10 μg/L could be used to indicate adequate iodine nutrition among SAC. In another cross-sectional study, reference ranges for DBS Tg were established among 6- to 12-year-old primary SAC in 12 countries (Zimmermann et al. 2013). On the basis of the findings of this study, Tg was proposed as a sensitive indicator of both low and excess iodine intake. It was recommended that a median Tg level < 13 μg/L and/or <3% of Tg levels >40 μg/L could be used to represent iodine sufficiency among primary SAC (Zimmermann et al. 2013). Tg reference ranges for population iodine sufficiency have not been established for other age groups or pregnant women (Pearce and Caldwell 2016).

Tg holds promise as a biomarker for ID, and it is increasingly being used as an index of population iodine status (Ma and Skeaff 2014). In prospective studies, DBS Tg has been shown to be a sensitive measure of iodine status, and it reflects intermediate response (weeks to months) iodine repletion (Zimmermann et al. 2006). However, there are some limitations associated with the use of Tg, and

several questions need to be resolved before Tg can be widely adopted as an indicator of iodine status (Zimmermann 2008). One disadvantage of the use of Tg as a biomarker of iodine status is the fact that it cannot be reliably measured in people with detectable anti-Tg antibodies (Spencer and Wang 1995). In addition, despite the development of a standard reference material for serum Tg, laboratory assay results for this biomarker remain highly method dependent (Schlumberger et al. 2007).

Policies and Protocols

The elimination of IDD is an important developmental indicator, and it should be a priority for governments and international agencies. In order to prevent, treat, and eliminate IDD, USI remains the key strategy recommended by the WHO, UNICEF, and ICCIDD. Moreover, national policies regarding salt iodization and the reduction of salt intake to <5 g/day are both essential, and they should be implemented with close collaboration. An effective ID control program should involve the following processes (as recommended by the WHO):

- Baseline assessment of the population iodine status
- Dissemination of findings
- Planning
- Achieving political will
- Implementation
- General program monitoring and evaluation

Sustainability is a critical issue in ID control programs. To be sustainable, programs must have an effective monitoring system that ensures that basic information is regularly collected regarding the iodization of salt and the population iodine status. It is recommended that population iodine status is assessed regularly in surveys that use universally accepted, practical, and cost-effective methods. Additionally, specific target groups (including pregnant women, nursing mothers, and newborns) should not be neglected, and so they should be included in the monitoring studies. UIC is a sensitive and universal indicator of iodine status in SAC and pregnant women. Also, serum or DBS Tg values can be used as population indicator of iodine status in SAC. For countries and regions that already have a system of neonatal TSH screening in place, neonatal TSH data can be analyzed as a supplementary assessment method (in addition to regular UIC screening), as the only additional costs incurred would be for the data analysis.

Dictionary of Terms

- **Goiter** – A thyroid gland that is enlarged.
- **Iodine** – A natural chemical element of the earth. It is an essential micronutrient for normal growth and development and essential component of the thyroid hormones produced by the thyroid gland.

- **Iodine Deficiency** – One of the most common nutritional disorders.
- **Iodine deficiency disorders** – Collective terminology of health consequences of ID including endemic goiter, cretinism, intellectual impairments, growth retardation, neonatal hypothyroidism, increased pregnancy loss, and infant mortality.
- **Screening** – An important concept in public health medicine and application of a specific diagnostic procedure in a population to detect asymptomatic disease.
- **Thyroglobulin** – Intrathyroidal protein synthesized only in the thyroid gland.
- **Thyroid-stimulating hormone** – Secreted by the anterior pituitary gland and it regulates thyroid hormone synthesis and secretion.
- **Thyroxine (T4) and tri-iodothyronine (T3)** – Thyroid hormones produced by the thyroid gland.
- **Total Goiter Rate** – Equivalent to the number of goiters of grades 1 and 2 (assessed by palpation) or thyroid volume $>$ 97th percentile (assessed by ultrasonography) detected in a population divided by the total number of individuals examined.
- **Universal Salt Iodization** – Iodization of all salt for human and animal consumption according to the internationally agreed recommended levels.
- **Urinary Iodine Concentration** – A sensitive indicator of recent iodine intake (days).

Summary Points

- ID is one of the most common nutritional disorders and, yet, it is an easily preventable cause of mental impairment worldwide.
- The global efforts to control ID have been highly successful, largely due to USI programs.
- In order to prevent and treat IDD, the best strategy has been the iodization of salt because it is widely available, consumed in regular amounts throughout the year, and also cost-effective.
- Baseline assessment and regular monitoring of population iodine status is crucial for effective surveillance of USI programs.
- The iodine status of populations should be assessed in surveys conducted every 3–5 years using valid and well-described methods.
- Four major methods are recommended to assess and monitor the iodine status of a population: UIC, total goiter rate (by palpation or ultrasound), serum Tg, and TSH levels in neonates.
- As $>$90% of ingested iodine is excreted in the urine, UIC is considered a sensitive biomarker of recent dietary iodine intake.
- Currently, the median UIC in spot urine specimens from a representative sample is the most common measure used to assess a population's iodine status.
- The median UIC is also recommended for assessing the iodine status of pregnant women (using specific cut-off values).
- TSH is a sensitive indicator of iodine status in newborns.

- For countries and regions that already have a system of neonatal TSH screening in place, as a supplementary assessment method, the data can be used to assess iodine status at the population level.
- Tg holds promise as a biomarker for ID, and it is increasingly being used as an index of population iodine status.
- The measurement of DBS Tg among SAC is recommended as a sensitive indicator of iodine status in a population, and DBS Tg can be used to monitor improvement in thyroid function after iodine repletion.
- In conclusion, UIC is the most universal, well-validated, readily applicable, cost-effective, and recommended indicator of population iodine status (including improvements in the iodine status after iodine repletion).

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Effects of Iron Deficiency on the Oropharyngeal Region: Signs, Symptoms, and Biological Changes

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Preeti Tomar Bhattacharya and Satya Ranjan Misra

Contents

Abstract

Iron is the most abundant essential trace element inside human body. Iron deficiency anemia is the most common nutritional deficiency worldwide. It reportedly causes reduction in working capacity, hampers mental development in growing children and young adults, and significantly worsens the quality of daily life. Nonpregnant women in reproductive age group and pregnant women are equally vulnerable to iron deficiency. Apart from systemic signs and symptoms

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like pallor, fatigability, palpitations, koilonychia, light headedness, and shortness of breath, oropharyngeal region can also provide important diagnostic clues to this systemic condition. Moreover, other oral conditions such as aphthous ulcers and burning mouth syndrome; premalignant conditions such as Plummer Vinson syndrome, oral lichen planus, and oral submucous fibrosis; and malignant disease such as oral squamous carcinoma have been associated with iron deficient state. This review appraises various signs and symptoms encountered within oropharyngeal region in the iron deficient state and underlying biological changes responsible for those clinical manifestations.

Keywords

Anemia · Atrophic · Cancer · Candidiasis · Cheilitis · Glossitis · Iron Deficiency · Oropharyngeal · Premalignant · Stomatitis · Syndrome

List of Abbreviations

ASHA	Accredited social health activist
BMS	Burning mouth syndrome
DNA	Deoxyribonucleic acid
Govt. & Govt. Aided	Government and government aided
IDA	Iron deficiency anemia
IFA	Iron/Folic acid
NFHS-3	National Family Health Survey-3
NRHM	National Rural Health Mission
OLP	Oral lichen planus
OSCC	Oral squamous cell carcinoma
OSMF	Oral submucous fibrosis
PVS	Plummer Vinson syndrome
RBC	Red blood cells
SLA	Sex-linked anemic
TfR	Transferrin receptor
WHO	World Health organization

Introduction

Iron is the most abundant essential trace element in the body and it is critical for the growth and differentiation of all cells. Around 3–5 g of iron is stored in the body with majority being in blood and rest in liver, bone marrow, and muscles in the form of heme. About 1–2 mg of iron is lost every day, through skin, enteric desquamation and minor blood losses. Intestinal absorption balances this loss. Iron plays an imperative role in oxygen transport, electron transfer, and serves as a cofactor in many enzyme systems, such as peroxide-generating enzymes and nitrous oxide-generating enzymes that are vital for immune cells to function normally (Bhattacharya et al. 2016b).

The status of iron inside body can vary from iron overload to normal iron status, to iron deficiency with no apparent signs, and finally to iron deficiency with anemia (World Health Organization recommendations 2001).

Iron deficiency can be defined as a condition in which there are no mobilizable iron stores and in which signs of a compromised supply of iron to tissues, including the erythron, are noted. The more severe stages of iron deficiency are associated with anemia. The mild to moderate level of iron deficiency may not result in clinically evident anemia but the cells are still functionally impaired. According to the World Health Organization criteria, men with Hb < 13 g/dL and women with Hb < 12 g/dL are defined as having Hb deficiency or anemia (WHO 2001). Additionally, as per Shine (1997) patients with serum iron level < 60 mg/dL are defined as iron deficient.

Iron deficiency anemia (IDA) is the most common, easily diagnosed and treated anemia and with women being more frequently affected than men. The prevalence of iron deficiency anemia varies widely with age, sex, and race. About 2% of adult men, 9–12% of white adult females, and roughly 20% of black adult women were found to be suffering from IDA in the study conducted by Johnson-Wimbley and Graham (2011). Similarly study by Shaw et al. (1999) found the incidence of iron deficiency in Taiwan to be 2.1% in males and 10.7% in females out of which only 0.2% males and 2.1% females were suffering from IDA.

Patients with IDA may have characteristic systemic symptoms such as pallor, fatigue, weakness, lightheadedness, exertional dyspnea, tachycardia, palpitations, postural hypotension, and neuropathy. Oral symptoms and signs may include mucosal pallor, atrophic glossitis, generalized oral mucosal atrophy, anemic stomatitis, angular cheilitis, tenderness or burning sensation of oral mucosa, various types of oral candidiasis, lingual varicosities, recurrent aphthous ulcers, dry mouth, and dysgeusia. Severe prolonged iron deficiency may result in Plummer Vinson Syndrome (Fig. 1).

Burning mouth syndrome has been described as the clinical condition characterized by burning sensation of the oral mucosa without any apparent mucosal changes. Burning mouth syndrome may also be associated with deficient iron status.

Apart from iron deficiency anemia, the role of iron deficiency in oral premalignant conditions has been extensively studied and the results suggest that deficient nutritional iron status may predispose to the development and progression of Oral lichen planus, and Oral submucous fibrosis. Low iron levels can result in increased oxidative stress thus playing some role in development of oral cancer.

The authors aim at discussing biological changes, signs, and symptoms associated with iron deficiency in relation to oropharyngeal region.

Signs and Symptoms of Iron Deficiency

Anemia

Per Benoist et al. (2008), WHO data (1993–2005), globally, anemia affects 1.62 billion people which corresponds to 24.8% of the population with highest prevalence noted in preschool-age children and lowest in men. Various reasons of iron

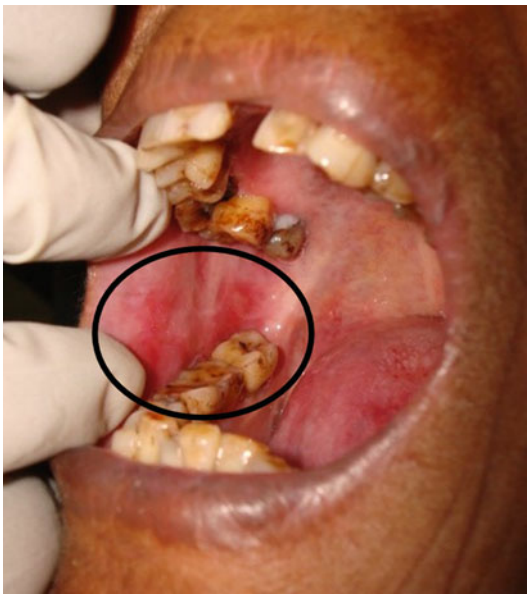

Fig. 1 Anemic stomatitis on right buccal mucosa. Photo shows erythematous atrophic right buccal mucosa or stomatitis in an anemic patient (Source: Personal collection)

deficiency include an increased demand for red blood cells (RBC) production during childhood growth spurts and during pregnancy; a decreased intake of dietary iron due to low socioeconomic status or negligence; reduced absorption of iron in patients with total gastrectomy or celiac sprue and chronic blood loss associated with excessive menstrual flow, gastrointestinal diseases, such as peptic ulcer, diverticulosis, or malignancies (Neville et al. 2009). Patients with IDA may have characteristic systemic symptoms such as fatigue, weakness, lightheadedness, shortness of breath, and palpitations. Oral signs of iron deficiency anemia include a myriad of conditions such as mucosal pallor, general mucosal atrophy, stomatitis, atrophic glossitis, cheilosis, lingual varicosities, angular cheilitis, oral lichen planus, various forms of candidiasis, and aphthous ulcers as outlined by Wu et al. (2014) The recommended daily dietary intake of iron in various countries has been listed in the Table 1.

Atrophic glossitis is a term used for "flattening of the tongue papillae" leading to a smooth and reddish tongue that may mimic geographic tongue or migratory glossitis. The bald appearance of the tongue dorsum results from the atrophy or loss of filiform papillae initially as they are most susceptible to nutritional deficiency followed by fungiform papillae. The circumvallate and foliate papillae on the posterior third of the tongue are spared. Samad et al. (2015) described the condition as being reversible on proper nutritional supplementation and regeneration of the lost papillae occurs in reverse order. In more severe cases, the tongue may be tender. Long et al. (1998) also reported cheilosis (dry scaling of the lips and corners of the mouth) as a common finding in patients of iron deficiency anemia (Fig. 2).

Angular cheilitis is a protean condition with several predisposing factors such as nutritional deficiency of vitamins and iron, lip-sucking, dehydration, overclosure of

Table 1 Reference intake of iron in different age groups and countries

		Females (Mg/day)							Males (Mg/day)				
Country/ region	Reference	1–3 years	4–8 years	9–13 years	14–18 years	19–30 years	Pregnancy	Lactation	1–3 years	4–8 years	9–13 years	14–18 years	19 years and above
USA	Otten et al. (2006)	7	10	8	15	18	27	10	7	10	8	11	8
India	Gopalan et al. (2011)	12	18	34	50	60	50	40	12	18	19	28	30
South East Asia	Institute of Medicine (US) panel (2001)	7	10	8	15	18	27	10	7	10	8	11	8
Africa	Vorster et al. (2013)	7	10	8	15	18	27	10	7	10	8	11	8
Europe	Montagnese et al. (2015)	7	10	8	15	18	27	10	7	10	8	11	8

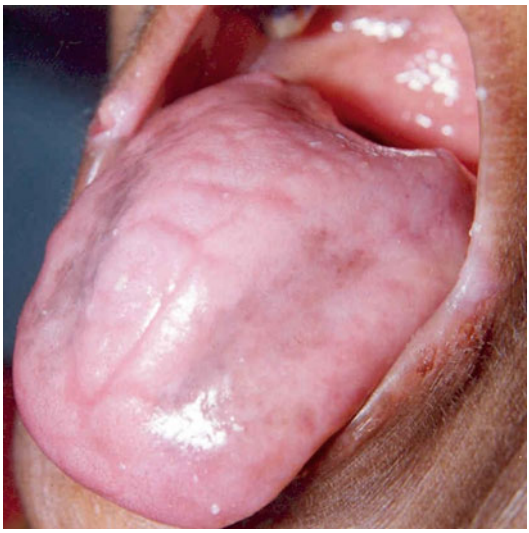

Fig. 2 Atrophic glossitis of tongue. Photo shows atrophic glossitis or bald tongue due to depapillation in an anaemic patient (Source: Personal collection)

the jaws, improper vertical dimension in artificial dentures, and mixed bacterial and fungal infections (Shafer et al. 2003). This lesion is characterized by wrinkling of epithelium at the corners of the mouth unilaterally or bilaterally which over the time becomes macerated, more pronounced to from one or more deep fissures which later ulcerate with or without exudative crusting. These fissures characteristically terminate at the mucocutaneous junction and do not involve the mucosal surface of the commissures of the mouth. With time, these fissures tend to get infected with bacterial and fungal strains such as staphylococci, streptococci, and candida species (Fig. 3; Shafer et al. 2003).

Shin-Yu Lu (2016) found in his study that iron deficiency led to a high incidence of Candida infection among the patients and resulted in a variety of clinical forms such as angular cheilitis, pseudomembranous candidosis, erythematous candidosis, median rhomboid glossitis, chronic mucocutaneous candidosis, papillary hyperplastic candidosis, and cheilocandidosis. Impaired cellular immunity along with epithelial changes may result in one or more types of candidal lesions. Candidiasis is an opportunistic infection caused commonly by yeast like fungus *Candida albicans* apart from other species such as C. tropicalis, *C. glabrata, C. parapsilopsis, C. guilliermondii, C. pseudotropicalis*, *C. stellatoidea*, and *C. krusei*. Acute and chronic forms of pseudomembranous candidiasis are the most common and characterized by the soft, white, elevated, curd-like scrapable plaques on buccal mucosa, gingivae, tongue, and floor of the mouth (Fig. 4). In erythematous form of candidiasis, the clinical manifestation is of red lesion involving palatal mucosa, buccal mucosa, and tongue (Fig. 5 and 6). When erythematous candidiasis involves tongue, it is referred to as median rhomboid glossitis (Fig. 5). Chronic mucocutaneous candidiasis presents with firm, white, nonscrapable plaques usually on the lips, tongue, and cheeks. The papillary hyperplastic candidiasis is the third stage of denture stomatitis. The first stage consists of numerous palatal petechiae and a

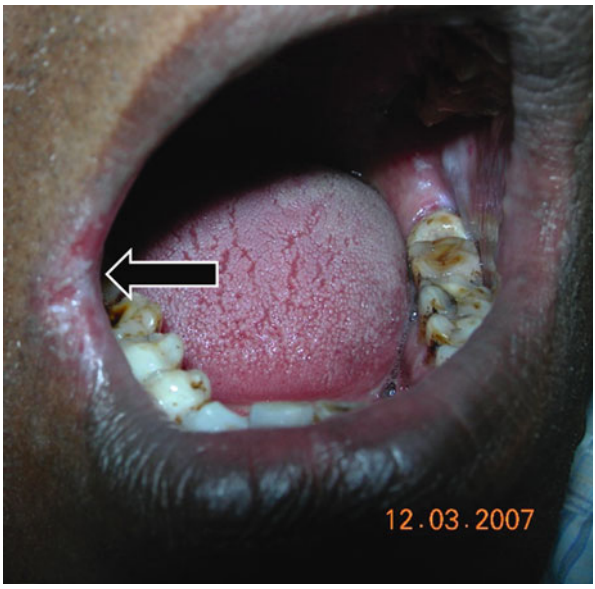

Fig. 3 Angular cheilitis. Photo shows fissuring and ulcerations in angle of mouth bilaterally in an anemic patient (Source: Personal collection)

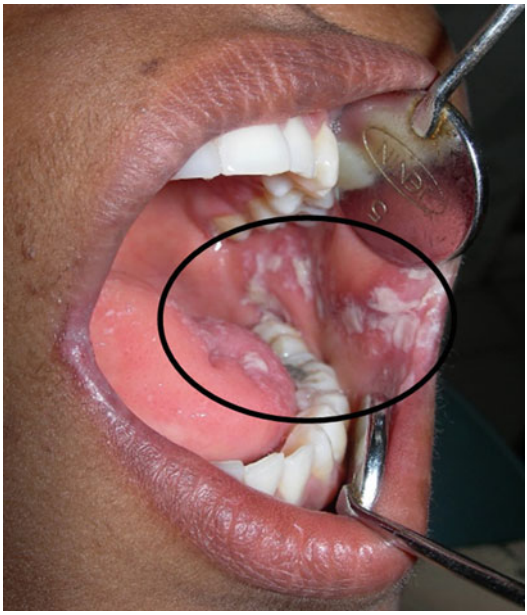

Fig. 4 Pseudomembranous candidiasis on left buccal mucosa and tongue. Photo shows white curd-like deposits on left buccal mucosa and tongue which are scrapable in an anemic patient (Source: Personal collection)

diffuse erythematous involvement of the denture-covered mucosa is seen in the second stage. The third stage shows the development of tissue granulation or nodularity (papillary hyperplasia) commonly involving the central areas of the hard palate and alveolar ridges (Greenberg et al. 2008).

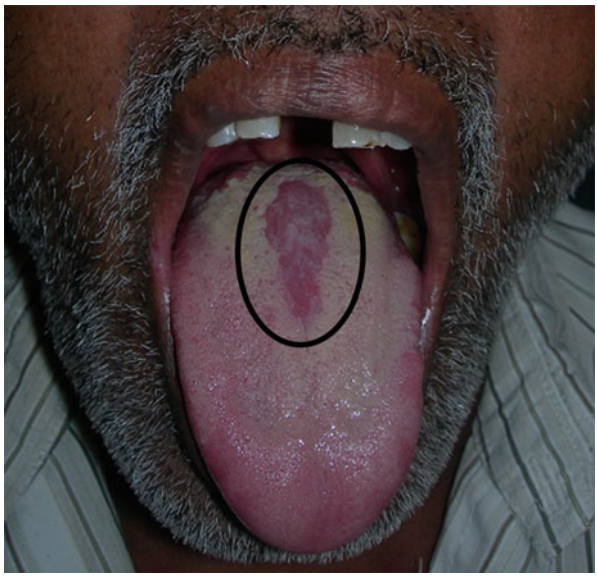

Fig. 5 Median rhomboid glossitis or erythematous candidiasis of tongue. Photo showing red rhomboidal shape lesion, i.e., median rhomboid glossitis on dorsum of tongue posteriorly is a type of erythematous candidiasis (Source: Personal collection)

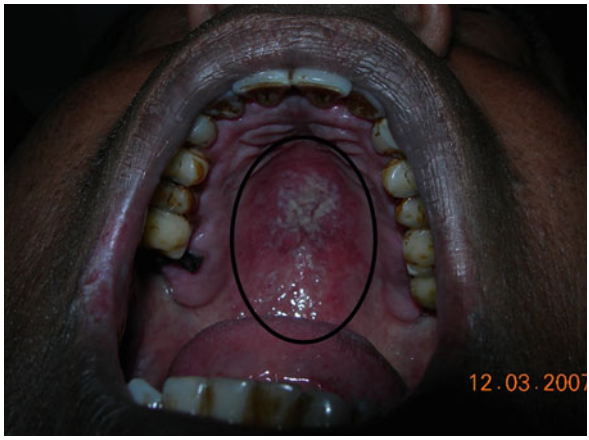

Fig. 6 Erythematous candidiasis on palate. Photo showing red lesion with few white components is a manifestation of erythematous candidiasis (Source: Personal collection)

Other miscellaneous oral manifestations found in patients of iron deficiency anemia in various studies include hyperpigmentation, recurrent oral ulcers, lingual varicosities, and oral lichen planus (Wu et al. 2014; Samad et al. 2015; Lu SY 2016). Varicosities are abnormally dilated and tortuous veins that are often found on the ventral surface of the tongue in aged individuals due to the age-related loss of connective tissue tone supporting the veins (Neville et al. 2009). Oral lichen planus (OLP) is characterized as presence of radiating white or gray, velvety, thread-like papules in a linear, annular, or retiform manner forming a typical lacy reticular patch known as Wickham striae or papules over the buccal mucosa, lips, tongue, and palate which may or may not be associated with erosion or ulceration (Fig. 7). Pica, a

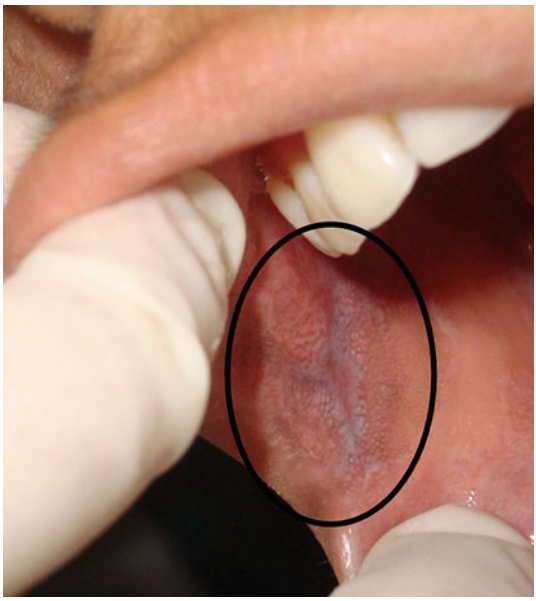

Fig. 7 Papular oral lichen planus on right buccal mucosa. Photo shows nonscrapable white granular lesion or oral lichen planus on right buccal mucosa in an anemic patient (Source: Personal collection)

behavioral disturbance comprising abnormal consumption of dirt or mud, also referred to as geophagia, is seen in iron deficiency patients (Louw et al. 2007). This results in poor oral hygiene; inflamed, erythematous gingiva; and staining of teeth.

Oral symptoms as outlined by Wu et al. (2014) chiefly due to atrophic mucosa and fungal infections included burning sensation of oral mucosa, pain, dry mouth, numbness of oral mucosa, recurrent ulcers, bad taste, and dysfunction of taste. The reason behind symptoms in iron deficiency anemia patients has been described in details under the biological changes section of the chapter.

Plummer Vinson Syndrome (PVS)

Also, known as Paterson Kelly syndrome or sideropenic dysphagia is a triad of microcytic hypochromic anemia, atrophic glossitis, and esophageal webs or strictures. Apart from these findings koilonychia or spoon-shaped nails and atrophic changes in the conjunctiva have also been associated with this syndrome.

PVS was first described by Paterson and Kelly in 1919. PVS is associated with iron deficiency anemia and has become increasingly rare due to early diagnosis and treatment of deficient iron status. Postmenopausal women are more commonly affected. One or more signs and symptoms of iron deficiency anemia may be present along with the above-mentioned triad. Apart from these, koilonychia or spoon-shaped nails of brittle nature are also noted. Dysphagia due to esophageal strictures is a characteristic finding in these patients. PVS is a potentially malignant disorder with the risk of development of squamous cell carcinoma of the esophagus due to the post cricoidal webs that are formed as described by Samad et al. (2015).

Burning Mouth Syndrome

Burning mouth syndrome (BMS) is a condition characterized by a burning sensation of the oral mucosa in the absence of clinically noticeable mucosal changes. BMS is described by 2–3% of adults and 14% of postmenopausal women. The prevalence is reported to have been more in advanced age, women, and Asians as compared to males and Caucasians, respectively. Lopez-Jornet et al. (2010) reported BMS as one of the most common disorders encountered in oral mucosal disease clinics. Systemic factors which predispose to BMS include iron deficiency anemia and PVS apart from others. The tip and lateral borders of the tongue are commonly involved.

Premalignant Conditions

Oral submucous fibrosis (OSMF) is an insidious, chronic, and debilitating condition encountered in South Asian population and has a malignant predisposition and can affect any part of the oral cavity and sometimes the pharynx (Jayasooriya et al. 2011). Epithelial atrophy characterizes OSMF along with changes in the connective tissue fibers of the lamina propria and deeper parts leading to stiffness of the mucosa causing trismus and inability to eat. Globally, estimates of OSMF shows a confinement to Indians and Southeast Asians, with an overall frequency of about 0.2–0.5% in India from 20 to 40 years' age range (Reddy et al. 2011). Angadi and Rao (2011) suggested strong association of OSMF with a risk of OSCC with a malignant transformation rate of 7.6% over a period of 17 years.

Interestingly, Bhattacharya et al. in 2016a described a very rare case of onset and subsequent treatment of OSMF secondary to iron deficiency anemia which supports the hypothesis that iron deficiency may have some role in the initiation and progression of this premalignant condition.

Oral lichen planus and Plummer Vinson syndrome as discussed earlier are potentially malignant conditions and have been associated with the iron deficient state.

Oral Cancer

Oral cancer is a disfiguring, potentially fatal disease that continues to rise in incidence among younger and older people alike. The annual incidence of oral and pharyngeal cancer is approximately 615,000 worldwide as reported by Mignogna et al. (2004). More than 90% of oral cancer is oral squamous cell carcinoma (OSCC). Richie et al. (2008) outlined cigarette smoking, chronic alcohol consumption, and low dietary nutrition as the major risk factors for onset of oral squamous cell carcinoma. Negri et al. in 2000 and Petridou et al. in 2002 found significant risk of oral cancer with decreased dietary intake of iron whereas Gupta et al. in 1999 found no significant association between oral premalignancy and low iron intake in Indian women. Nevertheless, in a case controlled study conducted by Richie et al. (2008), a significant association between low iron body stores and an increased risk of

oral cancer was appreciated. The possible reason of this association has been described in detail in the further biological changes section.

Biological Changes

Iron deficiency has profound effect on important bodily functions which has been demonstrated in numerous studies and was also observed by Camaschella (2015). Various biological changes underlying the iron deficient state have been outlined in Fig. 8.

Iron deficiency is the result of long-term negative iron balance within the human system. With decreased dietary intake or increased iron loss from the body, the stores of iron namely hemosiderin and ferritin are progressively reduced. This results in the compromised supply of iron to the transport protein apotransferrin. The overall condition leads to diminished saturation of transferrin and an increase in transferrin receptors in the circulation and on the surface of cells, including the erythron. Hence there is deficient supply of iron to all the tissues including erythron. Enhanced expression of transferrin receptors on cell surfaces is in proportion to actual iron need of the same. Consequently, insufficient supply of iron to all other tissues ensues due to compromised supply of iron to the erythron. Thus, lack of mobilizable iron stores in due course cause a demonstrable change in classical laboratory tests, such as measurement of hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, total iron-binding capacity, transferrin saturation, and zinc-erythrocyte protoporphyrin.

All physiologic effects of iron deficiency are dependent on the severity of anemia, decreased oxygen transporting capability of the blood and the iron-containing

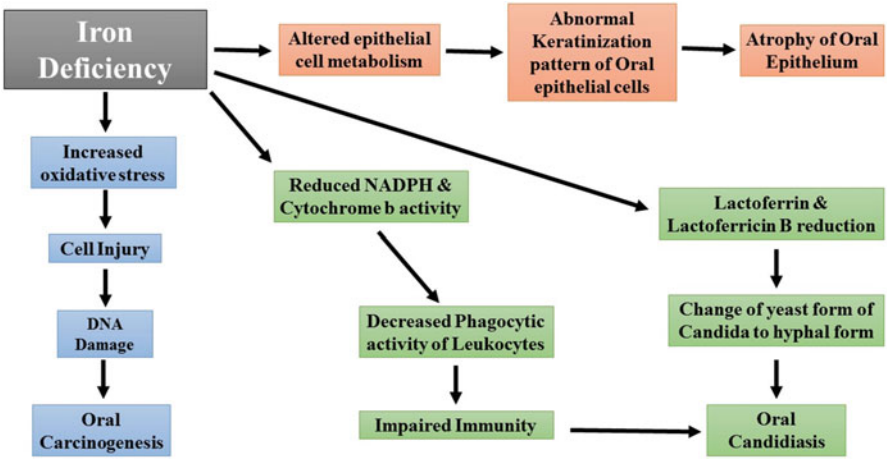

Fig. 8 Biological changes and clinical manifestations. Flowchart shows the most important biological changes and the resultant clinical manifestations in iron deficient status (Source: Personal collection)

proteins (Wu et al. 2014). Many reviews have described atrophy of the oral and gastric epithelium and nail changes in individuals as the foremost nonerythroid changes in humans and animals. The oral changes are the most prevalent and significant, with atrophic glossitis, angular cheilitis, and Plummer Vinson syndrome characterized by postcricoid dysphagia being the most common. Iron is an essential trace element for the growth and maturation of all cells. Rennie et al. (1982) in their study on the buccal epithelial cells in iron deficient subjects stated that a decidedly significant reduction in the total epithelial thickness and predominantly the thickness of the maturation compartment, along with low enzyme levels in the buccal epithelium, was noted. Richie et al. (2008) also confirmed similar findings by observing that the histological examination of the oral mucosa in iron deficiency anemia revealed epithelial atrophy with thinning of the lamina propria of the connective tissue. Sustained decreased levels of iron within blood result in reduced hemoglobin levels carrying inadequate supply of oxygen to oral mucosal tissues and finally leading to mucosal atrophy (Rennie et al. 1982; Wu et al. 2014). The biological changes in the blood profile, oral epithelium, defense cells, and enzymes system involving iron result in innumerable manifestations is evident in iron deficiency anemia. Per Montes et al. (2014) papillary atrophy of the tongue or atrophic glossitis results as a consequence to abnormalities in keratinization patterns and cellular structure of oral epithelium secondary to changes in the metabolism of oral epithelial cells. The thinned or atrophied oral mucosa is susceptible to ulcer formations.

Pigmentation in iron-deficiency anemia is evident due to excessive tyrosine formation which leads to abnormal melanosis. Ghosh K in 2006 elucidated the role of iron in melanin metabolism. Accordingly, enzymes phenylalanine hydroxylase and homogentisic oxidase are dependent on iron for their action on homogentisic acid and melanin quinines. In the scenario of iron deficiency, homogentisic acid cannot be metabolized and hence ensue excessive tyrosine formation.

There is no biological explanation for pica indulgence. Other miscellaneous clinical findings such as lingual varicosities and oral lichen planus have been reported in iron deficient cases without any logical explanation for the same.

The gastrointestinal tract is susceptible to iron deficiency; rapidly losing the iron-dependent enzymes because of its high cell turnover, causing degeneration of the mucosa, and predisposing to web formation (Chisholm 1974). Also, the resultant epithelial atrophy, changes in cell kinetics, and decreased repairing capacity of the mucosa amplify the effects of carcinogens and cocarcinogens and consequently predispose the oropharyngeal area to malignant transformation as described by Dinler et al. (2009). Dantas (1993) correlated the reason of dysphagia to decreased gastric motility in iron deficiency.

Iron has a significant role in immune-surveillance, due to its properties of promoting growth and differentiation inducing properties for leukocytes interfering with cell-mediated immunity and chemical mediators of inflammation as observed by Oppenheimer (2001). Iron deficiency adversely affects the normal defense system of the body as it compromises the body's immune system to act against pathogens. Iron deficiency also negatively influences the normal defense systems against infections. Ribonucleotide reductase is an iron-dependent enzyme required for DNA synthesis, hence Doherty

(2007) elucidated that in iron deficiency T lymphocyte formation is decreased thereby impairing the cell-mediated immunity. Besides, in iron deficiency even the phagocytic activity of the neutrophils is impaired thereby decreasing host defense against pathogens.

Loiarro et al. (2010) expounded the role of iron dependent co-enzyme nicotinamide adenine dinucleotide phosphate hydrogen oxidase and cytochrome b in immunity and illustrated that the activation of above mentioned enzymes does not occur in the iron deficient state and consequently the formation of free hydroxyl radicals within the leukocyte and the respiratory burst are affected thus decreasing the phagocytic capability of neutrophils. In a very noteworthy study, Soukka et al. (1992) found that lactoferrin, an iron binding glycoprotein, and lactoferricin B (LFcin B), an LF-derived antimicrobial peptide, inhibit the growth of Candida species, strains, not only in yeast form but also in hyphal form, and hence are important for the pathogenesis of this fungus. Therefore, the atrophied oral epithelium is colonized by the opportunistic candida species, normal commensals in the oral cavity, especially the tongue, and there is hyphal invasion of the stratum corneum.

The role of iron in collagen synthesis is well-documented. Iron is essential for collagen synthesis by enzymes proline hydroxylase and peptidyl lysine hydroxylase during hydroxylation of proline and lysine, respectively. Iron is a cofactor along with molecular oxygen alpha-Ketoglutarate and ascorbic acid that is used by peptidyl proline hydroxylase in this process. Ganapathy et al. (2011) reestablished that in oral submucous fibrosis there is iron deficiency as there is extensive uncontrolled collagen production which requires iron among other nutrients. Besides due to ulceration and epithelial atrophy patient is unable to chew adequately hence the deficiency is aggravated.

Per Lin et al. (2013), untreated dry mouth and iron or vitamin B12 deficiency over a period may lead to partial atrophic changes in the epithelium of the tongue as described earlier. In BMS patients, this change is so indiscernible that it is difficult to appreciate the same on clinical visual inspection. Thus, the spicy salivary components may diffuse through the atrophied oral tongue epithelium into the subepithelial connective tissue and irritate the free sensory nerve endings persuading burning sensation, pain, or numbness of the tongue.

Iron deficiency has been implicated in the pathogenesis of oral cancer as it induces oxidative stress. There is an equilibrium between the prooxidant and the antioxidant systems in the body; when the prooxidant systems predominate favoring oxidation, they cause direct oxidative damage to cellular molecules like the deoxyribonucleic acid (DNA) which is referred to as oxidative stress. Reactive oxygen species and free radicals are produced in iron deficiency which contribute to oxidative stress leading to cell injury (Vives Corrons et al. 1995; Halliwell 1994).

Policies and Protocols

The developed as well as developing nations need to draft strong policies and follow protocols vigorously to eradicate iron deficiency from the population especially in low socioeconomic areas.

Government of India has taken significant steps to achieve the same and laid National Guidelines for Control of Iron Deficiency Anaemia with following four purposes:

1. To bring to attention of program managers of health and health-related activities the serious negative consequences of anemia for the health and physical, mental, and economic productivity of individuals and populations
2. To layout iron and folic acid supplementation protocols across the life cycle (preventive strategy)
3. To define a minimum standard treatment protocol for facility-based management of mild, moderate, and severe anemia segregated by levels of care (curative strategy)
4. To broadly identify platforms of service delivery and indicate roles of service providers

Identification Nutritional anemia is a major public health problem in India and is primarily due to iron deficiency. The National Family Health Survey-3 (NFHS-3) data suggests that anemia is widely prevalent among all age groups, and is particularly high among the most vulnerable – nearly 58% among pregnant women, 50% among nonpregnant nonlactating women, 56% among adolescent girls (15–19 years), 30% among adolescent boys, and around 80% among children under 3 years of age.

Delivery Per Kapil and Bhadoria (2014) National Iron + Initiative will reach the following age groups for supplementation or preventive programming:

- Biweekly iron supplementation for preschool children 6 months to 5 years
- Weekly supplementation for children from 1st to 5th grade in Govt. & Govt. Aided schools
- Weekly supplementation for out of school children (5–10 years) at Anganwadi Centers
- Weekly supplementation for adolescents (10–19 years)
- Pregnant and lactating women
- Weekly supplementation for women in reproductive age

Monitoring

- Provision of iron and folic acid tablets to pregnant women will be during routine antenatal visits at subcenter/Primary Health Centre/Community Health Centre/ District Hospital.
- Accredited social health activist (ASHA) to ensure provision of IFA supplements to pregnant women who are not able to come for regular antenatal check-ups through home visits. She will also monitor compliance of IFA tablets consumption through weekly house visits.

Accountability The Ministry of Health and Family Welfare took a policy decision to develop the National Iron + Initiative and to take cognizance of ground realities,

evaluate the programs in various centers, and review the implementation on a monthly basis by program reports from the state health departments.

Recent Developments Food fortification programs to supplement nutrition with iron have not been very successful. One alternative solution is iron biofortification. Biofortification of crops is an interesting approach towards increasing the concentrations of bioavailable mineral elements such as iron in food crops. Two methods have been tried namely first involves application of mineral fertilizers and/or improving the solubilization and mobilization of mineral elements in the soil and second development of crops with increased ability to acquire mineral elements and accumulate them in edible tissues (White and Broadley 2009).

Dictionary of Terms

- **Accredited social health activist (ASHA)** – Community health workers instituted by the government of India's Ministry of Health and Family Welfare as part of the National Rural Health Mission (NRHM).
- **Anaemia** – Abnormally low level of hemoglobin in the blood.
- **Anganwadi Centre** – A shelter providing the basic health care in Indian villages.
- **Atrophy** – Decrease in the size of an organ or tissue.
- **Candidiasis** – A fungal infection due to any type of Candida (a type of yeast).
- **Epithelial cell** – A cell which lines various organs/structures in the body.
- **Iron deficiency** – Depleted/decreased of iron concentration in the body.
- **Phagocytosis** – Process by which a cell engulfs pathogens, debris, or foreign bodies.
- **Premalignant** – An altered condition of tissues which are more prone to develop cancer.
- **Protoporphyrin** – Chemical intermediate that combines with iron and protein to form hemoglobin, myoglobin, and certain respiratory pigments.
- **Stomatitis** – Inflammation of mouth mucosa.
- **Total iron binding capacity** – Measure of the binding capacity of transferrin for iron.
- **Transferrin receptor** – It is a carrier protein for transferrin.
- **Transferrin** – Protein synthesized in the liver that transports iron in the blood. It can bind two molecules of ferric iron.

Summary Points

- Iron is essential nutrient required for the functioning of various physiologic processes, which includes electron transfer reactions, binding and transport of oxygen, gene regulation, and regulation of cell growth and differentiation.
- Hemoglobin values below 80 g/L have been associated with impaired physical work capacity, reproductive efficiency, and cognitive and psychomotor development.

- Maternal hemoglobin concentrations at either the low or high end of the distribution during pregnancy (usually in the first or second trimester) are markers of increased risks of low birth weight and perinatal mortality.
- Iron deficiency has significant effect upon the oral mucosa.
- Oral effects of iron deficiency on oropharyngeal region varies from mucosal pallor, atrophic glossitis, generalized oral mucosal atrophy, anemic stomatitis, angular cheilitis, tenderness or burning sensation of oral mucosa, to various types of oral candidiasis, recurrent aphthous ulcers, dry mouth, and dysguesia. Severe prolonged iron deficiency may result in Plummer Vinson Syndrome.
- Atrophic changes in iron deficiency anemia are observed in the middle layers (progenitor compartment) of the epithelium.
- Impaired cellular immunity along with epithelial changes may lead to fungal infections within oral cavity.
- Burning mouth syndrome and premalignant conditions are other noticeable changes associated with iron deficiency.
- Increased oxidative stress has been suggested as a predisposing factor in initiation and progression of premalignant conditions and oral cancer.
- Regular iron screening of the vulnerable population and closely monitored iron supplementation in diet or tablet form are effective tools in combating ill effects of iron deficiency.

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Iron Deficiency in Women

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Abstract

The treatment of iron deficiency depends on correct diagnosis of iron deficiency with hemoglobin, ferritin, transferrin, sTfR estimations, the treatment of the reasons of iron deficiency, and the choice of effective iron preparations.

There are causes for iron deficiency in nonpregnant women like hypermenorrhea. The volume of blood loss is correlated with hemoglobin and ferritin values. Iron deficiency and anemia in pregnancy and postpartum are associated with maternal and fetal morbidity and mortality.

Beside the iron intake in the diet (daily requirement 2 mg) for treatment of iron deficiency, oral and intravenous iron preparations can be used. For parenteral iron treatment, three preparation types must be contemplated with different pharmacokinetics, toxicity, and side effects.

Keywords

Anemia · Fetal morbidity · Ferritin · Hemoglobin · Hemoglobinopathies · Hypermenorrhea · Iron deficiency · Iron dextrin · Iron hydroxide gluconate · Iron Hydroxide-sucrose complex · Iron therapy · Maternal morbidity · Transferrin

Introduction

Iron deficiency is known to be among the commonest nutritional deficiency states worldwide, with the physical sequelae and symptoms depending on the duration and severity of the iron deficiency. While countries with chronic malnutrition have a high prevalence of people with iron-deficiency anemia (50–80%), iron deficiency states without anemia are frequently found in countries with normal nutrition (prevalence up to 20%) (Milman 2011). Successful iron therapy depends, on the one hand, on correct diagnosis of the iron deficiency and, on the other hand, on the choice of effective iron preparations and treatment of the causes of the iron deficiency. Women naturally have a much higher risk of iron deficiency states than men. Women with regular periods have a prevalence of iron deficiency that is about ten times higher than men of the same age; in addition, it can be shown that among blood donors, for example, it is nearly always only women who have empty iron stores. The reason for this is the regular blood or iron losses during menstruation at the same time as often inadequate or insufficient daily iron intake in the diet (Akabas and Dolins 2005; Fraser et al. 2015).

Gynecological Bleeding as a Cause of Iron Deficiency and Iron-Deficiency Anemia

Gynecological bleeding causing iron deficiency includes recurrent hypermenorrhea, menorrhagia, or metrorrhagia. They all come under the heading heavy menstrual bleeding. It has been shown for many years that the rate of anemia significantly

increases with increasing menstrual blood loss. It has been shown that if menstrual blood loss is 61–80 mL per cycle, the rate of anemia is 10.3%, while the rate is 50% with menstrual blood loss of between 151 and 240 mL. A further study showed that with one definition of iron deficiency (hemoglobin less than 12 g/dL and a ferritin level less than 16 μg/L), the prevalence of iron-deficiency anemia was 0% with blood loss of less than 20 mL, while if blood loss was 60–80 mL, the prevalence was 17% and with blood loss of over 100 mL, the prevalence was 26%. Gynecological bleeding caused by diseases such as adenomyosis, uterine fibroids, or endometrial hyperplasia frequently results in considerable iron-deficiency anemia. In registration studies for a hormonal treatment of hypermenorrhea, in the baseline groups of 231 patients with hypermenorrhea, blood loss of a mean of 640 mL was demonstrated using the very specific alkaline-hematin method in a 90-day reference period. The amount of blood loss correlated well with low hemoglobin and ferritin levels (Akabas and Dolins 2005; Fraser et al. 2015; Gao et al. 1992).

Pregnancy and Postpartum

Anemia in pregnancy and the puerperium is associated with increased maternal and fetal morbidity and mortality depending on the severity and possible additional complications. It is among the commonest risk factors in obstetrics and perinatal medicine. According to the WHO, the estimated worldwide prevalence of anemia in pregnancy is 30–50%, depending on geographical area. Especially in developing countries, postpartum anemia continues to be among the commonest causes of death in women who have just given birth; in Europe, approximately 10% of women in the puerperium have moderate to severe anemia, adequate treatment of which without recourse to allogeneic blood is a current problem (Allen 1997; Bencaiova et al. 2012; Breymann et al. 2011).

Symptoms of Iron Deficiency

Symptoms of iron deficiency causing the woman considerable suffering are the main reason for the use of iron therapy. The therapeutic use of iron preparations for correction of low iron stores alone without symptoms should be rejected. Symptoms in iron deficiency (fatigue, headache, hair loss, poor concentration, reduced physical performance in general) are, among other things, a consequence of the iron deficiency in various enzyme systems such as oxidoreductases, mono-oxidases, dioxygenases, and especially decreased mitochondrial activity in the body cells (Milman 2011; Akabas and Dolins 2005; Hercberg et al. 2001; Munoz et al. 2011; Murray-Kolb 2012). Various placebo-controlled studies have shown a positive influence of iron administration on specific symptoms. In this context, the effect of iron does not correlate directly with the amount of iron administered or the ferritin level, which is a measure of iron stores. It is important to note that certain symptoms, such as fatigue, may only suggest iron deficiency but are not proof that it is present.

People without iron deficiency may have the same degree of fatigue as people with iron deficiency. The sensitivity of the symptom "chronic fatigue" for iron deficiency (ferritin <15 μg/L) is only 20%. Thus, if iron deficiency is suspected as the cause of certain problems, this always needs to be confirmed by specific tests (Beglinger and Breymann 2010).

Consequences in Pregnancy

Iron deficiency is known to influence several body functions such as physical and intellectual performance, enzyme functions (e.g., electron transport chain), thermoregulation, muscular functions, immune response, and neurological functions. Only a few of these possible effects have been specifically studied in iron-deficiency anemia. In general, iron-deficiency anemia leads to many symptoms such as fatigue, decreased physical and work capacity, increased cardiovascular stress (tachycardia, fall in blood pressure), reduced thermoregulation, and increased susceptibility to infection. In pregnant women, tolerance to peripartum blood losses is greatly reduced. Depending on the severity of iron-deficiency anemia, maternal mortality is increased. The reasons for this are an increased rate of cardiovascular insufficiency, a higher risk of hemorrhagic shock, higher infection rates in the puerperium, and poorer wound healing. Maternal morbidity is pos sibly linked to further factors such as socioeconomic status, medical care, nutritional state, etc. A general problem when interpreting the available studies is that maternal and fetal outcome are related to the severity of the anemia but not to the duration and time of first appearance of the anemia or severity, duration, and start of iron deficiency. Taking this limitation into account, some authors postulate a correlation between maternal mortality and degree of anemia, but there are no prospective studies on this issue and it is unclear what the critical hemoglobin level is in relation to maternal mortality. It appears to be levels below 8–9 g/dL, with the association between moderate anemia and maternal morbidity being unclear. There are currently no studies on the relationship between iron-deficiency anemia present prior to pregnancy and the course of pregnancy, and there are no prospective studies in large cohorts that show the effect of early interventions and treatment of anemia on maternal, fetal, and neonatal outcome (Allen 1997; Algarin et al. 2013; Bencaiova and Breymann 2014; Hercberg et al. 2000; Horowitz et al. 2013).

Nevertheless, the WHO summarized in 2001 "iron deficiency is associated with multiple adverse outcomes for both mother and infant, including an increased risk of hemorrhage, sepsis, maternal mortality, perinatal mortality, and low birthweight."

Diagnostic Principles

The basis of the diagnosis and consequent management of anemia in pregnancy is correct differentiation between the relative or physiological anemia in pregnancy due to the increase in plasma volume on the one hand and "true anemia" with its different

pathophysiological causes on the other hand. When defining the cut off value for anemia in pregnancy, the degree of changes in plasma volume depending on gestational age must be taken into account. Accordingly, hemoglobin levels of <11.0 g/dL in the first and third trimester and hemoglobin levels of <10.5 g/dL in the second trimester indicate possible anemia that should be further investigated. The pathogenesis of anemia in pregnancy may be multifactorial. Thus, it is not enough simply to diagnose anemia based on the hemoglobin level; rather the causes should always be investigated. Various etiological factors which are accompanied by reduced hemoglobin synthesis or increased hemoglobin breakdown or hemoglobin loss need to be considered in the differential diagnosis. In addition, combinations of decreased hemoglobin synthesis and increased cell death occur, e.g., in thalassemia syndromes, as a result of which the diagnosis and treatment may be additionally complicated. The most important dif ferential diagnoses include iron-deficiency anemia and its precursors (to which often too little attention is paid), hemoglobinopathies (thalassemias, sickle cell anaemia), anemia of infection, and renal anemia, e.g., in pregnant women who have had a kidney transplant or have renal failure (Breymann 2002; Breymann et al. 2011).

The first important steps to investigate anemia are a thorough history and clinical examination of the pregnant woman. This often already lays the foundation for correct diagnosis. The next investigations include a full hematological status and determination of specific biochemistry parameters, with the diagnosis of iron deficiency having been complemented in particular by determination of a range of additional promising parameters. The current gold standard for detecting iron deficiency is still the serum ferritin level. In addition, possible infection (chronic or acute) should always be ruled out as a cause. The pathogenesis of anemia is, as described, very varied. Thus, a diagnosis based on hemoglobin levels alone is usually insufficient. The reason for the reduced hemoglobin synthesis should therefore always be investigated, whether through a targeted history, clinical assessment or through investigations on top of the basic work-up (Gibson 1990; Letzky 1990; Milman et al. 1995).

Basic Work-Up

Hemoglobin and Red Cell Indices

The first test for the investigation of anemia mostly includes the hematological profile with the standard parameters:

- hemoglobin concentration
- hematocrit
- MCV, MCH

Although in everyday clinical practice the hemoglobin concentration usually gives the first indication of iron deficiency, it should be borne in mind that both

the hemoglobin level and the red cell indices such as the MCV and MCH have a very low sensitivity and specificity for detecting iron deficiency states and in most cases show significant changes only if iron deficiency has been present for some time. If suspected, more specific and more sensitive tests should be used especially for the early detection of iron-deficiency states and thus for the prevention of iron-deficiency anemia.

Differential Diagnosis and Additional Investigations

Iron-Deficiency Anemia and Specific Parameters of Iron Metabolism

Ferritin

Measurement of the serum ferritin level has the highest sensitivity and specificity for detecting iron deficiency. Ferritin levels of <20 μg/L are proof of iron deficiency, regardless of the hemoglobin level. Ferritin levels between 20 and 50 μ/L are regarded as a gray area, i.e., even if at these levels there are still low iron stores, it is assumed that a fair number of women already have symptoms of iron deficiency at these levels. If the ferritin levels are within the normal range (>50 μg/L), iron-deficiency anemia can be virtually ruled out, unless infection is suspected at the same time (Kirschner et al. 2016). In this situation, the ferritin levels may yield a false normal result, since apoferritin is, like C-reactive protein, an acute phase protein, and increases in infections as well as in inflammatory reactions (e.g., postoperatively). It is assumed that the serum ferritin levels once again correctly represent the iron stores about 6 weeks after surgical operations or childbirth. If the concomitant presence of iron deficiency and anemia is suspected, the presence of infection or inflammation (CRP measurement) must always be excluded in order to be able to draw a clear conclusion about the iron status. In special cases, iron investigations may be supplemented by various parameters (Munoz et al. 2011; Milman et al. 1995).

Serum Iron, Transferrin, Transferrin Saturation

In general, assay of serum iron levels and transferrin levels does not provide any additional benefit for investigating iron deficiency, even in pregnancy, since the serum iron levels in particular are subject to many influences such as diurnal, intraindividual, and interindividual variations. Conclusions as to prelatent iron-deficiency states are possible only in combination with transferrin levels by determining the transferrin saturation. If the ferritin levels are within the normal range, but the transferrin saturation is less than 15%, this is indicative of latent iron deficiency since now iron is being released in higher quantities from circulating transferrin to maintain erythropoiesis. However, it needs to be remembered that the fluctuations in the serum iron levels also affect calculation of transferrin saturation and thus may lead to incorrect interpretations (Milman et al. 1991).

Transferrin Receptors (sTfR)

Various studies have shown that serum transferrin receptors increase in iron-deficiency states or if there is an increased cellular iron requirement and thus are a sensitive and specific indicator of changes in iron kinetics. Transferrin receptors are probably also not affected in the context of infections, and thus they represent a good addition to ferritin assay. The first studies in pregnancy have already been conducted. Low sTfR levels at the start of pregnancy appear to be associated with inhibited erythropoiesis in the first trimester. The increase in sTfR in the course of pregnancy is attributed, on the one hand, to increasing stimulation of erythropoiesis and, on the other hand, to an increasing iron requirement of iron-dependent cell proliferation. It is not known whether inhibited erythropoiesis at the start of pregnancy negatively influences detection of concomitant iron deficiency through measurement of sTfR. There is nothing to suggest that the sTfR concentration is influenced by inflammatory reactions. Thus, this parameter would also be useful for the investigation of unclear situations in pregnancy (normal ferritin in the presence of an elevated CRP) and in the early puerperal phase. In our own studies, we showed that after labor, sTfR are not influenced by the inflammatory reaction at birth, in contrast to ferritin levels (Breymann 2002).

Prevention and Treatment of Iron Deficiency in Nonpregnant Women

Ideally, a woman can compensate for her iron losses through adequate iron intake in the diet. Iron intake in the diet depends on the iron content of the food, the amount ingested and lastly absorption of iron in the intestine and bioavailability of the iron. Foods high in iron such as meat contain up to 2 mg/100 mg, but absorption in the intestine ranges from 1% to 20%, depending on whether the food is of animal or vegetable origin. A daily requirement of 2 mg/iron per day is covered by 300 g of meat/fish but by about 1000 g of soya beans or 5000 g of spinach. This shows that compensating for higher iron losses through diet is unrealistic given current eating habits and quantities (Hercberg et al. 2001; Ahmed 2012; Verdon et al. 2003). On top of this is the fact that the population's knowledge about the iron content of food is usually very low, i.e., people do not know at all which foods are actually high in iron and how much they should consume. We have developed a smartphone application (MyIronfriend™) that aims to help women with this issue and to provide guidance when they do their dailyshopping (www.myironfriend.com). Both oral iron preparations (tablets or drops/ syrup) and intravenous iron preparations can be used for treatment. Oral iron preparations are available as Fe II salts or Fe III complexes, with the absorption of oral preparations being between 1% and 8% depending on the composition. An 80 mg tablet/day corresponds to just under 8 mg iron absorption/day. It can be shown from intervention studies (oral iron versus placebo) that even just daily doses of 20 mg of Fe II salts result in a significant improvement in symptoms of deficiency. As the dosage increases, the gastrointestinal side effects

of oral iron increase because of the toxic oxidative effect of iron in cells (Hidalgoa et al. 2013). This is usually at daily doses >100 mg/day, which results in a reduction in patient compliance. Irrespective of the preparation, almost 20% of women stop oral iron therapy sooner or later. In general, iron (III) complexes show better gastrointestinal tolerability, but are absorbed to a lesser extent (Ortiz et al. 2011). In iron deficiency states that do not respond to oral iron, it is possible to switch to an intravenous iron preparation (iron sucrose, ferric carboxymaltose, iron dextran, iron gluconate). In gynecology, most experience has so far been acquired with the iron sucrose and carboxymaltose preparations. These preparations distinguish themselves above all through their high safety even at high doses (ferric carboxymaltose up to 1000 mg/single administration). The rate of adverse reactions is approximately 5% (dizziness, feeling of heat, limb pain, flu-like symptoms), and severe allergic reactions are very uncommon with iron sucrose and ferric carboxymaltose. Extravasation must be avoided at all costs during parenteral iron administration since following this persistent skin discoloration may occur (Verdon et al. 2003; Krayenbuehl et al. 2011. There are now large randomized trials on the efficacy of iron sucrose and ferric carboxymaltose in females in the puerperal period, in pregnancy (iron sucrose) and in heavy uterine bleeding (Breymann et al. 2008; Favrat et al. 2014; Krayenbuehl et al. 2011; Munoz et al. 2008).

Sustained Iron Therapy

Iron therapy is only useful if the cause of the iron deficiency or iron losses is simultaneously treated. Thus, a woman may once again have empty iron stores a few months after intravenous iron therapy if iron losses are not corrected at the same time. Vegetarians who menstruate and consume little iron in their diet are, for example, not usually able to replenish their iron stores or maintain their replenished stores. Women with periodically high iron losses (menstruation, blood donors) or consumption (competitive sportswomen, pregnant women) are also not able to do this. Thus, in parallel to iron therapy, treatment of the cause of the bleeding disorders is of great importance as well as supportive measures from gynecologists and members of other specialties (e.g., internal medicine specialists, hematologists, dieticians).

Prevention and Treatment of Iron-Deficiency Anemia in Pregnancy

The treatment of anemia is directed at its cause and severity. Maternal and fetal risk factors that may be complicated by anemia also need to be taken into account. The time period that is available for the treatment of anemia (e.g., before childbirth) likewise plays an important role. Later complications can be prevented early on by adopting a prospective and preventive way of thinking, i.e., even milder forms of anemia need to be systematically treated to prevent any exacerbation and to reduce

peripartum complications if there are high blood losses. The administration of allogeneic blood should be avoided at all costs as a last resort in pregnant women and those in the puerperium; as an alternative, newer treatment strategies may be considered for severe iron-deficiency anemia, for example, especially use of well-tolerated parenteral iron preparations or, in special cases, recombinant erythropoietin (Breymann et al. 2011, 2000).

Prevention

There are three ways to improve the body's iron status: to reduce losses, limit consumption, and increase intake. Depending on existing iron stores immediately after pregnancy, sufficient intervals before further pregnancies may be used to replenish the iron stores. In the puerperium, worthwhile strategies to reduce or avoid major blood losses make a contribution at the time of birth. In pregnancy, however, only the option of increasing intake is available: via dietary intake or via targeted pharmacological supplementation.

Pharmacological Supplementation

The benefit of preventive iron administration, that is, general regular administration of iron preparations without actual knowledge of iron stores, continues to be a subject of debate. This approach is recommended and practiced particularly in the industrialized countries. Critical arguments against it include, on the one hand, the lack of evidence that the worldwide prevalence of iron deficiency and consequences of anemia is reduced and, on the other hand, possible harmful effects of nonselective iron administration on the mother. According to the last update to the Cochrane Database, there is no scientific or medical justification for prophylactic iron administration in pregnancy in countries with adequate nutritional resources since epidemiological data do not show any positive effect on the course of pregnancy and/or maternal and fetal outcome (Pena-Rosas et al. 2012). In terms of hematological data (ferritin levels, hemoglobin), randomized placebo-controlled trials do at any rate show a positive effect. Women with low iron stores at the start of pregnancy develop anemia less often if they receive iron substitution. In contrast, it has been shown that iron supplementation in countries with a high prevalence of iron-deficiency states is indeed justified (Murray-Kolb 2012; WHO 2012; Van den Broek 1998). However, it is unclear whether studies on iron substitution in developing countries can be extrapolated to industrialized countries. The correct dosage for pure prophylactic administration is unclear. Current guidelines on purely prophylactic iron supplementation are 60–120 mg elemental iron per day. Lower dosages appear to be less effective. At dosages from 120 mg, adverse reactions increase and patient compliance becomes poorer (Milman et al. 1991, 2014). As women with low iron stores benefit most from prophylactic iron administration, selective iron administration in pregnancy would be the optimum solution. This presupposes investigating the iron

stores since the hemoglobin level that is usually measured in isolation shows a poor correlation with iron stores. The ferritin level must be used for this. According to a study from Denmark, ferritin levels of less than 70 µg/L at the start of pregnancy are indicative of subsequent iron deficiency. However, as the individual iron metabolism during pregnancy can be very different, the best time for measuring iron stores in pregnancy is unclear (Milman et al. 1995, 1991).

Treatment of Iron-Deficiency Anemia in Pregnancy

The preceding sections show that iron-deficiency states and anemia should be treated. The change over time or worsening of the situation can often not be predicted even for mild forms of anemia, and maternal and fetal risks increase with increasing anemia. In deciding on the method of treatment, various factors such as time remaining until birth, severity of the anemia, additional risks (e.g., premature contractions), maternal comorbidity, and patient's wishes (e.g., refusal to have allogeneic blood in severe anemia) should be taken into account. Thus, a Jehovah's Witness with severe anemia 2 weeks before birth requires a different approach compared with a woman who has moderate anemia in the second trimester without additional risk factors. The current most important alternatives for the treatment of anemia are oral iron, parenteral iron, stimulation of hematopoiesis using growth factors (human recombinant erythropoietin), and administration of heterologous blood.

Oral Iron

Oral iron is the gold standard for the treatment of mild-to-moderate iron-deficiency anemia. It is unclear whether weekly or intermittent administration of oral iron is equivalent to daily administration or even better than it. Studies on this issue are currently underway. The ideal dosage for intermittent or weekly administration is also unclear. As the rate of absorption is inversely proportional to the administered dose, dosages between 100 and 200 mg iron daily are a compromise in relation to the hemoglobin increase and tolerability of iron. The recommended dosage is 80–160 mg elemental iron per day. If there is a good response to the oral iron therapy, reticulocytosis occurs within 3–5 days and increases until 8–10 days after treatment. The hemoglobin increase follows after a delay and is, at best, approximately 0.2 g/dL/day or approximately 2.0 g/dL within 3 weeks. Once the hemoglobin levels have returned to normal, oral iron should be continued for at least another 4–6 months until a target ferritin level of approximately 50 µg/L and a transferrin saturation of at least 30% have been reached (Pena-Rosas et al. 2012; Haider et al. 2013).

As already mentioned, gastrointestinal side effects such as constipation, heartburn, and nausea, which occur in up to 30% of female patients and which limit the dose that can be administered, are key disadvantages of oral iron preparations. In this case, the dose must either be reduced or a switch to a different preparation

tried. However, even then the often poor compliance remains a problem. It has been shown that only 36% of pregnant women regularly take oral iron despite being specifically instructed about the problem of iron deficiency. Studies from countries such as Tanzania and Indonesia also show compliance of only 36–42% (Milman 2011).

Parenteral Iron Preparations

Parenteral iron is the most important alternative to oral iron preparations. The indications for parenteral administration of iron include: Parenteral iron administration circumvents the natural mechanism of iron absorption via the intestine and thus protein binding. Nonprotein-bound, free iron is therefore able to circulate. Free iron is toxic since it promotes the formation of hydroxide and oxygen radicals, which in turn result in cell and tissue damage via peroxidation. Thus, parenteral iron should only be administered if the iron status is known, in order to prevent potential iron overload. In principle, a distinction needs to be made between three classes of preparations. They differ on the basis of their pharmacokinetics, complex stability, molecular mass, toxicity, and side effects.

Type I Complexes (Iron Dextrin, Iron Dextran)

These are stable iron complexes with a high molecular weight (>100,000 Da) and high stability (e.g., Imferon®). Iron is therefore released to the transport proteins slowly and competitively in relation to endogenous iron. The free iron is, on the one hand, bound to transferrin and then used for heme synthesis and, on the other hand, transported into the reticuloendothelial system. The plasma half-life of type I complexes is 3–4 days. While the stability and slowly released iron are to be viewed as favorable, it is assumed that the dextran components in particular can result in severe allergic reactions. This reaction appears to be less severe with dextrins. Patients who generally have an allergic reaction to drugs also have a high risk of iron dextran allergy. The reason for the severe allergic reactions has not been determined, but corresponds to an anaphylactic reaction with release of mediators from mast cells. A further hypothesis is that there is the formation of dextran antibody which can lead to severe reactions even at the first contact. Dextran chains form biological polymers of different sizes, which could be responsible for this. Also in favor of this is the fact that dextran 1, for example, a polymer of only 1000 Da, causes virtually no reaction (Chertow et al. 2006; Hoigne et al. 1998; Naim and Hunter 2010; Perewusnyk et al. 2002).

Type II Complexes (Iron Hydroxide-Sucrose Complex)

These are complexes with moderate stability and a molecular weight of 30,000–100,000 Da (Venofer®). After administration of a bolus, peak plasma levels are already reached after 10 min (30 mg/L). Twenty-four hours after administration, the plasma levels have once again reached pretreatment levels. The half-life is 5.5 h, and studies with PET indicate immediate accumulation in the bone marrow, in

parallel to the fall in the plasma levels. In patients, 70–97% of the iron is used for erythropoiesis, depending on the severity of the iron-deficiency anemia. In studies in humans, there were not noted to be either morphological organ changes or lipid peroxidation as a result of free radicals at the usual dosages (1–4 mg/kg body weight). In clinical practice, the substance is to be regarded as very safe; moreover, no biological polymers (see also dextrans) are formed; hence, anaphylactic reactions are extremely rare. The general (adverse) reactions include: metallic taste, feeling of heat, nausea, local irritation, and dizziness (Chertow et al. 2006; Hoigné et al. 1998; Naim and Hunter 2010; Perewusnyk et al. 2002).

Type III Complexes (Iron Hydroxide Gluconate, Iron Hydroxide Citrate, Iron Hydroxide Sorbitol)

Iron gluconates (e.g., Ferrlicit®) are the best-known substances from this group. They are unstable, labile complexes with a molecular weight of less than 50,000 Da. Because of the lower stability compared with iron dextrans and iron sucrose complexes, type III complexes are bound to transport proteins to a lesser extent, while free iron is released short term in higher quantities. Several studies have described maximum transferrin saturation after administration of iron gluconate. It is assumed that the free iron is stored in the organ parenchyma in increased amounts. Free radicals lead to lipid peroxidation and, compared with type I/II complexes, to higher tissue toxicity. With regard to allergic and anaphylactic side effects, iron gluconates, in particular, appear to be comparable to iron sucrose complexes and thus, like iron sucrose, have a better side-effect profile compared with iron dextrans (Chertow et al. 2006; Hoigne et al. 1998; Naim and Hunter 2010; Perewusnyk et al. 2002).

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Impaired Magnesium Status and Depression

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Nicola Veronese and Marco Solmi

Contents

Abstract

Magnesium (Mg) is an element present in everyday dietary plan of regular meals, but it has been shown that a large part of the population presents a low Mg status. Mg has a wide range of physiologic and protective functions within energy regulation and cellular, included neuronal, homeostasis. It obstacles excessive calcium flow into the cells, preventing cells' death, has anti-inflammatory properties, antioxidant action, and interacts with serotonin, a central neurotransmitter

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V. R. Preedy, V. B. Patel (eds.), *Handbook of Famine, Starvation, and Nutrient Deprivation*, https://doi.org/10.1007/978-3-319-55387-0_49

involved in depression pathophysiology. Epidemiologic studies have shown that low Mg status is associated with increased frequency of depression, with both cross-sectional and longitudinal designs. Promising evidence has shown that Mg has antidepressant activity similar to imipramine, and that it can be a valid supplementation to antidepressants in treatment resistant depression. However, at the present state of the art too few and small studies have investigated the role of Mg among other therapeutic means in depression, and any conclusion about its utility in clinical practice cannot be drawn. Future research should shed a light on such an important field needing more evidence.

Keywords

Magnesium · Hypomagnesemia · Depression · Depressive mood · Neurology · Psychiatry · Glutamate · NMDA · Magnesium supplementation · Epidemiology · Animal experiments

List of Abbreviations

BDNF	Brain-derived neurotrophic factor
Mg	Magnesium
NMDA	N-methyl-D-aspartate
RCT	Randomized controlled trial
RDA	Recommended daily allowance
SSRI	Selective serotonin reuptake inhibitors

Introduction

Magnesium (Mg) plays a fundamental role in several key metabolic pathways involved in energy expenditure and physiologic cell metabolism (Volpe 2013). Its deficiency is associated with several negative health outcomes in human beings, among which metabolic disorders such as increased sugar blood levels and cardiovascular diseases widely conceived (Veronese et al. 2016). Beyond such severe and frighteningly common conditions, the role of deficiency of Mg in the development of neurological and psychiatric diseases is more complex and ultimately less clear. Mg passes the blood-brain barrier and is physiologically involved in neuron signaling and brain function at a higher order. Thus, any imbalance in Mg status may affect regular neuronal signaling and brain network, in turn possibly playing a role in the onset of neurological and psychiatric diseases, such as dementia, severe mental illnesses (including major depressive disorder, bipolar disorder or schizophrenia, suicidal behavior), and stroke. (Veronese et al. 2015b; Volpe 2013; Carl-Albrecht 2010; Ruljancic et al. 2013).

Within severe mental illness, the role of Mg deficiency in depression is still debated, with some evidences suggesting it has a role in a wide range of pathogenetic steps of mood disorders (Table 1). It was hypothesized that Mg might underpin response to treatment as well, alongside with other factors.

Table 1 Evidences regarding magnesium and psychiatric diseases (others than depression)

Condition	Reference
Premenstrual syndrome	(Pearlstein and Steiner 2000)
Attention-deficit/Hyperactivity disorder	(Kozielec and Starobrat-Hermelin 1997)
Schizophrenia	(Nechifor 2008)

In this chapter, we will discuss the possible mechanisms through which Mg deficiency underpins the development of depression, the epidemiological evidences of such a relationship, and its clinical implications.

Role of Magnesium Deficiency in Depression: Pathological and Molecular Evidences

Animal models have suggested the possible role of Mg in depression according to several pieces of evidence. To mention a couple of studies, mice receiving low Mg diet for several weeks showed enhanced depression-like behavior, as reported by their poor performance in the forced swim test (Singewald et al. 2004), and other tests with depression and anxiety-like attitudes in these animals. Similarly, in rats, Mg deficiency was associated with depression-like and anxiety-related behavior and the administration of Mg supplementations was able to improve these symptoms. (Spasov et al. 2007).

Such evidence is not surprising, and a role of Mg deficiency in depression pathogenesis is to be expected in humans as well. Actually, the role that magnesium is supposed to play in depression can be essentially summarized in three main dimensions as represented in Fig. 1.

The first we will describe is the calcium-mediated excitoxicity, the second is the relationship between Mg and serotonin, and the third is the interplay of Mg and peripheral inflammatory cytokines, alongside with oxidative stress and BDNF.

Mg is a natural calcium antagonist. For this effect, Mg acts blocking the voltage-dependent N-methyl-D-aspartate (NMDA) channel (Fig. 1), which in turn physiologically regulates the entrance of calcium into the neuron (Iseri and French 1984). Physiologic Mg levels are believed to have neuroprotective properties leading to a reduction in the neuron death (Alzheimer 2012; Sobolevskii and Khodorov 2002). Such a beneficial action is supported by the fact that Mg deficiency damages neurons through the excessive opening of NMDA-coupled calcium channels leading to an excessive intraneuronal mitochondrial concentration of calcium, which finally activates some calcium-dependent enzymes regularly silenced in normal conditions. This excitotoxic pathway results in a series of mechanisms (e.g., cytoskeletal breakdown, failure to generate ATP, and production of free radicals) ultimately leading to neuronal death (Durlach et al. 1997). Another effect of Mg deficiency in the development of depression is probably linked to L-glutamate, which in turn can furtherly boost a calcium-mediated neuronal excitoxicity (Mark et al. 2001).

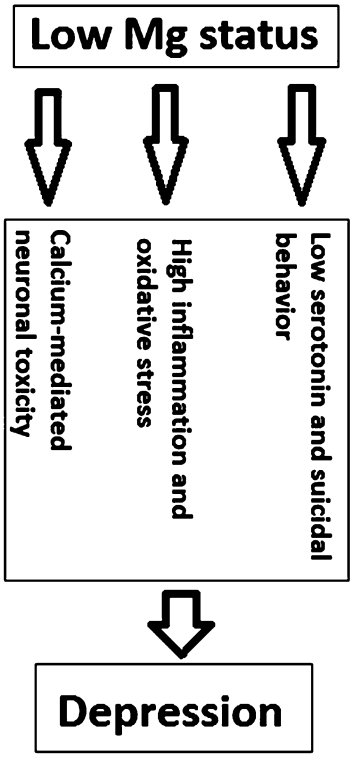

Fig. 1 Role and pathways of magnesium deficiency in depression

From a clinical perspective, as happens for ketamine's antidepressant activity, such a NMDA receptor blockade may result in a mood improvement, and theoretically this is the case also of Mg.

In particular it has been suggested that such low Mg levels' detrimental effects on mood disorders may be localized in particular in hippocampus (Durlach et al. 1997).

Secondly Mg seems to be implicated in the metabolism of different neurotransmitters involved in the pathogenesis of depression (Szewczyk et al. 2008; Durlach et al. 1997), and evidence showing a correlation between platelet magnesium, platelet serotonin, and suicidal behavior in a population of patients with depression furtherly supports a close Mg-serotonin interplay with clinical implications (Ruljancic et al. 2013). Such an interaction plays at a central level as well, as shown in animal models where the forced swim test performance is linked to serotonin receptors and is improved by concomitant administration of Mg and antidepressants (Cardoso et al. 2009). Moreover, Mg has shown to protect neurons in the cerebellum from hypoxic damage through an increase in BDNF (Golan et al. 2004).

Third, a subclinical Mg deficiency status has been demonstrated to be associated with chronic inflammatory state (Nielsen 2014), and Mg seems to able to decrease inflammatory cytokines. More in detail, in vivo Mg has shown to induce a decrease in TNF-alpha and IL-6, in vitro to reduce inflammatory cytokines' genes and

proteins (Sugimoto et al. 2012), and low Mg status has been associated with oxidative stress in subjects with type 2 diabetes mellitus (Araujo Sampaio et al. 2014). In other words, Mg deficiency seems to be associated with an increased inflammatory state and oxidative stress level (as shown by the increased levels of oxidative stress markers, e.g., lipid, protein, and DNA oxidative modification products). Finally, other research has suggested a potential relationship between Mg deficiency and reduction in antioxidant barriers (Zheltova et al. 2016). Since both inflammation (Kohler et al. 2017) and oxidative stress (Black et al. 2015) play a role in the development of depression, it is possible that deficiency in Mg contributes to depressive mood through affecting these pathways.

Epidemiological Evidences of Magnesium Deficiency and Depression

Mg deficiency is largely present in the general population (Veronese et al. 2014) and some individuals (such as diabetic subjects) are particularly prone to this condition (Veronese et al. 2016). Some research suggests that optimal Mg levels are associated with healthy life (Veronese et al. 2015a). However, despite such an important role of Mg and physical and mental well-being, more than half of the American individuals do not reach the suggested RDA for Mg (Whang 1987). Since several conditions associated with Mg deficiency (poor nutrition, gastrointestinal and renal diseases, insulin resistance and/or type 2 diabetes, alcoholism, stress, and certain medications) are also associated with depression (Serefko et al. 2016), the interest on Mg status in populations is increasing, and given the above-mentioned mechanisms, Mg deficiency as a putative risk factor for depression has been object of growing interest.

Table 2 summarizes the findings of epidemiological studies regarding Mg deficiency and depression in human beings. In a systematic review, Derom et al. found that a higher dietary intake of Mg is probably associated with lower prevalence and incidence of depression (Derom et al. 2013), suggesting a protective role of Mg. However, the same authors failed to find any significant association between other estimates of Mg status (e.g., blood and cerebrospinal levels) and the presence of

Table 2 Epidemiological evidence of Mg deficiency in depression

	Association	References
Dietary	Mg deficiency is associated with higher prevalence and incidence of depression	(Derom et al. 2013)
Serum/ plasma	Less evident association between Mg deficiency (hypomagnesemia) and depression	(Derom et al. 2013; Cheungpasitporn et al. 2015)
Other measurements	Not consistent results regarding cerebrospinal fluid Mg status, analyzed through phosphorous nuclear magnetic resonance spectroscopy, seems to be lower in depressed people	(Derom et al. 2013; Eby and Eby 2010)

depression. These latter findings are surprising and are in contrast with other investigations which reported that cerebrospinal Mg is probably low in both treatment-resistant suicidal depression and in patients that have attempted suicide (Eby and Eby 2010). Consistently with an association between depression and Mg deficiency, other investigations reported that serum Mg levels are lower in depressed people compared to healthy controls (Levine et al. 1999; Joffe et al. 1996). As evidence accumulated enough across years, other researchers recently and definitively confirmed a possible role of low Mg levels in predicting depression showing that hypomagnesemia increased the presence of depression of about 34% in a meta-analysis including almost 20,000 participants, with a low heterogeneity of results (Cheungpasitporn et al. 2015). Such an increased risk, even if with a statistically marginal significance, survived after sensitivity analysis according to cross-sectional study or longitudinal design (Cheungpasitporn et al. 2015).

While a magnesium deficiency may be considered among the causes that contribute to depression, it cannot be said on the other hand, at the present state of evidence, that a low Mg status should be expected in all subjects with depression. More probably, a subgroup of depression cases may have Mg deficiency as a contributing factor to onset or maintenance of the disease.

Moreover, beyond serum Mg levels, brain magnesium has been found low in depressed subjects as well, using neuroimaging techniques, such as phosphorous nuclear magnetic resonance spectroscopy (Eby and Eby 2010). However, the most consistent evidence regards in fact the association between low dietary Mg intakes and depression, or blood levels of Mg and depression, while other estimates of Mg (such as blood) are less used for epidemiological purposes, thus limiting any conclusion on the relationship between brain Mg and depression.

Clinical Implications of Mg Deficiency in Depression

Figure 2 summarizes the clinical implications of Mg deficiency as risk factor for depression. Animal and in vitro models suggest a relevant role of Mg deficiency for the development of depression, with response to antidepressants and anxiolytics drugs in response to experimentally induced Mg deficiency states (Szewczyk et al. 2008). Also, in these laboratory studies such a response to psychopharmacologic agents is augmented by Mg administration, suggesting an interaction between Mg, antidepressants, and anxiolytics (Szewczyk et al. 2008).

Data involving human subjects show similar results about the potential implications of Mg use as a therapeutic mean for depression. Mg supplementation should be considered as a safe intervention, with used doses in clinical trials ranging from 300 mg to 4 gr die (Du et al. 2016). Its use has been tested in a limited, but meaningful number of studies.

One randomized controlled trial (RCT) compared Mg efficacy with imipramine 50 mg, in 23 adults with depression, and hypomagnesemia and type 2 diabetes mellitus, showing equivalent efficacy between the two interventional arms (Barragan-Rodriguez et al. 2008). Another recent cross-over placebo-controlled

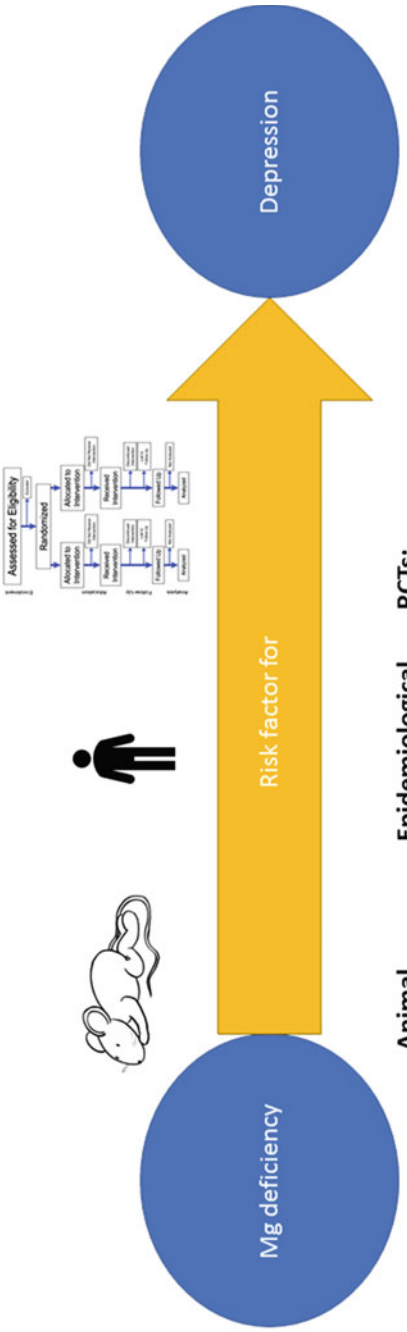

Fig. 2 Clinical implications of magnesium deficiency in depression

Table 3 Questions still open regarding magnesium and depression.

1. Should serum magnesium levels be assessed in all depressed individuals?
2. What is the precise role of magnesium in depression?
3. Can magnesium supplementation improve depressive symptoms?
4. Should magnesium supplementation be used as add-on therapy in depression?

study assessing Mg impact on depressive symptoms in treatment resistant depression, showed a correlation of 4 mg of Mg infusion and Patient Health Questionnaire-9 scoring, but not of Hamilton Rating Scale for Depression, after 7 days of treatment (Mehdi et al. 2016). A further investigation on a small group of patients described a notable response rate in patients with treatment resistant depression concomitantly treated with probiotics and Mg in addition to SSRI (selective serotonin reuptake inhibitors) (Bambling et al. 2017). Overall, however, a small total sample size and a limited number of studies have assessed effects of Mg on depression to draw any definitive conclusion.

It cannot be excluded that a subgroup of patients with low serum Mg may benefit the most from Mg supplementation, but whether routine Mg serum levels' first line screening are warranted or not in patients with depression is still a question to be addressed. Mg serum levels assessment, however, should be considered among concomitant factors maintaining depressed mood in cases showing partial or no response at all to standard antidepressant treatment instead. Vice versa patients with low Mg state should be monitored for an increased risk of depression, and Mg integration in those cases should be warranted as a preventive mean against mood disorders (Table 3).

Magnesium and Antidepressants

A different perspective should be used to evaluate concomitant administration of Mg and antidepressants. In this context, the association between Mg and medications is complex and may differentially affect drugs' efficacy according to the used compound, even beyond antidepressants class and depression (Eby and Eby 2006). For example, it was demonstrated that Mg supply decreased the intensity of morphine-induced physical drug dependence (Nechifor 2008), and that lithium, neuroleptics, and benzodiazepines treatment may require lower doses when supplemented with Mg in bipolar affective disorders (Heiden et al. 1999). Mg is supposed to play its antidepressant activity and to interact with antidepressants as NMDA antagonist, through GSK-3 inhibition, and boosting serotoninergic action rather than noradrenergic action of antidepressants (Szewczyk et al. 2008), as shown in Fig. 3. Such an interaction, has also shown to correlate with the risk of suicidal behavior, with low platelet serotonin and magnesium levels are present with higher frequency when self-harming occurs. Other specific data regarding antidepressants are, however, needed.

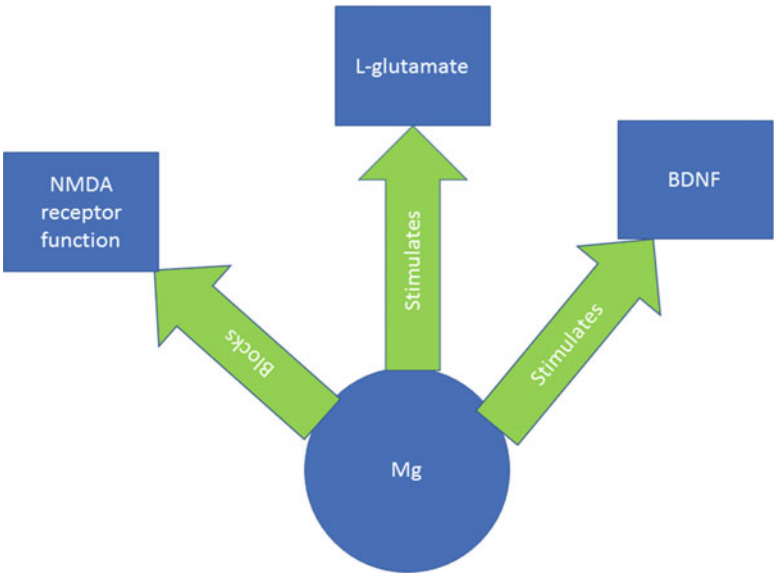

Fig. 3 Mechanisms of Mg action as antidepressant

Conclusion

Mg deficiency is a common condition in general population. The impact of this condition in the development of depression is probably important, but still too few studies use dietary Mg as proxy for Mg status. In the future more observational studies are needed to better characterize the relationship between Mg and depression, which ideally assess Mg status with more precise, valid and reliable methods than Mg dietary intake. The same is for the role of Mg supplementation of antidepressant treatment in patients suffering from depression. While promising evidence is available already, more studies should address several questions, with larger samples. In particular, it is still unclear whether Mg improves depressive symptoms in specific population such as patients with hypomagnesemia or treatment resistant depression only, or conversely if it may be a valid treatment coadjuvant for the whole population affected by depression.

Policies and Protocols

In this chapter, we have summarized the role of magnesium in depression. We have started describing the relevance of magnesium in cellular and neuronal homeostasis, and in a wide range of severe conditions. Secondly, we have focused on depression and described the possible mechanisms that may underpin a role of poor magnesium

status in increasing the risk of depression, identifying three main pathophysiologic domains. Then, we have moved to epidemiologic data supporting an association between depression and low magnesium status. Finally, we have summarized what is the state of the art of the efficacy evidence of magnesium in several depression figures, such as depression in presence of hypomagnesemia, and treatment resistant depression. We have ultimately concluded that evidence is not enough at the present time to confirm or exclude any relevant role of magnesium among treatment means for depression.

We suggest that more studies in the future focus on magnesium supplementation of depression treatment, and in particular that trials with specific features are needed. First of all trials should include larger sample sizes, and have a randomized double-blinded design, to minimize any possible risk of bias. Moreover, studies should focus on specific populations, in order to answer the question whether magnesium is helpful in treating depressive symptoms in case of hypomagnesemia only, or if its properties are useful in amplifying antidepressants' action in any figure of depression, regardless the magnesium state.

Summary Points

- Magnesium is an important physiologic regulator of cellular homeostasis, including neurons.
- Low magnesium status is frequent.
- Low magnesium status is involved in several severe medical conditions, such as diabetes and cardiovascular disease.
- Low magnesium status can contribute to depression pathogenesis or maintenance through calcium-mediated cellular toxicity, disruption of inflammatory cytokines homeostasis, and impaired serotonin balance.
- Low magnesium status increases the risk of depression, and can be frequent in a population affected by depression.
- Several animal studies suggest that hypomagnesemia leads to depression.
- Several animal studies suggest that magnesium supplementation has antidepressant activity.
- Several animal studies suggest that magnesium supplementation amplifies the action of antidepressants according to behavioral tests.
- Preliminary evidence suggests a role of magnesium supplementation in improving depression in patients with hypomagnesemia.
- Studies in humans are too few and with small sample sizes to draw any definite conclusion about the role of magnesium in depression, regardless the magnesium status.
- Further studies are needed to better characterize the role of magnesium in treating depression, with larger sample sizes, randomized double blind design, and subjects with either low or normal magnesium state.

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Magnesium Deficiency: Prevalence, Assessment, and Physiological Effects

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Jesse Bertinato

Contents

Abstract

Magnesium (Mg) is an essential mineral nutrient that functions as a structural component of organic molecules and cofactor in hundreds of enzymatic reactions. Mg is required for calcium and potassium ion transport, muscle contraction and

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relaxation, neurotransmitter release, protein biosynthesis, cellular energy production, mitochondrial functions, stabilization of cell membranes, transmembrane ion flux, genome stability, bone development, and glucose homeostasis. The intestine, kidneys, and bone are the major sites regulating whole-body Mg balance. Food, drinking water, and Mg-containing supplements are the main sources of Mg exposure. Recommended intakes for Mg have been established by scientific bodies, but values differ substantially. In North America, Mg intakes fall short of dietary recommendations for a large segment of the population. Overt Mg deficiency and marked hypomagnesemia can cause a wide range of symptoms and have serious health consequences; however, this degree of deficiency is rare in the general population. At present, the prevalence of Mg deficiency in the general population is unclear because of the uncertainty regarding Mg intakes needed for optimal health and the lack of an ideal biomarker of Mg status. Many observational studies have shown an inverse association between Mg intake and risk of diseases and health conditions, but there is a need for large, placebo-controlled, randomized trials to address the issue of causality. There is also a need for a comprehensive review of the existing evidence to determine if a reference interval for total serum (plasma) Mg concentration based on a health outcome can be established.

Keywords

Adequate intake · Dietary intake · Dietary reference values · Estimated average requirement · Hypomagnesemia · Magnesium deficiency · Metabolism · Nutritional biomarker · Serum magnesium concentration · Tolerable upper intake level

List of Abbreviations

AI	Adequate intake
AR	Average requirement
CCHS 2.2	Canadian Community Health Survey Cycle 2.2
DRVs	Dietary reference values
EAR	Estimated average requirement
EFSA	European Food Safety Authority
EU	European Union
IOM	Institute of Medicine
Mg	Magnesium
mo	Month
NHANES I	First National Health and Nutrition Examination Survey
PRI	Population reference intake
PTH	Parathyroid hormone
RBC	Red blood cell
RDA	Recommended dietary allowance
SCF	Scientific Committee on Food
UL	Tolerable upper intake level
y	year

Introduction

Magnesium (Mg) is an alkaline earth metal with atomic number 12. It is an essential mineral nutrient and the fourth most abundant cation in the human body and the second most abundant intracellular cation after potassium. The adult human body contains ~22–26 g of Mg, most of which is present in bone (~55%), muscle (~25%), and soft tissues (~20%) (Swaminathan 2003; Wester 1987). Only a small fraction of total body Mg is present in the extracellular fluid (<1%) and serum (~0.3%).

Within cells Mg functions as a structural component of organic molecules and a catalytic cofactor in hundreds of enzymatic reactions (Pasternak et al. 2010). Table 1 lists some of the many diverse biochemical processes that require Mg. A number of biological functions are related to Mg's role as a regulator of calcium and potassium ion transport including muscle contraction and relaxation, neurotransmitter release, action potential conductance, and regulation of vascular tone. Bound to ATP or GTP, Mg maintains a favorable conformation of the nucleotides which facilitates the release of phosphate for energy requiring reactions. Mg is also important for protein biosynthesis, cellular energy production, mitochondrial functions, stabilization of cell membranes, transmembrane ion flux, genome stability, bone development, and glucose homeostasis (Pasternak et al. 2010; Swaminathan 2003; Volpe 2013; Elin 1994; Wolf and Cittadini 1999).

Table 1 Magnesium-dependent biochemical processes

Cellular transport of calcium and potassium
Muscle contraction and relaxation
Synaptic transmission
Action potential conductance
Regulation of vascular tone
Protein biosynthesis
Glycolysis
Krebs cycle
β-oxidation
Mitochondrial function (ATP production)
Cell membrane stabilization
Transmembrane ion flux
Genome stability (DNA repair, DNA replication, chromosome segregation)
Bone development
Glucose homeostasis

Magnesium is required for many biochemical processes as a structural component of organic molecules or catalytic cofactor in enzymatic reactions

Key: *ATP* adenosine triphosphate, *DNA* deoxyribonucleic acid

Whole-Body Magnesium Metabolism

The intestine, kidneys, and bone are the major sites that regulate whole-body Mg balance (Fig. 1). Dietary Mg is absorbed from the small and large intestines by two processes: a passive transport system that involves solvent drag and a saturable active transport system. The efficiency of Mg absorption is ~40–60% but can vary considerably depending on the quantity of Mg ingested (Schwartz et al. 1984; Andon et al. 1996). Fractional absorption is inversely proportional to the amount of Mg ingested. Mg is mostly absorbed by the passive system when intakes are high, whereas the active system predominates when intakes are low.

The kidneys function to maintain Mg balance by regulating the reabsorption of filtered Mg. Mg excretion in the urine increases to eliminate excess body Mg, whereas urinary Mg excretion is reduced to conserve Mg under conditions of deficiency. Bone is a store for Mg that supplements the extracellular fluid and tissues when Mg levels decline. Diet, health conditions (e.g., digestive disorders, kidney diseases), or medications (e.g., hypermagnesuric diuretics) that alter gastrointestinal, renal, or bone Mg metabolism can lead to Mg deficiency or toxicity.

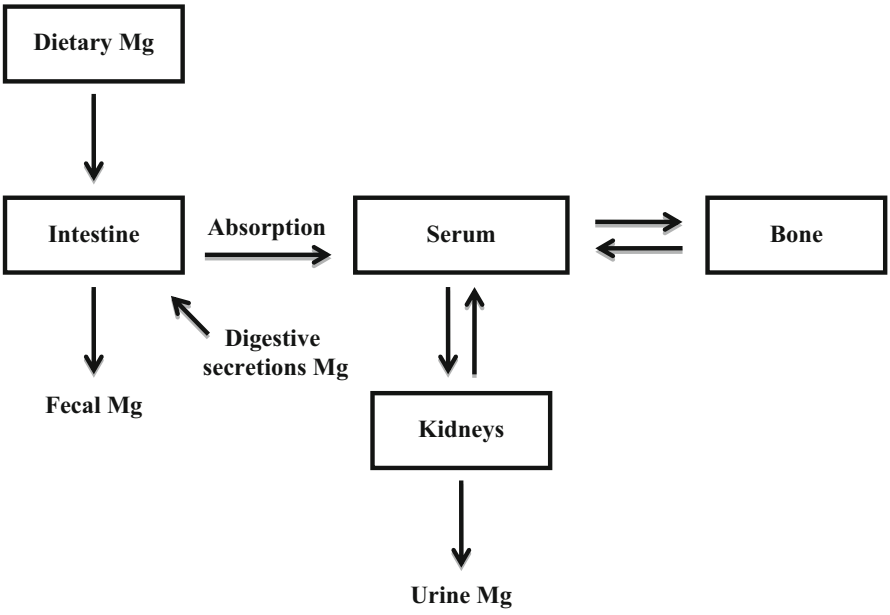

Fig. 1 Major sites regulating whole-body magnesium homeostasis. The intestine, kidneys, and bone are the main sites controlling whole-body magnesium balance. Magnesium is absorbed in the small and large intestines. Bone stores magnesium and supplements the serum under conditions of deficiency. The kidneys play a critical role in regulating body magnesium through the reabsorption of filtered magnesium. When magnesium is in excess, urinary magnesium excretion is increased. Under conditions of magnesium deficiency urinary excretion is reduced. Magnesium status is primarily affected by dietary magnesium intake and factors that alter intestinal magnesium absorption or urinary magnesium excretion Key: *Mg* magnesium

Dietary Reference Values (DRVs) for Magnesium

Mg is a mineral nutrient required in relatively large amounts. At present, Mg intakes needed for optimal health are not well defined. Recommended intakes and tolerable upper intake levels (UL) for Mg by life-stage group established for North America (Canada and United States) and the European Union (EU) are shown in Table 2. As part of the North American dietary reference intakes, the Institute of Medicine (IOM) established an estimated average requirement (EAR) for Mg for children, adolescents, and adults (Institute of Medicine 1997). The EARs were based on Mg balance studies and range from 65 to 350 mg day^{-1}. For children 1–3 y, 4–8 y, and 9–13 y EARs and Recommended Dietary Allowances (RDAs) were set the same for males and females. Values for males were set higher for adolescents and adults. For pregnancy the EARs were established at an additional 35 mg day^{-1} based on accretion of lean body mass and an adjustment factor for a bioavailability of 40%. For lactation recommended intakes were set the same as for nonlactating women because of the lack of evidence indicating that Mg requirements are increased during lactation. It was presumed that reduction in urinary Mg excretion and increased Mg resorption from bone provides the additional Mg needed for milk production. For infants 0–6 mo an Adequate Intake (AI) was established based on average intake of human milk (780 mL day^{-1}) and the average concentration of Mg in human milk (34 mg L^{-1}). The AI for 7–12 mo old infants was derived by adding Mg consumed from human milk and solid food.

The European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies (NDA) established DRVs for Mg for the EU (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) 2015). The panel noted the limitations of using balance studies to set DRVs and it was concluded that Average Requirements (ARs) and Population Reference Intakes (PRIs) could not be derived for any life-stage group and therefore AIs were established based on observed intakes in nine EU countries. AIs ranged from 80–350 mg day^{-1}. For infants aged 7–11 mo the AI was derived by extrapolating upward from the estimated Mg intake in exclusively breast-fed infants 0–6 mo and taking into consideration observed average Mg intakes from a limited amount of surveys. AIs for children, adolescents, and adults were based on observed intakes in healthy populations in the EU. Similar AIs were established for both sexes for children 1– $<$ 3 y and 3– $<$ 10 y. AIs for children/adolescents 10– $<$ 18 y and adults $\geq$18 y were set higher for males compared to females. It was concluded that there was a lack of evidence to support higher requirements during pregnancy or lactation and AIs for women aged $\geq$18 y were set the same as for non-pregnant or non-lactating women.

The IOM selected diarrhea as the critical endpoint for setting the UL for Mg. The UL for Mg is unique in that it applies only to Mg consumed from nonfood sources. A UL of 350 mg day^{-1} for supplementary Mg was established for persons aged $>$8 y. It was assumed that children were as vulnerable to the effects of supplementary Mg as adults and the ULs for children were derived from the adult UL by extrapolating downward based on body weight. It was concluded that there was no evidence to suggest that pregnant and lactating women are more vulnerable

Table 2 Reference values for magnesium

	North America[a]				European Union[b]	
Life stage group	AI (mg day^{-1})	EAR (mg day^{-1})	RDA (mg day^{-1})	UL (mg day^{-1})[c]	AI (mg day^{-1})	UL (mg day^{-1})[d]
0–6 mo	30	–	–	–	–	–
7–11 mo[e]	–	–	–	–	80 (80)	–
7–12 mo	75	–	–	–	–	–
1–<3 y[e]	–	–	–	–	170 (170)	–
1–3 y	–	65	80	65	–	–
3–<10 y[e]	–	–	–	–	230 (230)	–
4–8 y	–	110	130	110	–	–
≥4 y	–	–	–	–	–	250
>8 y	–	–	–	350	–	–
9–13 y[e]	–	200 (200)	240 (240)	–	–	–
10–<18 y[e]	–	–	–	–	300 (250)	–
14–18 y[e]	–	340 (300)	410 (360)	–	–	–
≥18 y[e]	–	–	–	–	350 (300)	–
19–30 y[e]	–	330 (255)	400 (310)	–	–	–

31–50 y[e]	–	350 (265)	420 (320)	–	–	–
51–70 y[e]	–	350 (265)	420 (320)	–	–	–
>70 y[e]	–	350 (265)	420 (320)	–	–	–
14–18 y (P)	–	335	400	350	–	–
19–30 y (P)	–	290	350	350	–	–
31–50 y (P)	–	300	360	350	–	–
≥18 y (P)	–	–	–	–	300	–
14–18 y (L)	–	300	360	350	–	–
19–30 y (L)	–	255	310	350	–	–
31–50 y (L)	–	265	320	350	–	–
≥18 y (L)	–	–	–	–	300	–

Key: *AI* Adequate Intake, *EAR* Estimated Average Requirement, *L* lactation, *mo* month, *P* pregnancy, *RDA* Recommended Dietary Allowance, *UL* Tolerable Upper Intake Level, *y* year

[a]Values were obtained from (Institute of Medicine 1997)

[b]Values for AIs were obtained from (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) 2015) and the value for the UL was obtained from (Scientific Committee on Food 2001)

[c]UL only applies to supplementary magnesium

[d]UL only applies to readily dissociable magnesium salts and compounds like magnesium oxide in nutritional supplements, water, or added to food and beverages

[e]Values for females are in parentheses

to the adverse effects of supplementary Mg. ULs for pregnancy and lactation were set the same as for non-pregnant or non-lactating women. A UL was not established for infants because of the absence of data on the effects of Mg supplements in infants.

The Scientific Committee on Food (SCF) established a UL for Mg for the EU based on a similar endpoint, a transient laxative effect (Scientific Committee on Food 2001). The UL does not apply to Mg normally present in foods and beverages and was established for readily dissociable Mg salts (e.g., chloride, sulfate, aspartate, etc.) and chemical compounds like Mg oxide in nutritional supplements, or added to food and beverages. The same UL of 250 mg day^{-1} was established for persons aged $\geq$4 y including pregnant and lactating women. A UL for children aged $\leq$3 y was not established given the lack of data on adverse effects for this age group and that it was deemed inappropriate to extrapolate from the UL for older children and adults based on body weight.

Sources of Magnesium Exposure

Food, Mg-containing dietary supplements, and drinking water are the main sources of Mg exposure. Mg can also be absorbed through the skin or by inhalation; however, for most persons this amount is negligible in comparison to total daily intakes. The Mg content of some Mg-rich foods available in Canada is shown in Table 3 (Health Canada 2015). The Mg contents were obtained from the Canadian Nutrient File (CNF) and are shown per serving size and 100 g of the food. Nuts, seeds, legumes, seafood, cereals, and dark green vegetables are rich sources of Mg.

Supplement use can make a substantial contribution to total Mg intakes. The most commonly used multivitamin-multimineral supplements sold in Canada and the United States usually contain 50–100 mg of Mg (Nielsen Market Track 2012; Ford and Mokdad 2003). A 100 mg supplement corresponds to 24–32% of the RDA for adults.

Drinking water can also be an important source of Mg intake. A survey of bottled water products and municipal water sources in Ottawa, Ontario, Canada, showed that Mg content varies widely among sources of drinking water (Bertinato and Taylor 2013). Values ranged from 0 mg L^{-1} in purified water up to 103 mg L^{-1} in mineral water. Consumption of 2.2 L day^{-1} for women and 3.0 L day^{-1} for men [approximately the amount of water consumed from all beverages for adults at the 50th percentile (Institute of Medicine 2005)] of drinking water containing the highest amounts of Mg can provide more than 20% of the RDA.

Some studies in rats and humans have suggested that the bioavailability of Mg from certain organic compounds may be greater compared to some inorganic compounds (Coudray et al. 2005; Walker et al. 2003). However, a study that fed rats similar amounts of Mg from three inorganic (Mg oxide, Mg sulfate and Mg chloride) and five organic (Mg citrate, Mg gluconate, Mg orotate, Mg malate and EDTA disodium Mg) Mg compounds (in the context of a high phytic acid diet) reported similar Mg bioavailability from all compounds (Bertinato et al. 2014b).

Table 3 Magnesium content of foods

Food	Serving size	Magnesium per serving (mg)	Magnesium per 100 g (mg)
Nuts and seeds			
Brazil nuts (without shell)	60 mL	133	375
Almonds, cashews, pine nuts (without shell)	60 mL	70–109	220–287
Hazelnuts, walnuts (without shell)	60 mL	47–52	157–173
Pumpkin or squash seeds (without shell)	60 mL	317	551
Sunflower seeds (without shell)	60 mL	112	345
Flaxseeds and sesame seeds	30 mL	56–78	295–366
Legumes			
Peas (cooked)	175 mL	121	96
Fermented soy products (cooked)	150 g	116	77
Soybeans (cooked)	175 mL	109	87
Hummus	125 mL	92	71
Peanuts (without shell)	60 mL	65	176
Peanut butter	30 mL	51	159
Black/lima/navy/adzuki/white kidney/ pinto/great northern beans	175 mL	60–89	–
Tofu (prepared with magnesium chloride or calcium sulfate)	150 g	45–80	30–53
Lentils, split peas (cooked)	175 mL	52	35
Fish and seafood			
Salmon, halibut (cooked)	75 g	80–92	107–123
Atlantic mackerel/pollock (cooked)	75 g	64–73	85–97
Atlantic snow crab (cooked)	75 g	47	63
Milk and alternates			
Soy cheese	50 g	114	228
Soy yogourt	175 g	70	40
Grain products			
All bran cereals	30 g	94–111	313–370
Wheat germ cereal (toasted)	30 g	96	320
Quinoa (cooked)	125 mL	47	48
Vegetables			
Spinach, Swiss chard (cooked)	125 mL	80–83	87
Artichoke	1 (medium)	77	60
Edamame/baby soy beans (cooked)	125 mL	52	55
Fruits			
Tamarind	125 mL	58	91
Avacado (raw)	1/2 fruit	36	24

(*continued*)

Table 3 (continued)

Food	Serving size	Magnesium per serving (mg)	Magnesium per 100 g (mg)
Banana (raw)	1 (medium)	32	27
Papaya (raw)	1/2 fruit	32	21

Average values for magnesium content of magnesium-rich foods available in Canada. Magnesium content is shown per serving size of the food source or per 100 g of the food. Data obtained from Health Canada. Canadian Nutrient File (2015). Available at http://www.hc-sc.gc.ca/fn-an/nutrition/fiche-nutri-data/index-eng.php

Rats were fed Mg at an amount inadequate to maintain optimal Mg status and the ability of each compound to preserve Mg status of the rats was examined. The results showed that Mg status of the rats (assessed by bone, serum and urine Mg concentrations) fed the different Mg compounds was similar after a 5 week feeding period indicating that any differences in the bioavailability of Mg among compounds were small and physiologically irrelevant.

Magnesium Intakes

In Canada and the United States, Mg intakes are lower than dietary recommendations for a large segment of the population (Ford and Mokdad 2003; Health Canada 2012). Mg intakes in the US population were estimated by 24 h dietary recalls from the National Health and Nutrition Examination Survey 1999–2000 (Ford and Mokdad 2003). Median intakes for adults aged ≥20 y were 326 mg day^{-1} (mean: 352 mg day^{-1}) for white men and 237 mg day^{-1} (mean: 256 mg day^{-1}) for white women. Median intakes were 237 mg day^{-1} (mean: 278 mg day^{-1}) for African American men and 297 mg day^{-1} (mean: 330 mg day^{-1}) for Mexican American men. Median intakes were 177 mg day^{-1} (mean: 202 mg day^{-1}) for African American women and 221 mg day^{-1} (mean: 242 mg day^{-1}) for Mexican American women. Racial differences were observed. Mean intakes were lower for African Americans compared to white Americans for both sexes. For the three racial groups examined, women had lower intakes compared to men.

More recent national estimates of usual Mg intakes were determined for the US population in the National Health and Nutrition Examination Survey 2005–2006 (U.S. Department of Agriculture 2009). Usual Mg intakes were based on 24 h dietary recalls from participants 1 y of age and over. More than half of Americans 19 y and over had Mg intakes from food and water below the EAR. A comparable shortfall in Mg intake exists in Canada. Results from the Canadian Community Health Survey, Cycle 2.2 (CCHS 2.2) conducted in 2004 showed that among Canadians 19 y and older greater than 34% had intakes from food and water below the EAR (Health Canada 2012). For several sex-age groups, estimates exceeded 40% below the EAR.

Mg intakes from food (fortified or not fortified) in nine EU countries (i.e., Finland, Germany, Italy, United Kingdom, France, Netherlands, Ireland, Sweden, and Latvia)

were analyzed by the EFSA Panel on Dietetic Products, Nutrition and Allergies for the purpose of setting AIs for Mg (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) 2015). The range of average Mg intakes (without consideration of Mg intake from dietary supplements) was 317–439 mg day^{-1} for men and 254–357 mg day^{-1} for women 18– < 65 y of age. For men and women aged 65– < 75 y, the range was 312–407 mg day^{-1} and 241–343 mg day^{-1}, respectively. The range of Mg intakes for older adults aged >75 y were 264–388 mg day^{-1} for men and 232–347 mg day^{-1} for women.

Assessment of Magnesium Status

Accurate assessment of physiological Mg status presents a challenge since an ideal nutritional biomarker is lacking. The most studied and commonly used biomarkers are listed in Table 4. The Mg loading test (also called the Mg retention test) is a sensitive method for evaluation of Mg deficiency (Rob et al. 1999). The method involves parenteral administration (usually intravenously) of a known quantity of Mg and determination of the amount of Mg excreted in the urine over a 24 h period. This allows calculation of the percentage of the Mg dose retained by the body. With increased severity of Mg deficiency, the percentage of the Mg load retained by the body increases. A strong negative correlation exists between percentage Mg retained and Mg concentration in bone. A shorter version of the Mg loading test that infused the Mg load over 1 h (instead of the standard 8 h) also proved useful for assessment of Mg deficiency (Rob et al. 1999). Although considered the "gold standard" for evaluating Mg status, the Mg loading test is complex and time consuming. Thus, this test is not ideal for use in large studies or for routine screening. In addition, it is unsuitable for persons with impaired renal function.

Total serum or plasma Mg concentration is the most frequently used method for assessing Mg status in humans. A population-based reference interval for total serum Mg concentration of 0.75–0.955 mmol L^{-1} was established based on results from the first National Health and Nutrition Examination Survey (NHANES I) conducted in the United States between 1971 and 1974 (Lowenstein and Stanton 1986). Thus, a serum Mg concentration lower than 0.75 mmol L^{-1} is usually considered as hypomagnesemia.

Table 4 Nutritional biomarkers of magnesium status

Magnesium loading (retention) test
Serum or plasma total magnesium concentration
24 h urine magnesium excretion
Red blood cell magnesium concentration
Bone magnesium concentration

Many biomarkers in various biological samples have been studied for their potential to measure magnesium status. Listed are the best characterized and most commonly used biomarkers.

Despite its widespread use, serum Mg concentration has certain limitations. Serum Mg concentration can be affected by changes in concentrations of serum proteins such as albumin. At low and high albumin concentrations, a linear relationship with serum Mg has been reported (Kroll and Elin 1985); however, the effect appears minimal when albumin concentrations are within the normal reference interval. Serum Mg concentration may not always accurately reflect intracellular Mg deficiency. Serum Mg concentration is tightly controlled primarily by renal Mg excretion. Bone also maintains serum Mg concentrations by supplementing the serum under conditions of deficiency. This strong homeostatic control may explain, at least in part, the poor association observed between dietary Mg intakes and serum Mg concentrations, in particular when Mg intakes exceed nutritional requirements.

Despite certain limitations, low-serum Mg concentrations usually indicate Mg deficiency. In animal models of dietary Mg restriction, serum Mg has been shown to be reduced in a dose-dependent manner demonstrating that serum Mg responds to dietary Mg deficiency and reduced Mg status (Bertinato et al. 2014a; Bertinato et al. 2014b). In humans, serum Mg responds to long-term changes in Mg intakes (Klevay and Milne 2002) and increases with Mg supplementation (Witkowski et al. 2011; Zhang et al. 2016a). A recent meta-analysis of randomized controlled trials of oral Mg supplementation showed that serum (plasma) Mg concentration increases in a time- and dose-dependent manner (Zhang et al. 2016a). Notably, increases were greater for persons with lower baseline serum Mg concentrations. It has been suggested that a serum Mg concentration below 0.75 mmol L^{-1} is a useful indicator of Mg deficiency, but a value greater than 0.75 mmol L^{-1} does not exclude deficiency (Arnaud 2008; Elin 2010).

Twenty-four hour urine excretion of Mg responds to Mg intake and may provide meaningful information on Mg homeostasis and status. Analyzes of results from Mg depletion and supplementation studies showed an overall response of 24 h urinary Mg excretion to dietary Mg intake (Witkowski et al. 2011). Analyzes of data from randomized controlled trials of Mg supplementation showed that 24 h urine Mg excretion was increased compared to the placebo groups and responded to the supplementation in a dose- and time-dependent manner (Zhang et al. 2016a). The response of 24 h urinary Mg to supplementation was greater for persons with higher baseline serum Mg concentrations or 24 h urine Mg excretion.

Mg depletion and supplementation studies show that red blood cell (RBC) Mg concentration (expressed as RBC volume) responds to Mg intake (Witkowski et al. 2011). Analyzes of nine randomized controlled trials of oral Mg supplementation showed that RBC Mg was increased compared to the placebo groups (Zhang et al. 2016a). A similar dose (i.e., 300 mg day^{-1}) was used in all these studies which prevented evaluation of a dose-response relationship.

Bone Mg concentration is a sensitive measure of total body Mg status and decreases in a dose-dependent manner in animal models of dietary Mg restriction (Bertinato et al. 2014a; Bertinato et al. 2014b). Bone Mg concentration is a valuable tool for evaluating Mg status in experimental animals, but the invasiveness of this method limits its use in humans.

A number of other potential biomarkers in various biological samples have been tested for suitability in assessing Mg status (Witkowski et al. 2011; Zhang et al. 2016a). However, for these biomarkers the number of studies is limited and thus conclusions regarding their value cannot be accurately assessed. Results from two trials did show that fecal Mg concentrations were higher in Mg supplemented versus placebo groups (Zhang et al. 2016a).

Physiological Effects of Magnesium Deficiency

Persons with Mg deficiency can be asymptomatic or present a number of clinical symptoms (Table 5). The metabolism of potassium and calcium is dependent on Mg. Low serum potassium concentration (hypokalemia) is a common manifestation of Mg deficiency and is most likely caused by increased potassium excretion by the kidneys. Low-serum calcium concentration (hypocalcemia) is often observed with Mg deficiency. Mg deficiency can impair secretion of parathyroid (PTH) hormone. Lower-circulating PTH reduces intestinal calcium absorption and calcium reabsorption from bone which can lead to hypocalcemia. There is also evidence to

Table 5 Clinical symptoms of magnesium deficiency

Altered mineral metabolism
Hypocalcemia (responsive to magnesium therapy)
Hypokalemia (responsive to magnesium therapy)
Cardiac abnormalities
Cardiac arrhythmias
Electrocardiogram changes
Neuromuscular symptoms
Tremor
Myoclonic twitches or jerks
Convulsions
Chvostek's sign
Trousseau's sign
Carpopedal spasm
Ataxia
Nystagmus
Dysphagia
Tetany
Muscle fasciculation
Muscle cramps
Psychiatric disturbances
Depression
Psychosis

Listed are clinical symptoms that may be observed in patients with overt magnesium deficiency and marked hypomagnesemia.

suggest end-organ resistance to the action of PTH when Mg deficient (Rude et al. 1976). Correction of the hypokalemia or hypocalcemia is dependent on Mg therapy.

Mg deficiency can have profound effects on the cardiovascular system. Cardiac arrhythmias and electrocardiogram changes can occur. In one study, postmenopausal women were fed a diet providing Mg at ~33% of the RDA. Five of the 14 women in the study developed heart rhythm changes which required Mg repletion before completion of the 78-day Mg depletion period of the study (Nielsen et al. 2007). Neuromuscular hyperexcitability is an early sign of clinical Mg deficiency. Neuromuscular symptoms can include tremors, myoclonic jerks, convulsions, Chvostek's sign, Trousseau's sign, carpopedal spasm, ataxia, nystagmus, dysphagia, tetany, muscle fasciculation, and muscle cramps (Flink 1981). Mg deficiency can also cause psychiatric disturbances such as depression and psychosis. It is important to mention that clinical symptoms of Mg deficiency can be secondary to metabolic changes in other minerals such as calcium or potassium.

Clinical symptoms of Mg deficiency are usually observed in patients with moderate to severe Mg deficiency and marked hypomagnesemia (<0.6 mmol L^{-1}). This degree of deficiency is rare in the general population. The effect of milder degrees of hypomagnesemia (Mg deficiency) on health is less understood. There is evidence, mostly from cross-sectional or prospective cohort studies, showing that low Mg intakes (compared to current dietary requirements) common in the general population are associated with increased risk of chronic diseases and health conditions.

Prospective cohort studies have shown a significant inverse relationship between Mg intake and incidence of type 2 diabetes. Meta-analyses have reported relative risks of 0.85 (95% CI: 0.79–0.92) (Larsson and Wolk 2007) and 0.86 (95% CI: 0.82–0.89) (Dong et al. 2011) for every 100 mg day^{-1} increment in Mg intake. In the Nurses' Health Study and the Health Professionals Follow-up Study, women were followed for 18 y and men for 12 y (Lopez-Ridaura et al. 2004). Comparing the highest with the lowest quintile of total Mg intake, the relative risk of type 2 diabetes was 0.66 (95% CI: 0.60–0.73) in women and 0.67 (95% CI: 0.56–0.80) in men.

Meta-analyses of eight cross-sectional and two prospective cohort studies showed a pooled relative risk of metabolic syndrome of 0.88 (95% CI: 0.84–0.93) for every 150 mg day^{-1} increase in Mg intake for adults (Ju et al. 2014). Meta-analysis of six cross-sectional studies reported that for every 100 mg day^{-1} increment in Mg intake the overall risk of having metabolic syndrome was decreased by 17% (Dibaba et al. 2014).

A qualitative summary of 29 observational studies relating dietary Mg intake to blood pressure suggests an inverse association (Mizushima et al. 1998). A meta-analysis of 34 randomized, double-blind, placebo-controlled trials indicate a causal effect of Mg supplementation on reducing blood pressure in adults (Zhang et al. 2016b). A significant reduction of systolic (2.00 mm Hg) and diastolic (1.78 mm Hg) blood pressure was observed with Mg supplementation at a median dose of 368 mg day^{-1} for a median duration of 3 months. An increase in serum Mg concentration by 0.05 mmol L^{-1} was also observed in comparison to the placebo groups and secondary analyses showed that serum Mg concentration was inversely associated with diastolic, but not systolic blood pressure.

A meta-analysis of prospective cohort studies showed a significant nonlinear inverse relationship between Mg intake and total cardiovascular disease events comprising stroke, coronary heart disease, and cardiovascular disease death (Qu et al. 2013a). The largest reduction in risk was observed when comparing intakes of 150–400 mg day^{-1}. In a separate meta-analysis of prospective cohort studies dietary Mg was not significantly associated with cardiovascular disease, but was associated with a 22% lower risk of ischemic heart disease (Del Gobbo et al. 2013). Dietary Mg and fatal ischemic heart disease showed a nonlinear inverse relationship, with the association detected up to ~250 mg day^{-1}. Another meta-analysis of prospective cohort studies failed to observe an association between Mg intake and total cardiovascular disease mortality, but an inverse relationship was found in females suggesting that sex may be an important modifier (Xu et al. 2013). Associations between sudden cardiac death and dietary Mg intake or plasma Mg concentration were examined prospectively in the Nurses' Health Study (Chiuve et al. 2011). Results showed a lower relative risk of sudden cardiac death in women in the highest versus the lowest quartile of dietary Mg intake or plasma Mg concentration.

A meta-analysis of eight prospective cohort studies revealed an inverse association between Mg intake and total or ischemic stroke (Nie et al. 2013). In a large Dutch cohort of adults aged 21–70 y, Mg intake was also associated with reduced risk of stroke (Sluijs et al. 2014). Associations between stroke and intakes of Mg, potassium, and calcium were examined in the Nurses' Health Study I and II (Adebamowo et al. 2015). For women in the highest compared to the lowest quintiles for total Mg intake, the pooled multivariate relative risk of total stroke was 0.87 (95% CI: 0.78–0.97). In that study high intake of potassium, but not calcium, was also associated with a lower risk of stroke.

A meta-analysis of eight prospective cohort studies showed a relative risk for colorectal cancer of 0.89 (95% CI: 0.79–1.00) when comparing the highest to the lowest category of Mg intake (Chen et al. 2012). A meta-analysis of seven prospective cohort studies showed a relative risk for colorectal cancer of 0.81 (95% CI: 0.70–0.92) when comparing the highest to the lowest Mg intakes (Qu et al. 2013b). The inverse association was nonlinear with the greatest reduction in risk occurring for Mg intakes between 200 and 270 mg day^{-1}.

Low Mg intake may decrease bone mineral density (Orchard et al. 2014) and impair physical performance (Veronese et al. 2014). Studies in rats suggest that adequate Mg intake is important for growth of lean body mass (Bertinato et al. 2016; Venu et al. 2005; Venu et al. 2008).

Prevalence of Magnesium Deficiency

Marked hypomagnesemia is common in hospitalized patients with reported incidence rates of 11% (Wong et al. 1983). Higher incidence rates of 65% have been reported among critically ill patients (Ryzen 1989). The primary causes are reduced Mg absorption and increased urinary excretion that can result from disease, malnourishment, or medications. Mg deficiency is common among alcoholics and

both type 1 and type 2 diabetics. Low Mg intake, reduced absorption, and increased urinary excretion contribute to the poor Mg status observed in alcoholics. It has been estimated that as high as 13.5–47.7% of type 2 diabetics are hypomagnesemic (Pham et al. 2007). The cause is multifactorial and includes increased loss of Mg in the urine from osmotic diuresis and possibly insulin resistance. A cross-sectional study in Ottawa, Canada, showed that serum Mg concentration was lower in South Asian Canadians compared to white Canadians (Bertinato et al. 2015). After controlling for use of diabetes medication, racial differences in serum Mg were no longer significant suggesting that the higher incidence of diabetes in South Asians makes them more vulnerable to Mg deficiency.

In North America, the increase in food processing most likely has contributed to the shortfall in Mg intakes compared to dietary recommendations. However, the prevalence of Mg deficiency in the general population is not known because of the uncertainty regarding Mg intakes needed for optimal health. At present, there are large discrepancies in DRVs for Mg established by authoritative scientific bodies (Institute of Medicine 1997; EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) 2015; Joint FAO/WHO Expert Consultation 2004).

The challenge in determining the prevalence of Mg deficiency in the general population is further compounded by the lack of a biomarker with a defined reference interval for health that can be used for routine evaluation of Mg status and in large studies. Total serum Mg concentration is the most widely used measure; however, the normal reference interval was based on serum Mg concentrations in the US population and not a health outcome. Thus, accurate assessment of the prevalence of Mg deficiency using this reference interval is not possible and can lead to large underestimates or overestimates.

Policies and Protocols

Research on the Health Effects of Low Magnesium Intakes and Nutritional Biomarkers of Magnesium Status

Overt Mg deficiency and marked hypomagnesemia can induce a number of clinical symptoms and have serious health consequences, but this degree of deficiency is uncommon in the general population. At present, there is a poor understanding of Mg intakes needed for optimal health and an ideal biomarker for assessment of Mg status is lacking, making it difficult to determine the incidence of Mg deficiency in the general population. Many studies have reported an inverse association between Mg intake and risk of chronic diseases and health conditions. However, much of the evidence comes from observational studies making it impossible to establish a causal relationship. There is a need for large, well-controlled, randomized clinical trials on the effects of Mg intakes on risk of chronic diseases and health conditions that will allow for evaluation of a dose-response relationship and address the issue of causality. Recent studies suggest that total serum (plasma) Mg concentrations can provide meaningful information on Mg status (Witkowski et al. 2011; Zhang et al.

2016a). Given that serum Mg can be easily measured at low cost, there is a need for a comprehensive review of the evidence to determine if a reference interval (or at minimum a lower cut-off value) for serum Mg based on a health outcome can be established. This will allow for accurate assessment of the prevalence of Mg deficiency and related health risk in populations which can be used to inform nutrition policies and regulations aimed at improving Mg status if deemed necessary.

Dictionary of Terms

- **Adequate Intake (AI)** – Recommended average daily intake based on estimates of nutrient intake of apparently healthy people that are presumed adequate.
- **Canadian Nutrient File (CNF)** – A food composition database that contains average values for nutrients in foods available in Canada.
- **Dietary Reference Values (DRVs)** – A set of recommended intakes for a nutrient that is often specific for life-stage groups.
- **Estimated Average Requirement (EAR)** – The average daily nutrient intake sufficient to meet the nutritional needs of 50% of the healthy persons in a particular life-stage group.
- **Hypocalcemia** – Low serum or plasma calcium concentration.
- **Hypokalemia** – Low serum or plasma potassium concentration.
- **Hypomagnesemia** – Low serum or plasma magnesium concentration.
- **Nutritional biomarker** – A quantifiable substance or characteristic in an organism that provides information on the physiological status of a nutrient.
- **Recommend Dietary Allowance (RDA)** – The average daily nutrient intake sufficient to meet the nutritional needs of 97.5% of the healthy persons in a particular life-stage group.
- **Tolerable Upper Intake Level (UL)** – The highest average daily nutrient intake that is likely to cause no adverse health effects to nearly all persons in a particular life-stage group.

Summary Points

- Magnesium is a mineral nutrient that functions as a structural component of organic molecules and a cofactor in hundreds of enzymatic reactions.
- Magnesium is required for calcium and potassium ion transport, muscle contraction and relaxation, neurotransmitter release, protein biosynthesis, cellular energy production, mitochondrial functions, stabilization of cell membranes, transmembrane ion flux, genome stability, bone development, and glucose homeostasis.
- The intestine, kidneys, and bone are the major sites regulating whole-body magnesium balance.
- Recommended intakes for magnesium established by scientific bodies differ considerably.

- Food, drinking water and magnesium-containing supplements are the major sources of magnesium exposure.
- Magnesium intakes fall short of dietary recommendations for a large proportion of the North American population.
- Total serum (plasma) magnesium concentration is the most commonly used biomarker for assessing magnesium status.
- Clinical manifestations of overt magnesium deficiency and marked hypomagnesemia can include hypocalcemia, hypokalemia, cardiac abnormalities, neuromuscular symptoms, and psychiatric disturbances.
- Magnesium deficiency and hypomagnesemia is common in hospitalized patients and persons with diabetes or alcoholism.
- The prevalence of magnesium deficiency in the general population is unknown because of the uncertainty regarding magnesium intakes required for optimal health and the lack of an ideal biomarker of magnesium status.
- Many observational studies have shown an inverse association between magnesium intake and risk of chronic diseases and health conditions.
- There is a need for large, placebo-controlled, randomized trials to examine the effects of a range of magnesium intakes on risk of diseases and health conditions to establish causal inference.
- There is a need for a comprehensive review of the existing evidence to determine whether a reference interval for total serum magnesium concentration can be established based on a health outcome.

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Selenium and Cognition: Mechanism and Evidence

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Dawd Gashu and Barbara J. Stoecker

Contents

Abstract

Selenium (Se) is important for cognition by way of its contribution to the reduction of oxidative stress. Reactive species (RS) are generated during the normal course of metabolism in the body. Evidence shows that RS are known to regulate cellular functions. They also participate in the immune system and biosynthesis of macromolecules. On the other hand, accumulated RS are highly toxic to the body and they are major concern in the pathogenesis of chronic diseases including cancers, cardiovascular disease, and neurodegenerative

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diseases. Every aerobic cell is vulnerable to attack by RS consequently, resulting in oxidative stress. However, the brain is particularly susceptible to oxidative stress due to its composition and physiology. Oxidative stress to the brain is associated with cognitive deficits, mood disorders, and behavioral problems. Evidence from animal and human studies suggest the importance of selenium for cognitive performance, mood, and behavior through protection against oxidative damage to substrates including low-density lipoproteins (LDL), hydroperoxide, hydrogen peroxide, peroxynitrite, and tert-butyl hydroxyperoxide (t-BHP). The dose response relationship between Se and cognitive performance is non-linear suggesting both deficiency and excess intake results in adverse neurobehavioral outcomes. In addition, through its role in thyroid metabolism, limited evidence suggests the importance of selenium in improving cognitive performance of persons with hypothyroidism.

Keywords

Alzheimer's disease · Antioxidant · Brain · Cognition · Depression · Oxidative stress · Mood · Reactive oxygen species · Selenoproteins · Selenium

List of Abbreviations

AD	Alzheimer's disease
ATP	Adenosine triphosphate
CSF	Cerebrospinal fluid
CuZn-SOD	Copper, zinc-superoxide dismutase
DNA	Deoxyribonucleic acid
GHS	Glutathione
GPx	Glutathione peroxidase
IL-6	Interleukin-6
LDL	Low density lipoprotein
MCI	Mild cognitive impairment
NBNA	Behavioral neurological assessment
PKU	Phenylketonuria
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RS	Reactive species
rT3	Reverse triiodothyronine
SeMet	Selenomethionine
SePP	Selenoprotein P
SOD	Superoxide dismutase
T_4	Thyroxin
T_3	Tri-iodothyronine
T_2	Di-iodothyronine
TBARS	Thiobarbituric acid reactive substances
t-BHP	Tert-butyl hydroxyperoxide
TrxR	Thioredoxin reductase

Introduction

Selenium (Se) is important for cognition by way of its contribution to the reduction of oxidative stress. Prooxidants and reactive species (RS) (oxygen and nonoxygen RS) are indispensable products of the normal body metabolism. Reactive species participate in the normal vascular physiology, cellular oxygen sensing, and genomic stability and transcription regulation (Alfadda and Sallam 2012). In addition, the immune system requires certain oxidative states to destroy pathogens and control inflammation. Reactive species are also implicated in the biosynthesis of thyroid hormones (Brieger et al. 2012). On the other hand, RS at higher concentrations are highly toxic to biological macromolecules such as lipid, protein, and DNA, and thus are major concerns in the pathogenesis of chronic diseases including cancers, cardiovascular disease, and neurodegenerative diseases (Datta et al. 2000).

Oxygen is essential for cellular respiration, but about 5% of oxygen in the body is converted into reactive oxygen species (ROS) thus all aerobic cells are prone to oxidative damage. Attributable to its physiology and composition, the brain, however, is at higher risk of oxidative stress (Halliwell 2006). The underlying factors for brain's susceptibility to oxidative stress are summarized in the paragraph below.

The brain requires significant ATP generation thus high oxygen (O_2) utilization which is estimated to be 20% of the basal O_2 consumption. This high O_2 consumption yields high RS relative to other organs. High dopamine concentration in the neurons can undergo oxidation to generate metabolites such as 6-hydroxydopamine that are toxic to the brain. Normally, glutamate is found in smaller amounts in the extracellular fluid of the brain. However, release of glutamate upon neuronal cell death and slow or inefficient clearance of this glutamate by glial cells results in over accumulation. In addition, inactivation of the enzyme glutamine synthase that converts glutamate to glutamine is another notable risk factor for the accumulation of glutamate. This over accumulation induces oxidative damage to the neurons in the brain. Furthermore, free metal ions such as iron in different parts of the brain stimulate the initiation of free radicals through Fenton-type reactions. The brain is also an organ vulnerable to oxidative stress because neuronal membranes are rich in polyunsaturated fatty acids that are extremely susceptible to lipid peroxidation. Finally, even though the brain is potentially highly susceptible to RS, it is equipped only with modest antioxidant systems (low concentrations of catalase, glutathione peroxidase, and vitamin E) (Halliwell 2006; Cui et al. 2004; Kim et al. 2000).

Unbalanced redox states in the body, where the intake and endogenous synthesis of RS exceeds the handling capacity of the antioxidant systems, result in the development of oxidative stress (Halliwell 2006). Oxidative stress in the brain has been suggested to negatively influence cognitive performance and cause mood disorder and behavioral deficits (Berr et al. 2000). Evidence from animal and human studies supports the benefit of antioxidant compounds in counteracting RS (Fukui et al. 2002; Keller et al. 2005). Selenium is a potent antioxidant nutrient. It exists in the active sites of several enzymes that catalyze reactions essential to the protection of tissues against oxidative damage and consequently has been associated with prevention of cognitive and behavioral deficits (Steinbrenner and Sies 2009).

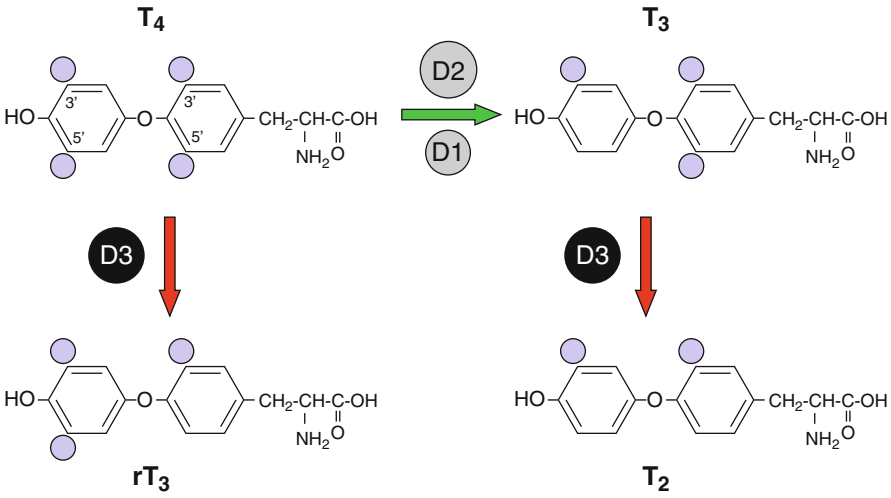

Fig. 1 Schematic presentation for the role of iodothyronine deiodinase enzymes in thyroid hormone metabolism (Adapted with permission from Pol et al. 2010)

Thyroid hormones regulate genes involved in myelination, synaptogenesis, and neuronal cell differentiation during early brain development (Bernal 2007). This process is thought to explain why deficiencies in iodine, the basis of thyroid hormones, contribute to delayed or reduced cognitive functioning. Selenium is an important cofactor for iodothyronine deiodinase enzymes involved in the conversion of the inactive thyroid hormone thyroxin (T_4) into the biologically active form triiodothyronine (T_3), or into the inactive metabolite reverse triiodothyronine (rT_3) and di-iodothyronine (T_2) (Fig. 1). In addition, the selenoenzyme thioredoxin reductase (TrxR) protects the autoxidation of the thyroid gland by hydrogen peroxide (H_2O_2) produced during the synthesis of thyroid hormones (Beckett and Arthur 2005). Thus, through its role in normal thyroid hormone synthesis and protection of the thyroid gland against oxidative damage, Se may influence cognitive performance. This review summarizes available evidence and underlying mechanisms on the relationship between Se nutrition and cognitive performance, mood and behavior.

Selenium and Selenoproteins

Selenium is a trace element needed by the body in small amounts. It is classified as a metalloid, exhibiting intermediate properties between metals and nonmetals. The element exists in organic forms associated with amino acids such as selenocysteine and selenomethione; and in inorganic forms as selenite (SeO_3^{-2}), selenide (Se^{2-}), selenate (SeO_4^{-2}), and elemental selenium (Se). Selenium enters the food chain through plants, which take it up from the soil. Soil Se distribution in the world is

Table 1 Range of selenium concentration in foods from different parts of the world (Navarro-Alarcon and Cabrera-Vique 2008)

Food item	Concentration range (ng/g)[a]
Meat[b]	27.0–770.0
Organ meats (pork kidney, pork liver)	256.0–1543.0
Fish (salmon, tuna, sardines)	270.0–810.0
Milk and milk products[c]	0.7–100.0
Fruits[d]	1.4–76.0
Vegetable[e]	3.0–180.0
Legumes (lentils and peanuts)	18.0–75.0
Refined cereals (bread, pasta)	5.8–131.8
Eggs	0.7–172.8

[a]Se concentration varies widely based on type of food and country of origin
[b]Beaf, lamb, rabbit, ham, oyster
[c]Cow's milk, cheese, yoghurt, butter, condensed milk, ice cream, buffolo milk
[d]Apple, kiwi, grapes, Sharon fruits, mango, orange
[e]Garlic, celery, lettuce, onion, green peas, pepper

highly variable (0.1–4.5 mg/kg) to the extent that deficiency and toxicity can occur among populations residing in nearby areas. Selenium status of populations is largely dependent on the concentration of phytoavailable Se (i.e., available for plant uptake) in the soil where staple crops are growing. Absorption of Se in humans is efficient and thus, deficiency mainly occurs in areas where dietary intakes are inadequate due to soil factors. Summary of range of Se concentration in foods from different parts of the world is indicated in Table 1. Phytoavailability of Se is influenced by soil physicochemical characteristics such as pH, soil mineral composition, organic matter content, soil textural class (clay, sand, silt fractions), and presence of Se-competing ions in the soil. In addition, even though Se has no role in crop nutrition, some species of plants are physiologically adapted to higher Se absorption and accumulation. Selenium through selenoproteins has diverse biological functions including thyroid hormone synthesis, DNA synthesis, immune function, fertility, and in the antioxidant defense system (Mehdi et al. 2013).

Selenium in the form of selenocysteine is a key component of the active site of diverse selenoproteins of essential biological functions. Selenoproteins predominantly play antioxidant roles in the body. So far, 25 selenoproteins have been discovered in human beings. Of the selenoproteins, selenoprotein P (SELENOP) and glutathione peroxidase (GPx) are the most studied in the context of their role in the prevention and control of oxidative damage. List and biological function of selenoproteins are summarized in Table 2. SELENOP is an extracellular glycoprotein containing 10 selenocysteine residues. About half of the circulating Se in blood plasma is found in the form of SELENOP which transports Se from the liver to different parts of the body and maintains a steady state of the element in the brain (Traulsen et al. 2004). For example, deletion of the SELENOP gene in animal models caused reduction in whole body Se by increasing urinary Se excretion (Burk et al. 2006). SELENOP also plays a critical role in protection of the body

Table 2 Common selenoproteins and their biological role (Brown and Arthur 2001)

Selenoproteins	Biological role
Glutathione peroxidase (GPx) Cytosolic GPx (GPx-1), gastrointestinal GPx (GPx-2), plasma GPx (GPx-3), phospholipid hydroperoxide GPx (GPx-4), epididymal GPx (GPx-5), olfactory epithelium GPx (GPx-6)	Reduce lipid hydroperoxides and H_2O_2 into alcohol and water
Iodothyronine deiodinases (deiodinase type 1, deiodinase type 2, deiodinase type 3)	Activation or deactivation thyroid hormones
Selenoprotein P (Se-P1, Se-P2, Se-P6, Se-P10)	Selenium supply from the liver to certain organs such as the brain, testis, and kidney Defense against oxidative injury
Selenoprotein W	Muscle metabolism in animals
Thioredoxin reductase (TrxR)	Catalyzes the reduction of thioredoxin substrate
Seleno-phosphate synthetase	Incorporation of selenocysteine into functional selenoproteins
Sperm capsule selenoprotein GPx4	Sperm mitochondrial capsule synthesis which aids sperm motility and protection against oxidative damage

against oxidative damage. In an in vitro system, Traulsen et al. (2004) reported the protection of SELENOP against oxidation of low-density lipoproteins (LDL). SELENOP, in human brain cell culture, was implicated in protecting astrocytes (ROS detoxifying cells in the brain) against oxidative stress induced by tert-butyl hydroxyperoxide (t-BHP) (Steinbrenner et al. 2006a). Similarly, SELENOP prevented *t*-BHP induced oxidative cell death by preventing disintegration of endothelial cell membrane (Steinbrenner et al. 2006b).

The other selenoprotein GPx is a family of enzymes known to reduce lipid hydroperoxides and H_2O_2 into alcohol and water, respectively. Eight isoforms of GPx have been identified. All isoenzymes have a catalytic center composed of glutamine and tryptophan residues. However, they show tissue and substrate specificity (Imai and Nakagawa 2003; Steinbrenner and Sies 2009). Of the eight isoforms, cytosolic glutathione peroxidase GPx (GPx-1), gastrointestinal GPx (GPx-2), plasma GPx (GPx-3), phospholipid hydroperoxide GPx (GPx-4), epididymal GPx (GPx-5), olfactory epithelium GPx (GPx-6) are selenoproteins with a selenocysteine active site (Brigelius-Flohé and Maiorino 2013).

Antioxidation Role of Se Compounds

The antioxidant potential of Se has been investigated by several researchers and nucleophilicity of the element is considered to be the basis for reactivity of selenocompounds and selenoproteins (Arteel and Sies 2001). Kunwar et al. (2007) investigated the in vitro antioxidant activity of a selenocysteine compound, 3,3-diselenobispropionic acid, and reported that the compound prevented the

depletion of erythrocyte glutathione (GSH) from free radical-induced hemolysis. A comparative study on superoxide radical scavenging capacity of Se (selenocarbamates) and sulfur (thiocarbamate) compounds by Takahashi et al. (2005a) indicated that the scavenging activity of selenocompounds ranged from 8.6–68.7% while activity ranged only between 2.9% and 29.5% for the tested thiocarbamates. In addition, tertiary selenoamide compounds (Takahashi et al. 2004), selenourea compounds (Takahashi et al. 2005b), and 1,3-selenazolidin-4-one compounds (Nishina et al. 2011) were reported to show superoxide anion scavenging capacity.

Free metal ions such as iron are important in initiating the generation of free radicals that attack macromolecules in the body. However, Se compounds are also known to effectively inhibit metal-mediated oxidative damage (Battin et al. 2011). In addition, some selenoenzymes exhibit metal coordination systems at their active site to inhibit metal-mediated oxidative damage. For example, molybdo-selenoprotein and Se-tungsten complexes at the active site of formate dehydrogenase enzymes are able to inhibit metal-mediated DNA damage (Battin et al. 2011).

Role of Selenoproteins in the Brain

The brain can retain Se at the expense of other tissues even during a state of Se depletion. It is also a priority organ for Se repletion indicating its key role for brain function. Selenium transport and homeostasis in the brain is carried out by SELENOP (Nakayama et al. 2007). Thus, a change in Se nutrition affects the activity of SELENOP. Conversely, an alteration to SELENOP affects Se levels in the body. An earlier study on the rate of clearance of Se from plasma after administration of ^{75}Se labeled SELENOP or ^{75}Se labeled GPx to Se deficient rats reported a fast clearance of ^{75}Se labeled SELENOP from plasma compared to ^{75}Se given as GPx, suggesting the transporting role of SELENOP. That study also reported that the brain was the priority organ for Se repletion (Burk et al. 1991). Similarly, target deletion of the SELENOP gene in mice resulted in Se deficiency in the brain and consequently neurological pathology (Hill et al. 2004). For example, disrupted spatial learning and synaptic dysfunction were reported among SELENOP deficient mice (Peters et al. 2006). Even interfering with the function of SELENOP by replacing it with the shorter version, SELENOP1$^{\Delta 240–361}$, or deletion of its receptor were associated with severe brain injury (Valentine et al. 2008).

Selenium and Cognitive Function

Selenium is important throughout the lifecycle, from fetal development through to the elderly. The formation, growth, and development of fetal brain cells are very rapid (Clouchoux et al. 2012). Certain nutrients including Se are thought to be especially important for optimal growth and development of the brain and consequently for cognitive function (Georgieff 2007). A recent

prospective mother and child cohort study investigated the impact of serum Se in different periods of pregnancy on child psychomotor functions at ages 1 and 2 years and indicated that maternal Se status during the first trimester of pregnancy was positively associated with motor development at 1 year, and Se levels in cord blood showed significant positive association with language development at 2 years. However, serum Se level at the second or third trimester had no significant association with any of the tested cognitive domains (Polanska et al. 2016).

Strong association between maternal erythrocyte Se at 27–28 weeks gestation and psychomotor development and language comprehension in young children at 1.5 years of age was also observed. Interestingly, the association was mainly apparent in girls (Skröder et al. 2015). Although, there is no clear evidence for an effect of gender on cognitive performance being mediated by Se, in female rats estrogen could influence transport and concentration of Se in the brain (Zhou et al. 2012). In another study in rats, long-term Se deficiency in mothers caused growth retardation to the offspring in utero and after delivery. Se deficiency was also associated with spatial learning and memory deficit in the offspring (Hong et al. 2006). A mother–new born dyad epidemiological study in China found an inverted 'U' relationship between cord blood Se and scores for the Neonatal Behavioral Neurological Assessment (NBNA). The authors reported 100 μg/L cord blood Se to be the maximum point on the U-shaped curve (Yang et al. 2013). Thus, the dose response relation between Se and cognitive outcome is not always linear, and both deficiency and excessive intake may result in adverse neurobehavioral outcomes. Therefore, interventions involving Se nutrition should be done with care and after thorough analysis of usual dietary intakes especially because Se has a narrow tolerable range between deficiency and toxicity (WHO 1996).

Controlling for potential confounders in the analysis such as micronutrient status (Fe, I, Zn), parental education, and anthropometric measurements, young children in Ethiopia with low serum Se had significantly lower scores on two reasoning subtests of the Wechsler Preschool and Primary Scale of Intelligence and the school readiness test than children with adequate Se (Gashu et al. 2016) (Fig. 2). Selenium deficiency can be due to inadvertent lack of Se in the diet or due to deliberate reduction of dietary components that also contain Se. For example, phenylketonuric (PKU) patients may be at greater risk of Se deficiency because they must consume low protein diets that consequently may be low in Se (van Bakel et al. 2000). A study on PKU patients that measured antioxidant cofactors (i.e., Se, retinol, tocopherol, coenzyme Q_{10}, a free radical damage marker in plasma (malondialdehyde)) and antioxidant enzymes in red blood cells (GPx, catalase, superoxide dismutase) reported significantly lower values of Se, coenzyme Q_{10}, and catalase in these blood components. In cognitive test analyses, more omission errors, fluctuating attention and inconsistency of response times, and slowing reaction time were recorded among the groups with lower plasma Se (Gassió et al. 2008).

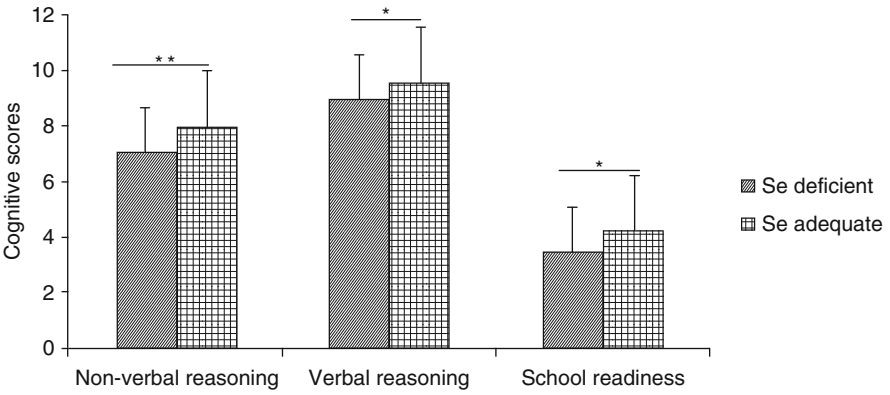

Fig. 2 Comparison of cognitive performance among selenium deficient young children and children of adequate selenium nutrition (*the difference is significant at $p < 0.05$, or **$p < 0.01$) (Adapted with permission from Gashu et al. 2016)

Selenium and Cognitive Decline in the Elderly

Cognitive decline is an important cause of disability and suffering particularly to the elderly and it contributes costs to healthcare systems. Despite progress in understanding the pathophysiology of the disease, it remains a significant challenge to modern science and medicine. Oxidative stress however is known to be a major player in the incidence of cognitive impairment and Alzheimer's type of dementia (Mecocci and Polidori 2012).

In a 9-year longitudinal follow-up study (n = 1389) of French elderly people (age 60–71 years) to evaluate the relationships between cognitive decline and oxidative stress, a decrease in plasma Se over time was associated with cognitive decline. Over the 9 years follow-up, participants with plasma Se level at the top tertile had a lower risk of developing cognitive decline. Subjects with the lowest plasma Se had greater odds (OR 1.58, 95% CI 1.08–2.31) of cognitive decline compared to participants with the highest plasma Se (Akbaraly et al. 2007; Berr et al. 2012). To investigate whether oxidative stress could explain cognitive decline in the elderly (60–70 years) in a cohort study of older subjects (n = 1166), plasma Se and carotenoids as antioxidant micronutrients, and thiobarbituric acid reactive substances (TBARS) as an indicator of lipoperoxidation were measured. Subjects in the highest percentile for TBARS had increased risk of cognitive decline emphasizing the importance of oxidative stress in cognitive decline and the protective role of Se (Berr et al. 2000). In a cross sectional survey of 2000 stable rural Chinese elderly (≥65 years), Se status was significantly associated with cognitive function. Also to best explain the dose-response relationship, the authors compared cognitive performance by nail Se quintiles and found a significant increase in performance on six out of seven cognitive tests as a function of Se quintile

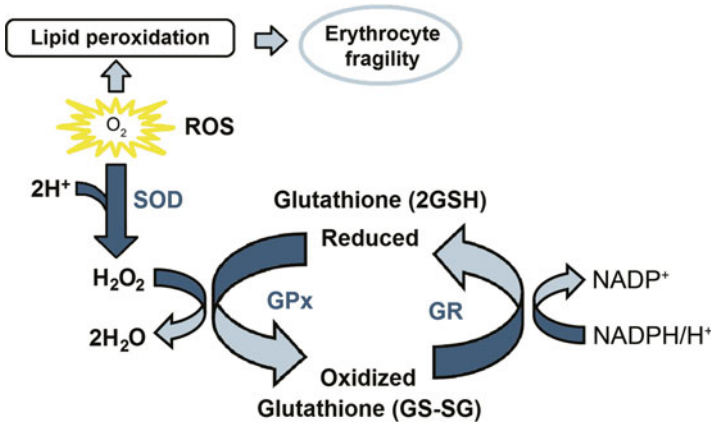

Fig. 3 Stepwise deactivation of reactive species with superoxide dismutase and glutathione peroxidase enzymes (Adapted with permission from Mineo et al. 2014). Superoxide dismutase catalyzes the dismutation of superoxide radical into H_2O_2 then glutathione peroxidase further converts H_2O_2 into H_2O

(Gao et al. 2007). In addition, among a sample of 484 (40–97 years) residents of communities in Texas and New Mexico, exposure to high ground water Se was associated with better memory function and reduced risk of cognitive decline (Hall et al. 2012).

The body has multiple protective antioxidative systems. Several of these are interdependent. Copper, zinc-superoxide dismutase (CuZn-SOD) catalyzes the dismutation of the superoxide radical (O_2^-) into hydrogen peroxide (H_2O_2) (Weydert and Cullen 2010). The Se-dependent GPx further converts H_2O_2 into H_2O (Fig. 3). The ratio of CuZn-SOD activity to GPx thus determines protection against oxidative damage from superoxide radical (O_2^-) and H_2O_2. To investigate the protective effect of antioxidant enzymes on the cognitive function of the elderly, Berr et al. (2004) measured red blood cell activity of CuZn-SOD and GPx. Subjects with cognitive decline exhibited significantly lower red blood cell GPx but higher SOD indicating high conversion rate of superoxide radical (O_2^-) into H_2O_2 but slow rate of H_2O_2 reduction.

Selenium and Alzheimer's Disease

Alzheimer's disease (AD) is a progressive brain disorder that leads to cognitive decline. Although the etiology of AD is unclear and believed to involve several factors, it has been commonly associated with the presence of oxidative stress in response to increased accumulation of oxidative products of nucleic acids (nuclear DNA, mitochondrial DNA, and RNA) (Moreira et al. 2008) and protein carbonyls (Aksenov et al. 2001) to areas of the brain important for cognition such as the neocortex, hippocampus, and other subcortical regions. In addition, amyloid β-peptide plays a role in the pathogenesis of AD (Butterfield et al. 2001) by

disrupting the blood-brain barrier consequently exposing the brain for T-lymphocytes invasion (Farkas et al. 2003; Ryu and McLarnon 2009).

Researchers have investigated various aspects of the postmortem damage seen in human AD patients using cell and tissue culture and animal models. An over-expression of SELENOP in postmortem human tissues with lesions of AD was documented (Bellinger et al. 2008). Another study of SELENOP and AD showed that compared to the control cells, SELENOP deficient cells had higher susceptibility for apoptotic cell death by amyloid β-peptide induced oxidative damage suggesting the protective role of SELENOP in AD (Takemoto et al. 2010). In addition, Se in the form of seleno-L-methionine (SeMet) improved survival of rat hippocampal neurons by preventing oxidative stress due to amyloid β-peptide accumulation (Xiong et al. 2007).

A significant positive correlation between serum trace element concentrations (Se, Fe, Cr, and Co) and cognitive scores was reported among a group of people with cognitive impairment nondementia (a subclinical phase of dementia) and a group of people with manifestation of AD. Patients with AD of the dementia type exhibited the lowest serum Se concentration compared to patients with cognitive impairment nondementia and control groups (Smorgon et al. 2004). A study comparing Se status in AD patients, subjects with mild cognitive impairment (MCI) and a control group, showed that the AD group had the lowest plasma Se (Cardoso et al. 2014). In addition, elderly subjects with AD had significantly lower Se concentration in plasma, erythrocyte, and nail samples compared to control groups of similar age, again suggesting the important relationship between Se deficiency and AD (Cardoso et al. 2010).

Selenium and Depression

Biomarkers and behavioral alterations that may be associated with depression have been investigated in animal models and human beings. Selenium deficiency may have a role in the pathophysiology of depression by diminishing dopamine concentrations in the cerebrospinal fluid (CSF) and brain regions, and by inducing inflammation to the brain through inflammatory cytokines (Miller et al. 2009; Castaño et al. 1997). In a nested case-control study of Se deficient and Se adequate women, higher odds of depression symptoms were reported among Se deficient women (Pasco et al. 2012). In aged mice, consumption of diets high in antioxidants showed promising effects on improving psychomotor performance by reducing interleukin-6 concentration in the brain (Richwine et al. 2005). However, Se supplementation to healthy individuals with adequate baseline Se with no symptom of mood disorder had no mood improving benefit (Hawkes and Hornbostel 1996). In a randomized control trial of the effect of Se on mood of 501 healthy UK elderly, supplementation with 100, 200, or 300 μg Se/day as high-Se yeast for 6 months significantly increased blood Se level but not mean total mood score. In addition, there was no difference among supplementation dose groups suggesting Se supplementation to healthy individuals with normal baseline Se status may not benefit mood and supplementation

should not be encouraged particularly given that the element has a narrow range between adequate and toxic dose (Rayman et al. 2006).

Role of Se for Cognition via Thyroid Metabolism

Thyroid hormone is an important factor in regulating the process of synaptogenesis, myelination, and neuronal cell differentiation throughout brain development (Ahmed et al. 2008). Selenium is an essential component of iodothyronine deiodinase enzymes important for normal thyroid metabolism, and it is a cofactor of the TrxR enzyme that protects the thyroid gland against oxidative stress by H_2O_2 produced during thyroid hormone synthesis (Beckett and Arthur 2005). The relationship between thyroid hormones and cognitive function is well established. Lower concentration of thyroid hormone, a condition called hypothyroidism, is an important risk factor for cognitive decline (del Ser et al. 2000; Klein et al. 2001). Based on its importance to normal thyroid metabolism, adequate Se nutrition is considered as a factor that might be important for cognitive performance. For example, a study designed to evaluate the possible benefit of Se supplementation on reversing cognitive dysfunction exposed to the antithyroid drug methimazole and exhibiting lower thyroid hormone production over the experimental period as evaluated using elevated plus maze, open field, and Morris water maze tests shows that hypothyroid rats in general exhibited high latency in locomotory performance and deficiency in memory and learning abilities compared to the control groups. Interestingly, Se supplementation reversed the hypothyroidism associated learning and memory deficits (Dias et al. 2012). However, there is a dearth of literature concerning possible roles for Se in cognition via its effects on thyroid hormone metabolism by the selenodependant enzyme, iodothyronine deiodinase.

Policies

- Selenium is an important component of the body's protection from oxidative damage and consequently is important in prevention and reversal of oxidative stress induced cognitive decline, mood, and behavioral deficits. However, Se supplementation to healthy individuals with marginal or adequate Se status is not beneficial and should be discouraged especially given that Se has a narrow margin between adequate and toxic concentration.
- Concentration of plant-available Se in soils largely determines staple crop Se concentrations and therefore the Se nutritional status of populations. However, Se distribution and availability in the soils is highly variable globally. Thus, soil mapping and integration with food system datasets can identify Se deficiency risks and inform the design of public health nutrition interventions. In addition, interventions must be carefully monitored because there is a narrow range between Se deficiency and toxicity.

- Selenium is implicated to play diverse roles in the human body including synthesis of macromolecules, fighting against viral infections, fertility, and prevention of chronic disease. It is also shown to be important for neurobehavioral development in the fetal period to cognitive performance through to old age. Selenium nutrition should get the desired attention in international micronutrient guidelines and recommendations.

Dictionary of Terms

- **Alzheimer's disease** – First identified by Dr. Alois Alzheimer during 1906. It is the commonest form of irreversible dementia characterized by severe difficulties in memory and thinking.
- **Amyloid plaque** – Abnormal deposition of toxic proteins called amyloid β peptides in neuron cells.
- **Antioxidants** – Exogenous or endogenous substances that inhibit oxidation of other molecules.
- **Cognition** – The mental processes of thinking, memory, problem-solving, reasoning, and other high-level functions of the brain.
- **Fenton reactions** – Reduction of hydrogen peroxide to produce reactive oxygen species under the catalytic action of trace elements, mainly iron.
- **Lipid peroxidation** – A metabolic degradation of unsaturated fatty acids by reactive oxygen species
- **Oxidative stress** – Damage to body tissues due to excessive reactive species beyond the capacity of the antioxidant defense system.
- **Prooxidants** – Xenobiotic or endogenous substances that themselves are reactive species or support generation of reactive species to induce oxidative stress to the body.
- **Selenocysteine** – Considered as the "21st amino acid" which is analogous to the amino acid cystein but containing selenium in place of sulfur.
- **Selenoproteins** – Proteins in the body containing one or more selenocystein amino acid residues.
- **Thyroid hormones** – Hormones (thyroxin and triiodothyronin) produced by thyroid gland.

Summary Points

- This chapter reviews the role of selenium nutrition on cognitive performance.
- Accumulation of reactive species generated during the normal course of metabolism is highly toxic to biological macromolecules and is a major concern in the pathogenesis of chronic diseases and a significant factor for cognitive decline.
- Even though every aerobic cell is at risk of oxidative damage, the brain is especially vulnerable and this condition is a significant factor for cognitive decline.

- Selenium compounds and selenoproteins exhibited a strong antioxidant role against oxidative substrates such as low-density lipoproteins, hydroperoxide, hydrogen peroxide perxynitrite, tert-butyl hydroxyperoxide, and several others. Selenium deficiency or altering the structure or deletion of selenoproteins is associated with severe brain injury.
- Maternal Se status is associated with cognitive ability of the new born. However, the relationship between maternal Se and cognitive ability is not linear (inverted "U") suggesting both deficiency and excessive Se intake may result in adverse neurobehavioral outcomes.
- Selenium nutrition in the elderly prevents the progression of Alzheimer's disease, cognitive decline, mood disorder, and depression. However, Se supplementation to healthy individuals with adequate baseline Se with no symptom of mood disorder has not been shown to improve mood.
- Hypothyroidism is associated with reduced cognitive performance. However, Se nutrition prevents and reverses hypothyroidism-induced cognitive deficits.
- Limited or no literatures are available on the role of Se in cognition through its effect on thyroid hormone metabolism thus new studies are recommended.

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Selenium Deficiency and Selenium Supplements: Biological Effects on Fibrosis in Chronic Diseases, from Animal to Human Studies

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Abstract

Selenium is a trace element, which is required for normal growth and development of animals and humans. It works by incorporating into proteins to make selenoproteins. These selenoproteins help to prevent free radicals from causing cellular damage, which may in turn lead to the development of various chronic diseases. Selenium deficiency, although is rare, can happen when the body does not have enough selenium. This chapter will review systematically the effects of selenium deficiency on fibrosis in various chronic diseases, such as cardiac fibrosis, liver fibrosis, kidney fibrosis, cystic fibrosis, thyroid fibrosis, oral submucous fibrosis, and pancreatic fibrosis in both animal and human studies. Moreover, their prevention and treatment with selenium supplement will be evaluated as well.

Keywords

Cardiac fibrosis · Cystic fibrosis · Fibrosis · Kidney fibrosis · Liver fibrosis · Oral submucous fibrosis · Selenium deficiency · Selenium supplement · Thyroid fibrosis · Pancreatic fibrosis

List of Abbreviations

CCL_4	Carbon tetrachloride
cGPx	Cellular glutathione peroxidase
ECM	Extracellular matrix
eGPx	Extracellular glutathione peroxidase
GPx	Glutathione peroxidase
HSCs	Hepatic stellate cells
HT	Hashimoto's thyroiditis
KBD	Kashin-Beck disease
KD	Keshan disease
MMP	Matrix metalloproteinase
SP	Selenium-enriched probiotics
TGF-β	Transforming growth factor-β
TIMP	Metalloproteinase inhibitor
WHO	World Health Organization

Introduction

Selenium is an important element, which is necessary in small amounts for the growth and development of animals and humans (Moghadaszadeh and Beggs 2006; Papp et al. 2007; Roman et al. 2014). Selenium is incorporated into proteins to form vital selenoproteins such as glutathione peroxidase (GPx) as a part of the active site (Lu and Holmgren 2009). In the ecosystem, bacteria, fungi, and plants serve as the primary producers, as they can easily integrate and convert inorganic selenium into organic selenium species. By consumption of the primary producers,

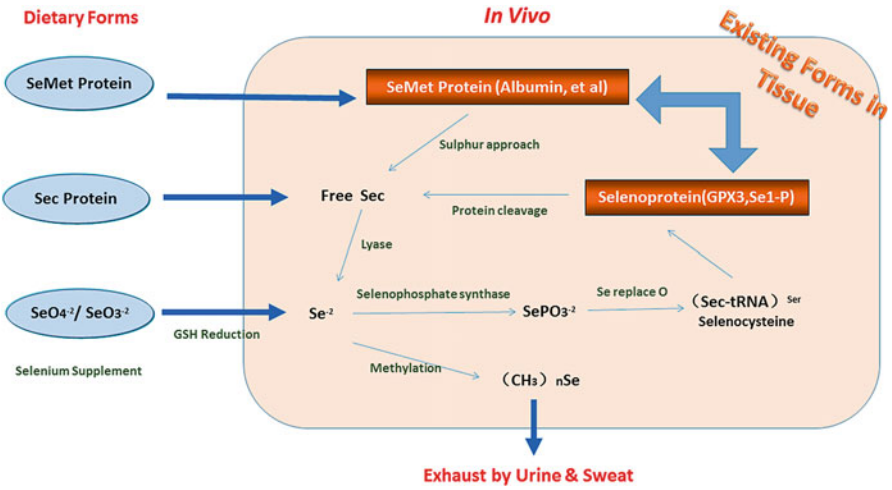

Fig. 1 Representative photo of selenium metabolism. Selenium can be in the dietary forms of SeMet, Sec proteins, and other selenium salts; when in vivo, selenium is incorporated into proteins to form vital selenoproteins, and the metabolite can be exhausted by urine or sweat

both forms of selenium enter the food chain and move to higher trophic levels (Chapman et al. 2010). In vivo, selenium is absorbed through the gastrointestinal tract in the form of selenite. Following its reduction to selenide by enterocytes, selenium is bound to albumin and transferred to the liver, where it is used for the synthesis of cellular glutathione peroxidase (cGPx), other selenoproteins, and metabolites (i.e., selenosugars). The selenium is then re-excreted into the bloodstream and transferred to the kidney, where it is degraded and utilized to synthesize extracellular glutathione peroxidase (eGPx) and selenosugars (Patterson et al. 1989; Suzuki and Ogra 2002; Swanson et al. 1991). Representative of the selenium metabolism is shown in Fig. 1.

Selenium deficiency is an issue faced worldwide, particularly in developing countries (Johnson et al. 2010; Spallholz et al. 2004). According to the World Health Organization (WHO), 40 countries encountered low-selenium or selenium-deficient problem, and China is one of them (Li et al. 2007). Although there are some areas with high-selenium intake in China, the selenium-deficient areas account for 72% of the total area, which translate to 70 million people facing the potential adverse health impacts as a result of selenium deficiency (Gao et al. 2011). A long-term selenium-deficient diet could lead to damages in multiple organs of the body (Han et al. 2016, 2017; Tan et al. 2002) and induce diseases, such as Keshan disease (KD, endemic cardiomyopathy) and Kashin-Beck disease (KBD, endemic osteoarthropathy) in China (Fig. 2). However, the relationship between selenium deficiency and tissue fibrosis in chronic diseases has not been fully explored. In this chapter, the studies on the relationships between various tissue fibrosis and selenium deficiency from animal and/or human will be reviewed systematically (Table 1). Furthermore, it will provide a comprehensive knowledge of the effects of selenium deficiency on fibrosis in

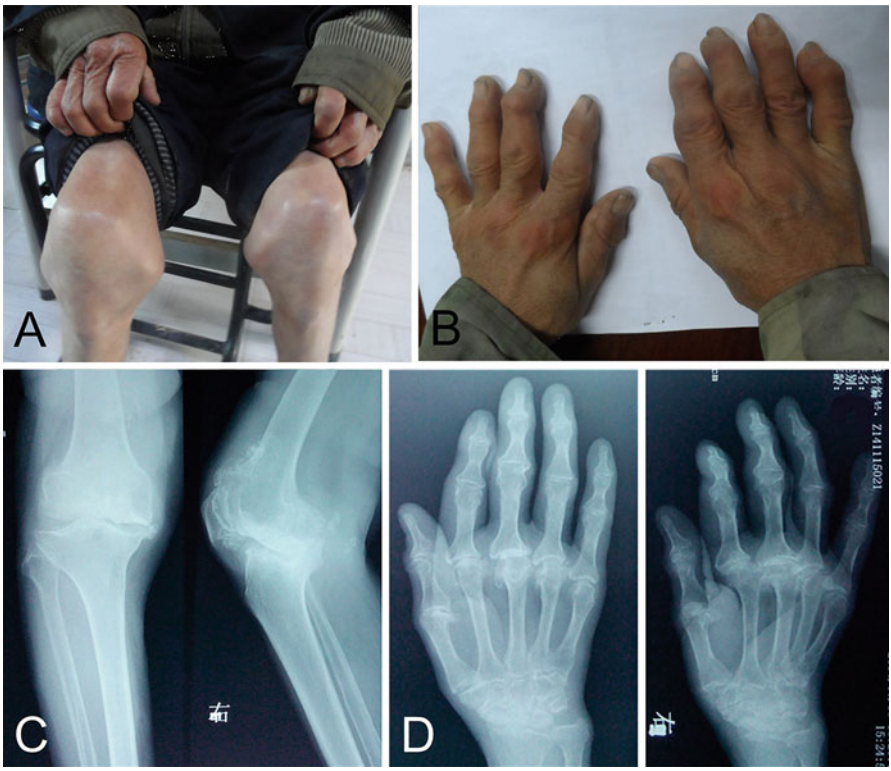

Fig. 2 Characteristics of KBD patients. (**a**) Deformed joints in two knees and (**b**) flexion of the terminal finger joints or deformed fingers of grade III KBD adult (male, 58 years age). Radiographic findings in the right knee (**c**) and right hand (**d**) of a patient with grade II KBD (female, 60 years age). (**c**) Obvious joint space narrowing and loosened bodies in the knee and (**d**) wrist joint crowding and space narrowing, phalanx thickening, and osteophyte formation (With permission of Han et al., Inflamm Res, 2015, 64:853–860)

chronic diseases. Lastly, the applications of selenium supplements for the prevention and treatment of fibrosis will be evaluated (Table 2).

Selenium Deficiency and Cardiac Fibrosis

Cardiac fibrosis, a pathological process common to most cardiac diseases, is often present at the end-stage heart failure caused by various factors, such as ischemia, pressure overload, and many others (Dai et al. 2013; Duerrschmid et al. 2013; Lopez-Andrès et al. 2012). Cardiac fibrosis is a tissue response to injury, characterized by an increased number of fibroblasts in the injured area and excess deposition of extracellular matrix (ECM) proteins, which results in scarring. This scarring leads to the distortion of the structure and function of the organs (Elnakish et al. 2013; Zambrano et al. 2013). It has been reported that selenium deficiency led to

Table 1 Selenium deficiency-related fibrosis in chronic diseases. The findings of fibrosis from animal and human studies related to selenium deficiency

Fibrosis	Animal studies	Human studies
Cardiac fibrosis	Myocardial fibrosis via effects on DNA methylation balance in mice (Metes-Kosik et al. 2012) Severe degenerative changes in aortic vessel wall's ultrastructure in spontaneously hypertensive rats (Ruseva et al. 2012)	Erythrocyte glutathione peroxidase activity was significantly lower than normal value; scattered and irregular areas of myocardial loss and fibrous replacement (Inoko et al. 1998) Subepicardial fibrosis in an "occidental" dilated cardiomyopathy patient (Johnson et al. 1981)
Liver fibrosis	Hepatic periportal fibrosis obvious thickening of blood vessel wall, increase in bile duct, and hyperplasia of collagen fiber; upregulated MMP1/3 and downregulated TIMP1/3 were observed in the liver from the low-selenium group (Han et al. 2016)	Selenium in serum was lower in cirrhosis than in controls and was more reduced in ascitic than in compensated patients (Casaril et al. 1989)
Kidney fibrosis	Significantly decreased body weight and selenium concentration in the kidney and damaged the ultrastructure of the glomerulus and tubules; increased MMP1/3 and decreased TIMP1/3 at the mRNA and protein levels in kidneys (Han et al. 2016)	
Cystic fibrosis		Mean plasma and erythrocyte selenium levels were reduced in cystic fibrosis children (Neve et al. 1983) Glutathione peroxidase activity (GSH-Px) was reduced in cystic fibrosis and in children with dietetically treated phenylketonuria (Ward et al. 1984) The marginally reduced intestinal absorption of ^{75}Se in two children with cystic fibrosis (Heinrich et al. 1977) Whole blood, plasma, and red blood cell selenium levels were significantly lower in patients with cystic fibrosis (Rea et al. 1979; Stead et al. 1985)
Thyroid fibrosis	The thyroids in selenium-deficient condition express more macrophage with obvious TGF-β immunostainings; the proliferation index of the epithelial cells decreased (Contempre et al. 1995)	The thyroid gland of a myxedematous cretin from the Himalayan region in 1908, which consisted of large and uniform fibrous strands with little remaining functional thyroid tissue (McCarrison 1909) Thyroid was atrophied, with loss of epithelial tissue, extensive fibrosis, and lymphocytic infiltration; selenium deficiency is a cofactor to the iodine deficiency (DeQuervain and Wegelin 1936) Selenium deficiency combine with

(*continued*)

Table 1 (continued)

Fibrosis	Animal studies	Human studies
		iodine deficiency increased necrosis, induced fibrosis, and impeded compensatory epithelial cell proliferation (Contempre et al. 1995)
Oral submucous fibrosis	Selenium can influence immune response by making immune cells more resistant to oxidative stress (Matés et al. 2012)	A significantly decreased selenium level in serum of oral squamous cell carcinoma patients as compared to the controls (Khanna et al. 2013)
Pancreatic fibrosis	Vacuolation and hyaline body formation in acinar cells and loss of zonation (Gries and Scott 1972)	

Table 2 Selenium supplements for prevention of fibrosis in chronic diseases. The findings of selenium supplements for prevention of fibrosis in animal and human studies

Fibrosis	Animal studies	Human studies
Cardiac fibrosis	Significantly reduced methylation potential, DNA methyltransferase activity, and DNA methylation (Metes-Kosik et al. 2012)	Selenium did not normalize the left ventricular dysfunction (Inoko et al. 1998)
Liver fibrosis	Selenium supplement showed positive effects on CCl_4 liver injury and fibrosis (Ding et al. 2010; He et al. 1999; Liu et al. 2014; Shen et al. 2005)	A negative correlation was found between serum selenium and aminoterminal peptide of type III procollagen, suggesting a protective role of selenium against fibrosis (Lu et al. 1986)
Cystic fibrosis		The improvement of lipid peroxidation markers was not related to the selenium supplementation (Portal et al. 1995)
Thyroid fibrosis		Preventing a reduction in thyroid echogenicity after 6 months of treatment and in reducing thyroperoxidase and thyroglobulin autoantibodies after 12 months (Nacamulli et al. 2010) Lower thyroid peroxidase autoantibody titers and a significantly higher improvement in well-being and/or mood (Toulis et al. 2010)
Pancreatic fibrosis	Acinar nuclei enlarged and became vesiculated, the cytoplasm filled the empty lumens as well; the fibrous tissue gradually disappeared (Gries and Scott 1972) Prevention of pancreatic degeneration and increasing the relative weight (Cantor et al. 1975)	

reactive myocardial fibrosis and systolic dysfunction accompanied by an increased myocardial oxidant stress, which might lead to myocardial fibrosis via effects on DNA methylation balance (Tao et al. 2013).

It has been reported that low-selenium intake caused extensive degenerative changes in the ultrastructure of the aortic vessel walls of spontaneously hypertensive rats. In addition, the histological examination found that multiple atherosclerotic spots spread along the thoracic aorta and even to the abdominal aorta (Ruseva et al. 2012). Plaques with fibrosis and hyalinosis predominated in the abdominal aorta, which were located near the branches of the arteries, and caused deformation and stenosis of the aorta lumen (Ruseva et al. 2012). Fibrosclerotic plaques with multiple necrosis and lipid drops in the media were found to be involved in the whole wall of the aortic vessel as well (Ruseva et al. 2012). The smooth muscle cells were functionally transformed to fibrocytes due to the degeneration and vacuolization. Consequently, the vessel wall thickened.

A case report study on a 38-year-old man with Crohn's disease showed that when he was at the age of 28, he developed rapidly progressing heart failure and ventricular premature beats associated with selenium deficiency following 16 years of total parenteral nutrition (Inoko et al. 1998). The concentration of his serum and erythrocyte selenium and his erythrocyte GPx activity were significantly lower than normal value. Although he had received selenium supplements and did not have heart failure symptoms for 11 years, he was diagnosed with congestive heart failure at 38 years old. After his death, microscopic examination of the myocardium revealed scattered and irregular areas of myocardial loss and fibrosis, which were located specifically in the midmural area of the left ventricular free wall (Inoko et al. 1998). Another case report by Johnson et al. (1981) mentioned an "occidental" patient whom selenium deficiency was associated with dilated cardiomyopathy. The autopsy also revealed midmural-localized subepicardial fibrosis, indicating that the midmural change was the characteristic of selenium deficiency-related cardiomyopathy. The damage to the left ventricle and the resulting dysfunction, if fully developed, may be irreversible despite the use of selenium supplements (Inoko et al. 1998).

Selenium Deficiency and Liver Fibrosis

Liver fibrosis is the scarring process that represents liver's response to injury. All chronic liver diseases can lead to liver fibrosis. An animal study (Han et al. 2017) of rats with long-term selenium-deficient diet showed that the ultrastructure of hepatocytes was changed, with clumped and condensed chromatin, random-shaped and enlarged nucleolus, evidently swollen mitochondria with a large decrease in electron density, and an unclear double-membrane structure of mitochondria. In a pathological analysis, the low-selenium rats showed hepatic periportal fibrosis, obvious thickening of blood vessel walls, increase in the size of the bile ducts, and hyperplasia of collagen fiber (Fig. 3). Alterations of MMP1/3 and TIMP1/3 expressions were also observed. More importantly, the difference in the

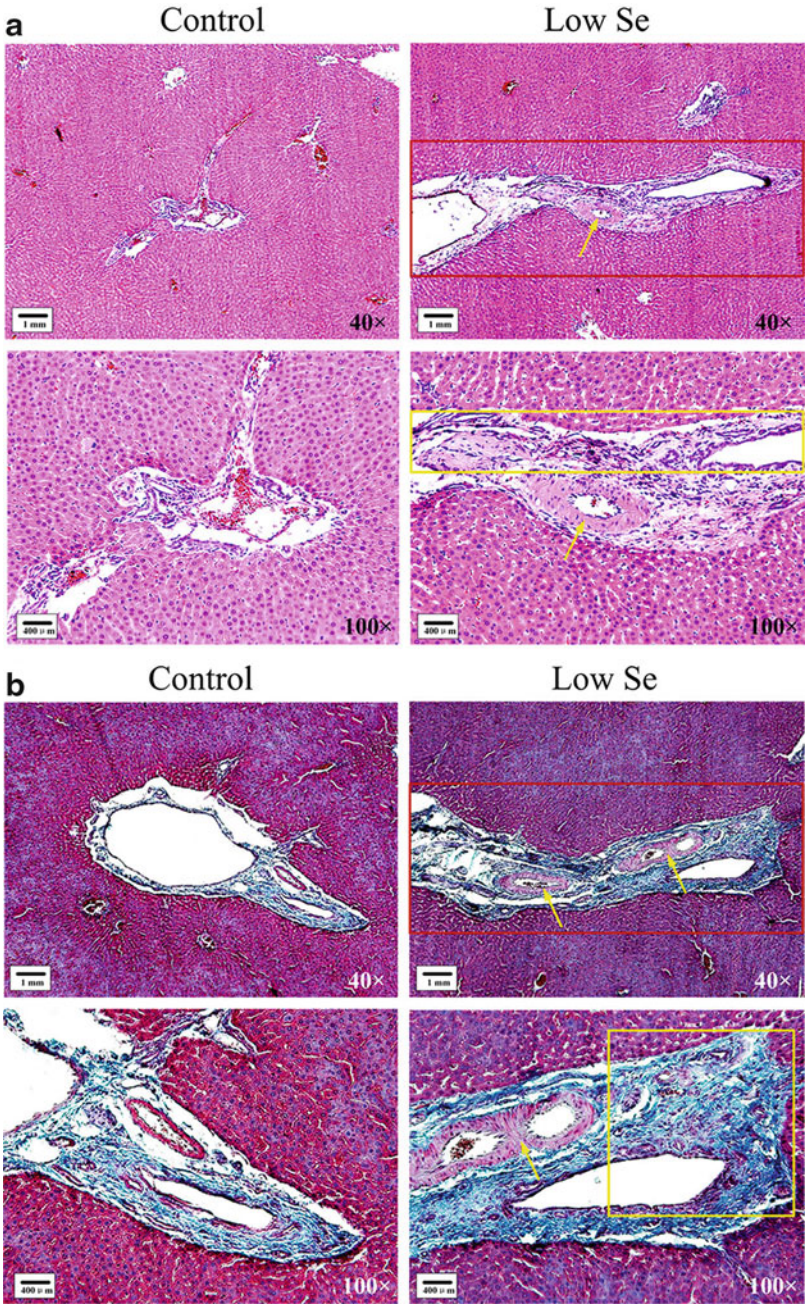

Fig. 3 Representative HE staining (**a**) and Masson's staining (**b**) of rat's liver from low-selenium and control groups. The pathological changes included fibrosis in hepatic periportal, thickening of blood vessel wall, increase in bile duct, hyperplasia of collagen fiber as well as bile duct in the hepatic portal of selenium-depleted group both from the HE and Masson's staining (With permission of Han et al., Biol Trace Elem Res, 2017, 175(2): 396–404)

expression of TIMP1/3 in the hepatic central vein and sinusoid comparing with the hepatic periportal area has been well-investigated in this study. In the hepatic central vein and sinusoid, upregulated MMP1/3 and downregulated TIMP1/3 were observed in the liver from the low-selenium group, which caused ultrastructure alterations of the hepatocyte. However, in the hepatic periportal area, both upregulated MMP1/3 and TIMP1/3 were detected and might be related to the fibrosis formed in the portal tracts. Thus, collagen fibers were formed in hepatic periportal except in the hepatic central vein and sinusoid. However, different cell sources, including endothelial cells, hepatic stellate cells (HSCs), or macrophages, may contribute to the differences of MMPs/TIMPs, which will be investigated in further studies.

A study on the comparison between 55 patients with alcoholic cirrhosis and 47 healthy individuals has been carried out to assess the concentration of selenium in the serum (Casaril et al. 1989). The selenium in serum was lower in patients with cirrhosis than in controls and was even lower in the ascitic patients than in compensated patients. A negative correlation that was found between serum selenium and aminoterminal peptide of type III procollagen suggested a protective role of selenium against fibrosis: selenium, as an important free radical scavenger, may protect the liver in alcoholic subjects from the oxidative damage that caused hepatic fibrosis (Lu et al. 1986).

Selenium Deficiency and Kidney Fibrosis

Kidney (renal) fibrosis is a direct consequence of the kidney with limited capacity to regenerate after injury. Renal scarring causes a progressive loss of the renal function, which eventually leads to the end-stage of the renal function failure. There was mounting evidences that suggested the importance of selenium in the development of chronic kidney disease (Zachara et al. 2006). The kidney, acts as a kind of selenium reservoir, is the organ with the largest concentration of selenium in the body (Loeschner et al. 2014). A previous study reported that the body weight and selenium concentration in the kidney of the rats with a selenium-deficient diet for 109 days significantly decreased (Han et al. 2016). The diet also caused damage to the ultrastructure of the glomeruli and tubules, which showed an obvious proliferation of the mesangial cells, the fusion of podocyte foot processes, and the thickening of the glomerular basement membrane. The glomeruli also had a slightly vacuolar degeneration of the tubule epithelial cells, an increase at the gaps between adjacent cells with edema fluid or protein solution, and edema of microvilli along with the increased gap and decrease of links between adjacent cells. Pathological changes with fibrosis around the renal hilum aorta and in the renal collecting duct were also observed in rats with selenium-depleted diet (Fig. 4). In addition, having a selenium-deficient diet caused an increased MMP1/3 and a decreased TIMP1/3 at the mRNA and protein levels in the kidneys (Han et al. 2016). The damage of the ultrastructure and ECM by the low amount of selenium in the kidney might affect the functions of the kidney.

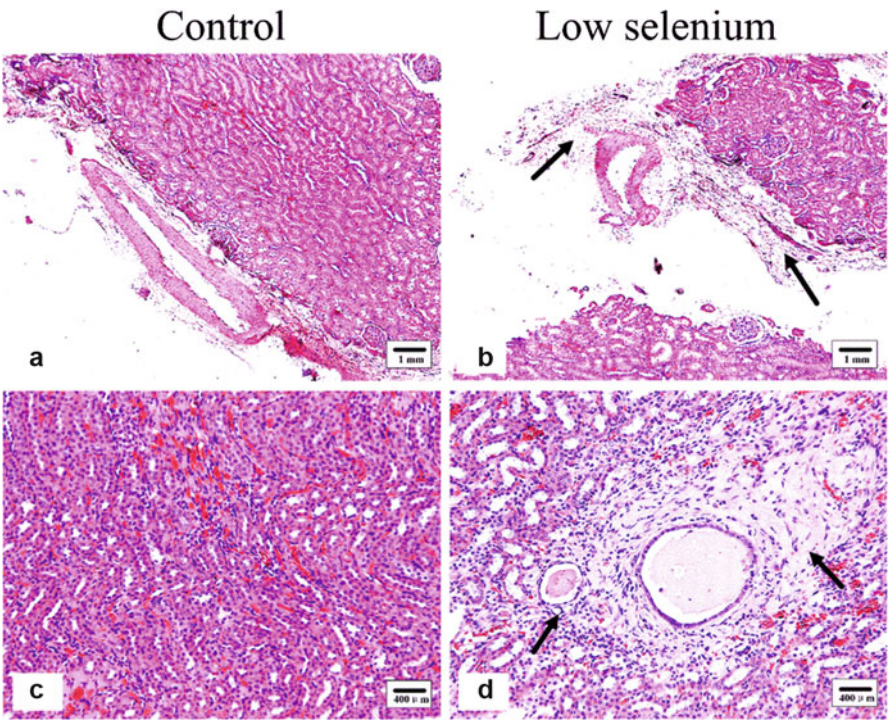

Fig. 4 Representative HE staining of rat's kidney from low-selenium and control groups. Control groups: (**a**) (40×), (**c**) (100×); low-selenium groups: (**b**) (40×), (**d**) (100×). In the low-selenium groups, increased fibers were observed around the renal hilum aorta (**b**) and increased fibrous tissues were detected in the renal collecting duct (**d**)

Selenium Deficiency and Cystic Fibrosis

Cystic fibrosis is a dangerous genetic disease that can cause extensive damage to the lung and digestive system, among other organs. A possible deficiency of the trace element selenium in cystic fibrosis has been well-investigated (Dworkin et al. 1987). Even though there were controversial results, it was evident that selenium deficiency was the primary factor that caused cystic fibrosis (Lloyd-Still and Ganther 1980; Castillo et al. 1981). The plasma and erythrocyte selenium of 20 children (aged 7–19 years) (Neve et al. 1983) with cystic fibrosis were measured and showed that the average of plasma and erythrocyte selenium levels (63 ng/ml ± 15 and 329 ng/g Hb ± 86) were markedly lower than the age-matched controls (82 ng/ml ± 13 and 404 ng/g Hb ± 116). Another study on a long-term selenium status in children from North East Scotland (Ward et al. 1984) also showed that the blood selenium content was significantly lower in the children with cystic fibrosis and coeliac disease than the age-matched control groups. Although younger patients (age 3–6 years) with cystic fibrosis did not present

clinical features of selenium deficiency, they expressed a reduced GPx activity. The lessened blood selenium in patients with cystic fibrosis, even when treated, could be due to malabsorption of selenium, which was shown in one study (Heinrich et al. 1977), where marginally reduced intestinal absorption of ^{75}Se in two children with cystic fibrosis was observed. The children with cystic fibrosis only absorbed 60% and 75% of the radiolabeled ^{75}Se from pork compared with 76% and 100% in healthy adults. It has been suggested that selenium deficiency might be one of the causes of cystic fibrosis (Wallach 1979). It must be noted that the lower blood selenium and GPx observed in younger patients (age 3–6 years old) may be a phenomenon attributed to the small sample sizes of those studies.

The study on the total selenium and selenium species in sera (Michalke 2004) showed that the sera from cystic fibrosis patients contained significantly lower values of total selenium, selenocysteine, and cationic/neutral selenium compounds in comparison to the sera of the healthy volunteers. The total selenium in the sera of cystic fibrosis patients was 58 ± 10 μ/L, which was notably lower than the sera in healthy persons (102 ± 12 μg/L). Selenocysteine in the cystic fibrosis was also much lower with a −75% difference from the healthy group. From these results, it could be concluded that the lowered selenocysteine values together with the lowered cationic/neutral selenium compounds in the cystic fibrosis patients indicated a selenium-depleted regulated pathway combined with a reduced capability of protective functions such as protection from peroxides.

The study by Dworkin et al. showed that the whole blood, plasma, and red blood cell selenium levels were significantly lower in patients with cystic fibrosis as compared to the age-matched controls. Plasma selenium levels were generally significant in short-term dietary selenium intake (Rea et al. 1979), while red blood cell selenium levels, which usually reflected the long-term dietary selenium intake, were more closely related to the activity of the selenoenzyme GPx. A similar drop in both plasma and red blood cell selenium levels suggested a chronic deficiency of selenium in these cystic fibrosis patients.

Lloyd-Still and Ganther (1980) found that the total blood selenium levels in patients with cystic fibrosis were only 55% of the normal blood selenium levels, which meant that the erythrocyte GPx levels were similar between the two groups. On the other hand, a study by Castillo et al. (1981) showed that the levels of serum selenium and GPx in patients with cystic fibrosis were similar as in the healthy cohorts. However, their patients underwent extensive nutritional counseling. Despite this, in their subgroup of cystic fibrosis patients with vitamin E deficiency, serum selenium was significantly lower than the control. Recently, Stead et al. (1985) reported that there was a significant diminished serum selenium level in young adults with cystic fibrosis. In the study, 20 young patients (age 19–34 years old) with cystic fibrosis, including two with cancer, had low serum selenium concentrations accompanied with low serum vitamin E concentrations. It was suggested that the low level of selenium in the blood was associated with an increased risk of carcinoma, especially when the reduced vitamin E level existed at the same time. Moreover, the older patients with cystic fibrosis might have even higher risk for the development of carcinoma (Stead et al. 1985).

Selenium Deficiency and Thyroid Fibrosis

McCarrison (1909) described the thyroid gland of a myxedematous cretin from the Himalayan region in 1908, as consisting of large and uniform fibrous strands with little remaining functional thyroid tissue. In 1936, DeQuervain and Wegelin (1936) described a very similar picture in Switzerland. The thyroid was atrophied, with loss of epithelial tissue, extensive fibrosis, and lymphocytic infiltration. It was suggested that both iodine and selenium deficiency were cofactors in the pathogenesis of myxedematous cretinism. Therefore, it was proposed that a remarkably increased level of the hydrogen peroxide in the iodine-deficient thyroid glands would be controlled by the hydrogen peroxide and its derived free radicals. A study (Contempre et al. 1995) was carried out to investigate the effect of the possibly impaired cellular defense mechanisms associated with selenium deficiency on thyroid necrosis and tissue repair. Pharmacological doses of iodide were administrated to induce necrosis in iodine-deficient thyroids, and it was also noted that the necrotic thyroid glands were accompanied with selenium deficiency, and the necrosis eventually led to fibrosis. Thus, the selenium deficiency paired with iodine deficiency increased necrosis, induced fibrosis, and impeded compensatory epithelial cell proliferation. These results were compatible with histological and functional descriptions of thyroid tissue from myxedematous cretins.

According to the above study, selenium deficiency-induced free radical damage and fibrosis of the thyroid are thought to be involved in the pathogenesis of myxedematous cretinism. A rat model has been replicated to explore the role of selenium deficiency in defective tissue repair and explain the relationship between selenium deficiency and tissue fibrosis (Contempre et al. 1995). It showed that the proliferation indexes of epithelial cells and fibroblasts were comparable between selenium-deficient and control groups. However, the thyroids in selenium-deficient condition expressed more macrophages. Consequently, these thyroids developed fibrosis. In the selenium-deficient rat model, the macrophages showed obvious transforming growth factor (TGF-β) immunostaining, and the use of anti-TGF-β antibodies restored the proliferation indexes of the epithelial cells and fibroblasts and completely prevented the thyroids from developing fibrosis. The results indicated that the inflammatory reaction and an increased TGF-β played a potential role in the active fibrotic process that occurred in the selenium-deficient thyroids (Contempre et al. 1995).

Selenium Deficiency and Oral Submucous Fibrosis

Oral submucous fibrosis is a chronic debilitating oral cavity disease, which is characterized by the inflammation and progressive fibrosis of submucosal tissues. Oral cancer is a major cause of cancer morbidity and mortality worldwide and is prevalent in most areas with high tobacco consumption. Selenium, among other trace minerals, plays an essential role in many biochemical reactions, and it has been demonstrated that the concentrations of various important trace minerals were

changed over the course of the malignant disease. Khanna and her colleagues investigated the selenium levels in the sera of patients with oral submucous fibrosis and oral squamous cell carcinoma in comparison with controls (Khanna et al. 2013) to identify predictors among these parameters for disease occurrence and progression. The study revealed significant lower levels of selenium in the sera of the cancer patients as opposed to the controls. Having known that selenium forms an integral part of the enzyme GPx, type I iodothyronine, deiodinase, metalloprotein, fatty acid binding protein, and selenoprotein P, selenium is considered as an antioxidant nutrient. The role of selenium is complex, which can be attributed to its immune modulating and antiproliferative properties to influence immune response by making immune cells more resistant to oxidative stress (Matés et al. 2012).

Selenium Deficiency and Pancreatic Fibrosis

Pancreatic fibrosis is a defining feature of chronic pancreatitis, which refers to the abnormal activation of stromal cells and deposition of ECM, which eventually impair the exocrine and endocrine functions of pancreas. One study on the day-old chicks fed with a selenium-deficient amino acid diet discovered that the chicks developed exocrine pancreatic degeneration and fibrosis (Gries and Scott 1972). In the study, the chicks were raised on a diet containing vitamin E and bile salts to maintain high plasma tocopherol levels. At 6 days old, the chicks started showing deficiency lesions, which exhibited vacuolation and hyaline body formation in acinar cells, as well as loss of zonation. Subsequently, the acinar cytoplasm started to shrink toward the nuclear end of the cell. In addition, an enlarging central lumen in the acinus and the appearance of fibroblasts were noticed in the interacinar tissue. Eventually, the pancreatic acinus consisted of a ring of cells composed mainly of small, dense-staining nuclei surrounding a lumen and embedded in cellular connective tissue. Some acinar necrosis was also noted.

Selenium Supplements for the Prevention of Fibrosis

Up to this point, it has been explained that a selenium-deficient diet may cause human chronic disease in the myocardium, liver, kidney, pancreas, and thyroid. Selenium is an essential trace element, which can be found in various forms and concentrations in the soil and in small amounts in certain foods and water. The human body requires very little amount of selenium; the minimum daily recommended dietary allowance is only 55 μg for adults. Although overadministration of selenium will highly increase the risk of disease, it is difficult to "overdose" selenium through food intake. Nevertheless, considering there is a possible risk of overadministration of selenium, proper intake of selenium supplement is important. Studies on the effects of selenium supplement on the prevention of pancreatic, myocardial, liver, thyroid, and cystic fibrosis in animals and humans will be discussed extensively.

In Animal Studies

Selenium supplements have been used effectively for the prevention of pancreatic fibrosis as demonstrated in several animal studies. One study on the prevention of pancreatic fibrosis in chicks (Gries and Scott 1972) showed that 4 days after supplying 0.1 ppm selenium to the deficient diets, acinar nuclei enlarged and became vesiculated, while cytoplasm filled the empty lumens. The fibrous tissue gradually disappeared due to the enlarged acini. After 2 weeks of having selenium supplement, the pancreas returned to the normal appearance. Another study (Cantor et al. 1975) investigated the efficacy of selenium dietary supplements in the form of sodium selenite, selenomethionine, selenocysteine, wheat, and tuna meal on the prevention of pancreatic fibrosis in chicks. Histological examination of the pancreases revealed that wheat and selenomethionine were the most effective sources of selenium. Among the three selenium compounds, selenomethionine was four times more effective than the others in preventing pancreatic degeneration and increasing the relative weight and selenium concentration of the pancreas.

Selenium supplements also had a positive effect on the prevention of liver damage as shown in animal studies. He and colleagues (1999) reported that selenium could be used as an antioxidant to preserve the liver tissue structure and function of the CCl_4-injured liver in rats and inhibit collagen synthesis in the human hepatocytes. In the study, there was increased blood selenium and GPx activity, as well as improvement of ultrastructure of the damaged liver, and a decrease of lipid peroxides content in the selenium supplementation group as compared to the control group. Another study on selenium supplementation (Ding et al. 2010) using CCl_4-induced mice damaged liver and fibrosis demonstrated that selenium decreased liver inflammation rather than causing necrosis by CCl_4. Selenium increased hepatocyte apoptosis, pro-apoptotic BAX and Bcl Xs/l proteins, and matrix metalloproteinase-9. Selenium also decreased stellate cell number and fibrosis after CCl_4 treatment. It was further indicated that selenium supplements could decrease hepatic fibrosis by lowering the number of collagen-producing stellate cells and accelerating collagen degradation. Liu and his group (2014) investigated the effects of selenium-enriched probiotics (SP) on the CCl_4-induced liver fibrosis in rats. Their results showed that SP significantly decreased serum alanine aminotransferase, aspartate aminotransferase, hepatic hydroxyproline, and malondialdehyde levels but increased GPx, superoxide dismutase, and glutathione levels in rats when treated with CCl_4. The SP could suppress both hepatic inflammation and necrosis induced by CCl_4. In addition, SP could reduce significantly the expression of α-smooth muscle actin, collagen, TGF-β 1, TIMP1, and inflammation-related gene, as well as the apoptosis of the activated HSCs in the rats. Their results also suggested that SP could protect the liver from fibrosis by attenuating hepatic oxidative stress, suppressing hepatic inflammation, and inducing apoptosis of HSCs. A study on the effects of dietary supplement with vitamin E and selenium on rat hepatic stellate cell apoptosis (Shen et al. 2005) showed that the combination of vitamin E and selenium supplements at a given level could inhibit CCl_4-induced activation and proliferation of HSCs and promote the apoptosis of the activated HSCs in acute damage phase. Vitamin E and selenium

could also effectively decrease the degree of hepatic fibrosis and promote the recovery process.

However, one animal study (Metes-Kosik et al. 2012) with adult C57/BL6 mice showed that the selenium supplementation reduced significantly methylation potential, DNA methyltransferase activity, and DNA methylation. In the study on mice fed with the selenium-supplemented diet, reactive myocardial fibrosis and diastolic dysfunction caused by altered myocardial matrix gene expression were observed in spite of lower oxidant stress and absence of myocardial hypertrophy. Their results indicated that both selenium deficiency and modest selenium supplementation led to a similar phenotype of abnormal myocardial matrix remodeling and dysfunction in a normal mice heart. This study suggested that more investigation would be required to focus on the important role of selenium in the maintenance of the balance between redox and methylation pathways while optimizing selenium status for the prevention and treatment of heart failure.

In Human Studies

Selenium supplementation has been shown to be very effective in the prevention of thyroiditis. In one study on autoimmune thyroiditis with 76 consecutive patients (Nacamulli et al. 2010), 30 cases were given no treatment, while 46 cases were treated with a single oral dose (80 μg/day) of sodium selenite for 12 months, showing that dietary supplementation with physiological doses of selenium seemed to be effective in preventing a reduction of thyroid echogenicity after 6 months of treatment and in reducing thyroperoxidase and thyroglobulin autoantibodies after 12 months. In a systematic review (Toulis et al. 2010), it was shown that 3-month administration of selenium supplement to the patients with Hashimoto's thyroiditis (HT) resulted in significant lower thyroid peroxidase autoantibodies titers and a notable higher improvement in well-being and/or mood when compared with the controls, suggesting that selenium supplementation could be useful as an adjunctive therapy to levothyroxine in the treatment of HT.

However, controversial results from the treatment of cystic fibrosis with selenium supplementation have been demonstrated. In the study by Portal and his group (1995), lipid peroxidation was assessed in 27 children with cystic fibrosis after a double-blind selenium supplementation. The results showed that the improvement of lipid peroxidation markers was not related to the selenium supplementation when compared with the control groups. They also reported that a decrease in the plasma level of fragile equilibrium and selenium concentrations was found in the patients administered with the placebo. On the other hand in another study, the plasma level of fragile equilibrium and selenium concentrations was increased when patients were administered with selenium supplements. In the study, the selenium status of cystic fibrosis patients was close to normal before any treatment in the Grenoble area, which could be explained by the progress in the nutritional nursing care of the children and by the addition of selenium to the diet. It has also been shown (Inoko et al. 1998) that after selenium supplementation to the patient with Crohn's disease,

he was free from symptoms of heart failure for 11 years. However, it did not normalize the left ventricular dysfunction, and he eventually suffered from heart failure at the age of 38 years (Inoko et al. 1998).

Policies and Protocols

Selenium is considered as a "double-edged sword" element because of its toxicity at concentrations slightly higher than what is required for normal physiological functions. There is a U-shaped association between selenium intake and health, and this may explain the contradictory results from clinical trials of the impact of selenium on disease. People with selenium-deficient diets may benefit from selenium supplementation in terms of disease/health outcomes, whereas populations with preexisting adequate or high-selenium status may suffer from detrimental health outcomes instead (Rayman 2012). Nevertheless, selenium is required in trace amounts for the development and well-being of humans and is vital in the maintenance of structures and functions of human organs. Therefore, moderate selenium supplements in diets, especially in selenium-deficient countries, are highly recommended. Selenium supplements are especially critical for normal development in children who are under the risk of selenium deficiency. The concentration of selenium supplement should be critically assessed. Selenium supplements could exhibit either antioxidant or oxidant function or even both. Thus, the toxicological behavior of selenium supplements should be tested before recommending appropriate selenium intake concentration in vivo. Organic selenium supplements with lower toxicity and higher bioactivities in vitro and in vivo may provide a better choice.

Dictionary of Terms

- **Fibrosis** – The formation of excess fibrous connective tissue as a reparative response to injury or damage. This can be a reactive, benign, or pathological state. Fibrosis may refer to the connective tissue deposition that occurs as part of normal healing or to the excess tissue deposition that occurs as a pathological process.
- **Chronic disease** – Persists for a long time, lasting 3 months or more, by the definition of the US National Center for Health Statistics. Chronic diseases which tend to become more common with age generally cannot be prevented by vaccines or cured by medication nor do they just disappear.
- **Selenium deficiency** – Lack of the essential mineral selenium, which can cause diseases such as Kashin-Beck disease and Keshan disease in China. Some chronic diseases are related to selenium deficiency. Treatment involves ensuring intake of the recommended dietary allowance of selenium.
- **Selenium supplement** – An increase of selenium-rich foods may be able to help prevent selenium deficiency diseases. It's believed that selenium can benefit these

by its ability to fight inflammation, reduce free radical oxidative stress, and help with antioxidant activity.

- **Trace element** – A chemical element whose concentration (or other measure of amount) is very low (a "trace amount"). The exact definition depends on the field of science: in analytical chemistry, a trace element is one whose average concentration of less than 100 parts per million (ppm) measured in atomic count or less than 100 μg/g. In biochemistry, a trace element is a dietary element that is needed in very minute quantities for the proper growth, development, and physiology of the organism. In geochemistry, a trace element is one whose concentration is less than 1000 ppm or 0.1% of a rock's composition.
- **Autopsy** – Postmortem examination in order to find the cause of death or the extent of disease.

Summary Points

- This chapter reviews the relationship between selenium deficiency and fibrosis in chronic diseases.
- This chapter also reviews the use of selenium supplements for the prevention and treatment of fibrosis in chronic diseases.
- Selenium deficiency causes cardiac fibrosis in both animal and human studies.
- Selenium deficiency is involved in liver fibrosis in both animal and human studies.
- Selenium deficiency causes kidney fibrosis in the animal study.
- Selenium deficiency causes cystic fibrosis in human studies.
- Selenium deficiency causes thyroid fibrosis in both animal and human studies.
- Selenium deficiency causes oral submucosa fibrosis in the human study.
- Selenium deficiency causes pancreatic fibrosis in laboratory animals.
- Effect of selenium supplement on pancreatic fibrosis in animal studies.
- Effect of selenium supplement on liver fibrosis in animal studies.
- Effect of selenium supplement on thyroid fibrosis in human studies.
- Effect of selenium supplement on cystic fibrosis in human studies.
- Effect of selenium supplement on cardiac fibrosis in animal and human studies.

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Analysis of the Relationship Between Zinc Deficiency, Androgen Disorders, and Lung

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Abstract

This review is focused on the adult lung in health and disease and the effect of zinc on both states. Pulmonary fibrosis (PF), chronic obstructive pulmonary disease (COPD), and asthma are some of the major health problems in the world. Zn is present in protein-bound and ionic forms, and plays important roles in mediating the function and structure of proteins, and in maintaining physiological homeostasis. It was found that zinc is involved in the regulation of sex hormones and that it also has effects on lung lipid metabolism and the immune system. It is known that sex affects lung development and physiology. Androgens enhance airway branching but delay alveolar maturation contributing to increased respiratory morbidity in prematurely born male infants. It was shown that there are sex-specific trends in the incidence, prevalence, and mortality of diseases, such as COPD and PF. In men, there is a gradual decline in testosterone secretion that begins after 30 years old, with the signs and symptoms of testosterone deficiency typically presenting after the age of 65. The absence of androgens induces oxidative stress and lipid peroxidation in lung, synchronically with changes in the expression of cytoprotective markers, what could be part of the mechanisms involved in the development of the previously mentioned diseases. Therefore, not only the levels of zinc, but also of testosterone are important in order to maintain the lungs healthy and functional.

Keywords

Lung · Androgens · Men · Lung diseases · Zinc · Sex hormones · Testosterone · Oxidative stress · Zinc deficiency · Androgen disorder · Decline testosterone · Pulmonary fibrosis · Chronic obstructive pulmonary disease

List of Abbreviations

AE2	Alveolar epithelial type II
AECs	Alveolar epithelial cells
AR	Androgen receptors
ARKO	AR knocks out
ASM	Airway smooth muscle
BHR	Bronchial hyperresponsiveness
CAT	Catalase
COPD	Chronic obstructive pulmonary disease
COX-2	Ciclooxigenase 2
DHT	Dihydrotestosterone
ECM	Extracellular matrix
EMT	Epithelial–mesenchymal transition
ER	Endoplasmic reticulum
GD17	Gestational day 17
GPx	Glutathione peroxidase
Hoxb5	Hox protein b5
Hsp27	Heat shock protein 27

Hsp70	Heat shock protein 70
IL-8	Interleukin-8
IPF	Idiopathic pulmonary fibrosis
LTB4	Leukotriene B4
MUC5B	Common genetic variant in mucin 5B
NF-κB	Nuclear factor κB
NOX	NADPH oxidase
PF	Pulmonary fibrosis
RCC	Renal cell carcinoma
RT	Radiotherapy
SMAD2P	Smad protein 2P
SMAD7	Smad protein 7
SPA	Surfactant protein A
SPC	Surfactant protein C
TBARS	Thiobarbituric acid reactive substances
TERC	Telomerase RNA component
TERT	Telomerase reverse transcriptase
TNF-a	Tumor necrosis factor-a
UPR	Unfolded protein response
Zn	Zinc

Introduction

A wide variety of pulmonary disorders can result in pathologies that affect the airway and/or lung stroma. Over the past years, numerous advances have been made in the understanding of lung diseases. Pulmonary fibrosis (PF), chronic obstructive pulmonary disease (COPD), and asthma are some of the major health problems and considered to be some of the most prevalent features of age- and sex-related diseases affecting millions of people in all countries. Besides that, other emerging conditions are also relevant, including smog (developing countries), sand storms, nanoparticles, and man-made fibers; while epigenetic modifications in general are acquired in response to environmental stimuli.

Disorders of the airways, lung, chest wall, and diaphragm will affect ventilation while pathologies of the lung and cardiovascular system, as well as peripheral tissues will affect respiration and consequently the exchange of gases (Goodman and Fuller 2015). Nasal, bronchial, and alveolar epithelia play critical barrier and homeostatic functions. Lung epithelium is the initial affected site of environmental and inflammatory stimuli since it constitutes the interface between the internal milieu and the external environment as well as being a primary target for inhaled respiratory drugs. It also responds to changes in the external environment by secreting a large number of molecules and mediators that signal to cells of the immune system and underlying mesenchyme (Knight and Holgate 2003).

Aging is associated with a physiologic reduction of appetite and food intake. Body weight decreases after 60 years, which is mainly due to muscle loss. The

combination of muscle loss and reduced food intake predisposes older people to undernutrition. In undernourished older people, combined treatment with testosterone and nutritional supplementation reduced the number of people hospitalized and duration of hospital admissions (Piantadosi et al. 2011). In addition, zinc is essential for immune response regulation and it is known that T cell function declines with age. In elders, inadequate stores of zinc might be a risk factor of pneumonia. Therefore zinc supplementation has the potential to reduce not only the number and duration of pneumonia episodes, but also all-cause mortality in older people. Based on a thorough review of the literature and given the upper safe limit of zinc, it is concluded that a dose of 30 mg elemental zinc per day for 3 months might be adequate to improve the immune function, and to reduce the risk of infections (Barnett et al. 2016).

Besides that, Zn is an important component of the testosterone-signaling pathway, since AR has two zinc finger motives. On this regard, it is known that sex steroids can affect different lung components, and they may play a role in important diseases such as asthma, COPD, pulmonary fibrosis, cancer, and even pulmonary hypertension. Sex-specific trends in the incidence, prevalence, and mortality of diseases such as COPD and PF have been reported. Multiple studies have suggested that the incidence and prevalence of PF is higher in males than females and females have better survival rates (Table 1). PF is a progressive interstitial lung disease leading to scarring. This pathology has a higher prevalence in males compared to females (Meltzer and Noble 2008) with faster progression, and less survival in males than females (Han et al. 2008; Olson et al. 2007). In contrast, the prevalence of chronic obstructive pulmonary disease (COPD) is increasing in females and the number of women dying from this disease now surpasses men in the United States (Han et al. 2008).

While numerous genetic, environmental, social, and behavioral factors are proposed to contribute to these trends, it is also likely that cellular and molecular mechanisms driving the diseases development and/or progression are not fully conserved between sexes (McGee et al. 2014). In contrast, asthma incidence and severity is greater in women than in men, and also women are more likely to develop corticosteroid-resistant or "difficult-to-treat" asthma. Puberty, menstruation, pregnancy, menopause, and oral contraceptives are known to contribute to the outcome of diseases in women, suggesting a role for estrogen and other hormones that affect allergic inflammation (Keselman and Heller 2015).

Table 1 Sex specific trends in lung stromal diseases. The effects of gender in lung stromal chronic diseases (pulmonary fibrosis and COPD: chronic obstructive pulmonary disease) varies. The incidence of pulmonary fibrosis is higher in males, although the survival rates are higher in women. The opposite occurs in COPD

	Lung stromal diseases	
	Pulmonary fibrosis	COPD
Incidence/ prevalence	Less than males (Meltzer and Noble 2008)	Increasing in females
Survival rates	Less than females (Han et al. 2008; Olson et al. 2007)	Less than males

Respiratory Diseases

Fibrogenesis is a dynamic process and was proposed to occur in all organs within four common phases: (i) initiation, due to injury of the organ/tissue; (ii) inflammation and activation of effector cells; (iii) enhanced synthesis of extracellular matrix (ECM); and (iv) deposition of ECM with progression to end-organ failure (Rockey et al. 2015).

Fibrotic lung disorders can be divided into diseases with known and unknown etiology (Fig. 1). Among those with unknown etiology, idiopathic pulmonary fibrosis (IPF) is a common diagnosis. It is a fatal disease with poor prognosis and limited therapeutic options. Alveolar epithelial type II (AE2) cells play a key role in lung fibrosis and they have a crucial role in epithelial regeneration and interaction with fibroblasts. An alternative explanation for the lack of confirmation that genetic mutations lead directly to fibrosis is that exposure to additional environmental injurious agents, in genetically predisposed individuals, is required for the development of lung fibrosis (Wolters et al. 2014).

The other disease is chronic obstructive pulmonary disease (COPD). It is a progressive pathology that includes emphysema and chronic bronchitis. Emphysema is characterized by damaged alveoli while chronic bronchitis is characterized by inflamed airways with excess mucus formation. Kamil et al. (2013) suggest sex-specific and race-related differences in the manifestation of COPD, although the mechanistic basis for such differences is not completely understood. This disease is characterized by chronic inflammation throughout the airways, parenchyma, and

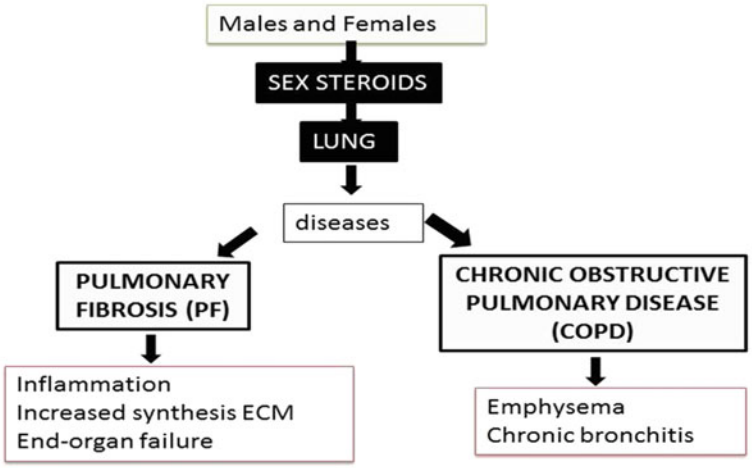

Fig. 1 Lung pathology and sex steroids. Fibrotic lung disorders are divided into diseases with known and unknown etiology. Wolters et al. (2014) thought that exposure to environmental injurious agents, in genetically predisposed individuals, is required for the development of lung fibrosis. Chronic obstructive pulmonary disease (COPD) is a pathology that includes emphysema and chronic bronchitis. Kamil et al. (2013) suggest sex-specific and race-related differences in the manifestation of COPD, although the differences are not completely understood

pulmonary vasculature. Activated inflammatory cells release a variety of mediators – including leukotriene B4 (LTB4), interleukin-8 (IL-8), tumor necrosis factor-a (TNF-a), and other compounds – capable of damaging lung structures or sustaining neutrophilic inflammation (Pauwels et al. 2001). Cigarette smoke exposure is associated with an increased risk of COPD.

Asthma involves both intrinsic and environmental factors suggesting that it is multifactorial in origin. Asthma is characterized by three major features: airway structural remodeling (involves both airway and epithelial cells); functional changes (airway hyperresponsiveness, edema, mucus production); and inflammatory changes (activation of inflammatory cells) (West et al. 2013; Wright et al. 2013). There is not a simple relationship between sex hormone levels and asthma symptomatology. Considerable evidence suggests that asthma is more common in prepubescent males compared to females, but following puberty, it is more common in females than in males (Melgert et al. 2007).

Zinc

Zinc (Zn) is well known as an essential trace element for a variety of biological activities. This ion is the second most abundant trace element in the body, and as a consequence, zinc deficiency is considered a public health problem. Zinc deficiency implies growth retardation and increased infectious disease morbidity such as diarrhea and pneumonia, especially in the first 2 years of life.

The body of an adult human (70 kg) contains about 2–3 g of zinc, which is absorbed from our dietary sources in the proximal small intestine, either the distal duodenum or proximal jejunum (Krebs et al. 1998), and released from there into the blood. However, the supply of zinc by our diet is dependent on its amount and bioavailability. It has been estimated that in a Western mixed diet, this bioavailability is about 20–30% of total contained zinc (Gallaher et al. 1988). The main sources of zinc are oysters, red meat, and poultry. Other good food sources include beans, nuts, certain types of seafood such as crab and lobster, whole grains, and dairy products (USDA 2011).

Zinc Biological Roles

In biological systems, Zn is present as protein-bound and ionic forms, and plays important roles in mediating the function and structure of proteins, and in maintaining physiological homeostasis. Plasma concentration of trace elements as zinc and copper are affected by physiological conditions such as age, gender, and nutritional status, as well as by pathophysiological conditions, like inflammation and the presence of cardiovascular risk factors (Ghayour-Mobarhan et al. 2005). Aging has been associated with a general decrease of plasma Zn, especially in elderly subjects with a suboptimal nutritional status (Prasad 2008).

Zinc plays an important role in lung functions and also functions as an antioxidant and anti-inflammatory agent with pro-survival effects. Zinc deficiency leads to oxidative damage in the airways by inducing the infiltration of inflammatory cells and increasing superoxide and nitric oxide production. These unique properties of zinc may have significant therapeutic benefits in human pulmonary diseases (Prasad 2008). In line with our findings, it has been shown that the peroxidation of lipid molecules induced by ROS, oxidative stress, and inflammation was significantly attenuated with zinc in a model of zinc-deficient rats. The localization of the abundant labile Zn in the apical cytoplasm of the airway epithelium is controlled by zinc transporters (Murgia et al. 2006). Zinc is also in the active site of ADAM33 metalloproteinase, which is expressed in pulmonary fibroblasts and bronchial smooth muscle cells (Orth et al. 2004), and it is important for extracellular matrix changes and airway remodeling after repeated damage and repair (Zou et al. 2004). It has been shown that Zn deficiency produces the nuclear translocation of Nuclear Factor-kβ (NF-kβ) in rat lungs and human airway epithelial cells (Gomez et al. 2006). NF-kβ is a cytoplasmic transcription factor that once translocated to the nucleus mediates the expression of pro-inflammatory cytokines (Oteiza and Mackenzie 2005).

The immune system requires essential micronutrients and trace elements such as iron, zinc, copper, and selenium for optimal function. In addition, Cu and Zn play a central or putative role in the development of important age-related diseases, including cardiovascular disease, cancer (Zuo et al. 2006), and type 2 diabetes (Mocchegiani et al. 2008), among others. Also, the Cu/Zn ratio seems to be of clinical importance as an inflammatory-nutritional biomarker and a sensitive predictor of mortality in elderly subjects aged 70 years and above. Cu/Zn ratio variability in elderly populations suggests that the decline of Zn circulating levels with age depend on physiopathological changes. Zn plasma concentrations have positive correlations with albumin, RANTES (Regulated on Activation, Normal T-cell Expressed, and presumably Secreted) after adjustment for multiple confounders. Cu/Zn ratio was positively associated with markers of systemic inflammation and age and negatively associated with albumin serum levels (Giacconi et al. 2016).

Regarding the immune system, zinc is indispensable for a suitable and sufficient immune response against pathogens. Zinc is crucial for normal development and function of cells mediating innate immunity, neutrophils, and natural-killer cells. Phagocytosis, macrophages, intracellular killing, and cytokine production are also affected by zinc deficiency. Human lung fibroblast cells cultured under zinc-deficient conditions undergo oxidative stress and DNA damage as well as cell's ability to repair this damage is also compromised (Ho et al. 2003). Patients with zinc deficiency show inadequate granulocyte migration (Hasan et al. 2016) and an inefficient T cell-mediated function (Barnett et al. 2010). This leads to increased susceptibility to a variety of pathogens; in particular, these patients are prone to develop pneumonia. Thus, zinc adequacy is necessary for maintaining DNA integrity and may be important in the prevention of DNA damage and cancer (Prasad 2008).

Implications of Zinc in Several Diseases

Elders' inadequate stores of zinc might be a risk factor of pneumonia. Zinc supplementation might be beneficial to Ancient with low serum zinc levels Such a measure has the potential to reduce the number and duration of pneumonia and the amount and duration of antibiotic use due to pneumonia. A dose of 30 mg of elemental zinc per day might be adequate to improve immune function, and reduce the risk of infections. However, it needs to be emphasized that in order to provide conclusive evidence, controlled studies are needed to determine the efficacy of zinc supplementation as a potential low-cost intervention to reduce morbidity and mortality due to pneumonia in this vulnerable population (Barnett et al. 2010). Elders that live in nursing homes were supplemented with 30 mg/day Zn for 3 months; however not all zinc-deficient elders reached adequate concentrations, but the increase in serum zinc was associated with the enhancement of T cell numbers (Barnett et al. 2016).

An involvement of zinc in the regulation of sex hormones can be concluded indirectly, as several alterations that can occur during pregnancy upon zinc deficiency. Zinc deficiency also alters testosterone levels and modifies sex steroid hormone receptor levels (Om and Chung 1996) (Table 2). The nuclear receptor for sex steroid contains zinc fingers motif in their protein structure that might explain zinc dependency of these hormonal systems (Sauer et al. 2016). Zn fingers are common among gene regulatory proteins (Richer et al. 1998). Specificity of HRE binding is determined by the more highly conserved hydrophilic first Zn finger (C1) (Green and Chambon 1987), while the second Zn finger (C2) is involved in dimerization and stabilizing DNA binding by ionic interactions with the phosphate backbone of the DNA (O'Malley 1990).

That is why Zn is rising as a very important trace element in lung, not only due to its role in the regulation of sex hormones but also due to its effects on lung lipid metabolism and the immune system. It is known that the major phospholipid component (at the air–liquid interface) is phosphatidylcholine (70–80%). Our own laboratory showed that a moderate Zn deficiency (in vivo) can induce a

Table 2 Levels of zinc and testosterone in child and man. This table shows the level of zinc and testosterone in men, at different ages according to the works of Rubio et al. (2007) and Rey et al. (2006)

	Child	Man
Zinc serum level (mg/day) (Rubio et al. 2007)	3 mg (1–3 years)	8 mg (9–13 years)
	5 mg (4–8 years)	11 mg (14–>70 years)
Testosterone serum level (ng/ml) (Rey et al. 2006)	1.00–3.00 (0–6 months)	2.68–5.60 (12–17 months)
	0.10–0.32 (6–9 months)	2.80–8.00 (adult)

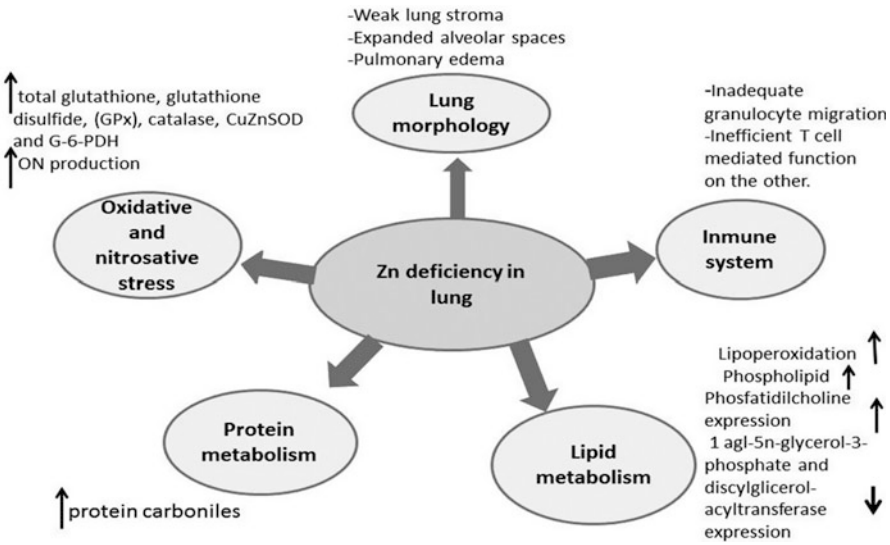

Fig. 2 Effect of Zn deficiency in lung. Zinc is necessary for maintaining the immune system, lipid metabolism, lung morphology, protein metabolism and, more important, it is the main actor in regulating the oxidative and nitrosative stress in the respiratory system

physiological stimulus that enhances phospholipid synthesis and changes especially the pattern of phospholipids, in adult rat lung. We found that the amount of 1-acyl-sn-glycerol-3-phosphate and the diacylglycerol acyltransferase expression decreases significantly in Zn-deficient lung, while phosphatidylcholine increases (Gomez et al. 2002, 2003). It is also known that Zn deficiency is accompanied by oxidative and nitrosative stress associated with significant morphological changes in lung parenchyma (Gomez et al. 2006; Fig. 2). What is more, Frey and collaborators (2000) demonstrated that fragmented phospholipids increase in the lung of patients with adult respiratory distress syndrome. All these results suggest that the lipid concentration in the lung (especially phospholipids) is modified directly or indirectly by a zinc-deficient diet.

Zinc also plays a role in systemic glycemic control through its effects on insulin biosynthesis within pancreatic β-cells and probably by modulating the insulinemic effects on target tissues. Kolahian et al. (2015) demonstrated that the supplementation of diabetic rats with leucine, zinc, and chromium, alone and in combination, was associated with significant improvements in the function and structure of respiratory system. The nutritional supplements enhance the enzymatic antioxidant activity of catalase, glutathione peroxidase, myeloperoxidase, and superoxide dismutase. Further, reinforcement of subjects with type 2 diabetes with these supplements may be an efficient method for preventing and/or treating diabetes-induced respiratory system dysfunction.

Androgens and Lung

Due to the above mentioned relationship between zinc and sex steroid hormones, our review will now focus on the adult lung in health and disease and the putative effect of sex steroids. However, it is also relevant to acknowledge the contribution of sex steroids in the developing lung and the influences in modulating postnatal lung growth and function.

Testosterone Biological Actions

Testosterone is the principal circulating androgen in males and this hormone is also secreted by ovaries and in small amounts by the adrenal gland (Mooradian et al. 1987). Testosterone can regulate the structure and function of nonreproductive organs including bone, lung, skeletal muscle, brain, liver, kidney, and adipocytes. Sex hormones exert their biological effects primarily through genomic mechanisms; however, they also elicit nongenomic effects. Testosterone and its active metabolite, 5α-dihydrotestosterone (DHT), exert their biological actions via androgen receptor (AR), also known as nuclear receptor subfamily NR3C4 (Chang et al. 2014). Data on sex steroid receptor expression in lung parenchymal cells is limited. The lung parenchyma comprises ~75% of cells, mainly in the alveoli. All the major sex steroid receptors have been found in the alveolar epithelium (Mikkonen et al. 2010). Additionally, the enzymes aromatase and 17β-hydroxysteroid dehydrogenase are localized in the same epithelium (Plante et al. 2009).

The biological activity of sex steroids depends on many factors including the availability of the unbound ligand, receptor expression and distribution, nuclear translocation, and signaling pathway. If the availability of free testosterone or the activity of androgen receptors (AR) and associated signaling pathways are altered during aging, then increasing the concentrations of testosterone may not be sufficient to fully reverse the effects of elders on different pathogenesis (vom Steeg et al. 2016).

Effects of Testosterone Decline in Human Health

In men there is a gradual decline in testosterone secretion that begins after 30 years old, with the signs and symptoms of testosterone deficiency typically presenting after the age of 65 (vom Steeg et al. 2016). Age-related reductions in testosterone production are associated with symptoms including decreased libido, erectile dysfunction, fatigue, depression, reduced strength, bone loss, and increased abdominal fat (vom Steeg et al. 2016).

In this regard, Lauretti et al. (2016) demonstrated that most patients with chronic obstructive pulmonary disease (COPD) share many risk factors and similar etiological agents with erectile dysfunction. Both conditions cause serious interference with the quality of life and sexual relationships (Lauretti et al. 2016). Additionally, fat-

free mass loss and physical inactivity are key features of COPD and are related to impaired muscular oxidative metabolism. The preservation of muscle mass and function through the protection of the mitochondrial oxidative metabolism is an important challenge in the management of COPD patients, where androgen therapy improves muscle mass and force but is insufficient, by itself, to improve lung function and clinical outcome (Samaras et al. 2014). Nevertheless, in combination with physical training and nutritional supplementations, androgen therapy is associated with increased fat-free mass and muscle force, together with increased peak workload and endurance time, and improved survival at least 1 year after the end of therapy. A short-term androgen therapy (3 months) could optimize the effects of rehabilitation in selected patients, but longer studies are warranted in order to identify whether mid- or long-term pulmonary rehabilitation could further improve the clinical outcome (Samaras et al. 2014). Smith and collaborators (2008) showed that testosterone is an efficacious vasodilator in pulmonary vasculature and this is not modulated by the patient's sex. This action suggests that testosterone therapy may be beneficial to male patients with pulmonary arterial hypertension. Unfortunately, androgen therapy could not be offered to female patients, due to the secondary male sexual characteristics that may develop as a result of the treatment.

Testosterone and Cancer Progression

Androgen/AR signaling has an important role in the initiation and progression of many hormone-related cancers. Chang et al. (2014) used various AR knock out (ARKO) mouse models in individual cells to study AR roles in various tumors. They led to very interesting yet distinct conclusions: while AR functions with positive roles to promote tumorigenesis in prostate, liver, bladder, kidney, breast, and lung cancers, AR functions differentially in either suppressing or promoting metastases in these tumors. AR functions as stimulator in bladder, kidney, and lung metastases since knockout of the AR in BCa urothelial cells, renal cell carcinoma (RCC) epithelial cells, and lung cancer cells led to decrease in metastasis as compared with their controls (Chang et al. 2014).

Although radiotherapy (RT) is an important cancer treatment modality, the cell killing induced by radiation is not tumor- or cell-type specific. The response to radiation is dynamic and involves several mediators of inflammation and fibrosis that are produced by macrophages, epithelial cells, and fibroblasts. Wu et al. (2009) found that androgen deprivation by castration augmented the radiation RT-induced inflammatory response. The increased NF-κB activity and subsequent elevated COX-2 by castration might be the underlying mechanism responsible for the increase in RT-induced inflammatory response. Data also indicate that RT-induced fibrosis is related to TGF-β1-induced epithelial-mesenchymal transition (EMT) and is probably mediated via the PI3K/Akt signaling pathway. These results suggest that sex differences play an important role in the inflammatory response, so that when androgen deprivation is concurrently used with irradiation

treatment, the modulating effects on the RT-induced inflammation and fibrosis should be taken into consideration for radiotherapy-associated complications (Wu et al. 2009).

Androgen Impact in Male Lung

Our experimental studies showed that androgen deprivation in male rats, over a period of 30 days, induced oxidative stress and lipid peroxidation in lung, synchronically with changes in the expression of cytoprotective markers (Alvarez et al. 2015). Some oxidative stress parameters [e.g., TBARS, catalase (CAT), and glutathione peroxidase (GPx) activities] were significantly increased in castrated rats, returning to the control values after the administration of testosterone. The same happened with NADPH oxidase (NOX) and GPx expression increased in castrated rats and they showed a decrease to control values in rats supplemented with testosterone. Immunohistochemistry showed a decreased expression of Hsp27 and increased expression of Hsp70i in castrated animals. During the androgen replacement period, Hsp70i was higher than the control group while Hsp27 showed a trend towards an increase in the same group. Taken together, all the results of this work suggest that the absence of androgens induces oxidative stress and lipid peroxidation in lung, synchronically with changes in the expression of cytoprotective markers. It is important to notice that some of the parameters recovered with the addition of testosterone (Alvarez et al. 2015).

Accumulating evidence suggests that sex affects lung development and physiology. Indeed, a higher incidence of respiratory distress syndrome is observed in male compared to female preterm neonates at comparable developmental stage. Experimental studies demonstrated an androgen-related delay in male lung maturation. Bresson et al. (2010) showed that there is a real delay in lung maturation between male and female in this period, the latter pursuing already lung maturation while the proper is not yet fully engaged in the differentiation processes at gestational day 17 (GD17). In addition, they provide a list of genes that are under the control of androgens within the lung at the moment of surge of surfactant production in murine fetal lung.

Androgens enhance airway branching but delay alveolar maturation contributing to increased respiratory morbidity in prematurely born male infants. Androgen effects on lung branching morphogenesis are partially mediated by Hoxb5 protein regulation. This level of regulation occurs partially through changes in cellular localization of SMAD2P and SMAD7 proteins and it involves interactions with TGF-β signaling (Volpe et al. 2013). This ability of androgen signaling to maintain the expression of Hoxb5 protein likely contributes to the known delayed airway epithelial maturation in male infants but positively contributes to the increased airway arborization and ultimate increased lung capacity in males compared to females.

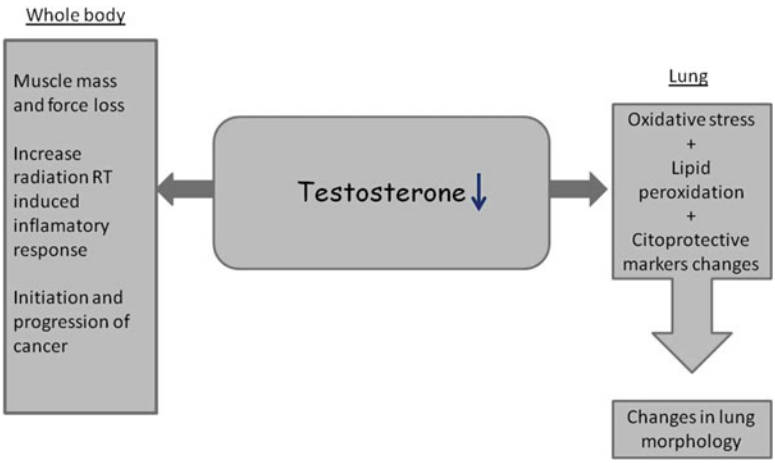

Fig. 3 Pathophysiological effects of testosterone decrease. On the right, the effects of testosterone decrease on the whole body can be seen. On the left, the *box* shows effects of a decrease in testosterone in lung

Conclusion

Considerable advances have been made in the understanding of the pathophysiology of lung diseases. The development of new treatment modalities is therefore critically important.

Besides that, we can conclude that not only the levels of testosterone, but also zinc levels are important in order to maintain the lungs healthy and functional (Fig. 3).

Policies and Protocols

For many years, little attention was paid to respiratory disorders and its relation with zinc. The knowledge presented in this chapter sheds light on the importance of zinc in the respiratory system.

Pulmonary fibrosis (PF), chronic obstructive pulmonary disease (COPD), and asthma are some of the major health problems in the world. It is known that sex affects lung development and physiology and also Zinc is involved in the regulation of sex hormones, specially testosterone, and the immune system (Fig. 4). Androgens enhance airway branching but delay alveolar maturation contributing to increased respiratory morbidity in prematurely born male infants. In men, there is a gradual decline in testosterone secretion that begins after 30 years old, however the signs and symptoms of testosterone deficiency typically

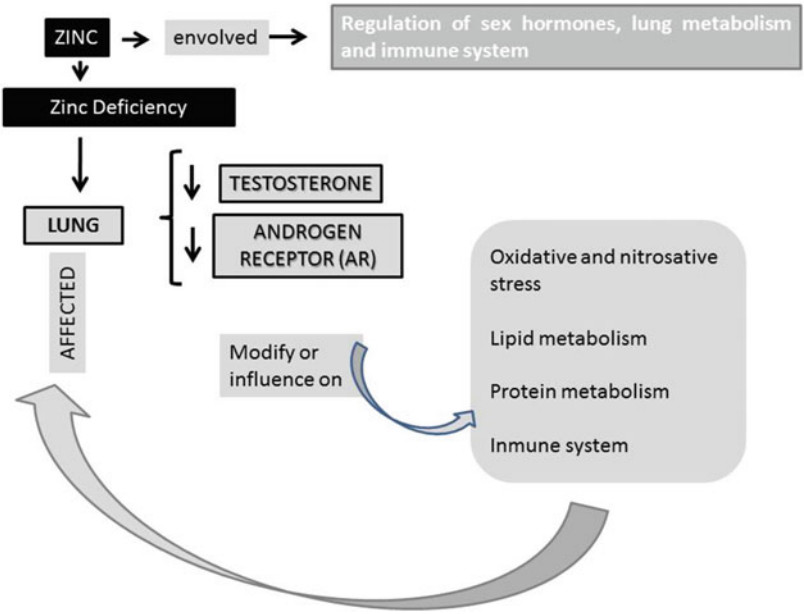

Fig. 4 Integrative image of the relation between lung, zinc, and testosterone. Zinc participates in the regulation of the synthesis of testosterone, in the regulation of the immune system, as it also directly affects the lung (it produces oxidative and nitrosative stress, modifies lipid and protein metabolism, and affects the immune system). In addition androgens also regulate lung function, so that the sum of the effect of zinc deficiency and androgen deficiency leads to a complex pathophysiological situation

presenting after the age of 65. The absence of androgens induces oxidative stress and lipid peroxidation in lung, synchronically with changes in the expression of cytoprotective markers.

Taken together this information points out the importance of Zn levels in the population. Zinc is supplied by our diet. It has been estimated that in a Western mixed diet, this bioavailability is about 20–30% of total contained zinc (Gallaher et al. 1988). The main sources of zinc are oysters, red meat, and poultry; beans, nuts, certain types of seafood such as crab and lobster, whole grains, and dairy products (USDA 2011). Therefore, it is crucial to have a well-designed diet in order to ensure the required level of zinc. Unfortunately, there are many countries with malnutrition and famine. Therefore, specific governmental policies aimed at counteracting these problems are required.

For the other way, in low- and middle-income countries "moderate zinc deficiency" increase and do not have symptoms. The reason of this situation is the use of the unbalanced diet (especially vegetarian and vegan diet). So these populations should have educational programs showing the importance of a balanced diet; pregnant mothers, infants, and aging people should be targeted as a priority group for education on nutritional aspects. Supplementation programs with

micronutrients would help to reduce the incidence of respiratory diseases in vulnerable population.

High-income countries also have health educational programs to raise awareness on the negative effects of unbalanced diets. Specific guidelines should be designed to promote a better design of the family menu.

Furthermore, high-income countries also need health education programs to raise awareness of the negative effects of unbalanced diets. Specific guidelines will be designed to promote a better family menu design. In addition, environmental health policies should be applied worldwide in order to reduce famine as well as its deleterious and mortal consequences on humanity.

Dictionary of Terms

- **COPD** – is a term used to describe progressive lung disease and the same is characterized by increasing breathlessness. Many people mistake their increased breathlessness and coughing as a normal part of aging.
- **Asthma** – is a chronic (long-term) lung disease that inflames and narrows the airways. Asthma causes recurring periods of wheezing (a whistling sound when you breathe), chest tightness, shortness of breath, and coughing. Affects people of all ages, but it most often starts during childhood.
- **Pulmonary fibrosis** – is one of a family of related diseases called interstitial lung diseases that can result in lung scarring. As the lung tissue becomes scarred, it interferes with a person's ability to breathe. In addition, in some cases, the cause of pulmonary fibrosis can be found. But most cases of pulmonary fibrosis have no known cause. These cases are called idiopathic pulmonary fibrosis (IPF).
- **Zinc deficiency** – It is a lowering from micronutrient "Zinc" in the body. The amount that the man requires depends on age, sex, and physiological state. Zinc deficiency could be mild, moderate, acute, or chronic. Any of them can lead to a health problem.
- **Androgens** – are a group of hormones that primarily influence the growth and development of the male reproductive system. The most active androgen is testosterone, which is produced by the testes. The other androgens are produced mainly by the adrenal cortex – the outer portion of the adrenal glands – and only in relatively small quantities.

Summary Points

- This review is focused on the adult lung in health and disease.
- Pulmonary fibrosis (PF), chronic obstructive pulmonary disease (COPD), and asthma are some of the major health problems in the world.
- Zn is present in protein-bound and ionic forms, and plays important roles in mediating the function and structure of proteins, and in maintaining physiological balance.

- Zinc is involved in the regulation of sex hormones.
- Sex affects lung development and physiology.
- Zinc also has effects on lung lipid metabolism and the immune system.
- Androgens enhance airway branching but delay alveolar maturation contributing to increased respiratory morbidity in prematurely born male infants.
- There are sex-specific trends in the incidence, prevalence, and mortality of diseases such as COPD and PF.
- In men, there is a gradual decline in testosterone secretion that begins after 30 years old, with the signs and symptoms of testosterone deficiency typically presenting after the age of 65.
- The absence of androgens induces oxidative stress and lipid peroxidation in lung, synchronically with changes in the expression of cytoprotective markers.
- Not only the levels of testosterone, but also of zinc are important in order to maintain the lungs healthy and functional.

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Anti-inflammatory and Antioxidant Effects and Zinc Deficiency

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Abstract

Over the past 20 years, many researchers have demonstrated the critical role of zinc (Zn), a group IIb metal, in diverse physiological processes, such as growth and development, maintenance and priming of the immune system, and tissue repair. Zinc nutritional importance has been known for a long time, but in the last decades, its importance in immune modulation has arisen. This chapter aims at describing the mechanisms involved in the regulation of zinc homeostasis and their effects on the immune response focusing on those that are implicated in the inflammation. Zinc functions as a modulator of the immune response through its availability, which is tightly regulated by several transporters and regulators. When this mechanism is disturbed, zinc availability is reduced, altering survival, proliferation, and differentiation of the cells of different organs and, in particular, cells of the immune system. Zinc deficiency causes a decrease in innate and adaptive immunity. In addition, during zinc deficiency, the production of pro-inflammatory cytokines increases, influencing the outcome of a large number of inflammatory diseases or worsening other pathologies.

Keywords

Zinc · Metallothioneins · Immunity · Inflammation · Cytokines · Immune diseases · Antioxidant · Oxidative stress

List of Abbreviations

FDA	Food and Drug Administration
HL-60	Human premyelocytic leukemia cell line
HUT-78	Human malignant lymphoblast cell line
IFN	Interferon
IL	Interleukin
MDA+HAE	Malondialdehyde + hydroxyalkenals
MMP	Matrix metalloproteinase
NF	Nuclear factor
NF-κB	Nuclear factor-κB (zinc-dependent transcription factor).
NK	Natural killer
RDA	Recommended daily allowance
ROS	Reactive oxygen species
SLC	Solute-linked carrier
STAT-4	Transcription factor
T-bet	Transcription factor
TD	T cell-dependent
TH	T helper cell
TI	T cell-independent
TPN	Total parenteral nutrition
VCAM-1	Vascular cell adhesion molecule-1
WHO	World Health Organization
ZIP (Zrt-Irt-Protein)	SLC 39a
ZnT	SLC 30a

Introduction

Zinc, an essential trace element and a member of one of the major subgroups of the micronutrients, has attained prominence in nutrition and health, especially for the growth and development of infants and children (Hambidge et al. 2010; Kaur et al. 2014). It is ubiquitously present in all biological systems and has unique and extensive functions within these systems (Bhowmik et al. 2010; Hambidge et al. 2010). The adult human (~70 kg weight) contains about 2–3 g of zinc in the body, with higher average in men than in women, 0.1% of which is replenished daily through dietary intake (Bhowmik et al. 2010). It is found in all organs, secretions, fluids, and tissues of the body, with 85% of the whole body zinc in muscle and bone, 11% in the skin and liver, and the remaining in all other tissues. In multicellular organisms, virtually all zinc is intracellular, 30–40% is located in the nucleus; 50% in the cytoplasm, organelles, and specialized vesicles (for digestive enzymes or hormone storage); and the remainder in the cell membrane (Bhowmik et al. 2010). It serves as a catalytic, structural, and protein interface component for diverse biological functions (Bhowmik et al. 2010). These include carbohydrates, lipids, and nucleic acid synthesis, sequence recognition, transcriptional regulation, cytoskeletal and membrane integrity, apoptosis, synaptic signaling, gene expression, cellular energy metabolism, cell division, hormonal storage and release, neurotransmission, memory, and visual process (Hambidge 2010; Bhowmik et al. 2010). It also has a specific role in enzyme functions – some of them are alkaline phosphatase, alcohol-dehydrogenases, Cu–Zn superoxide dismutase, dipeptidyl carboxypeptidase, DNA polymerase, RNA polymerase, and reverse transcriptase (Kumari et al. 2011), involved in most of the major metabolic pathways and consequently is necessary for a wide range of biochemical, immunological, and antioxidant functions (Maret 2009; Prasad 2014). The role of zinc as an antioxidant has gained importance as it prevents free radical formation, protects biological structures from damage, and functions against oxidative stress (Bhowmik et al. 2010; Kumari et al. 2011). This mineral stabilizes the quaternary structures of enzymes and structures of RNA, DNA, and ribosomes. Zinc finger proteins, the largest class of DNA binding proteins, provide a scaffold that organizes protein subdomains for the interaction with either DNA or other proteins (Kumari et al. 2011). From prokarya to eukarya, between 4–10% of the genome encodes zinc proteins, and in human cells this percentage corresponds to the remarkable number of about 3000 zinc proteins (Bhowmik et al. 2010; Hambidge et al. 2010). Zinc plays an important role in regulating the production and secretion of proteins at transcriptional and translational level. It is vital for a variety of hormonal activities including glucagon, insulin, growth hormone, as well as sex hormones. Its role in immune function is evident from thymic hormone, thymulin, which is zinc dependent and is required for T-cell maturation and differentiation (Bhowmik et al. 2010). It is also known for its anti-inflammatory (antiviral, antibacterial, and antifungal) and anticancerous properties and has been found to protect animals against otherwise lethal irradiation by neutrons (Bhowmik et al. 2010). The therapeutic roles of zinc in acute inflammation, diarrhea, acrodermatitis enteropathica, cognitive impairment, type 2 diabetes (Bhowmik et al. 2010; Maret 2013), prevention of blindness in patients with age-

related macular degeneration, and treatment of common cold have also been reported.

Zinc deficiency has become a global health problem that is generally recognized (Bhowmik et al. 2010; Hambidge et al. 2010; Hambidge 2010), and can be due to inadequate dietary intake, decreased absorption, increased requirements, increased loss, or genetic disease (Bhowmik et al. 2010). The ubiquitous nature of zinc in the human biological system indicates the widespread consequences and complexity of inadequate dietary supply of zinc and zinc depletion (Lowe et al. 2009).

The concentration of zinc in plasma is approximately 15 mM/L of which 84% is bound to albumin, 15% is tightly bound to a-2 macroglobulin, and 1% to amino acids (Seiler et al. 1988). Zinc deficiency is associated with a wide variety of clinical features such as growth impairment, alopecia, hypogeusia, hyposmia, diarrhea, dementia, delayed sexual development and impotency, eyes and skin lesions, emotional disorders, immune dysfunction, susceptibility to infections, impaired appetite, abnormal pregnancy, reduced glucose tolerance, and increased carcinogenesis (Tuerk and Fazel 2009; Hambidge et al. 2010).

It is estimated that more than 20% of individuals worldwide are zinc deficient, especially in developing countries. Zinc deficiency can be found in premature infants, children with poor nutritional intake, elderly patients, and in individuals with sickle cell anemia, anorexia nervosa, cystic fibrosis, liver and renal disease, diabetes, HIV infection, and chronic alcoholism (Hambidge et al. 2010; Hambidge 2010).

The RDA-recommended dietary intake of Zn is approximately 12 mg/day and this is obtainable from protein-rich foods, seafood, fresh fruit and vegetables, and dairy products.

Zinc possesses two main properties, which make it an ideal component in biological systems. First, Zn is virtually nontoxic as the homeostatic mechanism by which it is regulated is so efficient that no chronic disorders are known to be associated with excessive accumulation (Hambidge et al. 2010). Second, its physical and chemical properties enable it to interact with a variety of enzymes and other proteins that participate in cellular metabolism as well as in the control of gene transcription. In the body Zn exists in two main states: (i) a bound form that is held on to tightly by metalloproteins and Zn finger proteins and (ii) a more loosely bound labile form that participates in intracellular Zn fluxes and is readily depleted in Zn deficiency. Although all organs contain labile intracellular Zn, the following tissues are particularly labile Zn rich: hippocampus, testis, and secretory cells (e.g., pancreatic β cells and mast cells).

Zinc Metabolism

Zinc is tightly regulated within the body by transporters that work primarily in the duodenum, jejunum, and nephron (Hambidge et al. 2010; Hambidge 2010; Kaur et al. 2014). Zinc absorption occurs predominantly in the jejunum via the specific transporter Zip4 (Hambidge et al. 2010; Kaur et al. 2014) by passive diffusion or

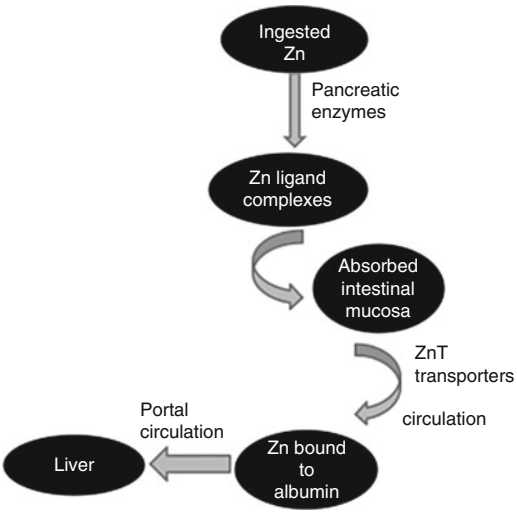

Fig. 1 Zinc metabolism: ingestion, absorption, and circulation up to the liver. Zinc-ligand complexes are absorbed into the intestinal mucosa, where ZnT transporters promote their release into the circulation; zinc is then bound to albumin and taken to the liver through the portal circulation

attached to the apical membrane of enterocytes, where transports are aided by metallothionein and cysteine-rich proteins (Hambidge et al. 2010; Hambidge 2010). It is then taken into the cell and either released into the blood or back into the intestine.

Absorption can be reduced through ingestion of zinc-binding substances such as phytates and fiber. The resulting insoluble complexes remain in the gastrointestinal tract and are excreted in the stool. Consumption of high amounts of iron and/or copper can potentially decrease zinc absorption (King et al. 2000). In the absence of these substances, ingested zinc is released from food by pancreatic enzymes (e.g., proteases, lipases) and forms complexes with amino acids, phosphates, and organic acids (King et al. 2000). These zinc-ligand complexes are absorbed into the intestinal mucosa, where ZnT transporters promote their release into the circulation (Liuzzi et al. 2004). The majority of zinc is then bound to albumin and taken to the liver through the portal circulation (Tuerk and Fazel 2009) (Fig. 1). Approximately 80–85% of zinc is stored in skeletal muscle and bone and has a slow turnover (Tuerk and Fazel 2009). Most zinc is lost through pancreatic secretions, feces, urine, sweat, menstrual fluid, semen, epithelial cells, and hair (Bridges and Zalups 2005). Because there is no specialized organ to regulate zinc in vivo, daily dietary intake is required to maintain a steady state.

Transport of Zinc and its Family Members

Zn belongs to the family of transition metals, which have low ionization energies and a wide range of oxidation states, or positively charged forms. Zn, cadmium (Cd), and mercury (Hg) have been classified as “group 12” metals. They share their way of

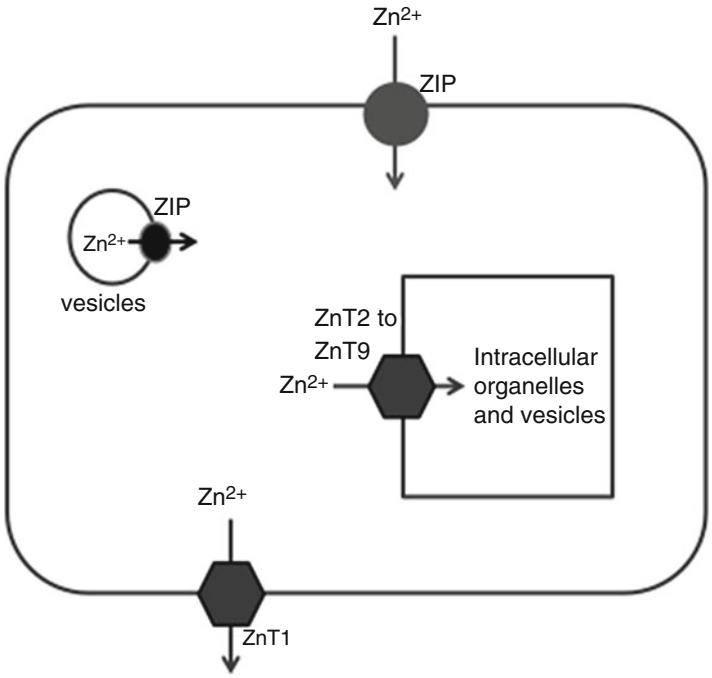

Fig. 2 Zinc transport: import by zip family transporters and export by ZnT family transporters. ZIP transporter import zinc from the extracellular fluid or from intracellular vesicles into the cytoplasm and ZnT family (ZnT1 to ZnT9) export zinc out to the extracellular space or sequestrate cytoplasmic Zn into intracellular compartments

transport in mammalian cells. They are taken up across cell membranes by active (MacDonald 2000) or facilitated diffusion, and this mode of transport in cells has been described as "ion mimicry" (Bridges and Zalups 2004).

There are two types of zinc transporters, coded from solute-linked carrier (SLC) gene families, which possess competing roles in zinc homeostasis. ZnT transporters (SLC30) function by reducing intracellular zinc, whereas Zip transporters (SLC39) function to increase intracellular zinc (Tuerk and Fazel 2009; Wang and Zhou 2010). There are 9 known ZnT and 14 Zip transporters in human beings identified to date with both transporter families being upregulated or downregulated based on zinc levels in vivo (Tuerk and Fazel 2009) and intracellular binding proteins, notably metallothioneins (MTs) are the main controllers of intracellular Zn concentration (Fig. 2).

Entry (Import)

Zn is taken up as a divalent cation and it has a single oxidation state and cannot be oxidized or reduced. The Zn2+ ions are hydrophilic, so they need specialized transporters to get into the cytoplasm. The ZIP family, in mammals, consists of 14

isoforms (ZIP1 to ZIP14) of transporters, which promote metal ion passage from the extracellular fluid or from intracellular vesicles into the cytoplasm (Liuzzi and Cousins 2004; see Fig. 2).

Most ZIP members have a similar protein structure with eight transmembrane domains and a cavity through which metals are transported (Eide 2006). Despite such similarities, different ZIP transporters are expressed on different cell types (Taylor et al. 2005). The absorption of Zn is reduced by increased levels of other cations, such as cadmium, copper, magnesium, calcium, nickel, and iron (Abdel-Mageed and Oehme 1990).

Exit (Export)

Flux of Zn away from the cytosol is needed to balance the input. Zn export is controlled by ZnT proteins. ZnT family consists of nine isoforms (ZnT1 to ZnT9) which contribute to the cytoplasmic Zn balance by exporting Zn out to the extracellular space or by sequestrating cytoplasmic Zn into intracellular compartments when cellular Zn levels are too elevated (Fig. 2). ZnT-1 is the member of the SLC30 family which is ubiquitously expressed and the only Zn transporter involved in Zn efflux across the plasma membrane in many different cells, thus conferring resistance to Zn (Qin et al. 2009). Moreover, among all transporters, ZnT-1 is the most highly regulated, with expression levels increased up to 20-fold during Zn supplementation. Low-dose of Cd induces ZnT1, while ZnT1 silencing enhances Cd toxicity (Fernandez et al. 2007). The other members of the ZnT family are localized on the membrane of intracellular organelles and secretory vesicles/granules. They sequester cytoplasmic Zn into various compartments for secretion, storage, or for supplying proteins that require Zn for their structure and function. For example, different ZnTs working together are necessary to supply Zn to alkaline phosphatases and other enzymes important in the early secretory pathway of proteins (Suzuki et al. 2005).

Homeostasis

Zn homeostasis is maintained by the regulation of uptake/elimination but also by intracellular sequestration in the so-called zincosome. Metallothioneins, which behave as Zn chaperones, are a major tool of this process. Zn is extensively chelated by MTs in the cytosol thanks to their large Zn-binding capacity (Maret and Krezel 2007), (Fig. 2). MTs have the capacity to bind heavy metals and Cd–MT complexes are 100- to 1000-fold more stable than Zn–MT complexes, and MTs were initially considered as Cd detoxification molecules (Carpene et al. 2007; Bell and Vallee 2009).

Zn acts indirectly when inducing the production of metallothionein (MT), which can bind metals with known pro-oxidant activity. Our groups showed that MT levels in lung were significantly higher in comparison with control rats of Zn-deficiency (ZD) diet, during two and 4 months of treatment (Gomez et al. 2003). We proposed

that the higher levels of MT in ZD lung probably reflect a protective response to low concentration of Zn. We were suggesting that high levels of MT are a response to Zn deficiency and exacerbated due to the direct contact of the lung with the environment (Gomez et al. 2003). The same work group determined gene transcription of MTs I and II. MT II mRNA transcription increased in the ZD group as compared to the control group. During the supplementation period, MT II gene transcription was decreased compared to the control group. The gene transcription of MT I was not significantly different among all groups (Biaggio et al. 2010).

It now appears that MTs are involved in, at least, two major types of reactions with Zn: Zn buffering and Zn muffling. Zn buffering is, under steady state conditions, a primary function of cytosolic Zn-binding proteins to buffer the Zn content in the picomolar range. The process of muffling differs from buffering process because it is slow compared with thermodynamic buffering process (Günzel et al. 2001). MTs, as well as some exporters, are transcribed via the metal responsive transcription factor-1 (MTF-1) (Gunther et al. 2012). MTF-1 is localized in both cytoplasm and nucleus. It is involved in cellular adaptation to various stress conditions, primarily exposure to heavy metals, but also to hypoxia and oxidative stress.

Antioxidant Effects of Zinc

Zinc has been shown to be an important antioxidant as excellently reviewed by Powell (2000) and this is best demonstrated in animal studies where there is increased organ susceptibility, such as: the lung (Taylor et al. 1997), liver (Parsons and Di Silvestro 1994), and testes. Recent studies in a rat model of Zn deficiency enhanced epithelial lesions in the gastrointestinal epithelium, which were blocked by a nitric oxide synthase inhibitor (Cui et al. 2000).

Zinc can act as an antioxidant by a number of mechanisms (Fig. 3), which may also be important in the respiratory system. First, Zn can directly act as an antioxidant by stabilizing and protecting sulfhydryl-containing proteins that are important in lung function (e.g., ciliary tubulin, kinases/phosphatases, and Zn finger transcription factors). This ion may protect proteins from thiol oxidation and disulfide formation. Second, Zn can displace Fe and Cu from cell membranes and proteins, which can otherwise cause lipid peroxidation and destruction of membrane protein lipid organization due to their ability to promote the generation of hydroxyl ion (OH) from H_2O_2 and superoxide. This is important as Zn has only one oxidation state (II) and therefore cannot undergo these redox reactions. In addition, Zn can accept a spare pair of electrons from oxidants, hence neutralizing their reactivity.

Zn is an important component of the major antioxidant enzyme Cu–Zn superoxide dismutase (Cu–Zn SOD), located in airway and alveolar epithelial cells cytoplasm. Cu–Zn SOD plays an important role removing superoxide anions. Larsen et al. (2000) recently demonstrated the important protective effect of Cu–Zn SOD in airway. Elevated levels of Cu–Zn SOD in lungs were found to be more resistant to allergen-induced hyperresponsiveness than their wild-type counterparts.

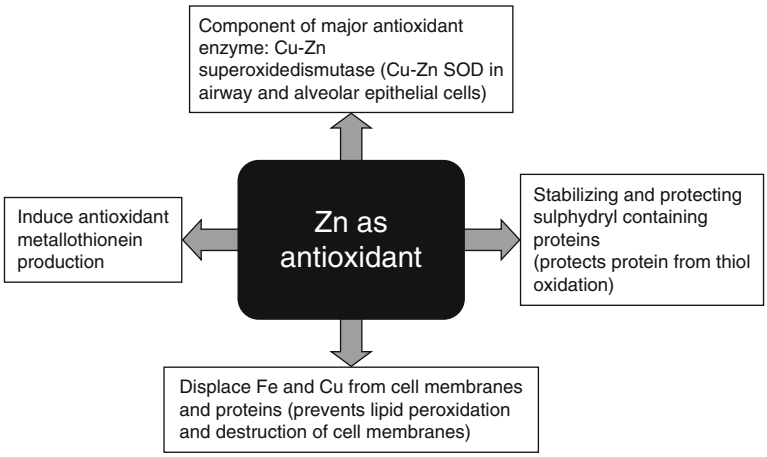

Fig. 3 Zinc and the function as antioxidant. Zinc is an important antioxidant in the respiratory system. Zinc acts as a component of the antioxidant enzyme Cu-Zn SOD (Cu-Zn superoxide dismutase), stabilizes and protects proteins, and induces the production of metallothionein while zinc stored in cells prevents lipid peroxidation and stabilizes membranes

It is known that Zn has a protective role in preventing cell death by apoptosis, in Zn deficiency. Zn and the protein Bcl-2 protect cells from oxidative stress, Zn acts as a cytoprotector of lung epithelium integrity during inflammatory stress and cellular depletion of Zn enhances susceptibility to apoptosis. During the apoptotic process, the molecular targets for Zn are the antiapoptotic Bcl-2-like and proapoptotic Bax-like, among others. Bax showed an increased expression in ZD group, suggesting the activation of apoptosis in the lung, whereas during the supplementation period Bax mRNA levels decreased (Biaggio et al. 2014) (Fig. 4).

Reactive oxygen species produce oxidative damage and have been shown to contribute to aging, neurodegeneration, carcinogenesis, and atherosclerosis (Lachance et al. 2001; Prasad et al. 2009). Zinc acts to prevent ROS formation by inhibiting enzymes that catalyze the formation of superoxide (Lachance et al. 2001). Further, it is a key component in superoxide dismutase, an enzyme capable of reducing hydroxide (Ho et al. 2001) and upregulates MTs (Lachance et al. 2001; Prasad et al. 2009).

Zinc Anti-Inflammatory Properties

Labile Zn plays a major role in inflammation control via a number of mechanisms. First, many inflammatory diseases, such as arthritis and asthma, are associated with an increase in the inducible form of nitric oxide (NO) synthase resulting in enhanced NO formation. Zn is able to inhibit lipopolysaccharide and IL-1β induced NO formation. Second, Zn is anti-inflammatory as it is also able to inhibit the activation of Nuclear Factor-κβ (NF-κβ), a transcription factor implicated in the expression of

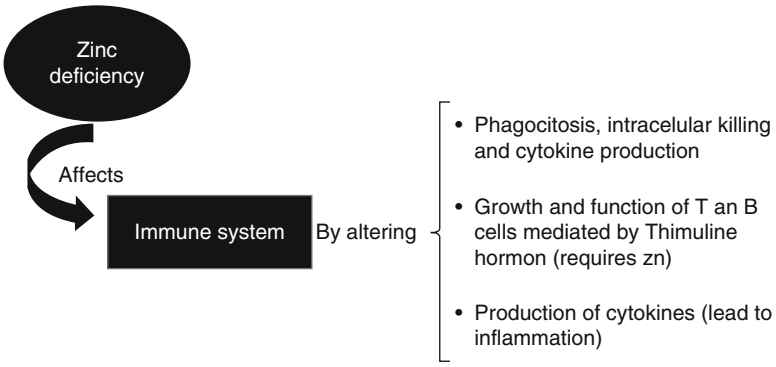

Fig. 4 Influence of zinc deficiency on immune system. Zinc deficiency affects immune system by altering phagocytosis, intracellular killing, and cytokine production. Deficiency leads to changes in growth and maturation of lymphocytes T and B

many pro-inflammatory genes. Zinc inhibits NF-κβ activation by blocking the phosphorylation and degradation of the inhibitory proteins Iκ (Jeon et al. 2000).

Other mechanisms, by which Zn may be anti-inflammatory in the respiratory tract, include: (i) blocking the binding of leucocytes to endothelial cells via the interaction between leucocyte-associated antigen 1 and intercellular adhesion molecule-1 (ICAM-1); (ii) blocking the docking of human rhinovirus on ICAM-1 of somatic cells, thereby preventing viral infections in the respiratory tract; and (iii) inhibiting the release of preformed mediators from mast cells and basophils (e.g., histamine) and eosinophils (e.g., eosinophil cationic protein).

While acknowledging that dietary changes over the past several decades have resulted in a decreased intake of fresh foods containing antioxidants, several intrinsic factors may contribute to a low Zn status in inflammatory diseases. Redistribution in plasma Zn to the liver can occur during excessive stress. This has been attributed to the release of leucocyte endogenous mediator from activated phagocytes, which then stimulates movement of Zn from plasma to hepatocytes, in allergic reactions. Second, the immune system is extremely dependent on the availability of Zn for maintaining its homeostasis. Inflammatory diseases can cause an increase in the demand for Zn as: (i) Zn is essential for producing the thymic hormone, thymulin, necessary for regulating T-cell development and activation; and (ii) Zn is crucial for the activation of natural killer cells, phagocytic cells and for granulocytes, such as mast cells and eosinophils. As a result, greater demand for Zn by the immune system could be a contributing factor to the Zn deficiency noted in inflammatory diseases.

Thymulin binds to high affinity receptors on T cells, induces several T-cell markers, and promotes T-cell function, including allogenic cytotoxicity, suppressor functions, and interleukin-2 (IL-2) production. After a mild Zn deficiency, serum thymulin activity, and mRNAs of IL-2 and IFNγ decreased. Thus, there is a shift from Th1 to Th2 functions affecting cell-mediated immunity adversely. A switch

from an initial cellular (Th1) to a more humoral and more pro-inflammatory (Th2) immune response occur in inflammatory diseases, such as asthma and can be reversed with Zn supplements.

Altered production of cytokines during Zn deficiency can lead to inflammation. It induces IL-1β release and subsequently inhibits the inflammation depending on the transcription factor NF-κβ (Summersgill et al. 2014). Moreover, simultaneous evaluation of circulating cytokines and Zn status showed that the reduced circulating Zn correlates with increased IL-6, IL-8, and TNFα level. In addition, the proliferative response of T and B lymphocytes following IL-6 and IL-2 stimulation increases in Zn deficiency, whereas it adversely influences the IL-4 signaling, causing an impairment of the immune system (Gruber et al. 2013).

Zinc deficiency itself is detrimental for inflammation as it results in dramatic increases in the number, size, and activation state of mast cells. This further exacerbates damage via increasing chemotaxis of eosinophils and neutrophils, which creates a continuous cycle of oxidative damage.

Zinc as an Antioxidant and Anti-Inflammatory Agent

Zn deficiency may increase oxidative and nitrosative stress in lung, which release several pro-inflammatory factors and provoke an infiltration of inflammatory cells (Gomez et al. 2006; Biaggio et al. 2010).

It is an important reminder at this point that zinc deficiency increased oxidative damage in the airways by causing infiltration of inflammatory cells and increased superoxide and nitric oxide production (Zalewski 2006; Gomez et al. 2006; Biaggio et al. 2010). Although reactive oxygen species are formed as a normal component of cellular respiration, in asthma and other inflammatory processes there is a reported imbalance between the flux of oxidants generated and the presence and/or activation of cellular antioxidant defense mechanisms. This especially relates to Cu-Zn SOD, and at least three studies have demonstrated a significant decrease in Cu-Zn SOD activity in erythrocytes (Tekin et al. 2000) and respiratory epithelial cells (Gomez et al. 2006; Biaggio et al. 2010). When zinc deficiency occurs in conjunction with acute lung injury or asthma, a more intense inflammation is produced. In addition, zinc is also able to restore chloride secretion in cystic fibrosis models (Zalewski 2006).

Oxidative stress and chronic inflammation are important contributing factors in several chronic diseases such as atherosclerosis and related vascular diseases, mutagenesis and cancer, neurodegeneration, and immunologic disorders. In addition, the aging process zinc deficiency per se resulted in increased generation of interleukin-1β (IL-1β) from the monocytes–macrophages. Zinc-deficient elderly and sickle cell disease patients also showed increased oxidative stress and they generated increased levels of inflammatory cytokines that decreased on zinc supplementation (Prasad et al. 2007; Bao et al. 2010).

Inflammation generates oxidative stress by increasing ROS, which results in oxidation of LDL. Oxidized LDL activates the NF-kβ-inducible kinase/IK-β kinase/NF-kβ signaling pathway and upregulates its downstream target genes such

as inflammatory cytokines, CRP, adhesion molecules, inducible nitric oxide synthase cyclooxygenase 2, fibrinogen, and tissue factor. These cytokines and molecules attract neutrophils, monocytes, macrophages, and platelets; induce coagulation; and initiate development of atherosclerosis. Zinc supplementation increased plasma antioxidant power, decreased inflammatory cytokines and oxidative stress biomarkers.

The NF-kβ pathway is known for its specific genes expression in response to inflammatory cytokines and oxidative stress. This pathway is also implicated in ROS perpetuation. Zinc decreased NF-kβ activation and its target genes such as TNFα, IL-1β, and VCAM increased the gene expression of A20 and PPAR-α. These zinc finger proteins have anti-inflammatory properties in HL-60 and THP-1 cells after ox-LDL stimulation. Thus, zinc decreased the expression of these cytokines and molecules by inhibition of NF-kβ activation via A20 and PPAR-α pathway.

Role of Zinc in Diseases

Malnutrition is one of the major public health challenges and the most common immune deficiency disease. Micronutrient deficiencies (as Zn and vitamin A) are the major risk factors for malnutrition, which are responsible of more than one third of death in children under five in developing countries. Moreover, malnutrition increases the risk of infections and affects every part of the immune system. Although dietary Zn deficiency was originally thought to be of rare occurrence, it is now estimated to affect more than 25% of the world's population. According to a World Health Organization report (WHO 2002), Zn deficiency ranks fifth among the most important health risk factors in developing countries and eleventh worldwide (Chandel et al. 2010). As Zn is widely required in cellular functions, abnormal Zn homeostasis causes a variety of health problems reaching different levels of severity. If chronic, severe and untreated, Zn deficiency can be fatal through immune defects leading to infections. Less drastic symptoms include infections, hypogonadism, weight loss, growth defects, dermatitis, alopecia, and delayed wound healing (Evans 1986).

Unfortunately, the importance of Zn in humans was only appreciated in the 1960s; before, only animal studies were conducted on this subject. An increased susceptibility to infections in patients was associated with Zn deficiency, indicating its importance for host immunity. Zn deprivation is associated with a decline of the immune system with increased inflammation leading to chronicity (Foster and Samman 2012). Several Zn supplementation studies were then conducted in Zn-deficient patients affected by different disease types (viral, bacterial, and parasitic infections or autoimmune diseases) (Prasad 2009).

Acrodermatitis enteropathica is one of the first diseases to provide the hallmarks of Zn importance. This rare genetic disorder is due to a recessive mutation in the gene encoding ZIP4, responsible for Zn uptake (Andrews 2008). This mutation leads to low plasma Zn concentration and to hyper-pigmented skin lesions, defective growth and, of major importance, to thymic atrophy and lymphopenia. Several

other diseases including infections, cancer, chronic diseases such as asthma, Alzheimer, and autoimmune diseases are related to alterations in Zn status.

Between others, rheumatoid arthritis (RA), characterized by a chronic inflammatory reaction, has been associated with deficiencies of Zn homeostasis. The first study linking Zn to RA reported improvements in joint swelling, morning stiffness, and walking in RA patients taking Zn supplements. However, these results on the antirheumatic effect of Zn were not confirmed by two other studies. But Zn supplementation was shown to modulate ex vivo phagocytosis and oxidative burst in phagocytes from RA patients (Filippin et al. 2008). Nevertheless, patients affected by RA show reduced Zn levels in sera, which negatively correlate with the levels of pro-inflammatory cytokines (TNFα and IL-1β) secreted by monocytes. Also, Zn deficiency increases the levels of TNFα, IL-1β, and IL-8 in a monocyte–macrophage cell line and Zn supplementation inhibits the LPS-mediated release of TNFα and IL-1β in monocytes (Filippin et al. 2008).

Besides its effects on the immune system, Zn regulates several other pathways involved in the development and perpetuation of RA. Zn is a component of matrix metalloproteases (MMPs), which are responsible for matrix remodeling and have a central role in bone destruction in RA. Lack of Zn and the related increase in IL-1β production stimulate the production of MMPs with consequent degradation of extracellular matrix components (Burrage et al. 2006). Lack of Zn has also been associated with an increased oxidative stress and an increased ROS production. Furthermore, Zn deficiency leads to an absence of SOD production and, subsequently, to a reduction in MT induction. Interestingly, local control of RA has been obtained following intraarticular administration of SOD. Zn also inhibits the activation of the transcription factor NF-κβ resulting in a decrease in the transcription of pro-inflammatory cytokines and adhesion molecules. However, the beneficial effects of Zn have not yet been directly demonstrated in RA patients.

In summary, Zn deficiency affects all aspects of the immune system indicating that the availability of Zn is essential for the proper development and function of the immune system, even if some mechanisms of action are not yet decrypted. Consequently several pathologies are characterized by imbalanced Zn homeostasis.

Conclusions and Perspectives

The nutritional importance of Zn is well known. Zn requires daily intake, and to achieve a steady-state level, the homeostasis of Zn inside the cells is tightly regulated by different transporters and regulators. Zn functions as a key structural or catalytic component and it is implicated at all levels of cellular signal transduction. In this way, Zn regulates also communication between cells, cell proliferation, differentiation, and survival. Furthermore, Zn involvement in the regulation of immune cells contributes to pathologies in which inflammation are presented. However, several mechanisms about Zn action on the immune system are still unclear. Further studies on the expression and regulation of transporters or on the interactions of Zn with other ions will also help to better understand the level of Zn and the evolution of

diseases, leading to potential new and innovative therapeutic avenues in the immunological field.

Policies and Protocols

To determine the dietary zinc requirement for a given age/gender group, it is necessary to define the relationship between absorption and intestinal losses and adjust by a constant for the nonintestinal losses in order to calculate the minimum quantity of absorbed zinc necessary to offset total endogenous losses. The factorial calculations used are based on metabolic/tracer studies in which participants are fed diets from which the bioavailability of zinc is likely to be representative of typical diets in Australia and New Zealand (NRVs -2006).

There are not many studies of this type in other parts of the world, so the available data do not take into account the food habits of many populations, nor the ethnicity.

For the above, we can say that a greater number of investigations on this subject are necessary.

Percentage of zinc required per day according to sex and stages of life

Age	AI (Adequate intake)	EAR (Estimated average requirement)	RDI (Recommended dietary intake)
Infant (0–6 months) (7–12 months)	2.0 mg/day	2.5 mg/day	3 mg/day
Children and adolescents			
All			
1–3 year 4–8 year		2.5 mg/day 3.0 mg/day	3 mg/day 4 mg/day
Boys			
9–13 year 14–18 year		5 mg/day 11 mg/day	6 mg/day 13 mg/day
Girls			
9–13 year 14–18 year		5 mg/day 6 mg/day	6 mg/day 7 mg/day
Adults			
Men			
19–30 year 31–50 year 51–70 year >70 year		12 mg/day 12 mg/day 12 mg/day 12 mg/day	14 mg/day 14 mg/day 14 mg/day 14 mg/day
Women			
19–30 year 31–50 year 51–70 year >70 year		6.5 mg/day 6.5 mg/day 6.5 mg/day 6.5 mg/day	8 mg/day 8 mg/day 8 mg/day 8 mg/day

Age	AI (Adequate intake)	EAR (Estimated average requirement)	RDI (Recommended dietary intake)
Pregnancy			
14–18 year		8.5 mg/day	10 mg/day
19–30 year		9.0 mg/day	11 mg/day
31–50 year		9.0 mg/day	11 mg/day
Lactation			
14–18 year		9 mg/day	11 mg/day
19–30 year		10 mg/day	12 mg/day
31–50 year		10 mg/day	12 mg/day

Nutrient reference values for Australia and New Zealand including recommended dietary intakes (NRVs) 2006. NHMRC publications. ISBN Print 1864962372. ISBN Online 1864962437

Dictionary of Terms

- **Oxidative and nitrosative stress** – produced when there is an imbalance between antioxidant and pro-oxidant. The most important free radicals (pro-oxidant) are oxygen reactive species (ROS) and nitrogen reactive species (RNS).
- **Antioxidant** – enzymatic and nonenzymatic antioxidants are molecules that protect cells against free radicals because they can damage cell structures. Organisms have an antioxidant defense system.
- **Inflammation** – this is induced by mediators produced by damaged cells as: cytokines, on the other. Pro-inflammatory cytokines increased during zinc deficiency and decreased on zinc supplementation.
- **Metalothionein** – belongs to the group of intracellular cysteine-rich, metal-binding proteins which are involved in diverse intracellular functions. They have high affinity for metals, like Zn and Fe, and whatever facilitates their detoxification and maintenance of metal homeostasis.
- **Zinc deficiency** – when the body does not absorb or get from food the necessary amount of zinc. This amount depends on age, sex, and physiological state. Zinc deficiency could be mild or moderate, likewise acute or chronic. Any of them can lead to a health problem.

Summary Points

- Zinc is an essential trace element important in human nutrition and health especially for the growth and development of infants and young children, elderly, and in pregnancy.
- It serves as a catalytic, structural, and protein interface component for diverse biological functions.
- The role of zinc in immune modulation has been lately studied.

- This chapter centers on mechanisms involved in regulation of zinc homeostasis and their effects on the immune response focusing on those that are implicate in the inflammation.
- Zinc, cadmium, and mercury share their way of transport in mammalian cells, taking up across cell membranes by active or facilitated diffusion.
- There are different types of zinc transporters, which possess competing roles in zinc homeostasis.
- Zinc homeostasis is maintained by the regulation of uptake/elimination but also by intracellular sequestration in the so-called zincosome with a key participant, the zinc chaperones: metallothioneins.
- Zinc has been shown to be an important antioxidant by a number of mechanisms, which may also be important in the respiratory system.
- Zinc deficiency may increase oxidative and nitrosative stress in lung parenchyma, which release several pro-inflammatory factors and provoke an infiltration of inflammatory cells.
- Zinc deficiency causes a decrease in innate and adaptive immunity.
- During zinc deficiency, the production of pro-inflammatory cytokines increases, influencing the outcome of a large number of inflammatory diseases.
- Zinc involvement in the regulation of immune cells and inflammation produced by zinc deficiency contributes to human pathologies.

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Zinc Deficiency and Stunting

101

Valeria Galetti

Contents

Abstract

This chapter reviews key aspects of human zinc nutrition providing an insight on the etiology of zinc deficiency and current policies on the assessment of the risk of zinc deficiency in populations using stunting rates and other indicators. Zinc is an essential trace element in human nutrition, and it is critical to various basic molecular functions. Zinc depletion of the organism thus virtually affects any organ system in the human body, and it encompasses a number of diverse biochemical changes resulting in a generalized metabolic dysfunctions. Marginal

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zinc deficiency mainly occurs when zinc intakes from the diet are inadequate to provide for increased requirements, increased losses, decreased absorption, or decreased utilization. This form of zinc deficiency carries most of the public health significance of zinc deficiency. Marginal zinc deficiency adversely affects physiological, biochemical, and immunological functions. Typical signs are growth retardation, hypogonadism in male adolescents, rough skin, poor appetite, mental lethargy, abnormal dark adaptation, abnormal neurosensory changes, and delayed wound healing. A more severe form of zinc deficiency can be inherited or acquired due to iatrogenic-induced zinc-free diet or severely impaired intestinal uptake. Acrodermatitis enteropathica is a rare autosomal recessive genetic disorder that results in impaired zinc absorption. In population surveys, the risk of zinc deficiency is considered to be elevated and of public health concern when the prevalence of low serum zinc concentrations is greater than 20%, the prevalence of inadequate intakes is greater than 25%, or the prevalence of stunting is at least 20%. In such cases, an intervention to improve population zinc status or increase dietary zinc intake is recommended. Approximately 17.3% of the world's population are at risk of inadequate intake of absorbable zinc, with the highest risk carried by countries in South and Southeast Asia, sub-Saharan Africa, and Central America. The global mortality burden due to zinc deficiency is of 116,000 deaths per year, the second most important cause of mortality due to micronutrient deficiency after vitamin A deficiency.

Keywords

Zinc · Zinc metabolism · Zinc requirements · Zinc deficiency · Zinc intake · Inadequate zinc intake · Zinc status · Plasma zinc · Serum zinc · Dietary zinc · Stunting · Height-for-age

Abbreviations

AE	Acrodermatitis enteropathica
CI	Confidence interval
DALY	Disability-adjusted life year
EAR	Estimated average requirement
EDTA	Ethylenediaminetetraacetic acid
EFZ	Endogenous fecal zinc
FAO	Food and Agriculture Organization
HAZ	Height-for-age z-score
IAEA	International Atomic Energy Association
IL	Interleukin
IOM	Institute of Medicine
IZiNCG	International Zinc Nutrition Consultative Group
PZn	Plasma/serum zinc
RCT	Randomized controlled trial
RDA	Recommended dietary allowance
RR	Relative risk *or* rate ratio

SD	Standard deviation
UFZ	Unabsorbed fecal zinc
UL	Tolerable upper level of intake
UNICEF	United Nations Children's Emergency Fund
WAZ	Weight-for-age z-score
WHO	World Health Organization
WHZ	Weight-for-height z-score
ZIP	Gene expressing for a Zip transporter
Zip	Zinc transporter, transports zinc into the cytoplasm
ZnT	Zinc transporter, transports zinc out of the cytoplasm

Introduction

In 1961, Prasad et al. described a clinical picture featuring dwarfism, hypogonadism, hepatosplenomegaly, and iron deficiency anemia in 11 Iranian boys (Prasad et al. 1961). The boys were aged 14–21 years, but their appearance was that of 8–15-year-old boys due to shorter-than-normal height; the absence of pubic, axillary, and facial hair; and infantile genitalia. Other key features of the disease included edema and skin and hair changes. Prasad pointed out that the clinical picture was not that of kwashiorkor, previously observed in populations of Africa, Jamaica, and India, because of missing salient features characterizing the newly observed syndrome. In addition, dietary patterns were dissimilar: while subjects developing kwashiorkor consumed a predominantly high-carbohydrate diet low in protein, both animal and vegetable, the Iranian boys consumed large quantities of wheat, which is one of the best cereals in respect to both quantity and quality of proteins. Conversely, these subjects' diet was deficient in animal proteins only. In one subject, Prasad and colleagues were able to determine a distinctly low plasma zinc (PZn) concentration, and by acknowledging similarities in the clinical manifestations of zinc-deficient rats, they concluded that the newly discovered syndrome might have been explained by zinc deficiency (Prasad et al. 1961). Further studies by Prasad and colleagues in other populations in Egypt and Iran described the clinical patterns in more detail and established that its primary cause was zinc deficiency (Prasad 1991; Prasad et al. 1963a, b). The discovery that patients with the syndrome acrodermatitis enteropathica (AE), an inherited autosomal recessive disorder causing zinc deficiency, had low PZn levels was the next key finding that allowed to identify the phenotypic symptomatology of the zinc deficiency syndrome without the presence of other nutritional deficiencies, such as iron deficiency like in the observed cases in Iran and Egypt.

The scope of this literature review is to provide an insight on the role of zinc as an essential trace element in human nutrition, on the etiology of zinc deficiency, and on the assessment of the risk of zinc deficiency using stunting rates and other indicators.

Zinc Metabolism

It is estimated that the human genome encodes for more than 3000 zinc proteins (Andreini et al. 2006). Zinc interacts with proteins in three ways: (1) as central atom in the catalytic center of metalloenzymes that participate in the synthesis and metabolism of molecules (proteins, nucleic acids, lipids, among others) and in cellular proliferation, differentiation, and growth, (2) in the maintenance of tertiary and quaternary structure of proteins (Maret 2013) (tertiary and quaternary structure of proteins), and (3) in the regulation of gene expression and protein synthesis, where zinc interacts with zinc metalloregulatory proteins (Maret 2006). Within the human body, zinc is present in all organs, tissues, fluids, and secretions, with a total body zinc of about 2500 mg in adult men (Iyengar 1998). Most of the zinc is found in the skeletal muscle mass (1400 mg), in the liver (700 mg), and in the bones (400 mg). The highest concentration of zinc is found in teeth (250 mg/kg), hair (200 mg/kg), and prostate (100 mg/kg). Plasma provides zinc to all tissues; therefore, maintenance of homeostasis and constant PZn level is essential to sustaining normal biological functions. The maintenance of homeostasis is achieved by efficiently regulated mechanisms of gastrointestinal absorption and excretion, at the organism level, and import and export, at the cellular level (King et al. 2000). Body zinc balance is the net yield of ingested zinc minus excreted zinc. Chronically low zinc intakes, generic disorders such as AE, or disease states can alter this balance. A physiological model for zinc homeostasis has been proposed (Fig. 1).

The process of absorption is considered as the influx of zinc from the intestinal lumen (mainly the distal duodenum or the proximal jejunum) into the enterocyte via a saturable active carrier mechanism mediated by zinc transporter Zip4 (found along the apical membrane and promoting import into the cytoplasm) and further through the basolateral membrane of the enterocyte via zinc transporter ZnT1 (promoting the export from the cytoplasm) into the portal circulation (Krebs 2000). At lower luminal zinc concentrations (i.e., up to an amount of about 7–9 mg of ingested zinc), import is mainly carried out by Zip4 (King 2010). In a mice model, it was observed that in conditions of zinc restriction, ZIP gene expression is upregulated, suggesting that efficiency of absorption increases with reduction in zinc intakes or reduction of zinc availability in the intestinal lumen (Dufner-Beattie et al. 2004). At higher zinc luminal concentrations, like in case of supplementation therapy, a non-saturable absorption mechanism possibly involving paracellular passive zinc diffusion becomes prominent (King 2010).

Although zinc absorption seems to be capable of coping with large fluctuations in intake, its response is slow. Rapid "fine-tuning" of net retention is guaranteed by a further level of homeostatic control through secretion, reabsorption, and excretion (King et al. 2000). Secretion of endogenous zinc occurs mainly from the pancreas, followed by bile, duodenum, and transepithelial flux from enterocytes and from mucosal cells that are sloughed into the gut (Krebs 2000). Maintenance of zinc balance intrinsically depends on the amount of secreted zinc that is reabsorbed, although it is not clear if this reflects a reduction in the amount of secreted zinc into the intestinal lumen and/or an increased reabsorption rate of endogenous zinc due to upregulation of the carrier-mediated process (King et al. 2000). A study of intestinal

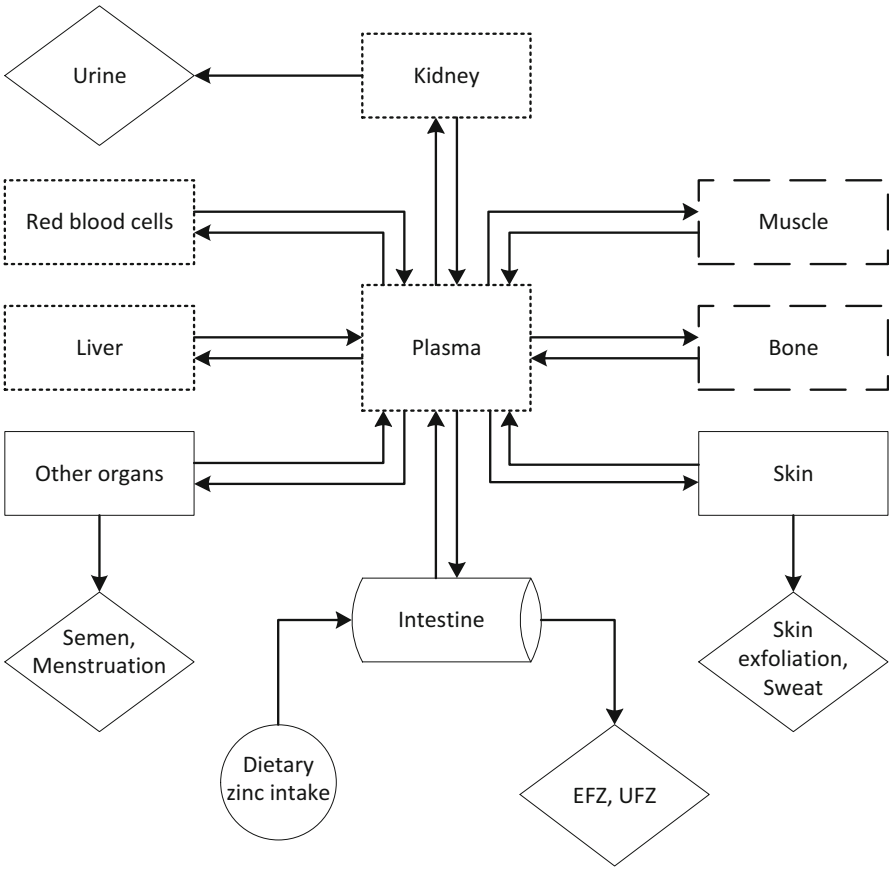

Fig. 1 Physiological model of zinc homeostasis. Dietary zinc is mainly absorbed in the duodenum and jejunum and transferred to plasma. From the plasma, zinc is distributed to the rapid turnover zinc pool tissues (dotted lines) and the slow turnover zinc pool tissues (dashed lines). Zinc is excreted through the intestine (*EFZ* endogenous fecal zinc, *UFZ* unabsorbed fecal zinc) and urine. Other zinc losses occur through skin exfoliation, semen, and menstruation

intubation showed that reabsorption of endogenous zinc continues more distally in the small intestine than absorption of exogenous zinc; however the mechanism of reabsorption remains unknown (Krebs 2000). The potential suggested route for the release of endogenous zinc into the gut is a Zip5-mediated zinc transport from the bloodstream to the enterocyte, with eventual release into the intestinal lumen via the apical transporter ZnT5 (Wang and Zhou 2010). Total zinc excretion represents the net gastrointestinal excretion (secretion minus reabsorption) plus the unabsorbed dietary zinc. The major route of excretion is feces (1–5 mg/day), but other losses include urinary excretion (0.4–0.6 mg/day) and surface losses (desquamation of skin, hair outgrowth, and sweat). Contribution from semen (1 mg) and menstrual losses (0.1–5 mg) is not negligible (Holt et al. 2012).

Dietary Sources of Zinc and Requirements

Zinc is found widely in the food supply. Zinc content is closely correlated with protein content, and rich sources include red meat, liver, kidney, heart, shellfish, crustacean, and cheese, with the highest concentration being found in oysters with 160 mg/100 g. Plant protein sources such as nuts and legumes (beans, chickpeas, peas, and lentils) are also good sources of zinc. In cereals, zinc is found in the bran and germ tissues. Highly refined cereal products, such as white rice or white breads, are poor sources of zinc because zinc-rich bran and germ are normally lost through the milling process. Dairy products and poultry are moderate sources of zinc, whereas poor sources are fruits, tubers, fats, and oils. Zinc-rich foods are not necessarily good contributors to dietary zinc intake. While zinc from animal sources is highly bioavailable, zinc from vegetable sources is generally less bioavailable due to the presence of phytate. Bioavailability describes the degree to which a nutrient in any food is absorbed, transported, and used physiologically. Bioavailability depends on the total zinc content in the diet as well as the food-matrix composition and interactions with other components of foods. Amino acids (e.g., histidine, methionine), organic acids (e.g., citrate), and low-molecular-weight chelators (e.g., EDTA) facilitate absorption by acting as zinc ligands (Lonnerdal 2000). Organic compounds such as phytate, the principal storage form of phosphorus in plants, form stable chelating complexes with zinc at intestinal pH reduce absorption. Certain levels of calcium and possibly iron increase zinc bioavailability by binding phytate and making it unavailable for chelating zinc (Miller et al. 2013). The content of phytate in the diet (also expressed as the phytate-to-zinc molar ratio), however, is the most important food-matrix factor determining zinc absorption (Lonnerdal 2000; Miller et al. 2013) (Fig. 2).

Different algorithms are proposed for estimating dietary zinc bioavailability by the WHO, US Institute of Medicine (IOM), International Zinc Nutrition Consultative Group (IZiNCG), and European Food Safety Authority (EFSA). WHO classifies diets into low, moderate, and high bioavailability with estimated zinc absorption of 15%, 30%, and 50%, respectively, irrespective of life stage and sex. IOM provides an estimated zinc bioavailability for mixed diets for children and adults. IZiNCG proposes revised estimates for mixed and unrefined diets for children, men, and women. EFSA differentiates between lactating women and other life stages (Table 1).

Dietary reference intake values include estimated average requirements (EARs), recommended daily allowances (RDAs), and tolerable upper levels of intake (ULs). The EAR is the median of the distribution of requirements for a specific sex and life-stage group of healthy individuals, and it meets the requirements of half the individuals in that population. The RDA, set at two standard deviations above the EAR, is the zinc intake level at which, theoretically, 97.5% of the population would meet the daily requirements of healthy individuals. The UL is defined as the highest level of zinc intake that is likely to pose no risk of adverse health effects for almost all individuals. The range between the RDA and the UL is considered as the range of safe intakes. Different sets of dietary reference intakes are proposed by WHO, IOM, IZiNCG, and EFSA (Tables 2, 3, and 4).

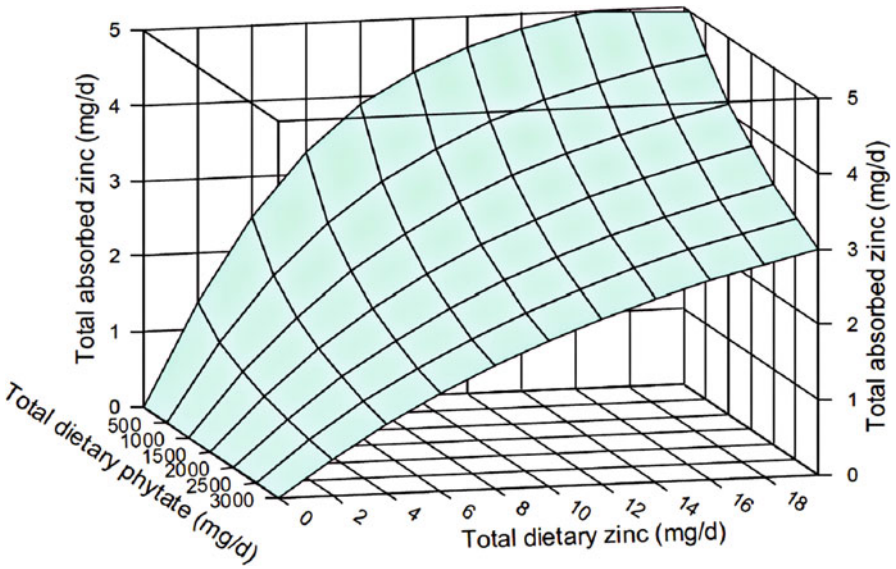

Fig. 2 Saturation response model. Trivariate saturation response model of total absorbed zinc as a function of dietary zinc and phytate intake (Source: European Food Safety Authority (EFSA 2014), with permission from Wiley Open Access under the Creative Commons license)

Risk Factors of Zinc Deficiency and At-Risk Populations

Risk factors for zinc deficiency include (1) dietary or socioeconomic factors that contribute to insufficient zinc intakes or interfere with absorption and bioavailability of dietary zinc, (2) physiological factors that contribute to increased demand owing to growth, and (3) pathological or genetic factors that contribute to increased zinc losses or impaired zinc absorption or utilization. Risk of zinc deficiency thus occurs during periods when zinc intakes from the diet are inadequate to provide for increased requirements, increased losses, decreased absorption, or decreased utilization. Population subgroups generally regarded to be at high risk of zinc deficiency are infants, children, adolescents, pregnant and lactating women, elderly, or any individual with a particular pathological condition, especially among populations from low-income strata or rural settings in developing countries but also among certain Western population subgroups (Brown et al. 2004).

Inadequate dietary intake of absorbable zinc is likely the primary cause of zinc deficiency in most situations (Brown et al. 2004). Risk factors, typical to most low-income rural settings, include poverty, limited food availability, or food choice driven by socioeconomic, cultural, or religious constraint (Gibson 2006). Geogenic factors can also contribute to low zinc content in locally grown foods (Nriagu 2011). In Western societies, reduction in total food intake in elderly (Brown et al. 2004) and consumption of vegetarian or vegan diets (Foster et al. 2013) might result in insufficient zinc intakes.

Table 1 Estimates of dietary zinc absorption as developed by the World Health Organization (WHO/FAO/IAEA 1996), US Institute of Medicine (IOM 2006), International Zinc Nutrition Consultative Group (IZiNCG) (Brown et al. 2004), and European Food Safety Authority (EFSA 2014)

	WHO			IOM			IZiNCG						EFSA	
Diet types	Highly refined	Mixed	Unrefined	Mixed			Mixed or refined vegetarian			Unrefined cereal based			Mixed	
Life stage, sex group	NA	NA	NA	≤18 years	>18 years M	>18 years F	1–18 years, lactating (14–18 years)	>18 years M	>18 years F, lactating (>18 years)	1–18 years, lactating (14–18 years)	>18 years M	>18 years F, lactating (>18 years)	Lactating	Other
Phytate: zinc molar ratio	<5	5–15	>15	NA	NA	NA	4–18			18–30			NA	NA
Zinc absorption	50%	30%	15%	30%	41%	46%	31%	26%	34%	23%	18%	25%	30	45
							40%		44%	32%		35%		

F female, *M* male

Table 2 EARs for dietary zinc by life stage as developed by expert committees of the World Health Organization (WHO/FAO/IAEA 1996), US Institute of Medicine (IOM 2006), International Zinc Nutrition Consultative Group (IZiNCG) (Brown et al. 2004), and European Food Safety Authority (EFSA 2014)

WHO[a]				IOM		IZiNCG			EFSA	
Age, sex	Highly refined diet (mg/day)	Mixed or refined vegetarian diet (mg/day)	Unrefined diet (mg/day)	Age, sex	Mixed (mg/day)	Age, sex	Mixed or refined vegetarian diet (mg/day)	Unrefined, cereal-based diet (mg/day)	Age, sex	Mixed (mg/day)
0–6 months	0.9[b]	2.3[c]	5.5[d]		–		–	–		–
7–12 months	0.7[b]/2.1[e]	3.4	7.0	2.5	6–11 months	3.0	4.0	7–11 months	2.4	2.5
1–3 years	2.0	3.4	6.9	1–3 years	2.5	1–3 years	2.0	2.0	1–3 years	3.6
4–6 years	2.4	4.0	8.0	4–8 years	4.0	4–8 years	3.0	4.0	4–6 years	4.6
7–9 years	2.8	4.7	9.3						7–10 years	6.2
	–	–	–	9–13 years	7.0	9–13 years	5.0	7.0	11–14 years	8.9
10–18 years, M	4.3	7.2	14.3	14–18 years, M	8.5	14–18 years, M	8.0	11.0	15–17 years, M	11.8
10–18 years, F	3.6	6.0	12.0	14–18 years, F	7.3	14–18 years, F	7.0	9.0	15–17 years, F	9.9

(*continued*)

Table 2 (continued)

WHO[a]				IOM		IZiNCG			EFSA	
Age, sex	Highly refined diet (mg/day)	Mixed or refined vegetarian diet (mg/day)	Unrefined diet (mg/day)	Age, sex	Mixed (mg/day)	Age, sex	Mixed or refined vegetarian diet (mg/day)	Unrefined, cereal-based diet (mg/day)	Age, sex	Mixed (mg/day)
>18 years, M	3.5	5.8	11.7	>18 years, M	9.4	>18 years, M	10.0	15.0	≥18 years, M	7.5–12.7[f]
>18 years, F	2.5	4.1	8.2	>18 years, F	6.8	>18 years, F	6.0	7.0	≥18 years, F	6.2–10.2[f]
Pregnancy (first, second, third trimester)	2.8, 3.5, 5.0	4.6, 5.8, 8.3	9.2, 11.7, 16.7	Pregnancy (14–18 years, >18 years)	10.5, 9.5	Pregnancy (14–18 years, >18 years)	9.0, 8.0	12.0, 10.0	Pregnancy	+1.3
Lactation (0–3, 3–6, 6–12 months)	4.8, 4.4, 3.6	7.9, 7.3, 6.0	15.6, 14.6, 12.0	Lactation (14–18 years, >18 years)	10.9, 10.4	Lactation (14–18 years, >18 years)	8.0, 7.0	9.0, 8.0	Lactation	+2.4

F female, *M* male

[a]Calculated values based on WHO recommended nutrient intakes according to a conversion factor equivalent to subtracting two standard deviations of the average nutrient requirement for a population subgroup (Allen et al. 2006)

[b]Exclusively human milk-fed infants. The bioavailability of zinc from human milk is assumed to be 80%

[c]Formula-fed infants. Applies to infants fed with whey-adjusted milk formula and to infants partly human milk-fed or given low-phytate feeds supplemented with other liquid milks

[d]Formula-fed infants. Applicable to infants fed with a phytate-rich vegetable protein-based formula with or without whole-grain cereals

[e]Not applicable to infants consuming human milk only

[f]Depending on level of phytate intake (from 300 to 1200 mg/day)

Table 3 RDAs for dietary zinc by life stage and diet type as developed by expert committees of the World Health Organization (WHO/FAO/IAEA 1996), US Institute of Medicine (IOM 2006), International Zinc Nutrition Consultative Group (IZiNCG) (Brown et al. 2004), and European Food Safety Authority (EFSA 2014)

WHO				IOM		IZiNCG			EFSA	
Age, sex	Highly refined diet (mg/day)	Mixed or refined vegetarian diet (mg/day)	Unrefined diet (mg/day)	Age, sex	Mixed (mg/day)	Age, sex	Mixed or refined vegetarian diet (mg/day)	Unrefined, cereal-based diet (mg/day)	Age, sex	Mixed (mg/day)
0–6 months	1.1[a]	2.8[b]	6.6[c]	0–6 months	2.0[d]		–	–		–
7–12 months	0.8[a]/2.5[e]	4.1	8.4	7–12 months	3.0	6–11 months	4.0	5.0	7–11 months	2.9
1–3 years	2.4	4.1	8.3	1–3 years	3.0	1–3 years	3.0	3.0	1–3 years	4.3
4–6 years	2.9	4.8	9.6	4–8 years	5.0	4–8 years	4.0	5.0	4–6 years	5.5
7–9 years	3.3	5.6	11.2						7–10 years	7.4
	–	–	–	9–13 years	8.0	9–13 years	6.0	9.0	11–14 years	9.4
10–18 years, M	5.1	8.6	17.1	14–18 years, M	11.0	14–18 years, M	10.0	14.0	15–17 years, M	12.5
10–18 years, F	4.3	7.2	14.4	14–18 years, F	9.0	14–18 years, F	9.0	11.0	15–17 years, F	10.4
>18 years, M	4.2	7.0	14.0	>18 years, M	11.0	>18 years, M	13.0	19.0	≥18 years, M	9.4–16.3[f]
>18 years, F	3.0	4.9	9.8	>18 years, F	8.0	>18 years, F	8.0	9.0	≥18 years, F	7.5–12.7[f]
Pregnancy (first, second, third trimester)	3.4, 4.2, 6.0	5.5, 7.0, 10.0	11.0. 14.0, 20.0	Pregnancy (14–18 years, >18 years)	12.0, 11.0	Pregnancy (14–18 years, >18 years)	11.0, 10.0	15.0, 13.0	Pregnancy	+1.6
Lactation (0–3, 3–6, 6–12 m)	5.8, 5.3, 4.3	9.5, 8.8, 7.2	19.0, 17.5, 14.4	Lactation (14–18 years, >18 years)	13.0, 12.0	Lactation (14–18 years, >18 years)	10.0, 9.0	11.0, 10.0	Lactation	+2.9

F female, *M* male

[a]Exclusively human milk-fed infants. The bioavailability of zinc from human milk is assumed to be 80%

[b]Formula-fed infants. Applies to infants fed with whey-adjusted milk formula and to infants partly human milk-fed or given low-phytate feeds supplemented with other liquid milks

[c]Formula-fed infants. Applicable to infants fed with a phytate-rich vegetable protein-based formula with or without whole-grain cereals

[d]Adequate intake (AI), i.e., insufficient evidence for establishing an RDA

[e]Not applicable to infants consuming human milk only

[f]Depending on level of phytate intake (from 300 to 1200 mg/day)

Table 4 ULs for dietary zinc by life stage as developed by expert committees of the World Health Organization (WHO/FAO/IAEA 1996), US Institute of Medicine (IOM 2006), and International Zinc Nutrition Consultative Group (IZiNCG) (Brown et al. 2004)

WHO		IOM		IZiNCG	
Age, sex	Upper limit (mg/day)	Age, sex	Upper limit (mg/day)	Age, sex	No observed adverse effect level (mg/day)
0–6 months	–	0–6 months	4.0	0–6 months	–
7–12 months	13.0	7–12 months	5.0	6–11 months	6.0
1–6 years	23.0	1–3 years	7.0	1–3 years	8.0
6–10 years	28.0	4–8 years	12.0	4–8 years	14.0
10–12 years, M	34.0	9–13 years	23.0	9–13 years	26.0
10–12 years, F	32.0				
12–15 years, M	40.0	14–18 years	34.0	14–18 years, M	44.0
15–18 years, M	48.0				
12–15 years, F	36.0			14–18 years, F	39.0
15–18 years, F	38.0				
>18 years, M	45.0	>18 years	40.0	>18 years	40.0
>18 years, F	35.0				
Pregnancy	35.0	Pregnancy (14–18 years, >18 years)	34.0, 40.0	Pregnancy	–
Lactation	35.0	Lactation (14–18 years, >18 years)	34.0, 40.0	Lactation	–

F female, *M* male

Increased requirements occur during the intense anabolic phase in periods of organism rapid growth due to the critical role of zinc in nucleic acid synthesis and protein metabolism (Gibson 1994). Requirements are thus increased during pregnancy for accrual zinc in fetal and maternal tissues (from 0.1 mg/day in the first quarter to 0.7 mg/day in the fourth quarter, totaling ≈100 mg for the whole gestation period), during lactation for the secretion of zinc in breast milk (4 mg/day in the first days, 1 mg/day at 1 month, and 0.7 mg/day at 6 months for exclusive breastfeeding), and during catch-up growth of premature infants or infants with a smaller content of zinc metallothionein at birth as a consequence of low birth weight or poor maternal zinc status (Brown et al. 2009; Gibson 2006; Swanson and King 1987). Increased demand for zinc is also suggested for weaning infants, children, and adolescents at the time of the pubertal growth spurt (Brown et al. 2004). Stress, trauma, obesity, and rehabilitation after starvation can also incur in increased requirements due to increased anabolism (Nriagu 2011).

Excessive zinc loss occurs via feces, urine, skin, and blood. Fecal losses, which can be of endogenous or exogenous origin, are mainly due to alteration of integrity, permeability, and absorptive capacity of the gastrointestinal mucosa or to increased chyme transit time. Gastrointestinal diseases such as diarrhea, parasitic infections

(i.e., hookworm), celiac disease, inflammatory bowel disease, and Crohn's disease and genetic disorders such as mucoviscidosis (cystic fibrosis), AE, and Wilson's disease can cause malabsorption or sustained loss of intestinal secretions (Holt et al. 2012). Morphological alterations of the intestine through surgical interventions as a strategy against obesity, such as gastric bypass, can promote malabsorption (Salle et al. 2010). Physiological mucosal changes in the intestine occurring during aging also affect zinc absorption and increase losses (Holt 2007). Chelating drugs such as phenytoin and tetracycline and pharmacologic doses of iron reduce zinc absorption (Solomons 1986; Weismann 1986). Increased zinc excretion through urine has been reported in a number of conditions, including liver diseases and cirrhosis, alcoholism, kidney diseases, diabetes mellitus, and chronic (i.e., thalassemia, sickle cell disease) or parasitic hemolysis (i.e., schistosomiasis, malaria) (Gibson 1994). Iatrogenic increase in urinary zinc excretion has been observed during treatment with some chelating agents such as penicillamine (Nriagu 2011). Conditions that induce catabolism are also associated with increased endogenous zinc losses: surgery, burns, multiple injuries, major fractures, and bone or muscle atrophy (Nriagu 2011). Increased losses can also occur through blood (i.e., excessive bleeding or menstruation) and semen (i.e., excessive ejaculation) and through exfoliation of skin and increased perspiration as a result of hot and humid climate or excessive exercise (Gibson 1994). Impaired utilization of zinc may occur in the presence of infection, which generally results in zinc sequestration by the liver, thus reducing levels of circulating zinc and availability to other tissues (Cousins and Leinart 1988).

Clinical Spectrum of Zinc Deficiency

Given zinc ubiquitous nature and implication in various basic molecular functions, zinc depletion virtually affects any organ system in the human body. Zinc deficiency, rather than manifesting a specific clinical condition, encompasses a number of diverse biochemical changes resulting in a generalized metabolism dysfunction (King 2011). Indeed, the variety of clinical manifestations exhibited by zinc deficiency illustrates the numerous roles that zinc plays in the body. Zinc deficiency ranges from marginal to severe: while marginal zinc deficiency is mainly due to suboptimal zinc intakes, severe states are consequent to disease-induced impaired absorption or iatrogenic-induced zinc-free diet. If unrecognized and not corrected, zinc deficiency can turn fatal (Dibley 2001).

Marginal zinc deficiency may occur during periods of increased zinc requirement, such as increased growth in infancy, childhood, adolescence, and pregnancy, mainly due to insufficient dietary zinc intakes. Typical signs of marginal zinc deficiency are growth retardation, hypogonadism in male adolescents, rough skin, poor appetite, mental lethargy, abnormal dark adaptation, abnormal neurosensory changes, and delayed wound healing (Tuerk and Fazel 2009). Depletion/repletion studies conducted by Prasad on male healthy volunteers aged 20–45 years revealed that marginal zinc deficiency in humans is characterized by decreased taste acuity (hypogeusia), decreased sperm count, decreased serum testosterone concentration,

hyperammonemia, decreased lean body mass, decreased serum thymulin activity, decreased IL-2 activity, decreased natural killer cell activity, and alterations in the T-cell subpopulations. The manifestations occurred during an experimental regime of zinc restriction (3–5 mg/day), which induced a total imbalance of ≈180 mg over the 6-month depletion period. All manifestations were corrected by zinc supplementation in the repletion period (27 mg/day) (Prasad 1991). These studies clearly established that even a marginal deficiency of zinc in humans adversely affects physiological, biochemical, and immunological functions.

Severe zinc deficiency can be inherited or acquired due to virtually no zinc in the diet or severely impaired intestinal uptake. Acquired zinc deficiency was observed in patients receiving total parenteral nutrition without zinc supplementation, whereas inherited zinc deficiency refers to subjects affected by AE. AE is a lethal rare autosomal recessive genetic disorder that results in a mutation of the Zip4 gene that encodes for the Zip4 zinc transporter (Wang et al. 2002). Zinc absorption thus results impaired as demonstrated in tracer studies (Weismann et al. 1979). AE occurs worldwide with an estimated incidence of 1 per 500,000 children, with no apparent predilection for race or sex (van Wouwe 1995), and usually develops days to weeks after weaning from breastfeeding or, if an infant is bottle-fed, within days from birth. It is likely that zinc is bound to different molecules in breast milk versus cow milk and that this might have an impact on absorbability; however reasons for this well-characterized difference in onset have not yet been clarified (Cousins and Smith 1980). Subjects affected by AE develop dermatologic manifestations (skin lesions in the acral and periorificial areas), ophthalmic manifestations (conjunctivitis, photophobia, and corneal opacities), neuropsychiatric manifestations (irritability, emotional instability, tremors, and occasional cerebellar ataxia), as well as growth retardation, anorexia, delayed puberty, and male hypogonadism (Maverakis et al. 2007). AE patients also show increased susceptibility to infections and severe gastrointestinal disturbances such as diarrhea, malabsorption, and lactose intolerance (Prasad 2013). Symptoms other than dermatitis seem to vary with age (Vanwouwe 1989). Before the beneficial effect of zinc therapy was recognized, AE was often a fatal disease. Patients on zinc-free total parenteral nutrition for prolonged periods commonly show manifestations similar to those by AE: severe dermatitis, alopecia, weight loss, wound healing problems, and intercurrent infections due to cell-mediated immune dysfunctions.

Assessment of Population Zinc Status

Nutritional indicators are dietary, biochemical, functional, or clinical indices that allow measuring the level of nutrient intake, nutrient exposure, nutrient status, or nutrient functional effects. Used either alone or in combination, they can characterize the stages of development of a nutritional deficiency (Gibson 2005). Several biochemical indicators of zinc status have been proposed and measured in a number of studies (such as zinc concentration in serum or plasma, erythrocyte, leukocyte, urine, hair, nails, to name a few), but the search for a reliable, sensitive, and specific

Table 5 Lower plasma zinc (μg/dl) cutoffs by age, sex, time of day, and fasting status as proposed by the International Zinc Nutrition Consultative Group (IZiNCG) (Brown et al. 2004)

	Children <10 years	Females ≥10 years	Males ≥10 years
Morning fasting	–	70	74
Morning nonfasting	65	66	70
Afternoon	57	59	61
		Pregnancy	
First trimester		56	
Second/third trimester		50	

biochemical index of zinc status at the individual level has been problematic and is still ongoing. Anthropometric or clinical indices, such as stunted growth, immune dysfunction, and skin lesions, are indicative of the functional effects of prolonged low zinc status (Lowe et al. 2009).

The functional outcome of choice for estimating the risk of zinc deficiency in populations is stunting (de Benoist et al. 2007). It is recommended to use the stunting prevalence in children under 5 years of age, i.e., the prevalence of children with height-for-age (HAZ) below-2SD of the age-specific median of the reference population based on the WHO Child Growth Standards (WHO 2006). The risk of zinc deficiency is considered to be of elevated public health concern when the prevalence of stunting among children aged <5 years is at least 20%. Although stunting may be associated with zinc status, it may also be caused by inadequate intakes of other nutrients including energy, protein, or micronutrients such as iron, zinc, and vitamins D, A, or C (singly or in combination). Consequently, HAZ is a valid qualificator of the likely risk of zinc deficiency ("high" versus "low" risk) but has limited value in quantifying the extent of the prevalence of zinc deficiency. Therefore, this assessment should be used as evidence suggestive for an increased likelihood that zinc deficiency is of public concern, whereby further assessment of zinc status using direct indicators is required, such as biochemical assessment of PZn and/or dietary assessment of zinc intake (Brown et al. 2004).

The biochemical indicator of choice is PZn, and lower cutoffs by age, sex, time of day, and fasting status have been proposed by IZiNCG (Brown et al. 2004) (Table 5). For population assessment, the percentage of individuals with PZn below the appropriate cutoff is calculated: <10% suggests that zinc deficiency is not of public health concern, 10–20% suggests that segments of the population may be at high risk of zinc deficiency and that stratified analysis for identification of at-risk groups is necessary, and >20% suggests that the population is at high risk of zinc deficiency, and programs at national level may be considered (Brown et al. 2004).

To assess the adequacy of population zinc intake as based on dietary surveys, IZiNCG suggests calculating the percentage of individuals with intakes below the EAR, using the EAR cut-point method: <15% suggests a likely low risk, 15–25% suggests an intermediate level of risk for zinc deficiency, and ≥25% suggests that there is an elevated risk of zinc deficiency in that population and that zinc deficiency is of public health concern (Brown et al. 2004).

Global Prevalence of Zinc Deficiency

Data is insufficient to classify many countries' national zinc status based on the population distribution of PZn or zinc intakes. In the absence of these direct indicators, in 2004 IZiNCG developed a method to derive estimates for a country's likely risk of zinc deficiency derived from two indirect indicators: (1) the national prevalence of stunting in children aged <5 years and (2) the national prevalence of inadequacy of zinc intakes based on the amount of absorbable zinc in each country's national food supply (Brown et al. 2004). The national prevalence of stunting in children aged <5 years is regularly assessed in most countries during surveys, and the national stunting prevalence is well documented in the WHO Global Database on Child Growth and Malnutrition. Estimated national and global prevalence of inadequate zinc intakes were estimated in 2005 and revised in 2012 (Wessells et al. 2012; Wuehler et al. 2005). Using these data, IZiNCG classified each country into one of the three categories of likely risk of zinc deficiency ("high," "moderate" and "low") using a composite index based on both indirect indicators: (1) prevalence of stunting ≥20% *and* estimated prevalence of inadequate zinc intake ≥25% to be suggestive evidence for "high" risk of zinc deficiency, (2) prevalence of stunting >20% *or* estimated prevalence of inadequate zinc intake >25% to be suggestive evidence for "moderate" risk of zinc deficiency, and (3) prevalence of stunting <20% *and* estimated prevalence of inadequate zinc intake <25% to be suggestive evidence for "low" risk of zinc deficiency. The global risk of zinc deficiency was thus estimated in 2004 (Brown et al. 2004) and was then revised in 2012 (Wessells and Brown 2012) (latest estimates are mapped in Fig. 3).

Absorbable zinc content of national food supplies may be inadequate to meet zinc requirements for approximately 15–20% of the world's population, with an estimate of 17.3% of global inadequacy of absorbable zinc intakes. Countries in South and Southeast Asia (22–29%), Sub-Saharan Africa (25%), and Central America (17%) were identified as being at highest risk of inadequate zinc intake. The mean prevalence of stunting in countries identified as being at low, moderate, and high risk of inadequate zinc intake was 19.6%, 28.8%, and 43.2%, respectively. The estimated prevalence of inadequate zinc intake significantly and positively correlated with the prevalence of stunting in children <5 years of age, although it was generally lower than stunting rates. This was explained by the fact that prevalence of zinc deficiency is indeed higher in young children than in adults and that prevalence of inadequate zinc intakes derived from food balance sheets is more likely to be reflective of adult dietary intakes than children (Wessells and Brown 2012).

The methodology applied in this approach has not yet been validated and should therefore be regarded as an interim measure. In fact, risk factors of stunting are not only limited to zinc deficiency but also include other micronutrient deficiencies and other forms of malnutrition, such as protein-energy deficiency, whereas national food balance sheet may not be representative for population groups in rural settings that are most vulnerable to stock price variation and meteorological conditions affecting subsistence farming. National health and nutrition surveys, although expensive and requiring a skilled staff, should be encouraged for the determination

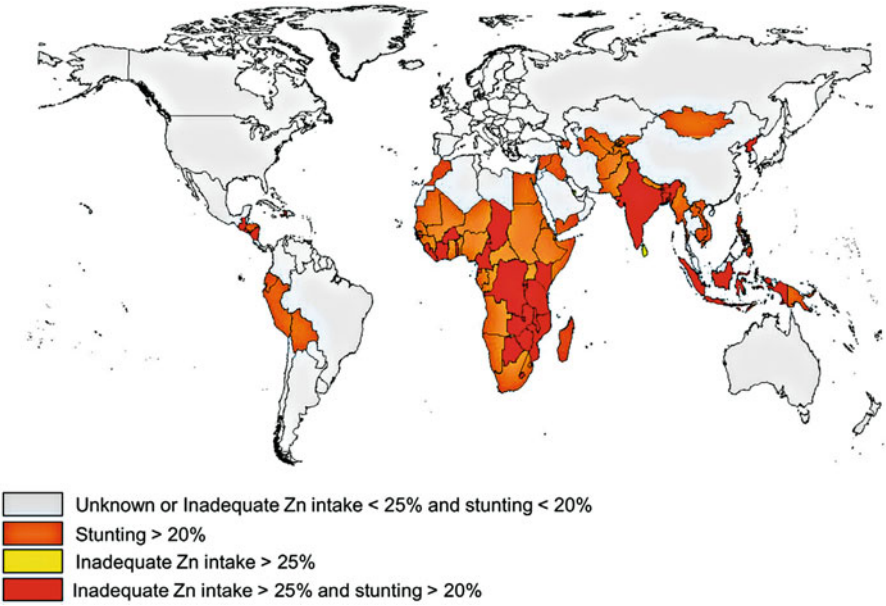

Fig. 3 National risk of zinc deficiency based on the prevalence of childhood stunting and the estimated prevalence of inadequate zinc intake. Stunting data (low height-for-age) are for children less than 5 years of age in 138 low- and middle-income countries. The estimated prevalence of inadequate zinc intake is based on the Food and Agriculture Organization national food balance sheet data from 188 countries (Source: Wessells and Brown 2012 with permission from PLOS under the Creative Commons license)

of prevalence of low PZn and inadequate zinc intake in nationally representative population strata. In recent years, more information has become available from national surveys in low- and middle-income countries on the distribution of PZn in nationally representative samples. A decent agreement was recently found comparing zinc deficiency risk estimation based on PZn with the estimation based on the IZiNCG methodology (Hess 2017).

Public Health Significance of Zinc Deficiency

Marginal zinc deficiency seems to carry most of public health significance of zinc deficiency, as it is more prevalent than more severe states but still shows clinically important manifestations (Hambidge 2000). The main cause of marginal zinc deficiency is dietary, thus due to the nature of diets prevalently consumed in settings of lower socioeconomic status more common to most developing countries. Results from several systematic reviews suggest that zinc deficiency substantially contributes to the morbidity and mortality of young children worldwide. A meta-analysis revealed that zinc deficiency in children aged <5 years is associated with a relative

risk (RR) of incidence of diarrheal disease of 1.28 (95% CI: 1.10–1.50), pneumonia of 1.52 (95% CI: 1.20–1.89), and malaria of 1.56 (95% CI: 1.29–1.89) and was estimated to yearly cause 176,000 diarrhea deaths, 406,000 pneumonia deaths, and 207,000 malaria deaths (Caulfield and Black 2004). The associated loss of disability-adjusted life years (DALYs) was estimated at more than 28 million, with the biggest share of disease burden found in countries in Africa, Eastern Mediterranean, and Southeast Asia. Revised estimates were proposed in 2008 by Black and colleagues in a meta-analysis of RCTs carried out in countries estimated to be at medium-high risk of zinc deficiency (Black et al. 2008). The pooled RR for morbidity associated with zinc deficiency was 1.09 (95% CI: 1.01–1.18) for diarrhea, 1.25 (95% CI: 1.09–1.43) for pneumonia, and 1.56 (95% CI: 1.29–1.89) for malaria. The RR for mortality in children aged 1–59 months associated with zinc deficiency was 1.27 (95% CI: 0.96–1.63) for diarrhea, 1.18 (95% CI: 0.90–1.54) for pneumonia, and 1.11 (95% CI: 0.94–1.30) for malaria. Zinc deficiency was estimated to yearly cause 450,000 deaths (4.4% of total death in children aged <5 years), and the associated loss of DALYs was estimated at more than 16 million (3.8% of total DALYs in children aged <5 years), more than iron and iodine deficiencies pulled together but less than vitamin A deficiency. These estimates were about 40% lower than those previously reported because included additional studies reporting lower RR. More recent estimates by Black and colleagues reported even lower figures with a total of 116,000 deaths attributable to zinc deficiency, mainly because the risk of pneumonia and diarrheal mortality was estimated starting at 12 months of age rather than 6 months of age, as previously done (Black et al. 2013). Although the share of global mortality burden due to zinc deficiency is only second to that of vitamin A deficiency among micronutrient deficiencies, zinc deficiency is responsible for the least proportion of global deaths when compared to other nutritional disorders: stunting (14.7%), underweight (14.4%), wasting (12.6%), fetal growth restriction (11.8%), suboptimum breastfeeding (11.6%), vitamin A deficiency (2.3%), and zinc deficiency (1.7%) (Black et al. 2013).

Policies and Protocols

WHO Policies

Stunting

Low HAZ occurs when an individual is short for his/her age and reflects either normal variation or a pathological process. When shortness is pathological, an individual is said to be "stunted." Stunting reflects a process of failure to reach linear growth potential as a result of long-term suboptimal health and/or nutritional conditions (WHO 1995). In contrast, low WHZ is referred to as "wasting" and describes the condition of gaining insufficient weight relative to height, implying recent severe weight loss. Low WAZ, or "underweight," refers to the gaining of insufficient weight relative to age implying stunting and/or wasting (WHO 1995). Since height deficit results from a long-term cumulative effect of socioeconomic,

health, and nutrition inadequacies, it was chosen as the operational anthropometric indicator of choice for predicting the level of risk with which these inadequacies occur, ultimately reflecting the level of development. When low HAZ is observed in children below 2–3 years, it probably reflects a continuing process of "failing to grow," whereas when observed in older children, it reflects a state of "having failed to grow" (WHO 1995). For these reasons, a current picture of the level of development of a population is given by the prevalence of stunting, i.e., HAZ <-2SD of the WHO Child Growth Standards (WHO 2006), in children below 5 years of age. Four ranges have been defined by WHO to classify levels of stunting for global monitoring purposes: <20%, 20–29%, 30–39%, and ≥40% representing low, moderate, high, and very high prevalence of stunting, respectively (de Onis et al. 1993). Stunting rates exceeding 20% are considered a public health concern (WHO 1995).

Zinc Status Indicators

In 2007, WHO/UNICAF/IAEA/IZiNCG, in an interagency meeting on zinc status indicators, reviewed methods of assessing population zinc status and endorsed the following recommendations for the use of specific biochemical, dietary, and functional indicators of zinc status in populations (de Benoist et al. 2007).

- The risk of zinc deficiency is considered to be elevated and of public health concern when the *prevalence of low serum zinc concentrations is greater than 20%*. In this case, an intervention to improve population zinc status is recommended.
- The risk of zinc deficiency is considered to be elevated and of public health concern when the *prevalence or probability of inadequate intakes is greater than 25%*. In this case, an intervention to increase population dietary zinc intake is required.
- The risk of zinc deficiency is considered to be of elevated public health concern when the *prevalence of stunting is at least 20%*. In this case, an intervention to improve population zinc status is recommended.

Dictionary of Terms

- Stunting. A form of failure to growth, also known as pathological shortness, occurring when an individual is short for his/her age, specifically when his/her height-for-age z-scores are below -2SD of the WHO Child Growth Standards.
- Kwashiorkor. A form of severe protein-energy malnutrition characterized by edema and an enlarged liver.
- Acrodermatitis enteropathica. A rare, lethal if untreated, inherited autosomal recessive genetic disorder causing zinc deficiency.
- Iatrogenic disease. An illness inadvertently induced by medical diagnostic procedures or treatment.

Summary

- This chapter reviews key aspects of human zinc nutrition providing an insight on the etiology of zinc deficiency and current policies on the assessment of the risk of zinc deficiency in populations using stunting rates and other indicators.
- Zinc is an essential trace element in human nutrition, and it is implicated in various basic molecular functions.
- Zinc depletion virtually affects any organ system in the human body, and it encompasses a number of diverse biochemical changes resulting in a generalized metabolism dysfunction.
- Zinc deficiency occurs when zinc intakes from the diet are inadequate to provide for increased requirements, increased losses, decreased absorption, or decreased utilization.
- Marginal zinc deficiency is mainly due to suboptimal zinc intakes and carries most of public health significance of zinc deficiency.
- Marginal zinc deficiency adversely affects physiological, biochemical, and immunological functions. Typical signs are growth retardation, hypogonadism in male adolescents, rough skin, poor appetite, mental lethargy, abnormal dark adaptation, abnormal neurosensory changes, and delayed wound healing.
- Severe zinc deficiency can be inherited or acquired due to virtually no zinc in the diet or severely impaired intestinal uptake. Acrodermatitis enteropathica is a rare autosomal recessive genetic disorder that results in impaired zinc absorption.
- The risk of zinc deficiency is considered to be elevated and of public health concern when the prevalence of low serum zinc concentrations is greater than 20%, the prevalence of inadequate intakes is greater than 25%, or the prevalence of stunting is at least 20%. An intervention to improve population zinc status or increase dietary zinc intake is recommended.
- Approximately 17.3% of the world's population are at risk of inadequate intake of absorbable zinc, with the highest risk carried by countries in South and Southeast Asia (22–29%), sub-Saharan Africa (25%), and Central America (17%).
- The global mortality burden due to zinc deficiency is of 116,000 deaths per year, only second to that of vitamin A deficiency among micronutrient deficiencies.

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Zinc Deficiency and Epigenetics

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Harvest F. Gu and Xiuli Zhang

Contents

Abstract

Zinc (Zn) is an essential micronutrient element. This element in relation with the structure and function of many proteins and enzymes is important for a variety of biological activities, including epigenetic regulations. Zinc deficiency is common in many parts of the world and particularly in poor populations. Accumulating evidence has demonstrated that several key enzymes and zinc finger proteins with

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zinc atom(s) in the reactive center and binding site play important roles in DNA methylation and histone modifications. Therefore, zinc deficiency may disrupt the functions of these enzymes and proteins and result in epigenetic dysregulation. Furthermore, zinc deficiency may enhance inflammatory response and subsequently alter DNA methylation status of the genes involved in inflammation. In this chapter, we first describe zinc dietary sources and deficiency, and then discuss direct and indirect effects of zinc deficiency in DNA and chromatin methylation alteration. Finally, we prospect a new zinc biomarker and further investigation on the effects of zinc deficiency in epigenetics.

Keywords

Betaine homocysteine methyltransferase · DNA methylation · Epigenetics · Histone modification · Methionine synthase · Oocyte epigenetic programming · Zinc · Zinc deficiency · Zn-dependent methyltransferases · Zinc finger proteins · Zinc food sources

List of Abbreviation

AI	adequate intake
BHMT	betaine homocysteine methyltransferase
DGLA	dihomo-γ-linolenic acid
dTMP	thymidylate monophosphate
DV	daily value of foods
FAO	food and agriculture organization
IL	interleukin
LA	linoleic acid
MTR	methionine synthase
RDA	recommended dietary allowance
RNI	recommended nutrient intake
SAMe	S-adenosyl methionine
SLC	solute-linked carrier
WHO	World Health Organization
ZFP	zinc finger protein

Introduction

Zinc is a chemical element with the symbol Zn^{2+} and atomic number 30. This chemical element is present in all body tissues and fluids as an essential component for approximately 1000 proteins (Dreosti 2001; Ho 2004; Chasapis et al. 2012). Of them, more than 300 enzymes participate in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients (Frassinetti et al. 2006; Prasad 2012, 2013). Except these enzymes, the rest mainly includes the proteins with a zinc atom in the reactive center and zinc finger proteins (ZFPs) (Laity et al. 2001). Therefore, zinc, in relation with the structures and functions of these proteins, is of importance in a variety of biological activities such as

apoptosis, signal transduction, transcription, differentiation, and replication in all organ systems and during embryonic development. Since zinc is involved in such fundamental and extensive biological activities, it most likely accounts for the essentiality of zinc for all life forms.

As an essential mineral, zinc has been perceived by the public as being of "exceptional biologic and public health importance." However, many peoples particularly in the developing countries consume less than the recommended nutrient intakes (RNIs) for dietary zinc (WHO and FAO 2004). Epidemiological study has reported that zinc deficiency affects about 2.2 billion people around the world and has been ranked 11th among global risk factors for mortality and 12th for burden of disease (Lopez et al. 2006). Clinical observation has demonstrated that zinc deficiency is associated with pathologic changes in many diseases (Frassinetti et al. 2006; Prasad 2012, 2013). Dysregulation of epigenetics due to zinc deficiency may be involved in the pathogenesis of the diseases.

Epigenetics is involved in many cellular processes. Within cells, there are three systems that can interact with each other to silence genes: DNA methylation, histone modifications, and noncoding RNA-associated silencing (Du et al. 2015). Zinc has been found to affect the activities of some key enzymes such as methionine synthase (MTR, also known as MS) and betaine homocysteine methyltransferase (BHMT) in the reaction of DNA methylation (Castro et al. 2008; Jing et al. 2015). ZFPs are the most abundant proteins in eukaryotes and fundamentally contribute to multiple layers of epigenetic regulation such as DNA methylation and histone modifications (Laity et al. 2001; Shimbo and Wade 2016). Furthermore, zinc homeostasis is involved in the immune cell signaling and activation. Zinc deficiency may enhance inflammatory response and subsequently alter DNA methylation status of the genes involved in the induction of a pro-inflammatory response (Wong et al. 2015). In this chapter, we briefly describe zinc dietary sources and deficiency, and then intensively discuss direct and indirect influences of zinc deficiency in DNA methylation changes. Finally, we prospect a new zinc biomarker and further investigation on the effects of zinc deficiency in epigenetics.

Zinc Food Sources and Deficiency

Zinc deficiency can occur not only in humans but also in soil, plants, and animals. In general, zinc deficiency is defined either qualitatively as insufficient zinc to meet the requirements of the body and thereby causing clinical manifestations or quantitatively as a serum zinc level below the normal range. In humans, the most common cause of zinc deficiency is reduced dietary intake, while other reasons include inadequate absorption, increased loss, or increased use (Wessells and Brown 2012; Wessells et al. 2012). Therefore, it is necessary to know the sources of zinc from foods.

A wide variety of foods contains zinc and the selected food sources of zinc are listed in Table 1. To help the consumers for comparison of the nutrient contents of products within the context of a total diet, the daily values of foods (DVs) in this table have been developed by the U.S. Food and Drug Administration. Oysters

Table 1 Selected food sources of zinc

Food	Mg per serving	Percent DV
Oysters, cooked, breaded, and fried, 85.05 g	74.0	493
Beef chuck roast, braised, 85.05 g	7.0	47
Crab, Alaska king, cooked, 85.05 g	6.5	43
Beef patty, broiled, 85.05 g	5.3	35
Breakfast cereal, fortified with 25% of the DV for zinc, ¾ cup serving	3.8	25
Lobster, cooked, 85.05 g	3.4	23
Pork chop, loin, cooked, 85.05 g	2.9	19
Baked beans, canned, plain or vegetarian, ½ cup	2.9	19
Chicken, dark meat, cooked, 85.05 g	2.4	16
Yogurt, fruit, low fat, 226.80 g	1.7	11
Cashews, dry roasted, 28.35 g	1.6	11
Chickpeas, cooked, ½ cup	1.3	9
Cheese, Swiss, 28.35 g	1.2	8
Oatmeal, instant, plain, prepared with water, 1 packet	1.1	7
Milk, low-fat or non-fat, 1 cup	1.0	7
Almonds, dry roasted, 28.35 g	0.9	6
Kidney beans, cooked, ½ cup	0.9	6
Chicken breast, roasted, skin removed, ½ breast	0.9	6
Cheese, cheddar or mozzarella, 28.35 g	0.9	6
Peas, green, frozen, cooked, ½ cup	0.5	3
Flounder or sole, cooked, 85.05 g	0.3	2

Mg milligrams; *DV* daily value. DVs were developed by the U.S. Food and Drug Administration to help the consumers for comparison of the nutrient contents of products within the context of a total diet

contain more zinc per serving than any other food. Oysters are unusual and delicious mollusks that provide the human body with a number of unique nutrients and minerals, particularly zinc (Murphy et al. 1975). The edible components are the meat inside the oyster, and once the shells have been cracked, we can cook this meat in a variety of ways, but they can also be eaten raw. The valves in oysters can actually cleanse entire ecosystems of pollutants and are a major benefit to the environment. In recent years, however, the oyster population of the world has dropped significantly, resulting in weaker overall ecosystems in the areas where oysters once flourished (Páez-Osuna et al. 2002; Lacerda and Molisani 2006). Red meat and poultry provide the majority of zinc in the diet. Other food sources, including beans, nuts, certain types of seafood (such as crab and lobster), whole grains, fortified breakfast cereals, and dairy products, are also good (WHO and FAO 2004). Comparatively, the bioavailability of zinc from animal foods is higher than that from grains and plant foods, because phytates are present in whole-grain breads, cereals, legumes, and other foods from plants and bind zinc and inhibit its absorption in foods (Wise 1995; Sandstrom 1997).

Table 2 RDAs for dietary zinc (mg/day) and the normative storage requirements from diets differing in zinc bioavailability

Group	Age	Assumed body weight (kg)	High bioavailability	Moderate bioavailability	Low bioavailability
Infants and children	0–6 months	6	l.l[a]	2.8[b]	6.6[C]
	7–12 months	9	0.8[a], 2.5[d]	4.1	8.4
	1–3 years	12	2.4	4.1	8.3
	4–6 years	17	2.9	4.8	9.6
	7–9 years	25	3.3	5.6	11.2
Adolescents	10–18 years (F)	47	4.3	7.2	14.4
	10–18 years (M)	49	5.1	8.6	17.1
Adults	19–65 years (F)	55	3.0	4.9	9.8
	19–65 years (M)	65	4.2	7.0	14.0
	65+ years (F)	55	3.0	4.9	9.8
	65+ years (M)	65	4.2	7.0	14.0
Pregnant women	First trimester	–	3.4	5.5	11.0
	Second trimester	–	4.2	7.0	14.0
	Third trimester	–	6.0	10.0	20.0
Lactating women	0–3 months	–	5.8	9.5	19.0
	3–6 months	–	5.3	8.8	17.5
	6–12 months	–	4.3	7.2	14.4

Source: Adapted from WHO and FAO 2004. Unless otherwise specified, the interindividual variation of zinc requirements is assumed to be 25%. Weight data interpolated from FAO, *Food and Nutrition Series,* No. 23, 1988). RNIs: recommended nutrient intakes; *Mg*: milligrams; *Kg*: kilograms; *F*: females; *M*: males; a: Exclusively human-milk-fed infants. The bioavailability of zinc from human milk is assumed to be 80%; assumed co-efficient of variation 12.5%. b: Formula-fed infants. Applies to infants fed whey-adjusted milk formula and to infants partly human-milk-fed or given low-phytate feeds supplemented with other liquid milks; assumed coefficient of variation 12.5%. c: Formula-fed infants. Applicable to infants fed a phytate-rich vegetable protein-based formula with or without whole-grain cereals; assumed coefficient of variation 12.5%. d: Not applicable to infants consuming human milk only

The current recommended dietary allowance (RDA) is the average daily level of intake sufficient to meet the nutrient requirements of nearly all (97%–98%) healthy individuals. The RDAs for zinc are summarized in Table 2. For infants aged 0–6 months, the food nutrition board at the Institute of Medicine of the National Academies established an adequate intake (AI) that is equivalent to the mean intake

of zinc in healthy, breastfed infants (Institute of Medicine 2001; WHO and FAO 2004; Ackland and Michalczyk 2016). AI is generally established when evidence is insufficient to develop an RDA and is set at a level assumed to ensure nutritional adequacy.

One previous report has demonstrated that zinc deficiency affects about 2.2 billion people around the world and is often caused due to poor diet consumption (Prasad 2001). Another recent study based upon on WHO growth standards has indicated that an estimated 17.3% of the world's population is at risk of inadequate zinc intake (Wessells et al. 2012). Country-specific estimated prevalence of inadequate zinc intake is negatively correlated with the total energy and zinc contents of the national food supply and the percent of zinc obtained from animal source foods (Wessells and Brown 2012; Wessells et al. 2012). Pregnant and nursing women are considered at higher risk of zinc deficiency, as are those with gut problems, babies born prematurely, or those who have consumed a high-grain or vegetarian diet (especially for a long period of time) (Ackland and Michalczyk 2016). The symptoms of zinc deficiency can vary and is often associated with the following problems: poor memory, weakened immune system or constant minor illnesses like colds, loss of taste or smell, sleep problems (zinc is needed to make melatonin), hair loss, loss of appetite, low libido, diarrhea, brain fog, slow wound healing, white spots on fingernails, and growth retardation in children (WHO and FAO 2004). However, most severe symptoms of zinc deficiency result from other factors including excessive alcohol use, liver diseases, malabsorption syndromes, renal disease, enteral or parenteral alimentation, administration of sulfhydryl-containing drugs, and sickle cell diseases (Evans 1986). Moreover, zinc deficiency is an important factor in the development and progression of cancers (Dhawan and Chadha 2010; Gumulec et al. 2011; Sharif et al. 2012**)** and metabolic disorders (Lin and Huang 2015; Wilson et al. 2016; Grüngreiff et al. 2016). Recent studies have provided evidence that zinc deficiency and zinc transports are involved in the pathogenesis of diabetes and diabetic complications (Gu 2015; Zhang et al. 2016a; Maret 2017). Taking together, the symptoms resulting from zinc deficiency are as diverse as the enzymes with which the element is associated. Although clinical observation of the symptoms caused by zinc deficiency is well documented, our knowledge concerning the mechanisms of zinc deficiency in relation with epigenetics is still limited. Herein, we mainly discuss the proteins, which are related with zinc in biological structure and function and possible problems in epigenetics caused by zinc deficiency.

Zinc Deficiency and the Oocyte Epigenetic Programming

It is well known that human pregnancy outcome is significantly influenced by the nutritional status of the mother and an optimal uterine environment. Quality of the maternal epigenome significantly contributes to promoting optimal embryonic development and postnatal health (Corry et al. 2009; Hales et al. 2011). The epigenome of the oocyte is dramatically remodeled during oogenesis. As the

oocyte nears ovulation, major changes in chromatin structure and biochemistry take place to prepare for fertilization and embryonic development (Debey et al. 1993; Zuccotti et al. 1995). Chromatin methylation is an important component of epigenetic programming during oogenesis.

Zinc deficiency during pregnancy causes abnormal embryo and fetal development and poor progeny health (Apgar 1985; Keen et al. 2003; Uriu-Adams and Keen, 2010). Several studies have implicated that zinc is an important factor necessary for regulating the meiotic cell cycle and ovulation (Kim et al., 2010; Bernhardt et al. 2011; Tian and Diaz, 2013). To investigate the effects of acute in vivo zinc deficiency before ovulation on oocyte epigenetic programming and embryonic development, Tian and Diaz (2013) have developed an animal model with zinc deficiency. Newly weaned 18-day-old female CD1 mice were given the zinc-deficient diet (zinc omitted from the mineral mix <1 mg zinc/kg), while the control mice have the diet of 29 mg zinc/kg. Diets are given for 3 or 5 days before ovulation. Results demonstrate that feeding a zinc-deficient diet (ZDD) for 3–5 days before ovulation (preconception) dramatically disrupts oocyte chromatin methylation and preimplantation development. Histone H3K4 trimethylation and global DNA methylation in zinc-deficient oocytes are significantly decreased. Furthermore, the H3K4 trimethylation can be restored by using supplementation of a methyl donor (s-adenosylmethionine) during in vitro maturation in oocytes from zinc-deficient mice. Methyl donor supplementation partially restores fertilization potential of zinc-deficient oocytes (Tian and Diaz 2013). Therefore, this study provides evidence that zinc deficiency leads to decreased oocyte chromatin methylation, and implicates that oocyte epigenetic programming during the period of final oocyte growth is important to perturbation in whole body zinc homeostasis.

Zinc Deficiency and the DNA Methylation Pathway

DNA methylation is one of epigenetic marks that regulates gene expression and suppresses transposon activity. The DNA methylation pathway is a process by which carbons are added onto folic acid from amino acids and redistributed onto other compounds throughout the body. Figure 1 illustrates the homocysteine recycling for DNA methylation. In the cycle, several key enzymes, including MTR and BHMT, are responsible for the formation of methionine, S-adenosyl methionine (SAMe), and thymidylate monophosphate (dTMP), and subsequently facilitate virtually every DNA methylation reaction in the body (Matthews and Goulding 1997; Smith and Denu 2009; Shimbo and Wade 2016). Zinc deficiency may disrupt their biological activities and consequently result in the decreased DNA methylation levels.

BHMT is mainly present in kidneys and liver (http://www.genecards.org/cgi-bin/carddisp.pl?gene=BHMT&keywords=BHMT). This enzyme and BHMT2 are the only enzymes that can catalyze the conversion of betaine and homocysteine to dimethylglycine and methionine, respectively. This reaction is considered the alternate or short route for methylation. BHMT uses zinc as a cofactor to catalyze the

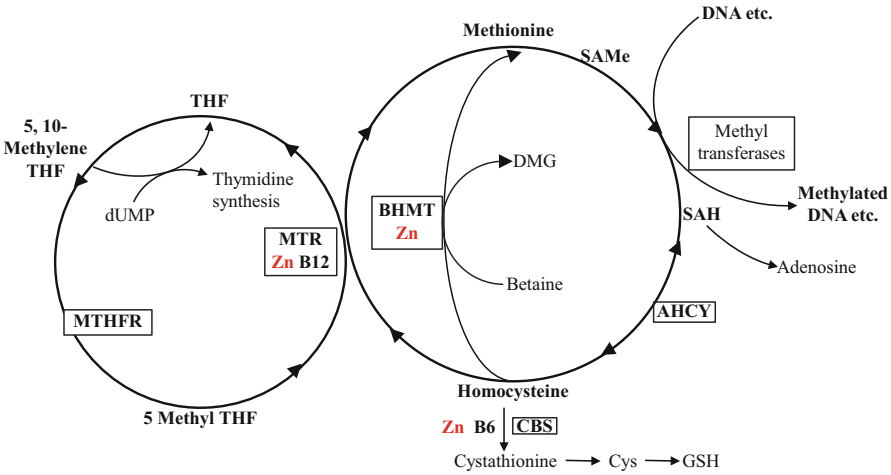

Fig. 1 Homocysteine recycling: involvement of zinc in MTR and BHMT for DNA methylation. This figure illustrates that methionine synthase (*MTR*) and betaine homocysteine methyltransferase (*BHMT*) are responsible for the formation of methionine, S-adenosyl methionine (*SAMe*), and thymidylate monophosphate (*dTMP*), and subsequently facilitate virtually every DNA methylation reaction in the homocysteine recycling. These two key enzymes are zinc-dependent methyltransferases. Zinc deficiency may disrupt their biological activities and consequently result in the decreased DNA methylation levels

transfer of a methyl group from betaine to homocysteine. Several studies have demonstrated that mutations in the BHMT gene may decrease the activity of this enzyme in the homocysteine recycling and DNA methylation. BHMT mutations in mothers may increase the risk of Down syndrome for their children. BHMT mutations may result in fatty liver and hepatocellular carcinomas (Matthews and Goulding 1997; Castro et al. 2008; Shimbo and Wade 2016).

MTR (also known as 5-methyltetrahydrofolate-homocysteine methyltransferase) is widely expressed in different organs and tissues. This enzyme facilitates the transfer of a methyl group from 5-methyltetrahydrofolate to homocysteine using zinc, cobalamin (B12), and MTRR enzyme as a catalyst. The end products of this reaction are the amino acid methionine and the vitamin tetrahydrofolate. Dysfunction of this enzyme due to mutations in the gene or disruption of zinc deficiency may lead to a lack of methionine and the accumulation of homocysteine in the body (hyperhomocysteinemia) (Matthews and Goulding 1997; Castro et al. 2008; Shimbo and Wade 2016). The pathological consequence of the gene mutation depends on how profoundly these methylation pathways are affected and the degree of homocysteine accumulation in the body.

Interestingly, Jing et al. (2015) have investigated the effects of dietary zinc (Zn) supply on homocysteine levels and expression of these two enzymes in the growing rats. Male weanling rats Sprague-Dawley are randomly assigned to four dietary groups for 3 weeks: Zn deficient (ZD; <1 mg Zn/kg); Zn control (ZC; 30 mg Zn/kg); pair fed (PF; 30 mg Zn/kg), and Zn supplemented (ZS; 300 mg Zn/kg). As expected,

several parameters, including feed intake, body weight gains, kidney weights, and serum and femur Zn concentrations, in the ZD rats are lower than those of ZC rats but not significantly different from the PF controls. The mRNA expression levels of the MTR gene are lower in liver and kidney of ZD rats compared to PF rats. However, hepatic and renal BHMT mRNA expression levels in ZD rats are not altered compared to controls. This study provides evidence suggesting that homocysteine homeostasis is disturbed by zinc deficiency but not zinc supplementation, and elevated serum homocysteine may be due to reduced expression of MTR but not BHMT during zinc deficiency.

Zinc Deficiency and Zinc Finger Proteins

Three classes of mammalian proteins recognize methylated DNA: MBD proteins, SRA proteins, and ZFPs. ZFP are a massive, diverse family of proteins and serve a wide variety of biological functions (Laity et al. 2001; Blattler et al. 2013). It is difficult to simply define what unites ZFPs due to their diversity. However, the common character of all ZFPs is that the functional domains of ZFPs require the coordination by at least one zinc ion. In general, the second structures (α-helix and β-sheet) of a ZFP, which are referred as "fingers" are joined by the zinc ion. For example, the Cys2His2 was the first domain discovered (also known as Krüppel-type). Figure 2 represents the structure of the Cys2His2 zinc finger motif consisting of α-helix and antiparallel β-sheet. The zinc ion (green) is located in the center and coordinated by two histidine residues and two cysteine residues. The Cys2His2 zinc fingers that bind DNA tend to have 2–4 tandem domains as part of larger protein. Cys2His2 proteins are the biggest group of transcription factors in most species. Lack of the zinc ion in the ZFP will result in the structural damage and functional

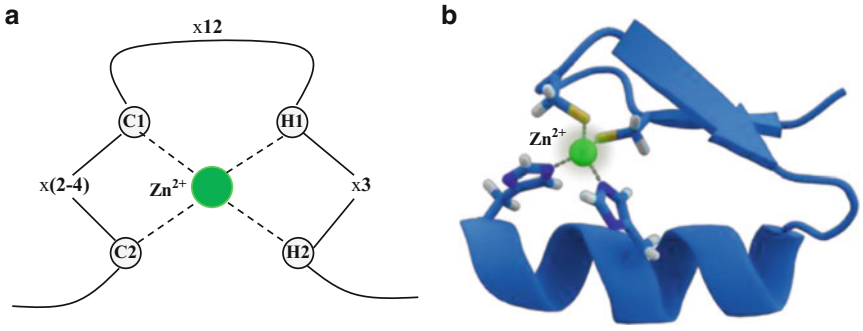

Fig. 2 A cartoon representation of the Cys2His2 zinc finger motif. The Cys2His2 zinc finger motif consists of α-helix and antiparallel β-sheet. Two histidine residues and two cysteine residues are coordinated by the zinc ion (in the *center* and labeled in *green*). (**a**): a schematic outline of the Cys2His2 type zinc finger domain. The number of x shows the intervals between the zinc binding residues. (**b**): a crystal of structure of the Cys2His2 zinc finger motif

Table 3 Zinc finger proteins, their recognition of methylated and specific DNA sequences

Protein name	Size molecular mass	Gene symbol	Aliases	DNA-binding specificity	
				Methylated sequence	Nonmethylated consensus
Zinc finger and BTB domain containing 33	672 AA 74484 Da	ZBTB33	KAISO	5′-CGCG-3’	5′-TCCTGCNA-3’
Zinc finger and BTB domain containing 4	1013 AA 105114 Da	ZBTB4	KAISO-L1	5′-CGCG-3’	5′-CTGCNA-3’
Zinc finger and BTB domain containing 38	1195 AA 134257 Da	ZBTB38	Croz	5′-CGCG-3’	5′-CATGTG-3’

loss (Wu 2002). Therefore, zinc serves to stabilize the integration of ZFP itself but is not involved in binding targets. Subsequently, zinc finger-containing domains typically act as interactors, binding DNA, RNA, protein, or small molecules (Laity et al. 2001; Wu 2002; Blattler et al. 2013).

Kaiso, ZBTB4, and ZBTB38 are three ZFPs can bind either methylated DNA in a sequence-specific manner or unmethylated consensus sequences. They may use a mode of binding common to other zinc-finger proteins, suggesting that many other sequence-specific methyl-binding proteins may exist. Table 3 represents the methylated DNA and unmethylated consensus sequences of Kaiso, ZBTB4, and ZBTB38.

Kaiso is originally described as a BTB/POZ zinc finger transcription factor and a p120-catenin-binding partner. This protein functions as a DNA methylation-dependent transcriptional repressor by binding to sequence-specific Kaiso-binding sites or to methyl-CpG dinucleotides (Blattler et al. 2013). Kaiso represses the expression of glucocorticoid receptor via a methylation-dependent mechanism and attenuates the antiapoptotic activity of glucocorticoids in breast cancer cells. Kaiso promotes cell migration and invasiveness through regulation of miR-31 expression in prostate cancer cells (Wang et al. 2016; Zhou et al. 2016). ZBTB4 (also known as Kaiso-L1) is found to be downregulated in breast cancer patients, and that its expression is significantly correlated with relapse-free survival. ZBTB4 functions as a novel tumor-suppressor gene with prognostic significance for breast cancer survival. CIBZ, was discovered to implicate in the spinal cord injury process for the first time. Further studies indicate that CIBZ is extensively distributed in various tissues, and the expression level is highest in muscle, followed by spinal cord, large intestine, kidney, spleen, thymus, lung, cerebrum, stomach, ovary, and heart, respectively. CIBZ gene is involved in secondary injury process and triggers the activation of apoptotic caspase-3 and bax genes independent of p53 (Cai et al. 2012). To date, the precise factors contributing to ZFPs-related zinc deficiency remain poorly defined.

Zinc Deficiency and Inflammation

As an essential micronutrient, zinc is required for many cellular processes, especially for the normal development and function of the immune system. There are remarkable similarities between the hallmarks of zinc deficiency and age-related immunological dysfunction because both are characterized by impaired immune responses and systemic chronic inflammation (Mocchegiani et al. 2000; Foster and Samman 2012; Prasad 2014). Moreover, zinc has anti-inflammatory properties and low zinc status is associated with increased susceptibility to infections and exaggerated inflammatory responses.

Interleukin 6 (IL-6) is an interleukin that acts as both a proinflammatory cytokine and an anti-inflammatory myokine. Wong et al. (2015) have recently demonstrated that zinc deficiency induces a progressive demethylation of the IL6 gene promoter in human monocytic cell line THP-1. The decreased IL6 gene methylation levels are correlated with increased IL6 expression. Evidence from this study suggests that zinc deficiency may have the effects in promoting inflammation and subsequently disrupt DNA methylation of the genes involved in the inflammation process. IL6 promoter methylation may also be reduced with age in human population. Most likely, there is a potential epigenetic link between zinc deficiency and age-related chronic inflammation (Bonaventura et al. 2015). However, it is necessary to include other proinflammatory cytokines such as IL1β, IL8, and tumor necrosis factor alpha (TNFα) for analysis before a conclusion is drawn.

Zinc Deficiency and Epigenetics

The effects of zinc deficiency in epigenetics are summarized in Fig. 3. *Zinc deficiency directly affects the key enzymes involved in the reaction of DNA methylation such as MTR and BHMT, and the proteins, in which zinc iron are constructed, including ZFPs, and consequently disrupts the DNA methylation and histone modification. Zinc deficiency also indirectly induces inflammation response and subsequently causes epigenetic alteration.*

Zinc transporters play an important role in regulating cellular zinc homeostasis. Members of Zip and ZnT zinc transporter families exhibit tissue- and cell-specific expression and possess differential responsiveness to dietary zinc, as well as to physiologic stimuli, including cytokines (Liuzzi and Cousins 2004; Bonaventura et al. 2015). Zinc transporters may also be involved into the indirect influence in epigenetics under the condition of zinc deficiency. Intrauterine growth retardation causes hypomethylation and hyperacetylation of genomic DNA while zinc deficiency is found to accompany fetal growth retardation in the patients with diabetes (Miao et al. 2013; Lin and Huang 2015). In alpha- and beta-cells of the islets of Langerhans, zinc has specific functions in the biochemistry of insulin and glucagon. When zinc ions are secreted during vesicular exocytosis, they have autocrine, paracrine, and endocrine roles. Solute carrier family 30 member 8 (SLC30A8, also known as ZnT8) is a zinc efflux transporter in the cell membrane and transports zinc

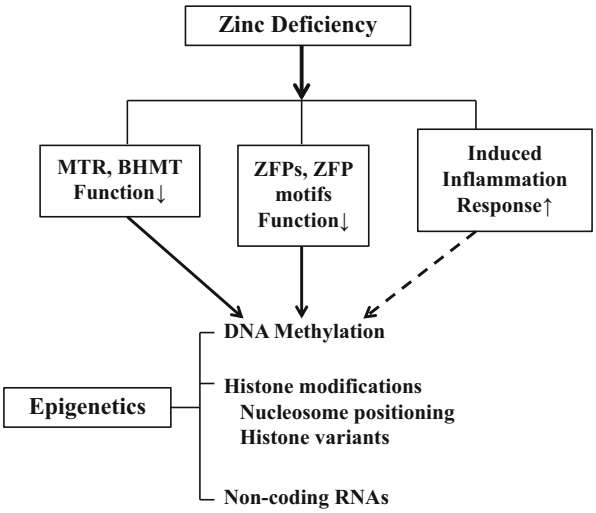

Fig. 3 Effects of zinc deficiency in epigenetics. Zinc deficiency can directly affect the activities of key enzymes such as MTR and BHMT and zinc finger proteins and consequently disrupt the DNA methylation and histone modification. Moreover, zinc deficiency can also indirectly induce inflammation response and subsequently cause epigenetic alteration. Other proteins such as zinc efflux transporters may also be involved into the indirect influence in epigenetics under the condition of zinc deficiency

ions into the insulin and glucagon granules and the subsequent crystallization of hexameric insulin. Pathological studies have demonstrated that the SLC30A8 gene expression levels are downregulated in pancreatic islets of diabetic mice (Fu et al. 2009). The downregulation of the SLC30A8 gene expression results in the reduction of insulin content and glucose-inducible insulin secretion (Chimienti et al. 2004; Gu 2016; Katsarou et al. 2017). Seman et al. (2015) have analyzed SLC30A8 DNA methylation with a bisulfite pyrosequencing protocol and found that DNA methylation levels of the SLC30A8 gene in type 2 diabetes patients are significantly higher compared to nondiabetic subjects. Interestingly, the average DNA methylation levels of the SLC30A8 gene in all studied subjects are high (~81.4%). However, the relationship between DNA methylation changes in this gene and zinc deficiency is still unknown. Furthermore, the recent studies have provided evidence that zinc deficiency enhanced albuminuria and extracellular matrix protein expression, associated with diabetic renal interstitial fibrosis by activation of renal interstitial fibroblasts and regulation of the expression of fibrosis-associated factors, which may be mediated by the activation of fibroblasts via the TGF-β/Smad signaling pathway (Zhang et al. 2016a). Zinc supplementation significantly inhibited the pathway (Zhang et al. 2016b). Therefore, zinc is one of the many factors in multiple gene-environment interactions that cause the functional demise of pancreatic beta-cells and renal interstitial fibrosis. Further epigenetic study is necessary to better understand between nutritional or conditioned zinc deficiency, environmental

exposures, and pathobiochemistry of the diabetes and diabetic complications including diabetic nephropathy.

Currently, the serum zinc concentration is used for evaluation of zinc deficiency. This biomarker for zinc status, however, may not be reliable because a decrease in serum concentration is only detectable after long-term or severe depletion. Therefore, it is necessary to develop new biomarker and protocol to more accurately detect dietary zinc deficiency. Dietary zinc deficiency affects blood linoleic acid: dihomo-γ-linolenic acid (LA:DGLA) ratio. The erythrocyte LA:DGLA ratio as a new zinc biomarkers has shown promise in preclinical and clinical trials and can be developed to more accurately detect dietary zinc deficiency (Knez et al. 2016). Consequently, the precise determination of zinc status will improve the basic and translation medicine researches on the effects of zinc deficiency in epigenetics.

Zinc is one of the many factors in multiple gene-environment interactions that cause the epigenetic alteration. Further investigation is of importance to better understand the inactive mechanisms of nutritional or conditioned zinc deficiency with zinc metabolism, environmental exposures and epigenetic regulation. Based upon the advanced knowledge concerning the relationship between zinc deficiency and epigenetic alteration, the possible interventions may include personalized nutrition, bioactive food, and pharmaceuticals targeting the control of cellular zinc in precision medicine.

Key Facts

Zinc is a chemical element with the symbol Zn^{2+}. The atomic number of zinc in periodic table of elements and chemistry is 30.

Zinc is publically considered as an essential micronutrient element because it exists in all body tissues and fluids and is included in the reactive centers and/or binding sites of many proteins and enzymes.

Zinc deficiency is defined either qualitatively as insufficient zinc to meet the requirements of the body and thereby causing clinical manifestations or quantitatively as a serum zinc level below the normal range.

An estimated global prevalence of inadequate zinc intake is 17.3% according to the data analysis based upon WHO growth standards. Zinc deficiency is actually common in many parts of the world and particularly in poor populations.

Zinc deficiency may lead to decreased oocyte chromatin methylation. The subsequent oocyte epigenetic programming during the period of final oocyte growth is important to perturbation in whole body zinc homeostasis.

Zinc deficiency may cause decreased DNA and chromatin methylation mainly due to dysfunction of MTR and BHMT in the reaction of DNA methylation.

Some zinc finger proteins (ZFPs) such as Kaiso, ZBTB4, and ZBTB38 bind either methylated DNA in a sequence-specific manner or unmethylated consensus sequences. These ZFPs may have the effects in epigenetic regulations under the condition of zinc deficiency.

Zinc deficiency is associated with immunological dysfunction, and may induce inflammation response and subsequently cause epigenetic alteration in the related genes.

Zinc transporters play an important role in regulating cellular zinc homeostasis. DNA methylation changes of some genes encoded for zinc transporters for example SLC30A8 has been found to be involved in the pathogenesis of diabetes.

Dietary zinc deficiency affects blood LA:DGLA ratio. This ratio can be used as a new zinc biomarker to more accurately detect dietary zinc deficiency.

Dictionary of Terms

- **Zinc deficiency** – It is defined either qualitatively as insufficient zinc to meet the requirements of the body and thereby causing clinical manifestations or quantitatively as a serum zinc level below the normal range. Zinc deficiency can occur in soil, plants, and animals. In humans, the most common cause of zinc deficiency is reduced dietary intake, while other reasons include inadequate absorption, increased loss, or increased use.
- **Homocysteine recycling** – Homocysteine is a sulfhydryl-containing amino acid, an intermediate product in the normal biosynthesis of the amino acids methionine and cysteine. In the methylation cycle, homocysteine is methylated to methionine, which undergoes S-adenosylation and forms S-adenosylmethionine (SAM). SAM is the principal methyl donor for all methylation reactions in cells. The reactions are controlled by two key enzymes named as methionine synthase (MTR) and betaine homocysteine methyltransferase (BHMT). MTR and BHMT are both zinc-dependent methyltransferases. Zinc deficiency may disrupt the biological activities of these two enzymes in the reaction of DNA methylation and consequently result in the decreased DNA methylation levels.
- **Zinc finger proteins** – They are a massive, diverse family of proteins that serve a wide variety of biological functions. These types of proteins are structured with a zinc finger, which is a small protein structural motif and characterized by the coordination of one or more zinc ions in order to stabilize the fold.
- **Zinc transports** – These proteins are encoded by two solute-linked carrier (SLC) gene families: SLC30 (ZnT) and SLC39 (Zip), and all have transmembrane domains. There are at least 9 ZnT and 15 Zip transporters in human cells. ZnT transporters reduce intracellular zinc availability by promoting zinc efflux from cells or into intracellular vesicles, while Zip transporters increase intracellular zinc availability by promoting extracellular zinc uptake and, perhaps, vesicular zinc release into the cytoplasm. Both ZnT and Zip transporter families exhibit unique tissue-specific expression, differential responsiveness to dietary zinc deficiency.
- **LA:DGLA ratio** – Linoleic acid (LA) is a polyunsaturated omega-6 fatty acid. Dihomo-γ-linolenic acid (DGLA) is a 20-carbon ω-6 fatty acid. The LA:DGLA in blood samples is closely responsible for dietary zinc deficiency. Thereby, this ratio is a potential new zinc biomarker for determination of dietary zinc deficiency.

Summary Points

- Current studies concerning the effects of zinc deficiency in diseases and in the relation with proteins or enzymes are extensive but our knowledge regarding the relationship between zinc deficiency and epigenetic dysregulation is still limited. Further basic and clinical investigation is necessary to better understand the roles of zinc and effects of its deficiency in epigenetic regulations. To summarize what we have discussed above, several points are listed as below: Zinc is an essential micronutrient element for health. Zinc deficiency is common in the developing countries and in the poor populations of the developed countries. The global prevalence of zinc deficiency is estimated by approximately 17.3%.
- MTR and BHMT are two key enzymes in the reaction of DNA methylation. Both are zinc dependent. Therefore, zinc deficiency leads to decreased DNA and chromatin methylation zinc finger proteins (ZFPs), mainly including Kaiso, ZBTB4, and ZBTB38, bind either methylated DNA in a sequence-specific manner or unmethylated consensus sequences. All these proteins are structured with a zinc finger, which is a small protein structural motif and characterized by the coordination of one or more zinc ions. Zinc deficiency may influence the function of ZFPs and subsequently result in epigenetic alteration.
- Zinc deficiency is found to induce inflammation response and subsequently cause epigenetic alteration in the genes encoded for inflammatory factors.
- Zinc transporters, including 9 members of SLC30 family and 15 members of SLC39 family in human play the important roles in regulating cellular zinc homeostasis. DNA methylation levels of these zinc transporter genes may be associated with dietary zinc deficiency.

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Impact of Low-Salt Diet

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Flávia Ramos de Siqueira, Karin Carneiro de Oliveira, Joel Claudio Heimann, and Luzia Naôko Shinohara Furukawa

Contents

Abstract

Studies in experimental animals and in groups of humans and epidemiological studies have shown that the sodium chloride or salt (sodium, Na, NaCl) plays an important role mainly in the regulation of blood pressure and represents an important environmental factor involved in the genesis of cardiovascular diseases. Therefore, salt intake in the population has been a constant concern. Variable blood pressure responses to different content in sodium intake are found in experimental hypertension models and in humans, and the reasons for such heterogeneity are not fully elucidated. The reduction of dietary sodium intake is recommended by public health as one of the non-medicated treatments for hypertension and consequently reducing the risk of cardiovascular diseases. However, some studies have demonstrated side effects of salt dietary restriction,

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reporting changes in glucose metabolism (hyperinsulinemia and insulin resistance), and these alterations are gender and time specific in experimental and population studies.

Keywords
Sodium · Salt · Low-salt diet · Insulin resistance · Chronic kidney disease · Coronary heart disease · Cardiovascular disease · Blood pressure · Hypertension · Atherosclerosis · Gestation

List of Abbreviations

CHD	Coronary heart disease
CI	Confidence interval
CKD	Chronic kidney disease
CVD	Cardiovascular disease
HOMA-IR	Homeostasis model of assessment
LS	Low-salt diet
RAAS	Renin angiotensin aldosterone system

Introduction

High dietary intake of sodium associated with elevated blood pressure is a major risk factor for cardiovascular disease (He and MacGregor 2009; WHO 2007). The United Nations, World Health Organization, Centers for Disease Control and Prevention, and other organizations have emphasized the relationship between dietary sodium and cardiovascular outcomes (He and MacGregor 2002, 2003; Moher et al. 1999; Alderman et al. 1997).

Studies conducted in many countries observed that 99.2% of adult population have estimated mean levels of sodium intake exceeding the World Health Organization recommendation of 2.0 g per day of sodium, and 88.3% of adult population exceed this recommended level by more than 1.0 g per day of sodium. Figure 1 shows estimated cardiovascular death related to exceeding sodium intake by 2 g per day in many countries in the world.

Based on this evidence, a lower intake of dietary sodium is recommended, expecting a reduction in cardiovascular morbidity and mortality.

Evidence Between Sodium Intake Reduction and Blood Pressure

Over the past years, it has been published several population and trial studies from centers of many countries around the world, and they have shown the effects of reduced sodium intake on blood pressure (Graudal et al. 2012). Therefore, huge data are available to analysis and generate other important consistent information.

Studies from Global Burden of Diseases Nutrition and Chronic Diseases Expert Group (NUTRICODE) found strong evidence of a linear dose-response relationship

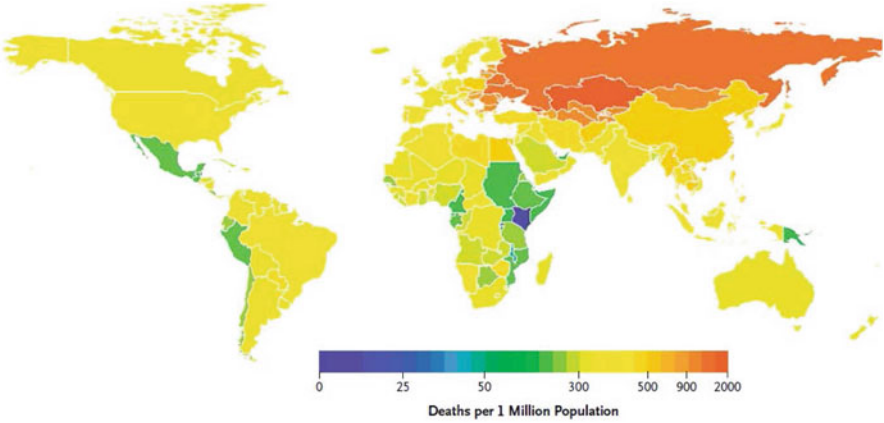

Fig. 1 Cardiovascular mortality (per one million of people) in each nation attributed to sodium consumption more than 2.0 g per day (Figure derived from Mozaffarian et al. 2014 Copyright © (2017) Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society)

between reduced sodium intake and blood pressure, where each reduction of 2.30 g of sodium per day was associated with a reduction of 3.82 mm Hg (95% confidence interval (CI), 3.08–4.55) in blood pressure (Fig. 2). They also observed that effects of reduction of sodium intake on blood pressure modify according to the population characteristics, for example, larger reduction on blood pressure among older than younger persons, among blacks than whites, and among hypertensive than normotensive persons. For a white and normotensive population of 50 years old, each reduction of 2.30 g per day in sodium intake lowered systolic blood pressure by 3.74 mm Hg (95% CI, 2.29–5.18). They did not find evidence of substantial blunting of the blood pressure-lowering effects of sodium restriction by antihypertensive drugs (Mozaffarian et al. 2014).

The evidence of a significant decrease in blood pressure of 1% (normotensive) and 3.5% (hypertensive) related to sodium reduction was demonstrated in randomized controlled trials allocating persons with normal or elevated BP irrespective of race and age to either a low- or a high-sodium diet and in which the sodium intake was estimated by the 24-h urinary sodium excretion (either measured on the basis of a 24-h urine collection or estimated from a sample of at least 8 h). However, this study also showed a significant increase in plasma renin, plasma aldosterone, plasma adrenaline, and plasma noradrenaline, a 2.5% increase in cholesterol, and a 7% increase in triglyceride. These biochemical changes usually correlate with reduction of sodium intake (Graudal et al. 2012).

In another study involving non-ill adult and children, a significant decrease of systolic and diastolic pressure levels with reduction of sodium intake was observed. Indeed, the decreased sodium intake had no significant adverse effect on blood lipids, catecholamine levels, or renal function in adults, a different result from the above study. Probably non-ill adult is less susceptive to sodium intake changes related to these biochemical parameters (Aburto et al. 2013).

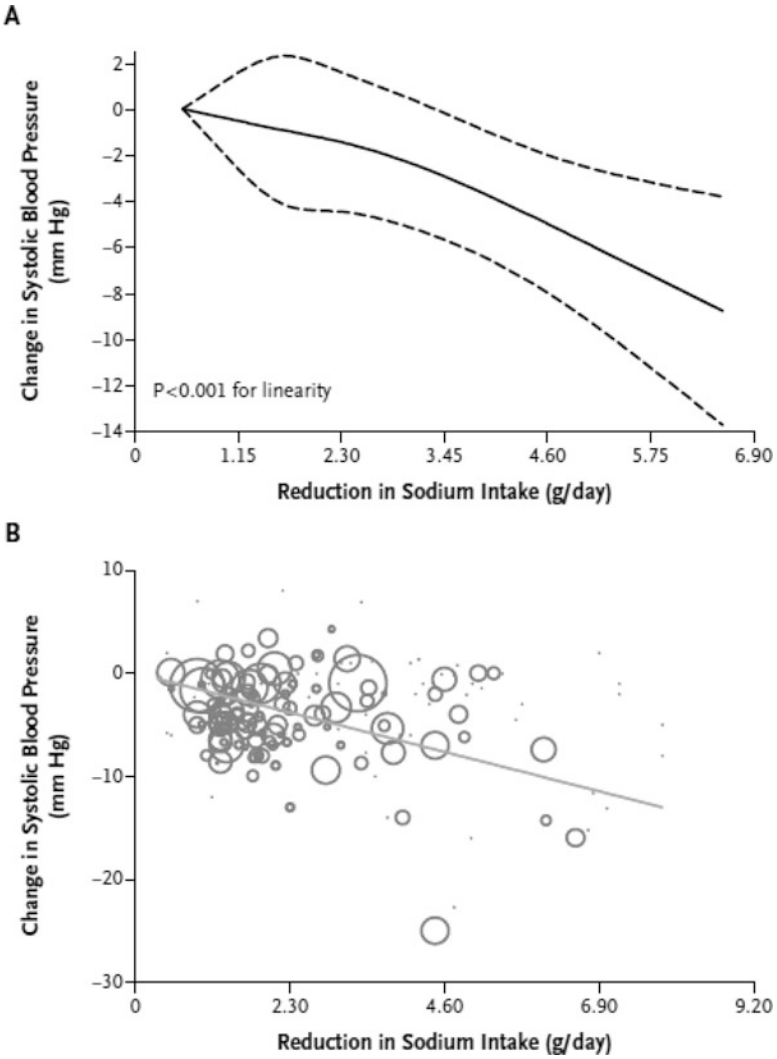

Fig. 2 Effects of sodium intake reduction on systolic blood pressure. Data are from 103 trials and included 6,970 persons. The reductions in sodium intake ranged from 0.53 to 6.56 g per day, the duration of the intervention ranged from 7 to 1,100 days, and the age of the participants ranged from 13 to 73 years. In (**a**) the effect of reduced sodium intake on systolic blood pressure was linear ($P < 0.001$ for linearity). The *solid line* represents the central estimate and the *dotted lines* the 95% confidence intervals [CIs]. The model is based on inverse-variance – weighted, restricted-cubic-spline regression adjusted for age, race, and presence or absence of hypertension. In (**b**) the inverse-variance is shown – weighted linear meta-regression. Each *circle* represents one randomized comparison of the intervention with the control group in each trial, and the size of the *circle* corresponds to its inverse-variance weight. The *fitted line* represents the effect of reduced sodium intake across all trials. Each reduction in sodium intake of 2.30 g (100 mmol) per day was associated with a reduction of 3.82 mm Hg (95% CI, 3.08–4.55) in systolic blood pressure (Graph derived from Mozaffarian et al. (2014) Copyright © (2017) Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society)

Effects of Sodium Intake Reduction on Cardiovascular Health and Kidney Disease

Most people with diabetes and chronic kidney disease (CKD) have hypertension characterized by enhanced sodium retention. Because blood pressure control is a key objective in management of diabetic kidney disease, antihypertensive agents, including angiotensin-converting enzyme inhibitors and angiotensin receptor blockers, are used to treat hypertension. In addition, reducing dietary sodium is critical to optimize the effectiveness of medication used for blood pressure control (KDOQI 2007). Patients with CKD have diminished their capacity to excrete sodium more than those with normal kidney; due to this, the blood pressure of these patients is more sensitive to high sodium intake (Johnson et al. 2002).

Many studies have shown decrease on blood pressure following reduction on sodium intake; however, it is not possible to quantify promptly its effect on cardiovascular health and kidney disease because in order to obtain these data, a long-term follow-up of the patients is necessary. Usually, cohort studies are followed for the maximum of 10 or 15 years; the reduced sodium intake is not habitual for the general population, and then, this reduction does not stand longer enough to measure the cardiovascular morbidity and mortality. Due to these factors, it drives researchers to evaluate cardiovascular and kidney disease based on a median of sodium urine excretion of individual participants measured during the study.

The study, based on sodium urine 24 h excretion and corrected by urinary creatinine, found a continued decrease in cardiovascular disease (CVD) events among those with sodium levels as low as 1,500 mg per day. The risk reductions among those at the lowest levels of sodium excretion were substantial, with a 32% reduction among those excreting less than 2,300 mg per day (Cook et al. 2014).

A low-salt diet physiologically activates the RAAS in kidney and elsewhere (Ingert et al. 2002).

Short- and long-term effects with sodium intake reduction on efficacy of renin angiotensin aldosterone system (RAAS) blockade to reduce blood pressure and proteinuria in patients with hypertension and nondiabetic and diabetic chronic kidney disease are observed (Fig. 3) (Vogt et al. 2008; Slagman et al. 2011; Ekinci et al. 2009; Kwakernaak et al. 2014).

Recent reviews have pointed that data are reliable and enough to confirm the strong association between high sodium intake and cardiovascular and renal disease risk. Furthermore, the beneficial effects of dietary salt reduction on pressure-independent effects on the heart, blood, vessels and kidney are being increasingly recognized (Susic and Frohlich 2012). A prospective cohort study of patients with CKD and with follow-up from May 2003 to March 2013 observed that among patients with CKD, a higher urinary sodium excretion was associated with increased risk of CVD. These findings, if confirmed by clinical trials, suggest that moderate sodium reduction among patients with CKD and high sodium intake may lower CVD risk (Mills et al. 2016). In addition, there is a study which could propose a projection that modest reduction in dietary sodium will reduce substantially the cardiovascular events and medical costs and potentially be a public health target.

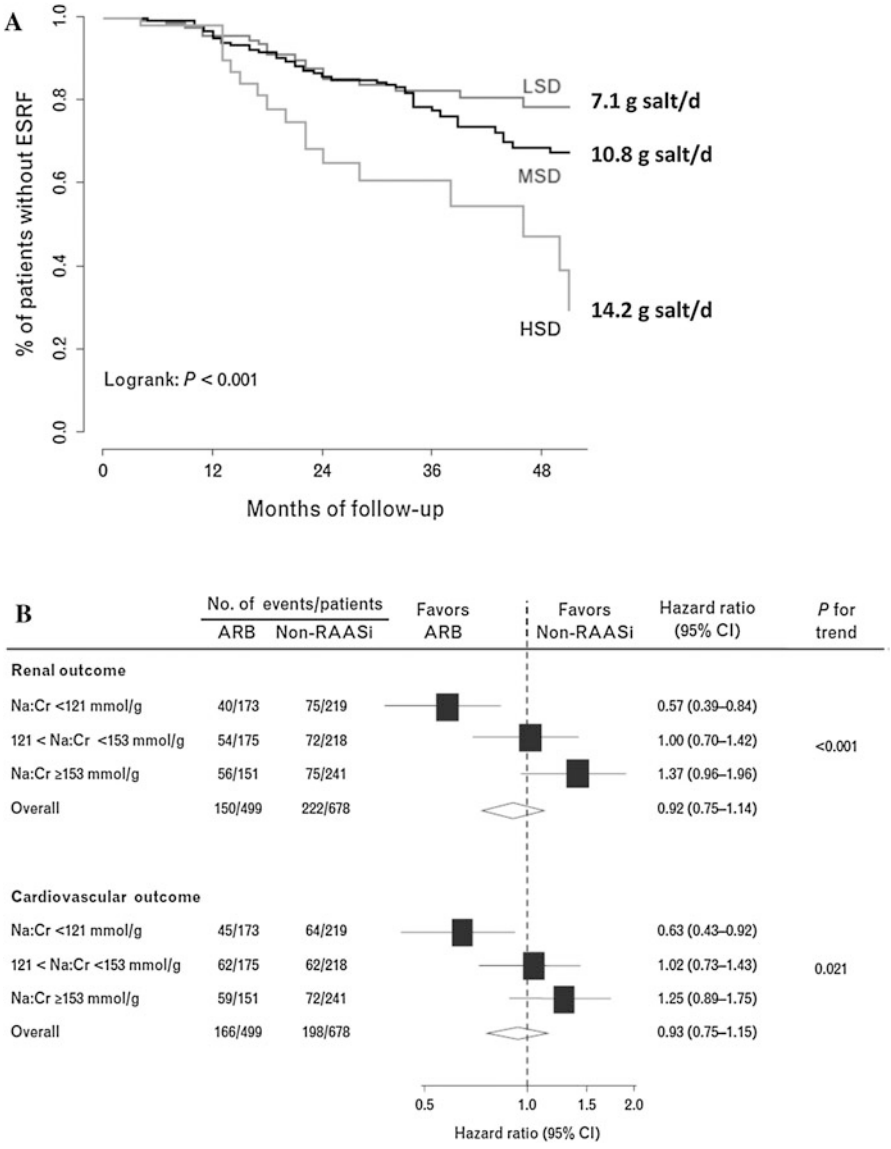

Fig. 3 Short- and long-term effects of sodium intake reduction. (**a**) End-stage renal failure (ESRF)-free survival according to high- (HSD), medium- (MSD), or low-sodium diet (LSD) in patients on angiotensin-converting enzyme inhibitor (ACEi) treatment, followed by 48 months. (**b**) Effect of sodium intake on renal and cardiovascular outcomes. *Na:Cr* sodium/creatinine, *ARB* angiotensin II receptor blocker, *Non-RAASi* non-renin angiotensin aldosterone inhibitor (Graph derived from Borst and Navis (2016) Copyright (2017), with permission from Elsevier)

They used the Coronary Heart Disease (CHD) Policy Model to quantify potentially achievable benefits in reducing dietary salt of up to 3 g per day (1,200 mg of sodium per day) on population-wide. They estimated the rates and costs of cardiovascular disease in subgroups defined by age, sex, and race; compared the effects of salt reduction with those of other interventions intended to reduce the risk of cardiovascular disease; and determined the cost-effectiveness of salt reduction as compared with the treatment of hypertension with medications (Fig. 4) (Bibbins-Domingo et al. 2010).

Negative Aspects of Reduction Sodium Intake

Although several studies have shown the benefits of reducing sodium intake on blood pressure and cardiovascular and kidney disease, there are some studies that demonstrate results with negative aspects of this intervention.

Some researchers are concerned about biochemical parameters, which are found increased in individuals submitted to reduction sodium intake. Renin angiotensin aldosterone activation and an increase in plasma adrenaline, noradrenaline, cholesterol, and triglyceride are observed and could interfere with blood pressure regulation and metabolic mechanism, contributing to the increase of cardiovascular risk (Graudal et al. 2012) and mortality (Cook et al. 2014).

Healthy subjects were submitted to low-sodium diet and high-sodium diet. After 7 days of low-/high-sodium diet, increased serum aldosterone, plasma renin activity, angiotensin II, and urine norepinephrine levels on individuals who received low-salt diet during 7 days compared to those that received 7 days of high-sodium diet are observed (Garg et al. 2011). The activated renin angiotensin aldosterone and sympathetic nervous systems were compared between low- and high-salt diet groups; however, it is not an adequate comparison because the sodium concentration of the high-sodium diet is extreme. Maybe, a plausible comparison of renin angiotensin aldosterone and sympathetic nervous systems activate would be between normal- and low-/high-sodium diet.

The increase of ANG II level is mainly to conserve sodium and regulates other natriuretic factors, including prostaglandins and bradykinin, under low-salt diet conditions (Kittikulsuth et al. 2012; Hocherl et al. 2002; Sivritas et al. 2008).

It is so important to pay attention at these findings because we still do not know the consequences of long-term sodium intake restriction in general population, considering people are accustomed to a high sodium intake for generations due to food industries, facility access to packaged and restaurant foods, etc.

In conclusion, high sodium intake is associated with high cardiovascular and kidney disease risk. However, the relation between low sodium intake and cardiovascular and kidney disease risk is not uniform and solid, because some are

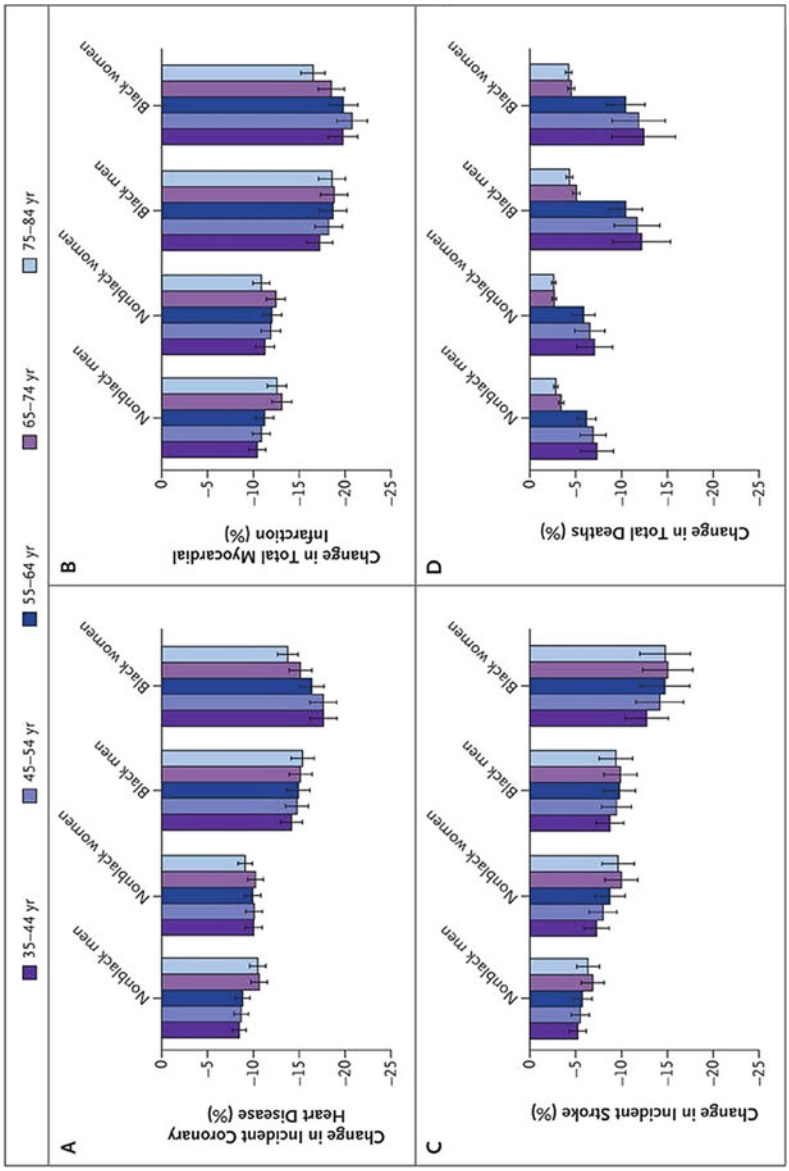

Fig. 4 (continued)

demonstrating awareness due to activation of renin angiotensin aldosterone and sympathetic nervous system and metabolic alteration; obstacle to decrease the sodium content from package food and restaurants is found; there is necessity of large public campaign.

Therefore, it will be interesting to program for long term to decrease little by little the sodium diet intake, until the minimum sodium intake related to low cardiovascular and kidney disease risk is reached.

Restriction of Salt from Childhood to Adulthood: Experimental Models

High sodium intake is associated with increased blood pressure levels in both normotensive and hypertensive individuals, as well as in laboratory animals. Likewise, lower intake of dietary sodium is related to a reduction in blood pressure levels. Persistent high blood pressure levels are associated with increased morbidity and mortality due cerebrovascular accident and coronary insufficiency. Several governmental organizations have recommend reduction of dietary salt intake. However, some changes in glucose, insulin, and lipid metabolism are found due to lower salt intake in the diet.

Decreased insulin sensitivity and lower glucose uptake are changes observed in animal models exposed to a low-sodium diet (0.06%) for 9 weeks compared to rats that received normal-sodium diet (0.50%) (Ruivo et al. 2006; Prada et al. 2000). In addition, animals submitted to salt restriction exhibited increased sympathetic nervous system activity and decreased nitric oxide-mediated vasodilatation (Ruivo et al. 2006). Also, it was observed that chronic salt restriction increased body weight and adipose tissue mass (Prada et al. 2000; Xavier et al. 2003; Okamoto et al. 2004). The decreased insulin sensitivity observed during chronic salt restriction is evidenced even when body weight is matched with animals fed with different salt contents (normal-salt and high-salt diet), suggesting that, in these animals, body weight is not a mechanism involved in insulin resistance.

It is well known that circulating renin angiotensin, a system is activated by low salt intake and plays an important role in blood pressure regulation, renal hemodynamics, fluid/electrolyte homeostasis, and cellular growth. In addition, angiotensin II is a well-characterized vasoconstrictor in systemic and uterine blood vessels.

Fig. 4 Annual projection of reduction in cardiovascular events defined a dietary salt reduction of 3 g per day (1,190 mg sodium) according to age group in nonblack men, nonblack women, black men, and black women. (**a**) shows changes in incident coronary heart disease, (**b**) changes in total myocardial infarctions, (**c**) changes in the incidence of stroke, and (**d**) changes in the rate of death from any cause. The projections were based on a reduction in dietary salt of 3 g per day (1,190 mg sodium) and on the high estimate for the effect of salt reduction on systolic blood pressure. Total myocardial infarction includes new and recurrent myocardial infarctions. I *bars* indicate standard errors of the Monte Carlo simulation (Graph derived from Bibbins-Domingo et al. (2010) Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society)

Rats fed with low-salt diet and treated with angiotensin-converting enzyme inhibitor (captopril) showed improvement in insulin sensitivity; however, treatment with angiotensin II AT1 receptor antagonist (losartan) did not change insulin sensitivity (Prada et al. 2000). The increased fasting blood glucose and plasma insulin levels and consequently the increase of HOMA-IR, which characterizes insulin resistance induced by low-salt restriction, are tissue-specific (Prada et al. 2005; Coelho et al. 2006). A possible mechanism by which low salt intake induces insulin resistance in the liver and muscle is through activation of protein JNK (protein kinase – c-jun N-terminal kinase) associated with a higher serine 307 phosphorylation of IRS-1 (insulin receptor substrate) (Prada et al. 2005). The likely mechanism for altering insulin signaling pathway in the liver and muscle is the higher serine 307 phosphorylation of the IRS-1 that is involved in inducing insulin resistance (Fig. 5). It is known that activation of the JNK protein induces IRS-1 serine 307 phosphorylation by blocking the interaction of the insulin receptor with its substrates and inhibiting the action of insulin in the cell (Aguirre et al. 2002).

Low-salt diet induced higher plasma concentrations of triacylglycerols and total cholesterol in Wistar rats, probably due to a 55% slower removal rate of triacylglycerols containing lipoproteins. Additionally low-salt diet increased plasma nonesterified fatty acid concentration, probably due to impaired lipoprotein lipase enzyme activity provoked by salt restriction (Catanozi et al. 2001). Similar finding was also demonstrated in LDL-receptor knockout mice and in apolipoprotein E knockout mice, indicating that low-salt diet elicits premature arterial lipid storage in hyperlipidemic normotensive animals. This finding leads to the speculation that low-

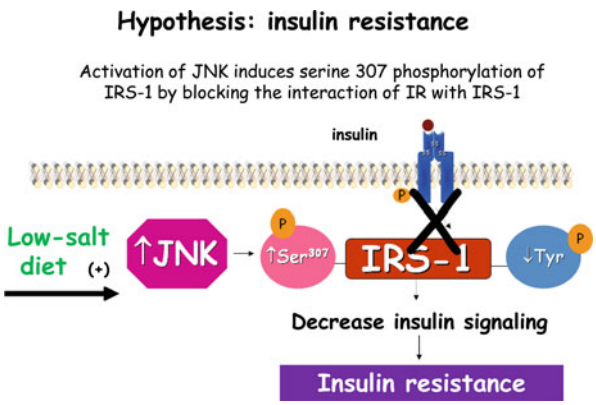

Fig. 5 Schematic representation of the molecular mechanism by which chronic salt restriction in the diet can induce insulin resistance in adult Wistar rats. The insulin receptor (IR) is a glycoprotein with intrinsic tyrosine kinase activity, and when activated by insulin, it autophosphorylates on tyrosine residues in the cytoplasmic domain. However, phosphorylation can occur on IR serine residues, which blocks the IR tyrosine kinase activity. The activation of the JNK protein may be one of the mechanisms of this block, since it stimulates IRS-1 serine 307 phosphorylation by decreasing the interaction of IR with its substrates and consequently impairing insulin signaling and contributing to insulin resistance

salt diet might have a greater impact on the development of atherosclerosis in hypertensive than normotensive animals (Catanozi et al. 2003). Recently, in a murine model of simultaneous hypertension and hyperlipidemia, the pleiotropic effect of chronic and severe sodium restriction (0.15% sodium chloride) elicited aortic damage even with reduced blood pressure. These negative effects on arterial wall were reduced by AT1 receptor antagonism, demonstrating the influence of angiotensin II in atherogenesis induced by a severely low-sodium diet (Fusco et al. 2017).

Salt Restriction During Pregnancy: Effects on Offspring

Many studies have demonstrated a link between the intrauterine environment and diseases in adult. The maternal diet is an essential factor for the health of offspring up to adulthood. Several experimental studies observed metabolic alterations on offspring at early after birth and at adulthood in response to maternal low-salt diet during pregnancy. These metabolic alterations depend on when maternal low-salt diet was administrated during the pregnancy. Sodium restriction during pregnancy induces low birth weight in both male and female offspring and alters lipid metabolism, and it is responsible for insulin resistance in adulthood offspring. Sexual dimorphism on some metabolic parameters is one of the consequences of maternal low-salt diet during pregnancy, and additional studies are necessary to clarify this phenomenon.

Wistar rats fed with low-salt diet (0.06% sodium – 0.15% NaCl) from the eighth week of life, during the gestational period until the end of lactation, showed an association between maternal sodium restriction diet and low birth weight of offspring and higher plasma levels of cholesterol and triacylglycerols in adulthood offspring (Vidonho et al. 2004). The insulin resistance that was observed in adulthood males, but not in female offspring, was assessed by hyperinsulinemic-euglycemic clamp method (Vidonho et al. 2004). In another study using the same experimental model described above, increased circulating leptin in males, while decreased in female adulthood offspring, and increased adiposity index only in female offspring were observed (Lopes et al. 2008). These data suggest that the level of dietary salt during intrauterine development can permanently alter mechanisms that regulate metabolism and body weight.

These effects might be related to the impact that sodium status has on hemodynamic and metabolic factors involved in uterine and placental perfusion, which has consequences for fetal nutrition. Although there were no significant changes in maternal food intake neither in uteroplacental blood flow, the placental and fetal weight were lower in response the maternal low salt intake during pregnancy. It was observed that inflammation and placental oxidative stress are not involved in the mechanisms of lower fetal and placental weights (Leandro et al. 2008).

There is evidence that low birth weight is due the low-salt diet intake during the second half of pregnancy that corresponds to the period of most rapid growth and

differentiation of key structures. One of the reasons that low-salt diet during pregnancy induces low birth weight appears to be an epigenetic mechanism. There is a negative correlation between low *Igf1* expression and high *Igf1* methylations levels in offspring with low birth weight (Siqueira et al. 2015).

The association between low birth weight and insulin resistance in adult offspring from mothers fed low-salt diet during gestation is related to maternal insult timing and the offspring's age as well as glucose intolerance (Siqueira et al. 2015). The amount of sodium consumed may alter glucose metabolism, while insulin concentrations or tissue sensitivity to this hormone may also interfere in the renal sodium control. The maternal low-salt diet intake during pregnancy does not modify the total renal volume, the volume of renal compartments, or the number of glomeruli in the newborns (Seravalli et al. 2016). However, when the maternal low-salt diet intake (0.07%) extends from gestation to lactation, the total number of glomeruli per kidney is lower in offspring with 1 and 12 weeks of age, probably due to a lower FGF-10 expression. Since removal of FGF-10 by knockout, the setting of GDNF signaling (Glial cell-derived neurotrophic factor vital for kidney development) stops kidney development.

In another experimental model, in which a maternal salt restriction of only 0.03% Na was given during the last 7 days of pregnancy, body, kidney, and cardiac ventricle weights were lower compared to the offspring of mothers fed with normal diet. At 12 weeks old, the body weight of the male offspring remained low, while high systolic blood pressure was present in both genders, starting at eighth week of age (Battista et al. 2002).

Unlike the maternal high-salt diet during pregnancy, which the transverse diameter of the nuclei of cardiomyocytes was higher in the left and right ventricles in the newborns, the maternal low-salt diet (0.06%) showed no cardiac and hemodynamic changes in the adult offspring even when they were submitted to high-salt diet in adult life (Seravalli et al. 2016; Alves-Rodrigues et al. 2013).

Therefore, maternal low-salt diet intake during pregnancy might have a protective effect on heart disease in adulthood. However, a lower expression of AT1 receptor in the kidneys and heart of males and lower renal expression of AT2 receptor in male and female offspring was shown.

Therefore, other studies are necessary to confirm if the observed results are reproducible in human beings in order to recommend low sodium level consumption during pregnancy.

Policies and Protocols

In this chapter we have described how dietary salt restriction is associated with metabolic and hemodynamic changes. The following protocols describe in detail the time, period, and diet concentration of salt in Wistar rats.

Male Wistar rats were fed a low-salt diet (0.06% Na or 0.15% NaCl), normal-salt diet (0.5% Na or 1.3% NaCl), and high-salt diet (3.12% Na or 7.94% NaCl) from weaning (3 weeks old) to adulthood (12 weeks old), in total 9 weeks of low-salt

dietary intake. All diets had 25% protein content and the only difference between the three diets was their sodium content. All animals were housed in a room with a controlled temperature and a 12 h light/dark cycle (with the lights on from 7 AM to 7 PM). They were given free access to food and water.

The maternal low-salt diet was also intake during pregnancy and lactation, and the offspring were studied after birth (first day of birth) or adulthood (12 weeks old). Twelve-week-old female Wistar rats were fed a low-salt (LS – 0.15% NaCl) or normal-salt (NS – 1.3% NaCl) diet during gestation (from first day of gestation until delivery) or a LS diet during either the first (LS10) or second (LS20) half of gestation.

Dictionary of Terms

- **Sodium urine 24-h excretion** – Urine collection for 24 h. Sodium from the diet is eliminated by the kidneys being excreted in varying daily amounts of 1–150 mEq, depending on the intake and total body sodium. Sodium is freely filtered by the glomeruli, and about 60% is reabsorbed in the proximal tubule. Sodium reabsorption also occurs along other segments of the nephrons. Urinary sodium dosage is important for the evaluation of hyponatremia due to renal loss (polycystic kidneys, proximal tubular acidosis), pre-renal, and renal oliguria. Indications: evaluation of hydroelectrolytic and basic acid disorders and differential diagnosis between pre-renal and renal oliguria. Clinical interpretation: increased urinary levels are found in adrenal insufficiency, salt-losing nephritis, and excessive sodium content in the diets, renal tubular acidosis, use of diuretics, and syndrome of inappropriate diuretic hormone secretion. Decreased urinary levels may exist in dehydration, congestive heart failure, liver disease, and nephrotic syndrome, situations that lead to a decrease in the glomerular filtration rate and in diets low in sodium.
- **Renin angiotensin aldosterone system (RAAS) –** Is closely related to the control of blood pressure and the regulation of the hydroelectrolyte balance. However, in the last decades, the alteration of the RAAS has been considered the main factor related to the development and the progression of diverse cardiovascular pathologies. The classic view of RAAS begins with the release of angiotensinogen from the liver, which undergoes the enzymatic action of renin, a proteolytic enzyme, secreted under conditions of hypotension and hypovolemia, originating the angiotensin I, which is then cleaved by the angiotensin-converting enzyme (ACE) in an octapeptide, angiotensin II (Ang II). The main physiological effects triggered by RAAS, such as vasoconstriction, synthesis and release of aldosterone, release of catecholamines, and control of water intake, cell proliferation, cardiac hypertrophy, oxidative stress, and inflammation, are mainly due to the classic actions of Ang II, through stimulation of the AT 1 receptor.
- **Angiotensin II** – Ang II is one of the main effector products of RAAS, which in turn plays its role through its angiotensin receptors, called AT1r and AT2r.

- **AT1 receptor** – AT1 is a G protein-coupled receptor responsible for triggering the activation of various intracellular signaling pathways, including phospholipase C, phospholipase D, phospholipase A2, adenylyl cyclase, ion channels, and others.
- **AT2 receptor** – The AT2 receptor, which is also a G protein-coupled receptor, induces vasodilation, inhibition of cardiomyocyte growth, inhibition of fibroblast proliferation, and extracellular matrix synthesis. Although the AT 2 receptor is highly expressed during fetal period, some pathological conditions increase its expression in the heart, such as ischemia, cardiac hypertrophy, hypertension, and heart failure.

Summary Points

- 99.2% of the adult population in the world have estimated mean levels of sodium intake exceeding the World Health Organization recommendation of 2.0 g per day of sodium.
- The reduction of sodium intake is recommended for the purpose of decrease cardiovascular morbidity and mortality.
- The evidence of a significant decrease in blood pressure of 1% (normotensive) and 3.5% (hypertensive) related to sodium reduction was demonstrated in randomized controlled trials.
- The reduction of sodium usually correlates with a significant increase in plasma renin, plasma aldosterone, plasma adrenaline, and plasma noradrenaline, a 2.5% increase in cholesterol, and a 7.0% increase in triglyceride.
- Systolic and diastolic pressure levels decrease significantly with reduction of sodium intake.
- Short- and long-term effects with sodium intake reduction on efficacy of RAAS blockade to reduce blood pressure and proteinuria in patients with hypertension are observed.
- There is a projection that modest reduction in dietary sodium will reduce substantially the cardiovascular events and medical costs.
- Some changes are found due to lower salt intake in the diet, such as glucose, insulin, and lipid metabolism.
- The low-salt diet induces to high plasma concentrations of triacylglycerols and total cholesterol in Wistar rats.
- Sodium restriction during pregnancy induces low birth weight in male and female offspring and alters lipid metabolism, and it is responsible for insulin resistance in adulthood offspring.

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Part XI

Modeling Systems, Cellular and Molecular Effects

The Role of the Central Nervous System in the Reduction of Food Intake During Infectious and Neoplastic Disease and in Eating Disorders: Experimental Approaches

104

Jan Pieter Konsman

Contents

Abstract

According to fixed set point models of energy balance, food intake increases when the organism is losing weight and decreases when it is gaining weight. Concomitantly reduced food intake and increased energy expenditure during infectious and neoplastic disease and in some eating disorders thus seem to contradict such models. Reduced food intake in infectious and neoplastic disease and anorexia nervosa may represent initially adaptive responses contributing to the organism's survival, even though chronically decreased food intake and increased energy expenditure lead to denutrition. The identification of cytokines and hormones mediating and the activation of brain structures in

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situations of concomitant reduced food intake and increased energy expenditure suggest that these are actively put in place by the organism. Furthermore, lesions or inhibition of some of the activated cerebral structures and pathways alleviate reduced food intake in animal models of infectious and neoplastic disease and anorexia nervosa. Thus, organisms seem capable of defending several set points, rather than just one fixed set point, of energy balance.

Keywords
Anorexia · Anorexia nervosa · Cancer · Central nervous system · Corticosteroids · Cytokines · Energy balance · Hypothalamus · Infection · Leptin

List of Abbreviations

IL-1beta	Interleukin-1beta
LPS	Lipopolysaccharide
MIC-1	Macrophage Inhibitory Cytokine-1
MRI	Magnetic Resonance Imaging
PET	Positron Emission Tomography
TNFalpha	Tumor Necrosis Factor-alpha

Introduction

The Lipostat Model of Food Intake and Body Weight Regulation

Although the glucostat and lipostat hypotheses of energy balance differ relative to what variable is detected by the central nervous system, they both propose that a "defended" set point maintains the organism's energy balance. Accordingly, food intake should increase when the organism is losing weight and, conversely, decrease when it is gaining weight. The discovery of the fat-derived hormone leptin that reduces food intake and is lacking in genetically obese mice (Zhang et al. 1994; Campfield et al. 1995) corroborated the lipostat hypothesis. Interestingly, leptin receptors are present on neuropeptide Y- and melanocortin-containing neurons of the arcuate hypothalamus (Hakansson et al. 1998), known to respectively stimulate and reduce food intake (Levine and Morley 1984; Fan et al. 1997). Thus, the identification of peripheral hormones and hypothalamic mechanisms that can be considered a feedback signal and output generator, respectively, has probably reinforced the idea that energy balance is regulated in function of a fixed set point.

Anorexia Can be Accompanied by Increased Energy Expenditure

The relationships between food and water intake, body weight and composition, and thermogenesis provide some test cases for fixed set point theories of energy balance.

Intraperitoneal administration of so-called endotoxin bacterial lipopolysaccharide (LPS) reduces food intake and body weight irrespectively of whether water was applied into the stomach (Miller et al. 1962). Moreover, treatment with sodium salicylate was found, as expected, to alleviate fever, but not the suppression of food intake after LPS administration (Mccarthy et al. 1984). These findings provide evidence against the idea that a reduction in food intake during infection would simply occur as a consequence of lower water intake or increased body temperature. However, mounting a fever response does require an increase in energy expenditure for organisms (Jennings and Elia 1987; Cooper et al. 1992). Since clinical data also report the cooccurrence of fever and loss of appetite in many infectious diseases (Gelston and Jones 1977; Mok et al. 1979; Pereira and Begum 1987), bacterial LPS-induced thermogenesis and reduced food intake seem to model nonspecific symptoms of infectious disease.

Reduced food intake and catabolism, reminiscent of what is observed in bacterial sepsis, but different from simple starvation in that body protein is not preserved, can be encountered in cancer denutrition or cachexia (Brennan 1977; Van Eys 1985). The cancer-associated anorexia-cachexia syndrome accounts for 10–30% of cancer deaths and complicates treatments (Inagaki et al. 1974; Dewys 1979; Theologides 1979). Interestingly, cancer-associated anorexia-cachexia can be reproduced in animals without chemo- or radiotherapy (Morrison 1974; Garattini et al. 1980) indicating that host-tumor interactions are sufficient to induce it.

In addition, reduced food intake "occurs in association with incubation, migration, molting, and hibernation, even though food is sometimes readily available" (Mrosovsky and Sherry 1980). Among these "animal anorexias," "activity-based anorexia" and "food restriction-induced hyperactivity" have been the subject of intense study (Pierce et al. 1986; Broocks et al. 1990). In these experimental paradigms, rodents with free access to food engage in wheel running that is covered for by energy intake, whereas animals for which access to food is limited in time show excessive running resulting in denutrition. Since increased physical activity occurs in 30–80% of anorexia nervosa patients (Davis et al. 1994) and food restriction in these patients is a factor contributing to increased physical activity (Holtkamp et al. 2004), these paradigms have been proposed as animal models of anorexia nervosa (Siegfried et al. 2003) So, during infectious and neoplastic disease and in some eating disorders, as well as in animal models of these conditions, increased energy expenditure is accompanied by reduced food intake in normal-weighted or weight-losing individuals.

Reduced Food Intake as a Biological Response

Potential Adaptive Value of Reduced Food Intake

The cooccurrence of increased energy expenditure and reduced food intake during infection and cancer as well as in some eating disorders can easily lead to the conclusion that these are due to dysfunctioning set point mechanisms. However,

both field observations and experimental work indicate that feeding during infection can be harmful and increases mortality while prior starvation decreases mortality (Murray and Murray 1979; Wing and Young 1980). Interestingly, intravenous or intragastric feeding of anorectic tumor-bearing rats also increases tumor growth and mortality (Cameron and Pavlat 1976; Cameron et al. 1979). Anorexia during infection and cancer has been proposed to favor host survival by eliminating infected or transformed host cells (Legrand 2000). Indeed, in a mouse model of intestinal neoplasia food restriction limits polyp growth and increases apoptosis (Kakuni et al. 2002). Moreover, food restriction eliminates preneoplastic cells through apoptosis in a drug-induced animal of liver cancer (Grasl-Kraupp et al. 1994). Interestingly, recent research indicates that reduced food intake improves host survival in response to bacterial inoculation and administration of bacterial LPS, but not after exposure to influenza virus, by protecting host tissues against the effects immune cell-mediated production of reactive oxygen species (Wang et al. 2016). Thus, bacterial infection-associated reduced food intake would allow host tissues to tolerate free radical stress better than bacteria.

The exercising subtype of anorexia nervosa has been proposed to reflect an evolutionary ancient response to flee famine or nutrient-poor or nutrient-depleted environments (Guisinger 2003). Accordingly, the encounter of energy-rich sources should reduce locomotor activity and stimulate intake. Testing of this hypothesis by providing energy-rich sucrose or equally sweet energy-free saccharine to food-restricted rats with access to a running wheel showed indeed that animals increase their sucrose intake, which, in turn, prevents hyperactivity and weight loss (Duclos et al. 2013).

Peripheral Signals Reducing Food Intake and Increasing Energy Expenditure in Nonoverweight Animals

For reduced food intake and increased energy expenditure to be considered biological responses, endogenous mediators and mechanisms need to be identified. Phagocytosis of bacteria promotes "the release of endogenous pyrogen from leukocytes" (Berlin et al. 1964), which, in turn, causes a negative nutrient balance during infection (Powanda and Beisel 1982). Indeed, this "endogenous pyrogen" coined Interleukin-1beta (IL-1beta) both induces fever and reduces food intake (Mccarthy et al. 1995). Conversely, peripheral administration of the naturally occurring Interleukin-1 receptor antagonist alleviates fever (Smith and Kluger 1992; Luheshi et al. 1996) and attenuates the reduction in food intake and body weight after bacterial LPS injection in rodents (Bluthé et al. 1992; Swiergiel et al. 1997). The available literature thus clearly supports the idea that IL-1 is an endogenous mediator of fever and reduced food intake after exposure of host tissues to bacterial LPS.

Based on their anorectic and catabolic properties, IL-1 and its functionally related, but structurally different, Tumor Necrosis Factor-alpha (TNFalpha), initially known as cachectin, have been proposed to play a role in cancer-associated anorexia-cachexia (Moldawer et al. 1987). Indeed, TNFalpha activity was found to

be increased in the serum of cachectic sarcoma-bearing rats (Stovroff et al. 1989). Moreover, neutralizing TNF action increases food intake in weight-losing sarcoma-bearing Fischer 344 rats (Smith et al. 1993; Torelli et al. 1999). Although some studies have shown a slight benefit of TNF-blocking strategies in cancer patients, these were found to be ineffective against anorexia and weight loss in advanced disease (Dezube et al. 1993; Eisen et al. 2000; Jatoi et al. 2007).

However, in autologous animal models, such as Morris hepatoma in Buffalo rats, and in many patients with cancer-associated anorexia increased plasma concentrations of IL-1beta or TNFalpha have not been observed (Ruud and Blomqvist 2007; Pourtau et al. 2011; Ezeoke and Morley 2015). Other cytokines, such as Macrophage Inhibitory Cytokine-1 (MIC-1), may play a role in cancer anorexia. Increased MIC-1 expression is found in cancer cells and in serum in many aggressive cancers (Mimeault and Batra 2010). This cytokine not only stimulates tumor growth and modulates immune responses (Mitchell and Bucala 2000; Candido and Hagemann 2013) but also constitutes an endogenous regulator of food intake as mice genetically deficient in MIC-1 display increased food intake and become obese (Tsai et al. 2013). Interestingly, MIC-1 serum concentrations correlate with prostate cancer-associated anorexia-cachexia and administration of a MIC-1 antiserum attenuates the reduction in food intake induced by xenografts of prostrate cancer in immunodeficient mice (Johnen et al. 2007). In addition, during autologous tumor growth in immunocompetent animals increased MIC-1 plasma concentrations correlate negatively with food intake (Borner et al. 2016a). Tumor-derived MIC-1 thus seems to constitute a mediator of cancer-associated anorexia.

Several tumors, including the Morris hepatoma 7777, that reduce food intake in rat produce the anorectic cytokine-like hormone leptin (Howard et al. 2010; Pourtau et al. 2011). In the Morris hepatoma model, plasma leptin levels in anorectic tumor-bearing rats are lower than in free-feeding tumor-free animals, but still higher than in food-restricted weight-matched animals (Pourtau et al. 2011). Interestingly, circulating concentrations of the orexigenic hormone ghrelin display the exact inverse relationships (Pourtau et al. 2011). So, in cancer-associated anorexia increased concentrations of anorectic mediators such as MIC-1 can be observed along with the absence of the expected leptin and ghrelin responses to weight loss.

Even though ghrelin administration alleviates cancer-associated anorexia in the Morris hepatoma model (Borner et al. 2016b), cachectic animals bearing other tumors seem less sensitive to its appetite-stimulating effects (Wang et al. 2006; Chance et al. 2008). Moreover, the ghrelin agonist anamorelin increases lean body mass, but not handgrip strength, in patients with advanced non-small-cell lung cancer (Garcia et al. 2015; Temel et al. 2016). Ghrelin administration may therefore alleviate some aspects of cancer-associated anorexia, but its impact on patient functioning remains to be established.

Anorexia nervosa is accompanied by increased cortisol concentrations, which are associated with physical activity (Walsh et al. 1978; Klein et al. 2007). In the activity-based anorexia animal model, corticosterone is synergistically increased by food restriction, and exercise and has been proposed to contribute to body weight loss and suppression of food intake (Broocks et al. 1990; Burden et al. 1993).

Moreover, in rat lines known to differ in hypothalamic-pituitary-adrenal axis (re) activity, higher food restriction-induced hyperactivity was associated with increased prefeeding corticosterone levels (Duclos et al. 2005). Finally, hyperactivity during food restriction is suppressed by adrenalectomy and restored by acute administration of corticosterone (Duclos et al. 2009). Thus, increased corticosterone plays an important role in food-restriction-induced hyperactivity. Leptin concentrations are low in anorexia nervosa patients, as well as in animals displaying food restriction-induced hyperactivity, and correlate negatively with physical activity (Exner et al. 2000; Frey et al. 2000; Holtkamp et al. 2003). These observations, along with the finding that leptin treatment can reduce food restriction-induced hyperactivity (Exner et al. 2000), have led some authors to call for clinical trials evaluating leptin treatment of anorexia nervosa (Hebebrand and Albayrak 2012). So, in animal models of infectious and neoplastic disease and eating disorders, host mediators have been identified that reduce food intake and increase energy expenditure.

Brain Structures Activated During Concomitantly Reduced Food Intake and Increased Energy Expenditure in Nonoverweight Individuals

The activation of common brain structures during reduced food intake and increased energy in infectious and neoplastic disease and eating disorders provides additional weight to the idea that these symptoms are actively put in place rather than being due to a loss of function. While noninvasive functional neuroimaging techniques, such as deoxyglucose Positron Emission Tomography (PET) and blood oxygen-level dependent Magnetic Resonance Imaging (MRI) are employed in humans, postmortem detection of the transcription factor c-Fos has often been used in animals to assess activation of brain structures. c-Fos mRNA and protein expression peak, respectively, around 30 and 120 min after exposure to the excitatory neurotransmitter glutamate, growth factors or increases in intracellular calcium, thus making it a suitable cellular marker for transcription activation (Morgan and Curran 1990).

Several patterns of c-Fos induction are observed after intraperitoneal IL-1beta or LPS administration in rodents (Fig. 1). The first, 1–2 h after injection, includes midline brainstem structures, the paraventricular and preoptic hypothalamus, and the central amygdala (Brady et al. 1994; Konsman et al. 1999). Between 3 and 8 hours after IL-1beta or LPS administration c-Fos expression in these structures is accompanied by a wave of induction in brain circumventricular organs, such as the area postrema and median eminence that lack a functional blood-brain barrier. From these organs labeling seems to spread to adjacent brain structures, for example, the arcuate nucleus of the hypothalamus (Brady et al. 1994; Konsman et al. 1999). In addition, increased c-Fos expression is observed in the nucleus accumbens in the ventral striatum during this time window after peripheral LPS administration (Frenois et al. 2007).

Human brain imaging approaches lack the cellular resolution of c-Fos studies in animals and reveal metabolic rather than transcription activation. In spite of these differences, human brain imaging studies indicate activation of some brain structures

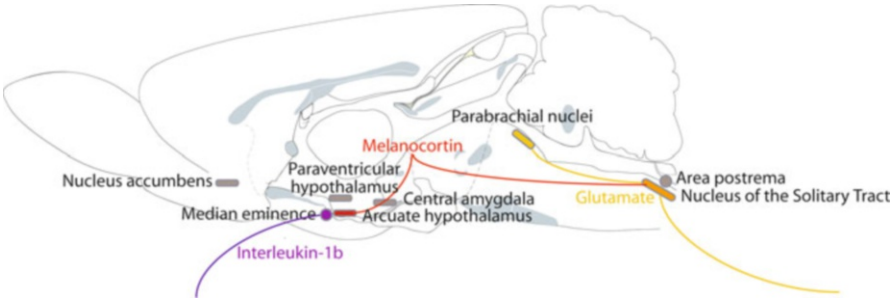

Fig. 1 Brain circuits associated with bacterial lipopolysaccharide-induced reduced food intake. Peripheral administration of bacterial lippolysaccharides results in the rapid systemic production of the pro-inflammatory cytokine Interleukin-1beta and induction of the cellular activation marker c-Fos in, among other structures, the brainstem nucleus of the solitary tract and parabrachial nuclei, the paraventricular hypothalamus, and the central amygdala as compared to saline injections. At later points, the area postrema, the arcuate nucleus of the hypothalamus, and the nucleus accumbens also show increased c-Fos expression. Brainstem glutamate-producing and brain melanocortin-containing circuits underlie early and sustained reduced food intake, respectively. Colors indicate different peripheral and central pathways reducing food intake

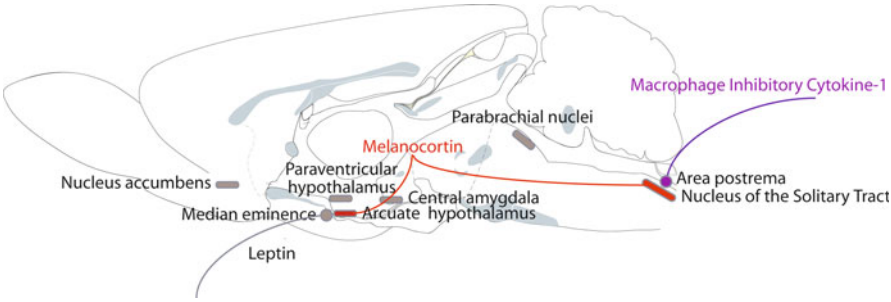

Fig. 2 Brain circuits associated with tumor-induced reduced food intake. Peripheral inoculation of tumor cells in rodents results in higher circulating leptin concentration, the systemic production of the macrophage inhibitory cytokine-1 and induction of the cellular activation marker Fos in, among other structures, the brainstem nucleus of the solitary tract, area postrema and parabrachial nuclei, the arcuate and paraventricular hypothalamus, the central amygdala, and nucleus accumbens as compared to food-restricted and free-feeding controls. Brain melanocortin-containing circuits play a role in reducing food intake. Colors indicate different peripheral and central pathways reducing food intake

that also showed increased c-Fos expression in animals after peripheral LPS administration. Indeed, volunteers receiving low doses of intravenous LPS show increased glucose and oxygen consumption in the pons and amygdala (Hannestad et al. 2012; Harrison et al. 2013; Labrenz et al. 2016). In addition, these human brain imaging studies have shown LPS-induced increases in metabolic activity in the cingulate and insular cortices (Hannestad et al. 2012; Harrison et al. 2013; Labrenz et al. 2016).

The pattern of c-Fos expression obtained during cancer-associated anorexia in the Morris hepatoma rat model (Fig. 2) is in part reminiscent of that observed after LPS

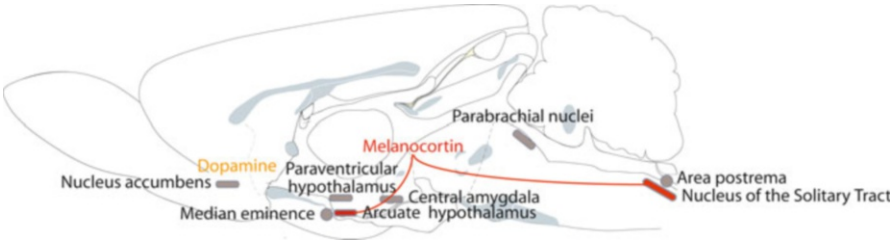

Fig. 3 Brain circuits associated with activity-based reduced food intake. Activity-based anorexia and food-restriction-induced hyperactivity paradigms result in increased c-Fos expression in, among other structures, the nucleus of the solitary tract; arcuate, supraoptic, and paraventricular hypothalamus; and nucleus accumbens. Dopamine-producing and melanocortin-containing circuits underlie reduced food intake in these paradigms. Colors indicate different peripheral and central pathways reducing food intake

injection (Konsman and Blomqvist 2005; Ruud and Blomqvist 2007). Interestingly, oxygen extraction measured by MRI was also found to be higher in the hypothalamus of lung cancer patients than that of healthy controls, but lower in anorexic compared to nonanorexic patients (Molfino et al. 2016). The distribution of c-Fos in the brains of rodents that display food restriction-induced hyperactivity or activity based anorexia (Fig. 3) also shows overlap with that observed after systemic LPS injection (Duclos et al. 2013; Scharner et al. 2016). Using PET imaging, striatal structures showed reduced glucose consumption in the activity-based anorexia animal model, but increased glucose consumption in anorexia nervosa patients (Herholz et al. 1987; Delvenne et al. 1999; Van Kuyck et al. 2007). So, in different animal models of concomitant decreased food intake and increased energy expenditure the same brainstem, hypothalamic, and ventral striatal structures display increased expression of the cellular activation marker c-Fos. Unfortunately, it is at present impossible to determine if the same structures are also activated in the clinical conditions these animal models are supposed to mimic as human brain imaging studies using different modalities.

Brain Circuits Mediating Reduced Food Intake and Increased Energy Expenditure

The brainstem mediates short-term regulation of food intake and activation of brainstem glutamatergic projections from the nucleus of the solitary tract to the parabrachial nuclei reduce food intake (Wu et al. 2012). We have shown that brainstem metabotropic glutamate receptor antagonism attenuates hypophagia and increases food intake after peripheral LPS injection to a greater extent than in vehicle-treated animals (Chaskiel et al. 2016). In parallel, intrafourth ventricle administration of the metabotropic glutamate receptor antagonist also reduces c-Fos expression in the nucleus of the solitary tract and lateral parabrachial nuclei (Chaskiel et al. 2016). These findings suggest that brainstem glutamatergic circuits

are part of the neuronal substrates that rapidly reduce food intake under inflammatory conditions (Fig. 1), but also negatively regulate food consumption in the absence of sickness.

In the hypothalamus, the arcuate nucleus expresses IL-1 receptors (Ericsson et al. 1995). Interestingly, neonatal excitotoxic lesions of arcuate neuropeptide Y/Agouti-related protein and proopiomelanocortin arcuate neurons exacerbate IL-1beta-induced hypophagia (Reyes and Sawchenko 2002). However, only part of the arcuate neuropeptide Y/Agouti-related protein containing neurons and none of the proopiomelanocortin-expressing neurons express IL-1 receptors (Scarlett et al. 2008; Chaskiel et al. 2014). But, even though proopiomelanocortin arcuate neurons do not express IL-1 receptors, central melanocortin antagonism has been found to attenuate hypophagia from 8 h onwards after the peripheral administration of either IL-1beta or LPS (Fig. 1) (Huang et al. 1999; Whitaker and Reyes 2008). In addition, our recent findings show that lesioning of arcuate IL-1 receptor-expressing cells alleviates the reduction in food intake after peripheral IL-1beta injection (Chaskiel et al. 2014). Therefore, besides the activation of brainstem glutamatergic projections between the nucleus of the solitary tract and the parabrachial nuclei mediating hypophagia after peripheral LPS injection, neurons in the arcuate nucleus of the hypothalamus seem to be target of systemic IL-1beta to reduce food intake.

The nucleus of the solitary tract and pontine parabrachial nuclei receive, among other sources, input from neurons in the area postrema, the brainstem circumventricular organ lacking a functional blood-brain barrier. Although lesions of the area postrema do not attenuate the reduction in food intake observed after LPS administration (Weingarten et al. 1993), its removal does alleviate cancer-associated anorexia in two rodent tumor models (Bernstein et al. 1985; Borner et al. 2016a). As neurons containing anorexigenic and catabolis melanocortins are present both in the brainstem nucleus of the solitary tract and hypothalamic arcuate nucleus, it is of interest to test their implication in cancer-associated anoxia cachexia. Several studies have shown that central administration of the endogenous melanocortin antagonist Agouti-related Protein or that of a synthetic antagonist attenuates anorexia and weight loss in experimental cancer models (Marks et al. 2001; Wisse et al. 2001). So, activation of brain circumventricular organs and melanocortin pathways plays an important role in cancer-associated anorexia (Fig. 2).

In the case of activity-based anorexia, central administration of Agouti-related Protein also increases food intake and decreases physical activity (Hillebrand et al. 2006). In addition, antagonizing dopaminergic neurotransmitter pathways also increases food intake and reduces physical activity in activity-based anorexia (Verhagen et al. 2009; Klenotich et al. 2015), whereas inhibition of serotinergic transmission lowers physical activity without improving food intake (Atchley and Eckel 2006; Klenotich et al. 2012). However, the selective serotonin reuptake inhibitor fluoxetine, but not the less serotonin-selective reuptake inhibitor imipramine, attenuates the reduction in food intake and the development of hyperactivity in acidity-based anorexia (Altemus et al. 1996). These findings indicate that CNS melanocortin and dopamine pathways mediate activity-based anorexia (Fig. 3),

whereas the exact role of serotonin remains to be elucidated. There is thus ample evidence to suggest that the activation of specific brain structures reduce food intake in different animal models of infectious and neoplastic disease or eating disorders. Although some brain pathways may be specific to a single condition, activation of melanocortin neurons seems to contribute to the reduction of food intake in several conditions of reduced food intake and increased energy expenditure in normal-weighted or weight-losing individuals.

Conclusion

Situations of concomitant reduced food intake and increased energy expenditure observed during infectious and neoplastic disease as well as in some eating disorders seem to contradict a fixed set point hypothesis of energy balance. Reduced food intake in infectious and neoplastic disease and anorexia nervosa may represent initially adaptive responses contributing to the organism's survival, even though chronically decreased food intake and increased energy expenditure lead to denutrition. The identification of cytokines and hormones mediating and the activation of common set of brain structures in these different situations of concomitant reduced food intake and increased energy expenditure suggest that these responses are actively put in place by the organism. Furthermore, lesions or inhibition of some of the activated brain structures and pathways alleviate reduced food intake in animal models of infectious and neoplastic disease and anorexia nervosa. Thus, organisms seem capable of defending several set points, rather than just one fixed set point, of energy balance.

Dictionary of Terms

- **Brainstem** – Ventrocaudal part of the animal brain that includes the midbrain, the pons, and the medulla, the latter of which controls respiration, heart rhythm, and blood glucose
- **Hypothalamus** – Ventromedian part of the animal brain containing "controllers" for thermoregulatory, ingestive, reproductive, and defensive behaviors
- **Neoplastic** – Pertaining to a tumorous condition or tumor formation

Summary Points

- Reduced food intake contributes to survival in experimental models of infection and cancer.
- Reduced intake of scarce energy-low food along with increased activity may favor the discovery of new energy-rich nutrients.
- Cytokines play an important role in reducing food intake in animal models of infection and cancer.

- Corticosteroids have been proposed to play a role in activity-based anorexia and mediate food restriction-induced hyperactivity in animals.
- The same brainstem and hypothalamic structures show increased expression of the cellular activation marker c-Fos in animal models of infection, cancer, and anorexia nervosa.
- Activation of brain melanocortin pathways contributes to the reduction of food intake in animal models of infection, cancer, and anorexia nervosa.

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Role of VPS34 Complexes in Starvation-Induced Autophagy

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Abstract

Autophagy, a catabolic pathway that degrades cellular components not needed by or harmful to cells, enables nutrient recycling and is critical for cell survival in starvation conditions. Starvation-triggered signals, including diverse protein-protein interactions and numerous posttranslational modifications, modulate autophagy, and many of these signals regulate the vacuolar protein sorting (VPS34) complexes responsible for key stages of autophagy. Autophagy plays a major role in numerous human diseases including cancers, neurodegenerative diseases, heart diseases, and infectious diseases. Therefore, substantial ongoing research focuses on developing therapeutic methods to modulate autophagy. Short-term starvation may constitute a readily accessible mechanism to induce autophagy.

Keywords

Starvation · Autophagy · VPS34/PI3KC3 · BECN1/Beclin 1/VPS30/ATG6 · ATG14 · VPS38/UVRAG · VPS15/p150 · Posttranslational modification · Protein-protein interaction · Cancer · Neurodegenerative diseases · Heart diseases · Longevity

List of Abbreviations

ADP	Adenosine diphosphate
AMBRA1	Activating molecule in BECN1 regulating autophagy 1
AMP	Adenosine monophosphate
AMPK	AMP-associated protein kinase
ATG	Autophagy related protein
ATP	Adenosine triphosphate
BARAD	β-α repeated autophagy-specific domain
BATS	BARKOR/ATG14 autophagosome targeting sequence
BCL2	B-cell lymphoma 2
BECLIN 1/BECN1	BCL2-interacting, coiled-coil protein
BH3D	BCL2 homology 3 domain
BIF1	BAX interacting factor-1
CCD	Coiled-coil domain
DLC1	Dynein light chain 1
HMGB1	High mobility group box 1
IDR	Intrinsically disordered region
LC3	Microtubule-associated protein light chain 3
(m)TORC1	(Mammalian) target of rapamycin complex 1
NF-κB	Nuclear factor κ-light-chain-enhancer of activated B cells

PI	Phosphatidylinositol
PI3KC3	Class III phosphatidylinositol 3-kinase
PI3P	Phosphatidylinositol 3- phosphate
PTM	Posttranslational modification
ROS	Reactive oxygen species
RUBICON	Run domain Beclin 1-interacting and cysteine-rich domain-containing protein
SNAP29	Synaptosome-associated protein 29
STX17	Syntaxin 17
SUMO	Small ubiquitin-like modifier
TAB2/3	TAK1-binding proteins 2/3
TRIM28	Tripartite motif containing protein 28
ULK	UNC-51-like autophagy-activating kinase
UVRAG	UV radiation resistance associated gene protein
VPS	Vacuolar protein sorting protein
WASH	Wiskott-Aldrich syndrome protein and SCAR homolog

Introduction

Most organisms live in substantially harsher natural environments than that encountered by humans living in civilized societies. One of the most primitive and commonly encountered deficiencies in normal environments is the lack of nutrients. Therefore, eukaryotes have evolved mechanisms that enable them to survive starvation conditions. Key among these is autophagy, a lysosomal degradation pathway wherein cytoplasmic components not needed by or harmful to the cell, such as damaged organelles, aggregated proteins, or pathogens, are engulfed in membrane vesicles called autophagosomes which fuse with lysosomes to enable degradation of autophagosomal components and recycling of nutrients such as amino acids, nucleotides, and energy-providing molecules into the cytoplasm (Kroemer et al. 2010). Notably, extensive or unregulated autophagy can cause cell death.

Autophagy is accomplished by several autophagy-related (ATG) proteins in four distinct stages: (1) autophagy induction, (2) autophagosome nucleation, (3) autophagosome elongation, and (4) autophagosome maturation (Kroemer et al. 2010). Autophagy induction involves the UNC-51 like autophagy-activating kinase (ULK) 1 signaling complex composed of ULK1/2 kinase, ATG13, and focal adhesion kinase family interacting protein of 200 kD (Mizushima 2010). Vesicle nucleation requires vacuolar protein sorting protein 34 (VPS34), also called the Class III phosphatidylinositol 3-kinase (PI3KC3), which phosphorylates phosphatidylinositol (PI) to generate phosphatidylinositol-3-phosphate (PI3P). VPS34 lipid kinase activity and PI3P production is up-regulated by formation of VPS34 Complex I (Obara and Ohsumi 2008). Subsequently, autophagosome elongation enlarges and completes the autophagosome, while maturation involves fusion with lysosomes enabling degradation of autophagosomal contents. VPS34 Complex II plays a role in autophagosome-lysosome fusion (Liang et al. 2006).

Starvation-Triggered Upstream Signaling Mechanisms That Induce Autophagy

The availability or lack of nutrients activates several signaling pathways, many of which regulate autophagy. Nutrient levels sensed via altered cellular energy levels, amino acid availability, and starvation-induced oxidative stress impact autophagy levels. These signals are often transduced to VPS34 complexes via serine/threonine kinases such as AMP-activated protein kinase (AMPK), target of rapamycin complex 1 (TORC1), and the ULK1/2 complexes.

Cellular Energy Levels

ATP is the major energy-storing molecule in most living organisms and therefore is often called the energy currency of life. The availability of carbon sources such as glucose directly affects relative cellular concentrations of ATP, ADP, and AMP as the hydrolysis of glucose results in ATP biosynthesis from ADP/AMP. Elevated ADP and AMP levels reflect energetic stress; therefore, the ATP:ADP+AMP ratio is a key indicator of cellular energy levels. At high concentrations, ADP and AMP bind to and activate AMPK (Laderoute et al. 2006). Activated AMPK phosphorylates and activates ULK1, and both phosphorylate VPS34 complex proteins and VPS34 complex regulators, to up-regulate autophagy (Kim et al. 2011, 2013) (Fig. 1). Activated AMPK also up-regulates autophagy by phosphorylating and inactivating mammalian (m)TORC1, which prevents mTORC1-mediated phosphorylation and repression of ULK1 (Kimura et al. 2003; Kim et al. 2011) (Fig. 1).

Amino Acid Availability

TORC1 activation is also regulated by cellular levels of Leu, Gln, and Arg (Bauchart-Thevret et al. 2010; Duran et al. 2012; Han et al. 2012; Zhu et al. 2015). At high Leu concentrations, leucyl-tRNA synthetase interacts with and activates Rag GTPase which then activates mTORC1, leading to suppression of autophagy by phosphorylation of ULK1/2 and AMPK (Han et al. 2012) (Fig. 1). Gln and Arg are also implicated in mTORC1 activation, but how they are sensed is unknown (Duran et al. 2012; Zhu et al. 2015).

Starvation-Induced Mitochondrial Oxidative Stress

Nutrient depletion leads to malfunction of mitochondrial electron transport. Electrons leak from NADH-Coenzyme Q oxidoreductase and Coenzyme Q-Cytochrome c oxidoreductase, resulting in partial oxygen reduction to superoxide radicals ($O_2^{\bullet -}$), the precursor of most other reactive oxygen species (ROS). These superoxide radicals may be converted to hydrogen peroxide (H_2O_2) by mitochondrial

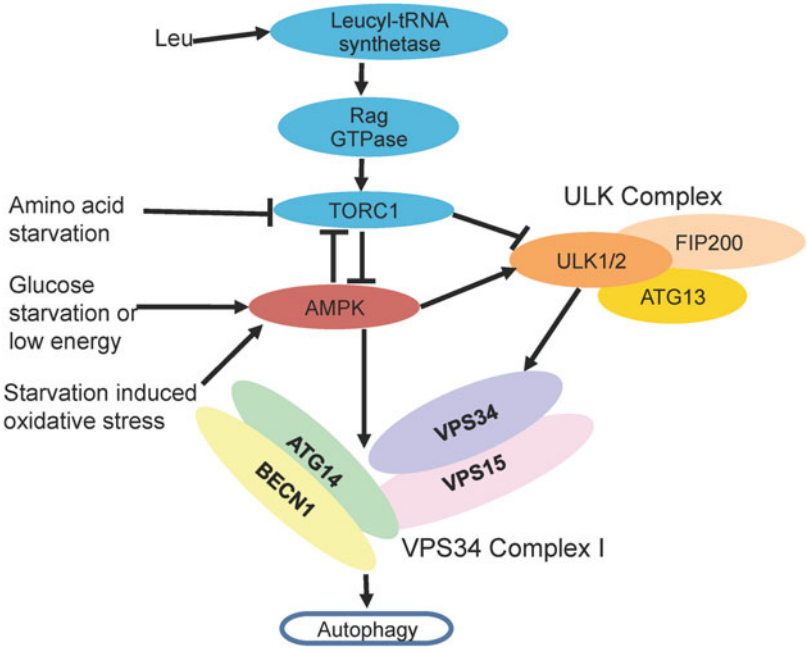

Fig. 1 Upstream signals that modulate autophagy in response to starvation. Proteins are represented as ovals, with VPS34 complex proteins in different colors

superoxide dismutase and released from the mitochondrial matrix to the cytoplasm. Starvation-induced H_2O_2 may activate AMPK, which may be redox-sensitive (Shao et al. 2014). Activated AMPK induces autophagy as discussed above (Fig. 1).

Organization of VPS34 Complexes

The availability or lack of nutrients activates many signaling pathways that directly or indirectly regulate function of the VPS34 complexes to regulate autophagy. These signals may regulate the assembly, cellular localization, and/or activity of VPS34 complexes, constituting major mechanisms by which starvation triggers autophagy. Human VPS34 is an 875-residue protein with three domains: an N-terminal C2 domain, an intrinsically disordered region (IDR), a central helical domain, and C-terminal lipid kinase domain (Backer 2008; Mei et al. 2014) (Fig. 2). VPS34 forms an obligate heterodimer with an N-terminally lipid-linked serine/threonine kinase, VPS15/p150, which consists of an N-terminal kinase domain, a central helical domain, an IDR, and a C-terminal WD40 domain (Rostislavleva et al. 2015; Mei et al. 2014) (Fig. 2). Interaction of the VPS34 C-terminal lipid kinase and C2 domains with the VPS15 N-terminal kinase and helical domains, respectively, mediates VPS34:VPS15/p150 heterodimerization. VPS34:VPS15 heterodimers,

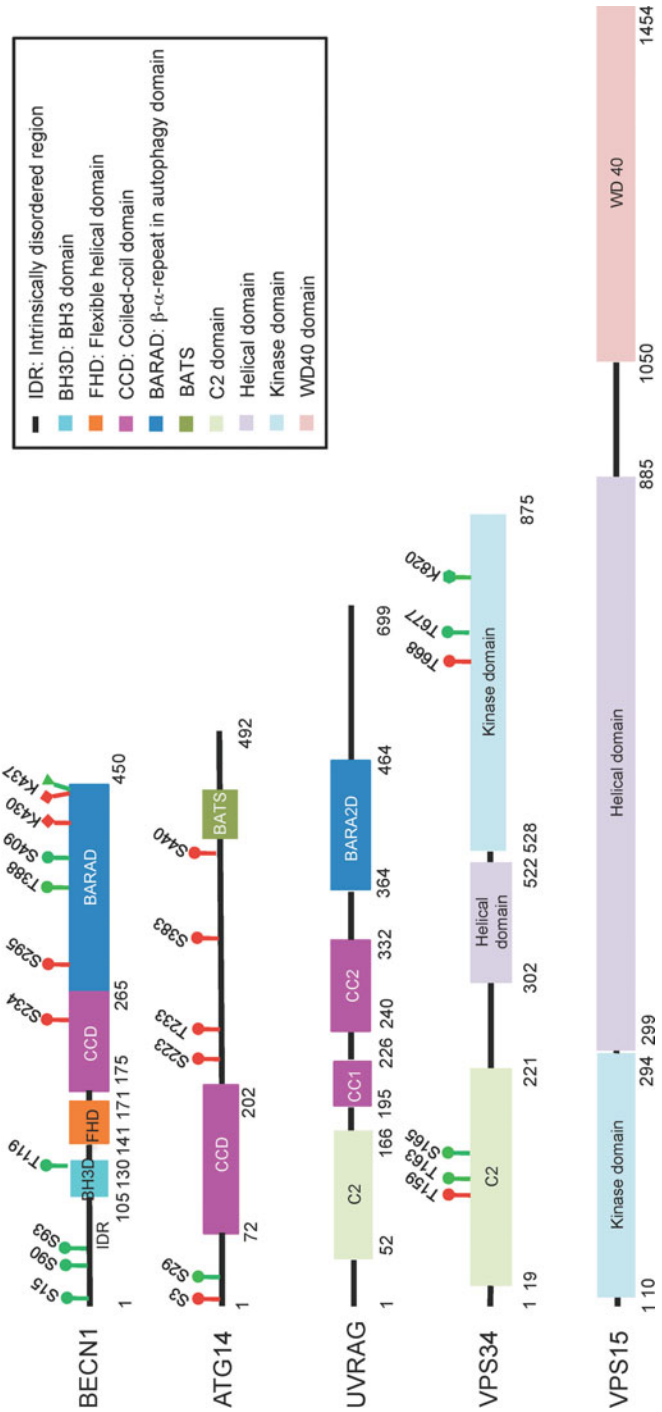

Fig. 2 VPS34 complex proteins: domain architecture and starvation-related PTMs. PTMs that promote or inhibit autophagy are colored green or red respectively. Small circles, diamonds, triangles, and hexagons indicate phosphorylation, acetylation, ubiquitination, and SUMOylation, respectively

often called non-autophagic VPS34 complexes, have basal PI3KC3 activity that likely enables low levels of autophagy.

PI3KC3 activity is substantially up-regulated by association of BECN1 and either ATG14 or UVRAG to form the autophagic complexes, Complex I or II, respectively (Itakura et al. 2008). Formation of these autophagic complexes is markedly up-regulated in starvation conditions.

BECN1, a core VPS34 complex component, is a 450-residue protein in humans. BECN1 homologs from diverse eukaryotes share between 29% and 99% identity. It consists of at least four domains/regions (Mei et al. 2016a): an intrinsically disordered region, a flexible helical domain, a coiled coil domain (CCD), and a β-α repeated, autophagy-specific domain (BARAD) (Fig. 2). Mammals have a second BECN1 homolog, BECN2, that also functions in starvation-induced autophagy (He et al. 2013). BECN2 shares 56.6% sequence similarity with BECN1 and is predicted to have structure and domain architecture similar to BECN1. Besides its role in autophagy, BECN2 regulates nutrient intake and ligand-induced endolysosomal degradation of several GPCRs. In the absence of BECN2, the cannabinoid 1 receptor is overexpressed, increasing nutrient intake resulting in weight gain and insulin resistance in cells. Each BECN domain interacts with diverse regulatory proteins (Mei et al. 2016a). BECN homologs are conformationally very flexible, which likely enable diverse interactions with concomitant conformational changes in BECN that impact assembly of VPS34 complexes.

ATG14, a less well-conserved core protein of VPS34 Complex I, helps regulate localization of Complex I (Matsunaga et al. 2010). Human ATG14 is a 492-residue protein comprised of an N-terminal cysteine-rich repeat region, a CCD, an IDR, and a C-terminal Barkor/ATG14 autophagosome targeting sequence (BATS) (Matsunaga et al. 2010; Mei et al. 2014) (Fig. 2). ATG14 and BECN CCDs heterodimerize with at least tenfold tighter affinity relative to BECN1 homodimerization (Mei et al. 2016b; Su et al. 2017). Mutation of critical ATG14:BECN1 interface residues destabilize the heterodimer and down-regulate starvation-induced autophagy (Li et al. 2012; Mei et al. 2016b). The N-terminal cysteine-rich domain is essential for targeting the VPS34 complex in mammals (Matsunaga et al. 2010), while the BATS senses and preferentially binds highly curved, PI3P-rich membranes (Fan et al. 2011). UVRAG, a core component of VPS34 Complex II, is a 699-residue protein comprised of an N-terminal proline-rich sequence, a C2 domain, two central CCDs, an IDR, and a C-terminal BARA2 domain (Liang et al. 2006; Mei et al. 2014) (Fig. 2). The UVRAG CCD and BARA2 domain interact with analogous BECN1 domains.

Complex I and II have very similar architecture, with an overall V-shape, with VPS34 together with VPS15/p150 forming one arm of the V-shape (Baskaran et al. 2014; Rostislavleva et al. 2015) (Fig. 3). The VPS15/p150 kinase domain interacts with the VPS34 activating loop, regulating its activity. The other arm of the V-shape is formed by a parallel heterodimer of VPS30/BECN1 and either ATG14 in Complex I or VPS38/UVRAG in Complex II. The C-terminal domains of these proteins, e.g., the VPS30/BECN1 BARAD and VPS38/UVRAG BARA2D, are located at the tip of the arm. The base of the "V" shape is formed by the VPS15/p150 helical domain,

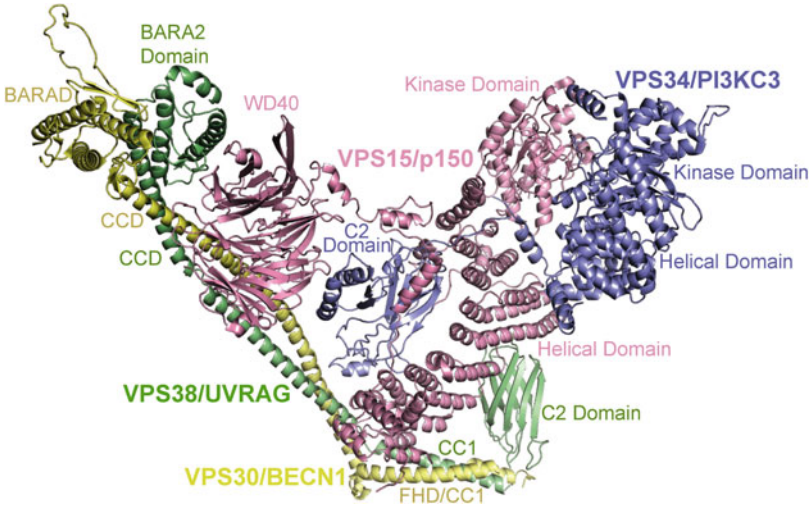

Fig. 3 Structure of VPS34 Complex II (PDB ID: 5DFZ). Each protein main-chain is traced in ribbon, colored according to Fig. 1. Domains of each protein are labeled

the VPS30/BECN1 IDR, and the N-termini of either VPS38 in Complex II or ATG14 in Complex I. The VPS34 C2 domain is buried in the center of the "V" shaped complex (Fig. 3), unlike the C2 domains of class I PI3Ks that pack between the helical and kinase domains and bind to membranes through Ca^{2+}.

In addition to interactions among protein components of VPS34 complexes, VPS34 activity is also regulated by interactions with other proteins and posttranslational modifications (PTMs).

VPS34 Regulation

VPS15/p150 binding triggers conformational changes required for VPS34 activity and starvation-induced autophagy (Rostislavleva et al. 2015). Besides direct interactions with VPS15/p150s, VPS30/BECN1, and either ATG14 or VPS38/UVRAG, in response to starvation, VPS34 activity is also regulated by the following PTMs.

VPS34 Phosphorylation

In response to starvation, the serine/threonine kinases AMPK and protein kinase D (PKD) up-regulate VPS34 catalytic activity and autophagy (Fig. 4). Glucose starvation-triggered increases in ADP and AMP levels activate AMPK, which phosphorylates VPS34 T163 and S165 (Kim et al. 2013), decreasing lipid kinase activity of nonautophagic VPS34 complexes, but increasing lipid kinase activity of autophagic

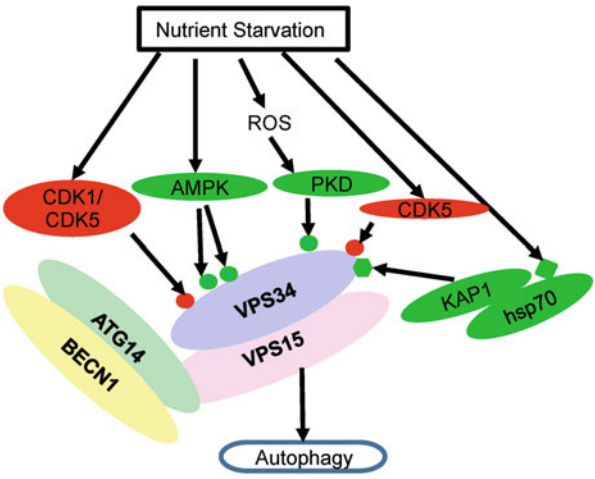

Fig. 4 VPS34 PTMs that regulate autophagy in response to starvation. The VPS34 complex is shown as in Fig. 1 and PTMs as in Fig. 2. Interacting proteins and PTMs that promote or inhibit autophagy are colored green or red, respectively

VPS34 complexes (Kim and Guan 2013). This is biologically reasonable, since VPS34 also participates in other cellular pathways and the inhibitory phosphorylation by AMPK in response to glucose starvation may down-regulate these other pathways, while simultaneously increasing the lipid kinase activity of VPS34 Complex I and II, to increase autophagy. On the other hand, in response to ROS, PKD phosphorylates the VPS34 catalytic domain T677, directly activating VPS34 and up-regulating autophagy (Eisenberg-Lerner and Kimchi 2012) (Fig. 4).

Conversely, autophagy is down-regulated in response to starvation by the cyclin-dependent kinases, CDK1 and CDK5, which are serine/threonine kinases that regulate essential cellular processes such as cell-cycle progression, development, and intracellular signaling. Starvation during mitosis triggers CDK1- and CDK5-mediated phosphorylation of VPS34 C2 domain T159 (Fig. 4), which disrupts VPS34:BECN1 association, thereby decreasing VPS34 activity (Furuya et al. 2010). CDK5 also phosphorylates the VPS34 catalytic domain T668, directly inhibiting VPS34 activity (Furuya et al. 2010) (Fig. 4). CDK-mediated down-regulation of starvation-induced autophagy during mitosis is biologically important, because increased autophagy due to normal starvation-triggered up-regulation would degrade organelles, proteins, or DNA, thereby disrupting mitosis.

VPS34 SUMOylation

Tripartite motif containing protein (TRIM)28, also called KAP1, is an E3 ligase that directs ligation of the small ubiquitin-like modifier protein (SUMO) (Ivanov et al. 2007). Amino acid deprivation promotes heat shock protein (HSP)70 acetylation. Acetylated HSP70 binds to VPS34 complexes and also recruits KAP1/TRIM28, which SUMOylates VPS34 at K820, enhancing VPS34 lipid kinase activity, thereby increasing autophagy (Yang et al. 2013) (Fig. 4).

BECN1 Regulation

BECN1-mediated autophagy is regulated in response to starvation by multiple protein interactions and PTMs.

BECN1 Interactions That Up-regulate Autophagy

Activating molecule in BECN1-regulated autophagy (AMBRA)1 binds BECN1, acting as an adaptor between the VPS34 and dynein motor complexes (Di Bartolomeo et al. 2010) (Fig. 5i). In nutrient-rich conditions, activated mTORC1 phosphorylates AMBRA1 S52 (Nazio et al. 2013) enabling binding to dynein light chain 1 (DLC1) of the dynein motor complex, thereby anchoring AMBRA1 to cytoskeletal microtubules and sequestering BECN1 and the VPS34 complex (Di Bartolomeo et al. 2010). Upon starvation, ULK1 phosphorylates AMBRA1 causing dissociation from DLC1, and VPS34 complex transport to the endoplasmic reticulum (Fig. 5i). Subsequent PI3P production at the endoplasmic reticulum initiates autophagosome nucleation.

High mobility group box (HMGB)1, a chromatin-associated nuclear protein, up-regulates autophagy by binding BECN1 (Tang et al. 2010). ROS produced upon prolonged starvation triggers HMGB1 translocation from nucleus to cytoplasm. Under oxidative conditions, an intramolecular disulfide bond forms between C23 and C45 of cytoplasmic HMGB1, enabling HMGB1 binding to BECN1, and competitive inhibition of BECN1 interaction with antiapoptotic B-cell lymphoma 2 (BCL2) proteins (Fig. 5ii). Moreover, cytoplasmic HMGB1 also promotes phosphorylation and activation of the extracellular signal-regulated kinases (ERK)1/2, which then phosphorylate BCL2 proteins, preventing them from binding BECN1 (Tang et al. 2010) (Fig. 5ii).

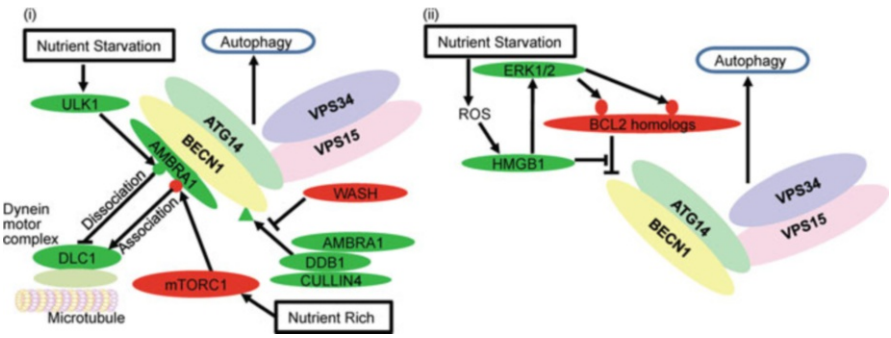

Fig. 5 Regulation of BECN1-mediated autophagy by (i) AMBRA1 (ii) HMGB1. Colors and symbols are as in Fig. 4.

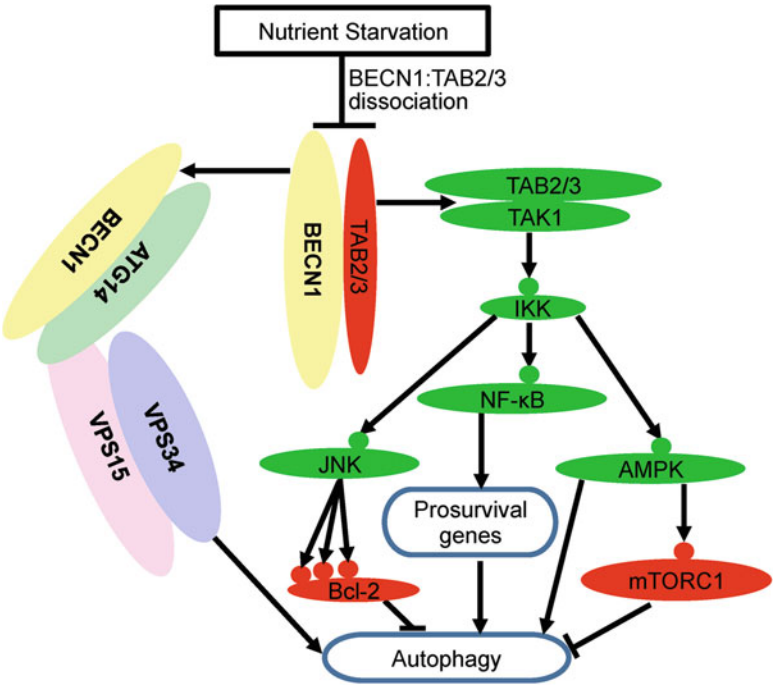

Fig. 6 Regulation of autophagy by TAB2/TAB3. Colors and symbols are as in Figs. 4 and 5

BECN1 Interactions That Down-regulate Autophagy

BCL2 proteins are characterized by the presence of poorly conserved BCL2 homology (BH) domains. Pro-apoptotic BCL2 proteins include BH3-only proteins such as BIM and BAD, as well as three-BH (BH1, BH3, and BH2) domain proteins: BAX, BAK, and BOK. Humans encode at least six, antiapoptotic, multi-BH domain (BH4, BH1, BH3, and BH2) proteins: Bcl-2, Bcl-X_L, Mcl-2, Bcl-w, A1, and Bcl-B. Many antiapoptotic BCL2 proteins bind to the BECN1 BH3D (Sinha and Levine 2008) decreasing VPS34 activity and starvation-induced autophagy (Pattingre et al. 2005). However, the mechanism(s) by which BCL2:BECN1 interactions down-regulate autophagy is unknown. Starvation induces c-Jun N-terminal protein kinase (JNK)1-mediated Bcl-2 phosphorylation at T69, S70, and S87, which disrupts interaction with BECN1, thereby stimulating starvation-induced autophagy (Wei et al. 2008a, b).

Under nutrient-rich conditions, transforming growth factor β activated kinase (TAK) 1-binding proteins, TAB2 and TAB3, bind via their CCDs to the BECN1 CCD, preventing BECN1 association with ATG14 or UVRAG, thereby down-regulating autophagy (Criollo et al. 2011) (Fig. 6). Starvation triggers dissociation of BECN1 from TAB2 and TAB3, enabling TAB2/3 binding to TAK1 via their CCDs. Consequent TAK1 activation causes TAK1-mediated phosphorylation and activation of IκB kinase

(IKK), which also up-regulates autophagy through the nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB), AMPK and JNK1 (Criollo et al. 2009) (Fig. 6).

Lastly, the Wiskott-Aldrich syndrome protein and SCAR homolog (WASH) binds BECN1 and suppresses autophagy by impacting BECN1 ubiquitination as described below, (Xia et al. 2013) (Fig. 5i).

BECN1 Phosphorylation

BECN1 phosphorylation by various starvation-activated serine/threonine kinases often improves association with VPS34, increasing VPS34 lipid kinase activity and inducing autophagy. ULK1, activated by amino acid deprivation or via TORC1 inhibition, phosphorylates BECN1 S15, enhancing VPS34 activity (Russell et al. 2013). Starvation-activated AMPK phosphorylates BECN1 S90, S93, and T388, with phosphorylation at S90 and S93 substantially increased when ATG14 or UVRAG are present (Kim et al. 2013; Zhang et al. 2016). BECN1 phosphorylation at these sites decreases BECN1:BCL2 interaction and enhances interactions with ATG14 and VPS34 (Zhang et al. 2016). Similarly, mitogen activated protein kinase (MAPK)-activated protein kinases (MKs), MK2 and MK3, of the p38 MAPK family, phosphorylate BECN1 S90, inhibiting BECN1:BCL2 interaction (Wei et al. 2015). Notably, AMPK also phosphorylates BECN1 at S90 (Kim et al. 2013). Since AMPK is mainly activated by glucose deprivation, while MK2/3 are activated by amino acid starvation, they have complementary functions implemented via phosphorylation of the same residue. Lastly, the calcium/calmodulin-dependent death-associated protein kinase (DAPK)1, which can be activated by starvation (Kang and Avery 2010), phosphorylates BECN1 BH3D T119, disrupting BECN1:BCL2 binding, thereby promoting BECN1 association with VPS34 (Zalckvar et al. 2009).

Two other serine/threonine kinases, protein kinase B (PKB) or AKT, and casein kinase (CK)1γ2, phosphorylate BECN1 under nutrient-rich conditions to down-regulate autophagy. PKB/AKT phosphorylates BECN1 at S234 and S295, enhancing BECN1 interaction with the 14-3-3 protein and vimentin intermediate filaments (Wang et al. 2012), which likely reduces the BECN1:VPS34 interaction. CK1γ2 phosphorylates BECN1 at S409 facilitating BECN1 acetylation by p300 (Sun et al. 2015) (Fig. 7).

BECN1 Acetylation

Under nutrient-rich conditions, CK1γ2-mediated BECN1 phosphorylation facilitates p300-mediated BECN1 acetylation at K430 and K437 (Fig. 7). BECN1 acetylation alters VPS34 complex structure and enables association of the run domain BECN1-interacting and cysteine-rich domain-containing protein (RUBICON), a negative regulator of autophagosome maturation (Sun et al. 2015).

BECN1 Ubiquitination

BECN1 BARAD K437 undergoes K63-linked ubiquitination (i.e., covalent linkage to chains of K63-linked ubiquitin proteins) by the E3 ubiquitin ligase

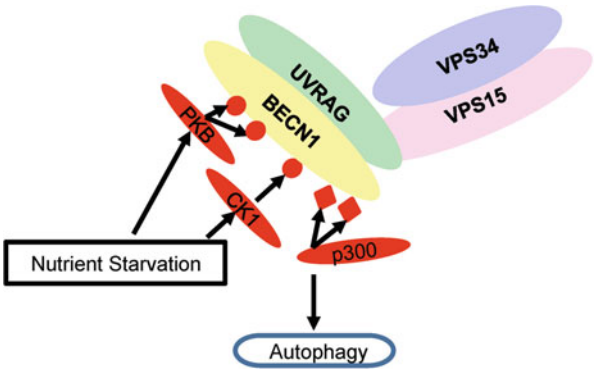

Fig. 7 PTMs that down-regulate BECN1-mediated autophagy. Colors and symbols are as in Fig. 4

complex, DNA damage-binding protein 1-CULLIN4 (DDB1-CULLIN4) complex, enabled by binding of AMBRA1 to both DDB1-CULLIN4 and BECN1 (Fig. 5i). This ubiquitination increases BECN1:VPS34 association and VPS34 lipid kinase activity and is required for starvation-induced autophagy (Antonioli et al. 2014). WASH binding to BECN1 abolishes K437 ubiquitination by the AMBRA1-DDB1-CULLIN4 complex and likely prevents excess autophagy during early embryonic development (Xia et al. 2013) (Fig. 5i).

ATG14 Regulation

ATG14 is regulated by protein interactions and PTMs.

ATG14 Interactions

Upon starvation, Syntaxin 17 (STX17), a target-soluble NSF attachment protein receptor (SNARE), along with synaptosome-associated protein 29 (SNAP29), is recruited to autophagosomes and facilitates fusion of autophagosomes with endosomes/lysosomes (Itakura et al. 2012). CCDs of each protein mediate ATG14 interaction with STX17, and with the STX17:SNAP29 binary complex, stabilizing the STX17:SNAP29 binary complex, which primes autophagosome:lysosome fusion (Diao et al. 2015).

ATG14 Phosphorylation

In nutrient-rich conditions, activated mTORC1 directly phosphorylates ATG14 S3, S223, T233, S383, and S440 (Fig. 2), decreasing VPS34 lipid kinase activity, thereby down-regulating autophagy (Yuan et al. 2013). Upon amino acid depletion, mTORC1 activity is inhibited, resulting in unphosphorylated ULK1, which is active and phosphorylates ATG14 at S29, increasing VPS34 lipid kinase activity, thereby increasing autophagy (Wold et al. 2016).

UVRAG Regulation

UVRAG function is regulated by interaction with RUBICON, as mentioned earlier, and Bax-interacting factor (BIF)1 or endophilin B1. RUBICON interacts with the VPS34 complex via UVRAG, inhibiting autophagosome maturation, thereby down-regulating starvation-induced autophagy (Matsunaga et al. 2009; Sun et al. 2011). However, the precise mechanism by which RUBICON binds to and regulates the UVRAG:BECN1 complex is unknown.

BIF1 consists of an N-terminal bin-amphiphysin-Rvs (N-BAR) domain and a C-terminal Src-homology 3 (SH3) domain. In response to starvation, BIF1 localizes to autophagosomes (Takahashi et al. 2007). The BIF1 SH3 domains bind to the UVRAG N-terminal proline-rich region, while the BIF1 N-BAR domain binds to membranes and drives membrane curvature. BIF1 BAR and SH3 domains are both required for PI3KC3 activation and autophagy.

Potential Health Benefits of Autophagy Triggered by Short-Term Starvation

Autophagy has significant health benefits as it protects against diseases like cancer, heart diseases, and neurodegenerative diseases and increases lifespan (Levine and Kroemer 2008). Indeed, several therapeutics currently being developed target autophagy proteins (Levine et al. 2015). Short-term starvation may confer similar benefits by inducing autophagy. Notably, short-term fasting, which is an established practice in many cultures, may have long-term health benefits that are yet been fully appreciated.

The Role of Autophagy in Cancer

Autophagy is either pro-tumoral or anti-tumoral depending on the stage of cancer development: initiation, promotion, progression, and metastasis. During cancer initiation, autophagy serves as a tumor suppressor pathway, for instance, by preventing ROS-induced DNA and protein damage leading to inhibition of tumorigenesis. However, in later stages of cancer, i.e., promotion, progression, and metastasis, autophagy is pro-tumoral, as insufficient oxygen and starvation in the core of the tumor triggers autophagy, enabling tumor cell survival and proliferation (Levine 2006; Mathew et al. 2007; Galluzzi et al. 2015).

The Role of Autophagy in Heart Diseases

Autophagy plays a dual role in cardiac diseases (Cao et al. 2009; Matsui et al. 2007; Rifki and Hill 2012). Inadequate blood flow to the heart due to blockage of heart arteries causes cardiac ischemia, leading to oxygen and nutrient shortage. This increases cellular ADP and AMP levels which activates AMPK, thereby triggering

autophagy and protecting myocardial cells from cell death. Conversely, restoration of blood flow to myocardial cells during reperfusion often results in ROS production, which further increases autophagy in cardiomyocytes, leading to a detrimental hypertrophic response and/or excessive self-digestion of cellular proteins and organelles leading to death.

Neuronal Autophagy Protects Against Neurodegenerative Diseases

Autophagy is important for removing misfolded and mutant proteins associated with several neurodegenerative disorders (Levine and Kroemer 2008). Mutations in proteins regulating autophagy cause accumulation of mutant huntingtin, α-synuclein, or Tau protein aggregates in neurons, causing cellular toxicity which culminates in neurodegenerative diseases such as Huntingon's, Parkinson's, and Alzheimer's, respectively (Nixon 2013; Shibata et al. 2006; Lynch-Day et al. 2012). Increased autophagy reduces these aggregated proteins in sick neuron cells (Webb et al. 2003). Short-term starvation dramatically increases autophagy in Purkinje and Cortic neuronal cells (Alirezaei et al. 2010).

Short-Term Starvation Increases Longevity

Elimination of damaged organelles and misfolded or aggregated proteins is a common feature of many lifespan-extending manipulations (Madeo et al. 2015). Short-term starvation inhibits the AKT/TORC1 pathway, thereby triggering autophagy and DNA repair pathways and increasing lifespan in several types of organisms (Madeo et al. 2015). BECN1/ATG6 are essential for autophagy induction and lifespan extension in *Caenorhabditis elegans* (Jia and Levine 2007). Under caloric restriction, decreased TOR signaling mediates lifespan extension in yeast (Powers et al. 2006). Thus, scientific evidence increasingly indicates that short-term starvation may be a readily accessible method of promoting longevity.

Therapeutic Regulation of Autophagy

Several small molecules have been shown to regulate autophagy. Rapamycin, a small molecule known to target mTORC1, up-regulates autophagy. Tamoxifen up-regulates BECN1 levels and induces autophagy. 3-Methyladenine and wortmannin target PI3KC3 and inhibit autophagy (Rubinsztein et al. 2012). Bafilomycin A1 activates mTORC1 and inhibits autophagosome fusion with lysosomes and also induces BECN1 binding to BCL2, decreasing autophagy while also promoting apoptotic cell death in pediatric patients with B-cell acute lymphoblastic leukemia (Yuan et al. 2015). Similarly, chloroquine prevents autophagy by blocking autolysosome formation and has been used to target colon cancer, malignant melanoma, hepatocellular carcinoma, low-grade glioma, and high

grade astrocytoma (Kimura et al. 2013). Small molecules like Verapamil, loperamide, and nimodipine up-regulate autophagy, enhancing clearance of soluble mutant huntingtin exon 1-encoded protein and reducing its aggregation and toxicity in neuroblastoma cells (Williams et al. 2008). Cell-permeable peptides or peptide mimetics derived from the BECN1 BH3D have been shown to selectively disrupt inhibitory interactions between BECN1 and antiapoptotic BCL2s, activating VPS34 complexes and up-regulating autophagy (Malik et al. 2011; Su et al. 2014). Another cell-permeable peptide derived from BECN1 residues 267–284 was shown to be a potent autophagy inducer that triggers autophagy to prevent replication of West Nile and Chikungunya viruses and may also target HIV-1 (Shoji-Kawata et al. 2013).

Implications of Short-Term Starvation for Human Health

The potential of short-term starvation as a therapeutic measure activating autophagy has not yet been investigated in a systematic, scientific manner in humans. However, recent scientific studies indicate significant health benefits of short-term starvation (Anton and Leeuwenburgh 2013). Caloric restriction without malnutrition extends life span and delays onset of age-related disorders in rhesus monkeys, which likely translates to human health (Mattison et al. 2017). In humans, glucose and protein-restricted diets for only 5 days each month lead to reduced body weight and body fat, lowered blood pressure, and decreased Insulin-like growth factor-1 (IGF-1) level with positive implications on aging and disease (Wei et al. 2017). Additionally, research on over-weight individuals shows that caloric restriction increases life expectancy, reduces cardiac risk factors, improves insulin-sensitivity, and prevents mitochondrial production of free radicals (Anton and Leeuwenburgh 2013).

Notably, short-term starvation by means of occasional fasting is an established ritual in various religions across the world, including Buddhism, Christianity, Hinduism, Islam, Judaism, Jainism, and Taoism. The frequency and duration of fasting varies widely, from a few hours to days, and may involve several weeks where food is consumed only at night or early morning, or selected foods are prohibited. Indeed, some cultures even fast for nonreligious reasons; for instance, in Switzerland, a day of fasting is marked as a way of remembering famines, plagues, and wars. The correlation of starvation practices, autophagy, and human health is unclear, as many interconnected cellular pathways work in conjunction, but together all evidence suggests that short-term starvation rituals may up-regulate autophagy, thereby facilitating removal of cytotoxins, improving health, and extending lifespan.

Dictionary of Terms

- **Dimer/Homodimer/Heterodimer** – Dimerization is the association of either two identical (homodimer) or different (heterodimer) polypeptides chains to form a functional protein complex.

- **Domains** – Independently folding structural units of a polypeptide chain/protein that are often the units of evolution.
- **Homolog** – Domains that share the same ancestry and three-dimensional structure and often, significant sequence similarity as well as similar function. Multidomain proteins may be homologous to other proteins over their entire length or over only specific domains.
- **Lysosome** – A cellular organelle comprising a vesicle containing hydrolytic enzymes in an acidic environment
- **Posttranslational modification (PTM)** – The covalent modification of protein side chains, or C- or N- termini, during or after polypeptide biosynthesis. PTMs facilitate cellular signaling by changing protein stability, conformation, function, and/or interactions. The most common PTMs are phosphorylation, acetylation, glycosylation, methylation, hydroxylation, and ubiquitination.
- **Serine/threonine kinase** – An enzyme that phosphorylates substrate proteins at specific amino acids, in this case at serine or threonine side-chain hydroxyls.

Summary Points

- Major starvation signals, such as reduced cellular energy, amino acid starvation, and oxidative stress, induce autophagy, which is essential for cellular recycling of nutrients.
- VPS34 complexes play an essential role in mediating autophagy.
- Starvation signals are transduced via many interacting proteins and posttranslational modifications to VPS34 complexes to regulate autophagy.
- Starvation-induced autophagy has potential health benefits such as in combating neurodegenerative diseases, cancer, heart diseases, and in lifespan extension.
- Thus, short-term starvation or caloric restriction rituals established in several religions and cultures may have beneficial effects on health and longevity.

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Autophagy as a Physiological Response of the Body to Starvation

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Abstract

Macroautophagy is a cellular degradation pathway that deliver cytoplasmic components such as damaged organelles, misfolded proteins, and pathogens to the lysosomes for degradation. Autophagy is associated with the survival of the cell under stress conditions and infections. Nutrient deprivation is one of the main inducers of autophagy, which recycles cytoplasmic components to provide building blocks required for cell survival and maintains cellular homeostasis. Due to its cytoprotective effects, autophagic responses are necessary in resisting diseases and ensuring health. Understanding the regulation of autophagic responses in mammalian cells is required to improve human health through innovations in treatment strategies. This chapter focuses on recent findings about autophagy mechanisms and their role in the body's response to starvation as well as the current knowledge of autophagy-related malnutrition disorders.

Keywords

Autophagy · Starvation · Stress · mTOR · AMPK · LC3 · ULK1 · ATG · Metabolic disease · Lysosome

List of Abbreviations

3HB	3-beta-hydroxybutyrate
AcAc	Acetoacetate
ATG	Autophagy-related proteins
CD	Crohn's disease
CMA	Chaperone-mediated autophagy
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
IBMPFD	Inclusion body myopathy with early-onset Paget disease and frontotemporal dementia
LC3	Microtubule-associated protein 1A/1B-Light chain 3
PE	Phosphatidylethanolamine
PI3K	Phosphatidylinositol 3-kinase
SLE	Systemic lupus erythematosus
Th2	Helper T cell 2
Ub	Ubiquitin
VLDL	Very-low density lipoprotein

Introduction

Autophagy is the primary cellular response of cells to starvation. In mammalian cells, survival responses are mainly dependent on hormonal and neuronal deregulations from stress signals. Mammalian cells respond to starvation by activating lipolysis and proteolysis, which are regulated by autophagic activation pathways upon nutrient deprivation (Hosokawa et al. 2009; Petibone et al. 2017). Activation of

lipolysis is required to provide fatty acids through the breakdown of triacylglycerols as an energy source for different tissues and organs during nutrient deprivation (Cockburn and Coore 1995; Angelini et al. 2016). Moreover, glycerol, which is a side product of lipolysis, can be utilized in the liver to be used as a glucose source under starvation conditions. When cells are exposed to long-term nutrient deprivation and energy loss, an excess amount of lipolysis results in the production of ketone bodies, which are the end product of free fatty acid oxidation reaction and can be used as an energy source in muscle and neurons. Since not all cells have the ability to use ketone bodies as an energy source, the cells that cannot utilize these small molecules activate proteolysis as a response to starvation (Luo et al. 2017; Lee et al. 2017). Proteolysis can be activated in two different ways, depending on the tissue type and stress duration. The first is the ubiquitin-mediated proteolysis system, which selectively degrades the nonfunctional or misfolded proteins which are linked to ubiquitin markers. Ubiquitin-mediated proteolysis is mostly active in skeletal muscle cells. The second pathway is the lysosomal pathway, which is activated upon long-term starvation and is responsible for the nonselective degradation of proteins through enzymatic activity in lysosomes.

Mammalian Autophagy

Autophagy is a well conserved lysosomal catabolic pathway that is required for the recycling of cellular compartments and proteins. However, this chapter is focused on macroautophagy, which is the predominant form in mammals. There are two additional types of autophagy: microautophagy and chaperone-mediated autophagy (CMA). Microautophagy occurs via the direct engulfment of cytoplasmic cargo by lysosomes by which it is degraded by enzymatic activity. CMA is a selective type of autophagy which occurs by chaperone-mediated selection and direct translocation of proteins into lysosomes without any need for the formation of double membrane structures. Macroautophagy (hereafter referred to as autophagy) is mostly activated upon stress signals in mammals; however, it also occurs under normal conditions in order to maintain cellular homeostasis by providing the turnover of long-lived, damaged, nonfunctional proteins in the cell (Erbil et al. 2016; Mathiassen et al. 2017). Upon stress, autophagy starts with the initiation and elongation of the little membrane structures in the cytosol. These double membrane structures engulf the cargo and form the autophagosomes. Lysosomes are then fused with the autophagosomes in order to form autolysosomes, by which the cargo is degraded by lysosomal enzymes and byproducts are released back to the cytosol to be utilized as an energy supply in the cell.

Molecular Signaling Pathways in Starvation-Induced Autophagy

Identification of the autophagy-related (ATG) proteins leads to an improvement of the clarification of the molecular signaling pathways of autophagy. Recently, 36 ATG proteins have been discovered in the core machinery of mammalian autophagy.

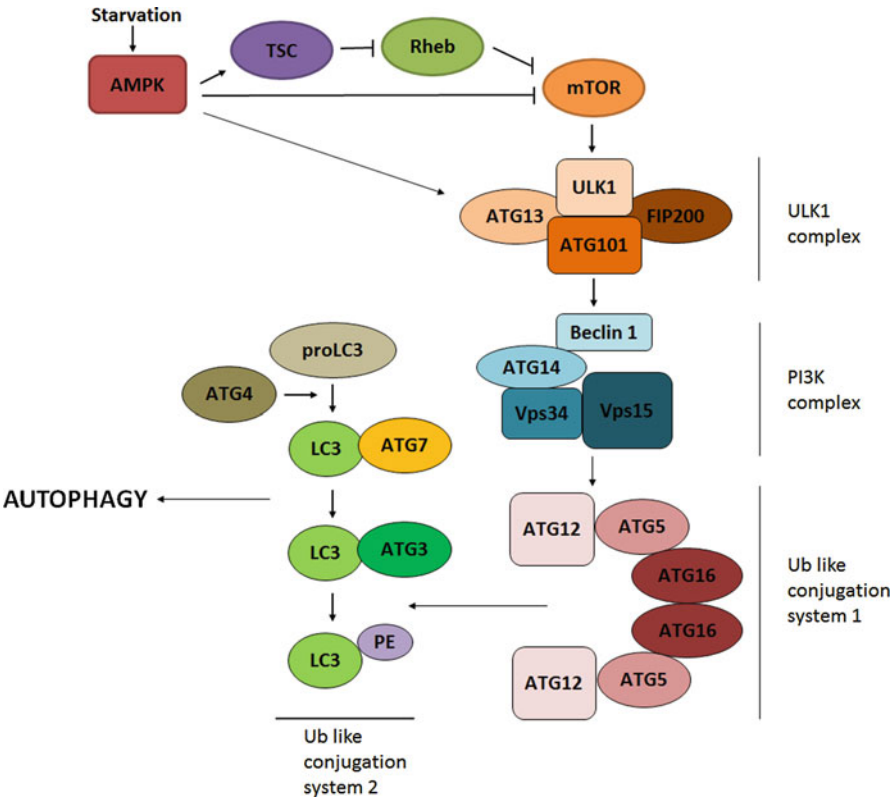

Fig. 1 **Signaling regulation of starvation-induced autophagy**. In mammals, starvation-induced autophagy starts with the activation of AMPK and TSC followed by the inhibition of Rheb and mTOR through phosphorylation events. When nutrients are limited, the phosphorylation of ULK1 by mTOR is inhibited due to mTOR inactivation. Upon dephosphorylation, ULK1 can interact with the other complex elements which mediate the formation of PAS leading to the initiation of autophagosome structures. Further nucleation steps require a PI3K complex which is formed by VPS34 and VPS15 and their regulatory subunits Beclin 1 and ATG14. Membrane elongation and closure requires two different Ub-like conjugation systems. Ub-like conjugation systems 1 and 2 together lead to PE conjugation of LC3 that is essential for the elongation of the membrane structures. Moreover, membrane-bound LC3 is required for the recognition of the cargo through adaptor proteins as well as the lysosome fusion to the autophagosomes. The resulting structure is named as autolysosomes and the cargo is degraded by enzymatic activity inside these structures. Key: PAS, phagophore assembly site; TSC, tuberous sclerosis complex; VPS34 and 15, vacuolar protein sorting 34 and 15; Ub, ubiquitin; PE, phophatidylethanolamine; LC3, microtubule-associated protein 1 light chain 3

These proteins are involved in different functional complexes in autophagycascade. The main four complexes are demonstrated in Fig. 1: ULK1 and phosphatidylinositol 3 kinase (PI3K) complexes, Ubiquitin (Ub)-like conjugation systems 1 and 2. Table 1 summarizes their key roles in the activation of autophagy as the cellular response to starvation in mammals.

Table 1 Key protein complexes acting in starvation-induced autophagy in mammalian cells. ULK1 complex, PI3K complex, Ub-like conjugation system 1, and Ub-like conjugation system 2 are the key protein complexes that act in starvation-induced autophagy. ULK1 complex consists of ULK1, ATG13, FIP200, and ATG101; PI3K complex consists of Beclin1, ATG14, Vps34, and Vps15; Ub-like conjugation system 1 consists of ATG5, ATG12, and ATG16; Ub-like conjugation system 2 consists of ATG3, ATG4, ATG7, and LC3 proteins. Key: PI3K, phosphatidylinositol 3 kinase; ATG3, 4, 5, 7, 12, 13, 14, 16, and 101, autophagy-related proteins 3, 4, 5, 7, 12, 13, 14, 16, and 101; VPS34 and 15, vacuolar protein sorting 34 and 15; Ub, ubiquitin; LC3, microtubule-associated protein 1 light chain 3

Complex	Complex elements	Function
ULK1 complex	ULK1 ATG13 FIP200 ATG101	This complex is negatively regulated by mTOR. Upon autophagy induction by starvation signals received by mTOR, ULK1 is activated by self-phosphorylation and translocates into the early autophagic structures. In addition to its self-phosphorylation, ULK1 phosphorylates ATG13 and FIP200 due to complex activation
PI3K complex	Beclin 1 ATG14 Vps34 Vps15	This complex is negatively regulated by Bcl-2 Beclin 1 interaction. Upon autophagy induction by starvation signals, Bcl-2 disassociates from Beclin 1 and forms the complex with other elements on the ER membrane. This complex is effective in the later autophagic events, as the inhibition of this complex result in the impairment of autophagosome and lysosome fusion
Ub-like conjugation system 1	ATG5 ATG12 ATG16	This complex is necessary for LC3 and phophatidylethanolamine (PE) conjugation which is essential for the elongation of the membrane structures
Ub-like conjugation system 2	ATG3 ATG4 ATG7 LC3	Deconjugation of LC3 form ATG4 is important for the LC3-PE formation and the closure of the autophagosome membrane. Since LC3-PE is localized both in the inner and the outer membranes of the autophagosome, its formation by Ub-like conjugation system 2 is important for the adaptor protein binding, such as p62, for selective autophagy

AMPK is the main energy sensor of the cell, and it plays a crucial role in the induction of starvation-induced autophagy in mammalian cells (Garcia and Shaw 2017). When a nutrient level is limited, AMPK activation leads to mTOR inhibition through TSC activation and Rheb inhibition. Therefore, AMPK indirectly activates the ULK1 complex which is in the downstream of mTOR and kept inactivated through phosphorylation events. Recent studies have showed that in addition to mTOR, AMPK can also phosphorylate ULK1 directly to lead to its activation upon starvation signals. Following phosphorylation, ULK1 interacts with the other complex elements, FIP200, ATG101, and ATG13, to form the ULK1 complex and phosphorylates them to activate the complex (Petherick 2015). The active ULK1 complex translocates to the ER membrane and activates the PI3K complex which consists of Beclin 1, ATG14, and vacuolar protein sorting 34 and 15 (VPS34 and 15). The PI3K complex recruits Ub-like conjugate system 1, formed by ATG12-5-16 proteins, which plays a role in the elongation and closure of the isolation membrane (Zhong et al. 2017). Moreover, Ub-like conjugate system 1 is also required for the

activation of Ub like conjugate system 2, which is necessary for phosphatidylethanolamine (PE) conjugation to LC3 (microtubule-associated protein 1A/1B-light chain 3) protein. The cytosolic form of LC3 is called pro-LC3. Due to the induction of autophagy, pro-LC3 is cleaved by ATG4, and then the cleaved form of LC3 is conjugated with PE by the activities of E1-like enzyme ATG7 and E2-like enzyme ATG3. PE-conjugated LC3 is membrane-bound, and it is present both in the inner and outer sides of the autophagosome (Nath et al. 2014). Therefore, it not only plays a role in membrane elongation and closure but also serves as the recognition site for adaptor proteins such as p62 (Klionsky et al. 2010).

Autophagy at Organ and Tissue Level and Affected Human Diseases from Their Impairment

Dysregulation of autophagy has been implicated in a range of diseases. Starvation-induced autophagy impairments mostly cause liver, pancreas, and muscular tissue disorders (summarized in Fig. 2).

Liver

Basal autophagy is required to maintain liver homeostasis through the clearance of protein aggregates and damaged mitochondria in order to prevent hepatocyte swelling (Wang et al. 2015). Liver autophagy is mainly controlled by hormones. While insulin, aminoacids, and glucose inhibit liver autophagy, glucagon activates the process. Activation of autophagy contributes to lipophagy through fatty acid β-oxidation, ketone body production, and glucogenesis in the liver. Moreover, liver autophagy is involved in the control of very-low-density lipoprotein (VLDL) and plasma glucose concentrations upon nutrient deprivation (Sparks et al. 2013). Since liver and liver autophagy has an extremely important role in homeostasis, the impairment of autophagy in this organ causes vital disorders such as alcoholic liver disease, fatty liver disease, hepatocellular carcinoma (HCC), liver fibrosis, and viral infection in the liver (Niu et al. 2016).

Alcohol consumption activates autophagy in the liver through the AMPK pathway in hepatocytes (Wang et al. 2016). Autophagy activation leads to the removal of lipid droplets and damaged mitochondria, thus preventing cell death. However, if alcohol consumption becomes chronic, excess amounts of ethanol inhibit hepatocellular autophagy by blocking both AMPK activation and vesicular transport that is required for autophagosome formation (Cho et al. 2017). As a result, the accumulation of aggregated proteins and damaged mitochondria leads to cell death. Additionally, lipid droplets that cannot be removed through lipophagy causes steatosis and fatty liver disease.

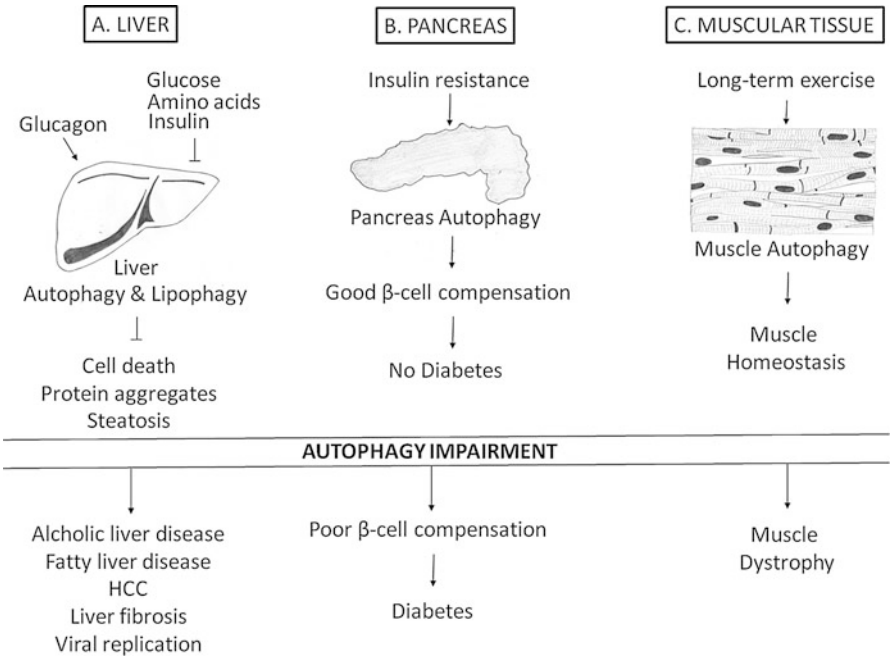

Fig. 2 The role of autophagy in liver, pancreas, and muscular tissue disorders. (**a**) Liver autophagy is inhibited by glucose, insulin, and amino acids under normal conditions. It is induced as the cytoprotective mechanism and increased glucagon level which lead to the inhibition of cell death, steatosis, and the accumulation of protein aggregates. Impaired liver autophagy may result in alcoholic and fatty liver disease, HCC, liver fibrosis, or viral replication in the liver. (**b**) Pancreatic autophagy is activated against insulin resistance to prevent poor β-cell compensation and diabetes. Since autophagy-defective cells cannot compensate the β-cells, diabetes occurs as a result of insulin resistance. (**c**) Muscle autophagy is activated after long-term exercise and maintain muscle cell homeostasis. Autophagy impairment in the muscular tissue result in macular dystrophy. Key: HCC, hepatocellular carcinoma

Hepatitis B and C viruses (HBV and HCV, respectively) require autophagy for their replication in hepatocytes. The viral proteins activate the PI3K complex and trigger autophagosome formation for their benefits (Vescovo et al. 2016). Therefore, regulation of autophagy in the liver is vital for viral infections that may end up with HCC.

Pancreas

Pancreatic β-cells are responsible for the storage and release of insulin (Morita et al. 2017). Pancreas autophagy has a significant role in maintaining β-cell homeostasis by keeping their mass and function properly. Impairment of autophagy results in dysfunctional β-cell and type-2 diabetes which is characterized by insulin resistance.

In vivo studies in ATG7 -/- mice have revealed that autophagy-defective β-cells lose their mass and insulin content, which causes suppressive insulin release (Rivera et al. 2014). Moreover, since autophagy is necessary for the clearance of damaged mitochondria, impaired autophagy in pancreatic β-cells leads to the rise of insulin resistance and impaired glucose tolerance (Xu et al. 2016).

Muscular Tissue

Similar to other organs, in muscle cells, autophagy is vital for organelle and protein aggregate clearance. However, excess amount of autophagy activation cause muscle weight loss. Impaired autophagy was also found as a main reason of malfunctional and dystrophic muscles after long-term exercise or starvation (Fiacco et al. 2016).

Autophagy is one of the major pathways of muscle homeostasis. It preserve myofiber integrity and prevents muscle damage. Autophagy impairment has been reported in several muscle diseases such as inclusion body myopathy with early-onset Paget disease and frontotemporal dementia (IBMPFD) (Bayraktar et al. 2016), Pompe Disease (Lim et al. 2017), and Danon Disease (Nascimbeni et al. 2017) (please see "Dictionary of Terms" section). In ATG7 null muscle cells obtained from ATG7-/- mice, distension of sarcoplasmic reticulum and random nonfunctional membrane structure accumulations were demonstrated (Mizushima and Komatsu 2011). All these effects may be the reason for muscular dystrophies which directly result in patient death due to myofibre necrosis in respiratory and cardiac systems.

Other Human Diseases Affected by Mutations in Starvation-Induced Autophagy Genes

Emerging molecular techniques and accumulated data about autophagy pathways allow researchers to carry out specific analyzes. Therefore, the involvement of autophagy, particularly on the molecular level, in human diseases is an attractive topic for clinical research. These diseases include asthma, systemic lupus erythematosus (SLE), Crohn's disease (CD), several types of cancers, and obesity (The diseases found to be directly related to the starvation-induced autophagy gene mutations are listed in Table 2.).

Asthma

Asthma is a common respiratory tract inflammation disease that causes high rates of death all over the world. Using a mice model, *in vivo* studies have showed that autophagy has a role in the clearance of pathogens in canonical ways, as well as its effect on helper T cell 2 (Th2) activation due to infection. Moreover, polymorphisms in ATG5 gene result in asthma symptoms in mice models (Martin et al. 2012; Pham et al. 2016).

Table 2 Known human diseases affected by mutations in starvation-induced autophagy genes. Impairments of starvation-induced autophagy resulted from ATG5 polymorphism, ATG16L T300A mutation, Beclin1 monoallelic deletion, mutations in PI3K and TSC1, or loss of ATG7 are the known causes of human diseases such as asthma, systemic lupus erythematosus, several types of cancer, and obesity. Key: ATG5, 7, and 16L, autophagy-related proteins 5, 7 and 16 like 1; T300A, threonine300 mutation to alanine; PI3K, phosphatidylinositol 3 kinase; TSC1, tuberous sclerosis complex 1

Mutation	Associated human disease
ATG5 polymorphism	Asthma (Martin et al. 2012), systemic lupus erythematosus (Zhou et al. 2011)
ATG16L T300A mutation	Crohn's disease (Massey and Parkes 2007)
Beclin 1 monoallelic deletion	Breast, ovarian, colorectal, and prostate cancers (Liang et al. 1999)
Gain of function mutations in PI3K	Cancer (Cully et al. 2006)
TSC1 mutation	Benign hamartomatous growths in multiple organs (Schwartz et al. 2007)
Loss of ATG7	Obesity (Yang et al. 2010)

Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is an autoimmune disease that is described by the self-reaction of the immune system to healthy tissues and organs (Gavand et al. 2017). The pathogenesis of the disease is not clear yet; however, 50% of patients are affected genetically. In the Chinese population, rare variants ATG5 were shown to be linked to SLE. In other words, one of the reasons for SLE disease is ATG5 polymorphism (Zhou et al. 2011). Therefore, autophagy proteins could be a potential therapeutic target for SLE treatment.

Crohn's Disease

Crohn's disease (CD) is an inflammatory bowel disease characterized by multiple gastrointestinal pathology from mouth to anus (Stappenbeck et al. 2011). However, it is not classified as an autoimmune disease; it is an immune system disorder. Although genetic and environmental factors, infections, and defective autophagy are reported in CD, the causal etiologic factors are still unknown. Analyzing 735 CD patients' genotypes in comparison to healthy controls showed that the T300A mutation of ATG16 protein is responsible for Crohn's disease (Massey and Parkes 2007). However, it is not the only reason for CD.

Cancer

Genetic link between cancer and autophagy has been shown in various cancer types, including human breast, prostate, colorectal, and ovarian cancer (Sumis et al. 2016). Most of the studies have showed that Beclin 1 gene is monoallelically

deleted in most of the cancer types (Liang et al. 1999). Also, PI3K mutations cause tumor formation and TSC1 mutation leads to benign hamartomatous growths in multiple organs (Cully et al. 2006; Schwartz et al. 2007). As an evident to anticancer effect of autophagy, several studies have reported that the overexpression of ATG genes in cancer cells lead to tumor suppression (Wesselborg and Stork 2015). Although, autophagy is indeed a tumor suppressor pathway, the mechanisms and molecules playing part behind it remain largely unclear.

Obesity

Obesity is the medical condition, in which the body's fat rate is too much that negatively affects the body health (Yilmaz 2017). It is an excess amount of energy flow which disrupts the energy sensing and the utilizing mechanisms of the body. The role of autophagy in obesity was first studied in mice hepatocytes (Yang et al. 2010). In the study, obese mice hepatocytes represent low levels of ATG7 expression, while the restoration of ATG7 resulted in elevated insulin levels (Zhang et al. 2009). However, it is known that environmental conditions trigger tendency to obesity, which seems to lead autophagy impairments.

Policies and Protocols

Measuring autophagic activity at cellular level is crucial to identify therapeutic targets for diseases affected by autophagy impairments. In order to detect autophagy activation or inhibition, several molecular techniques are developed based on immunoblotting, immunofluorescence, or isotope release as described in Table 3.

Detection of autophagosomes and autolysosomes by electron microscopy can be used as a conventional autophagy procedure. By using immunoblotting technique, the conversion of cytosolic nonlipidated LC3-I into membrane-bound lipidated form of LC3-II is commonly used for analyzing autophagy induction. However, LC3 is not the direct target of autophagy, as it is degraded in autolysosomes after long-term autophagy. Therefore, LC3 turnover assay is carried out using autophagy inhibitors in order to estimate the level of LC3 protein degradation. Similarly, the GFP-tagged LC3 protein is overexpressed in mammalian cells and the amount of generated free GFP protein can be measured for autophagic activity estimation.

By using immunofluorescence, counting the average number of GFP-LC3 punctate structures on the autophagosome membranes or endogenous LC3 protein are other well-known autophagy techniques. RFP-GFP LC3 protein construct can be used to distinguish the number of autophagosomes and autolysosomes, since GFP signal is quenched by the acidic environment of autolysosomes.

Lastly, long-lived protein degradation can be measured by tracing the radioactive isotope released from the cells indicating active autophagy state.

Table 3 Protocols for detecting autophagy in mammalian cells. Immunoblotting, immunofluorescence, and isotope release are the three main protocols for detecting autophagy in mammalian cells. LC3 conversion, LC3 turnover, and GFP-LC3 cleavage tests can be carried out and the amount of p62 in the cell can be detected by using immunoblotting procedures. Number of LC3 puncta/cell, RFP-GFP LC3 color change, and the amount of p62 in the cell can be measured by using immunofluorescence techniques; while lysosome-dependent long-lived protein degradation tests can be carried out by using isotope release protocols. Key: LC3, microtubule-associated protein 1 light chain 3; GFP, green fluorescent protein; RFP, red fluorescent protein

Immunoblotting	Immunofluorescence	Isotope release
1. LC3 conversion 2. LC3 turnover 3. Amount of p62 in the cell 4. GFP-LC3 cleavage	1. Number of LC3 puncta/cell 2. Amount of p62 in the cell 3. RFP-GFP LC3 color change	1. Lysosome-dependent long-lived protein degradation

Dictionary of Terms

- **Cellular stress** – Anything that creates a challenge or a constraint to cell health. Cellular stress can be intrinsic, such as DNA damage, misfolded protein accumulation, and damaged organelles; or extrinsic, such as starvation, toxins, pathogens, toxins, heat, etc.
- **Danon disease** – An X-linked glucagon storage disorder that is characterized by weakened skeletal and cardiac muscle combined with mental retardation.
- **Glucagon** – The hormone that is produced and secreted by pancreatic α-cells. Its role is opposite to insulin, as it increases the glucose levels in the blood.
- **Glucogenesis** – The production of glucose by the breakdown of glycogen that is stored in the liver.
- **Homeostasis** – Disposition of a physiological system to preserve its internal stability and to keep vital functions active
- **IBMPFD** – Inclusion body myopathy with early-onset Paget disease and frontotemporal dementia, which is a rare multisystem disease characterized by the weakness of muscles and bones in combination to intellectual disability.
- **Insulin** – The hormone that is produced in pancreatic β-cells and secreted into blood stream where it meets with glucose and enables it to enter the cells where needed. If glucose level is higher than needed in the blood, pancreatic β-cells produce and secrete more insulin in order to store the excess amount of glucose. Therefore, insulin is the key hormone that keeps blood sugar level stable.
- **Insulin resistance** – The pathological condition in which the cells do not recognize insulin and the blood sugar cannot be utilized by the cells. As a result of insulin resistance, the blood sugar level gets higher than the normal.
- **Ketone bodies** – The water-soluble molecules that are produced in the liver, as the end product of fatty acid oxidation due to energy limitation in the body. There are two main types of ketone bodies: acetoacetate and 3-beta-hydroxybutyrate

and the spontaneous breakdown product of them is acetone which can be counted as the third type of ketone bodies that are present in the blood.

- **Lipolysis** – Breakdown of lipid molecules by the hydrolysis of triglycerides to free fatty acids and glycerol.
- **Pompe disease** – A genetically inherited glucose storage disorder that result in the accumulation of glycogen in muscle cells disabling them to function normally.
- **Proteolysis** – Breakdown of proteins into aminoacids or smaller aminoacids by protease enzyme activity.

Summary Points

- This chapter focuses on the recent findings about autophagy mechanism and its role in the body response to the starvation as well as the current knowledge of autophagy-related malnutrition disorders.
- Autophagy is a cellular degradation pathway that deliver cytoplasmic components such as damaged organelles, misfolded proteins, and pathogens to the lysosomes.
- Autophagy is associated to the survival of the cell under stress conditions and infections. The role of autophagy in cell survival is summarized in Fig. 3.

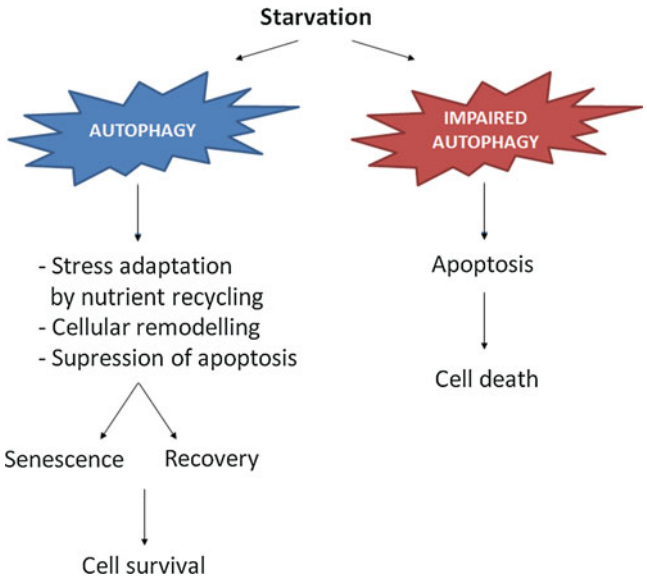

Fig. 3 The role of autophagy in cell survival. (*Left*) Autophagy acts as a survival mechanism of the cell supporting the cellular adaptation under stress conditions by nutrient recycling and cellular remodeling; (*Right*) impaired autophagy result in the rapid cell death since apoptotic pathway cannot be inhibited

- Nutrient deprivation is one of the main inducers of autophagy that recycle cytoplasmic components to provide building blocks required for cell survival and maintain cellular homeostasis
- Autophagic responses are necessary for resisting diseases and maintaining health.
- Understanding the regulation of autophagic responses in mammalian cells are required for improving human health by innovations in treatment strategies.

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Mitophagy in Starvation

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Shun-ichi Yamashita and Tomotake Kanki

Contents

Abstract

Autophagy is a cellular degradation system and is drastically induced under starvation. In contrast to the ubiquitin–proteasome system, autophagy is a non-selective bulk degradation system. In the last decade, autophagy has been found

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to degrade cargo in a selective manner. Mitochondria can be degraded through mitochondria-specific autophagy, which is termed mitophagy, and several important factors have been identified in yeast and mammals. Atg32, a mitochondrial membrane protein, functions as a receptor during mitophagy under starvation in yeast. Post-translational modification of Atg32 induces mitochondrial degradation. In mammals, several different factors for mitophagy have been reported from different research groups. In this review, we summarize the molecular mechanisms of mitophagy in yeast and mammals with respect to Atg32 in yeast and its functional homolog in mammals.

Keywords

Autophagy · Mitophagy · Mitochondria · *Saccharomyces cerevisiae* · Nitrogen starvation · Atg32 · Casein kinase · MAP kinase · Hypoxia · Tor kinase

List of Abbreviations

AIM	ATG8 interacting motif
ALS	Amyotrophic lateral sclerosis
ATG	Autophagy-related
BCL2L13	Bcl2-like13
BNIP3	BCL2/adenovirus E1B 19-kDa-interacting protein 3
BNIP3 like	BNIP3L
CALCOCO2/NDP52	Calcium binding and coiled-coil domain 2
CK2	Casein kinase 2
Drp1	Dynamin-related protein 1
ERK	Extracellular signal-regulated kinase
FUNDC1	FUN14 domain-containing protein 1
IMM	Inner mitochondrial membrane
LIR	LC3-interacting region
MAPK	Mitogen-associated protein kinase
MEK	MAPK/ERK kinase
MFF	Mitochondrial fission factor
MFN	Mitofusin
MiD	Mitochondrial dynamics protein
MKK	MAP kinase kinase
MLK	Mixed-linage protein kinase
mtROS	Mitochondrial ROS
NBR1	Neighbor of BRCA1 gene 1
OMM	Outer mitochondrial membrane
OPA1	Optic atrophy 1
OPAG	Primary open angle glaucoma
OPTN	Optineurin
PAS	Pre-autophagosomal structure
PI3K	Phosphatidylinositol 3-kinase
PI3P	Phosphatidylinositol 3-phosphate
PINK1	PTEN-induced putative kinase 1

RAF	Rapidly accelerated fibrosarcoma
ROS	Reactive oxygen species
TAX1BP1	TAX1 binding protein 1
TOR	Target of rapamycin

Introduction

In eukaryotic cells, there are two major pathways for protein degradation called autophagy and the ubiquitin–proteasome system. Generally, autophagy is a non-selective bulk degradation pathway, whereas the ubiquitin–proteasome system is selective (Hershko et al. 2000; Coux et al. 1996). This non-selective autophagy is generally termed macroautophagy, which functions not only at the basal level of protein turnover but also in considerable degradation of cytoplasmic components under starvation. Macroautophagy contributes to maintaining cellular amino acid pools for basal protein synthesis for cellular survival (Takeshige et al. 1992; Nakatogawa et al. 2009). However, recently, some types of autophagy were found to degrade specific substrates selectively. Protein aggregates, organelles, and invading bacteria are degraded by autophagy selectively (Johansen and Lamark 2011; Komatsu et al. 2007). Additionally, mitochondria are also known to be degraded by selective autophagy, which is termed mitophagy (Lemasters 2005) and is conserved from yeast to mammals.

Mitochondria are double membrane-bound organelles and play a pivotal role in cellular metabolism, including ATP synthesis, calcium buffering, regulation of apoptosis, β-oxidation of fatty acids, and thermogenesis (Mcbride et al. 2006; Brookes et al. 2004; Wallace 2005; Wanders et al. 2010; Gambert and Ricquier 2007). Therefore, quality control of mitochondria is important for maintaining cellular activity. Mitochondrial activity is maintained by mitochondrial interaction through their fusion and fission (Nakada et al. 2001). When inactive portions or damaged portions of mitochondria emerge, they can be complimented by fusion with a healthy mitochondrial portion. Additionally, when the mitochondrion is severely damaged, the damaged portion of the mitochondrion is excluded from the healthy mitochondrial network by fission and is then degraded by mitophagy (Twig et al. 2008). Accordingly, mitophagy might prevent accumulation of damaged mitochondria within the cells and prevent cellular damage by reactive oxygen species (ROS) produced from damaged mitochondria. Mitochondrial ROS are potentiated in mitophagy-defective yeast cells and cause mitochondrial DNA deletion and growth defects (Kurihara et al. 2012).

In budding yeast, Atg32 is the master regulator for mitophagy (Kanki et al. 2009; Okamoto et al. 2009). Atg32 is an outer mitochondrial membrane (OMM) protein and binds with cytosolic Atg11. Atg11 is an adaptor protein for selective autophagy that allows selective degradation of mitochondria (Kanki et al. 2009; Okamoto et al. 2009).

In contrast to yeast, several types of mitophagic pathways have been reported in mammalian cells. One of the well-studied pathways is PTEN-induced putative

kinase 1 (PINK1)-Parkin-mediated mitophagy. PINK1 and Parkin are causative genes of familial Parkinson's disease. Recent studies have shown that PINK1 and Parkin accumulate on damaged mitochondria and induce its degradation (Narendra et al. 2008). Another type of mitophagy is mitochondrial receptor-mediated mitophagy. Several mitophagy receptors have been identified, including BCL2/adenovirus E1B 19-kDa-interacting protein 3 (BNIP3) (Zhu et al. 2013; Thomas et al. 2011), BNIP3L/NIX (Schweers et al. 2007; Sandoval et al. 2008), FUN14 domain-containing protein 1 (FUNDC1) (Liu et al. 2012), and Bcl2-like 13 (BCL2L13) (Murakawa et al. 2015). Unfortunately, these receptors were evaluated by mutually different methods. Therefore, the priority and importance of these proteins in several situations and tissues remain unclear. In this review, we summarize the molecular mechanisms, signal transduction, and physiological relevance of mitophagy in yeast and mammals.

Molecular Mechanisms and Signaling of Autophagy

Autophagy is a subcellular degradation pathway and is drastically induced by starvation. Upon starvation, a membranous structure termed the isolation membrane/phagophore first emerges at the initiation site of autophagosome formation and then elongates and engulfs the cargo. Eventually, a double membrane-bound structure (termed an autophagosome) containing the cargo is formed and then fuses with vacuoles/lysosomes. Many proteins required for autophagy have been identified. Autophagy proceeds through the functions of autophagy-related proteins termed ATG proteins. More than 40 proteins belonging to this family have been identified (Itakura and Mizushima 2010; Koyama-Honda et al. 2013). ATG proteins can be divided into several functional groups, including the ATG1/ULK1 complex (ATG1/ULK1/2, ATG13, ATG17/FIP200, ATG101) (Hosokawa et al. 2009; Jung et al. 2009), ATG9 vesicle, the class III phosphatidylinositol 3-kinase (PI3K) complex (VPS34, VPS15/p150, ATG14, ATG6/Beclin1), phosphatidylinositol 3-phosphate (PI3P)-binding proteins and their interaction partner proteins (ATG18/WIPIs, ATG2, DFCP1), and two ubiquitin-like conjugation systems (ATG12, ATG7, ATG10, ATG5 and ATG8, ATG7, ATG3). These core ATG proteins are conserved from yeast to mammals and required for almost all autophagic pathways (listed in Table 1).

In the process of autophagosome formation (Fig. 1), the ATG1/ULK1 complex and ATG9 are initially recruited to the site for initiation of autophagosome formation, termed the pre-autophagosomal structure (PAS). The PAS is adjacent to vacuoles in yeast or a site close to the endoplasmic reticulum in mammals (Nakatogawa et al. 2009; Hamasaki et al. 2013). ATG9 localizes on the vesicle, shuttling between the Golgi apparatus, endosomes, and the initiation site for autophagosome formation (Young et al. 2006; Orsi et al. 2012). After ATG1/ULK1 complex and ATG9 vesicle recruitment to the initiation site, the PI3K complex localizes at the site and forms PI3P. PI3P-binding proteins localize at the site through binding to PI3P and contribute to elongation of the isolation

Table 1 Autophagy-related proteins in yeast and mammals

Yeast	Mammal	Function
Atg1/ULK1 complex		
Atg1	ULK1/2	Serine/threonine proteinkinase
Atg13	Atg13	Component of Atg1/ULK1 complex
Atg17	FIP200	Component of Atg1/ULK1 complex
	Atg101	Component of Atg1/ULK1 complex
Phophatidylinositol 3-kinase complex		
Atg14	Atg14L	Component of Class III PI3K complex
Atg6/ Vps30	Beclin1	Component of Class III PI3K complex
Vps34	Vps34	Catalytic subunit of Class III PI3K complex
Vps15	Vps15	Component of Class III PI3K complex
Phosphatidylinositol 3 phosphate binding proteins and Atg9 vesicle cycling		
Atg18	WIPI1/2/3/4	WD40 repeat protein, PI3P binding protein
Atg9	Atg9A, Atg9B	Transmembrane protein
Atg2	Atg2A, Atg2B	Atg9 vesicle cycling
Ubiquitin-like conjugation system		
Atg8	LC3A/B/C, GABARAP, GABARAPL1/2/3	Conjugated to phosphatidylethanolamine
Atg12	Atg12	Conjugated to Atg5
Atg4	Atg4A/B/C/D	Cysteine protease for C-terminal region of Atg8 family proteins
Atg7	Atg7	E1-like enzyme for Atg8 family and Atg12 conjugation system
Atg3	Atg3	E2-like enzyme for Atg8 family proteins
Atg10	Atg10	E2-like enzyme for Atg12 family proteins
Atg5	Atg5	Conjugated by Atg12
Atg16	Atg16L	Component of Atg12-Atg5-Atg16 complex

membrane (Polson et al. 2010; Proikas-Cezanne et al. 2004). Two ubiquitin-like conjugation systems then mediate closure of the isolation membrane. Finally, the autophagosome fuses with a vacuole/lysosome and the cargo is degraded by vacuolar/lysosomal proteinases. The autophagosome–lysosome fusion step is mediated through an interaction between syntaxin 17, an autophagosomal soluble *N*-ethylmaleimide-sensitive factor activating protein receptor, and the homotypic fusion and protein sorting-tethering complex (Itakura et al. 2012; Jiang et al. 2014). Starvation signals enter the initial complex of autophagy, the ATG1/ULK1 complex, via nutrient-sensing kinases. Under nutrient-rich conditions, target of rapamycin (TOR) kinase/mammalian TOR (mTOR) kinase inhibits the ATG1/ULK1 complex by phosphorylation of ULK1 and ATG13. Under starvation conditions, TOR/mTOR kinase is inactivated, resulting in dephosphorylation and activation of ULK1 and ATG13 (Fujioka et al. 2014; Hosokawa et al. 2009; Kamada et al. 2000).

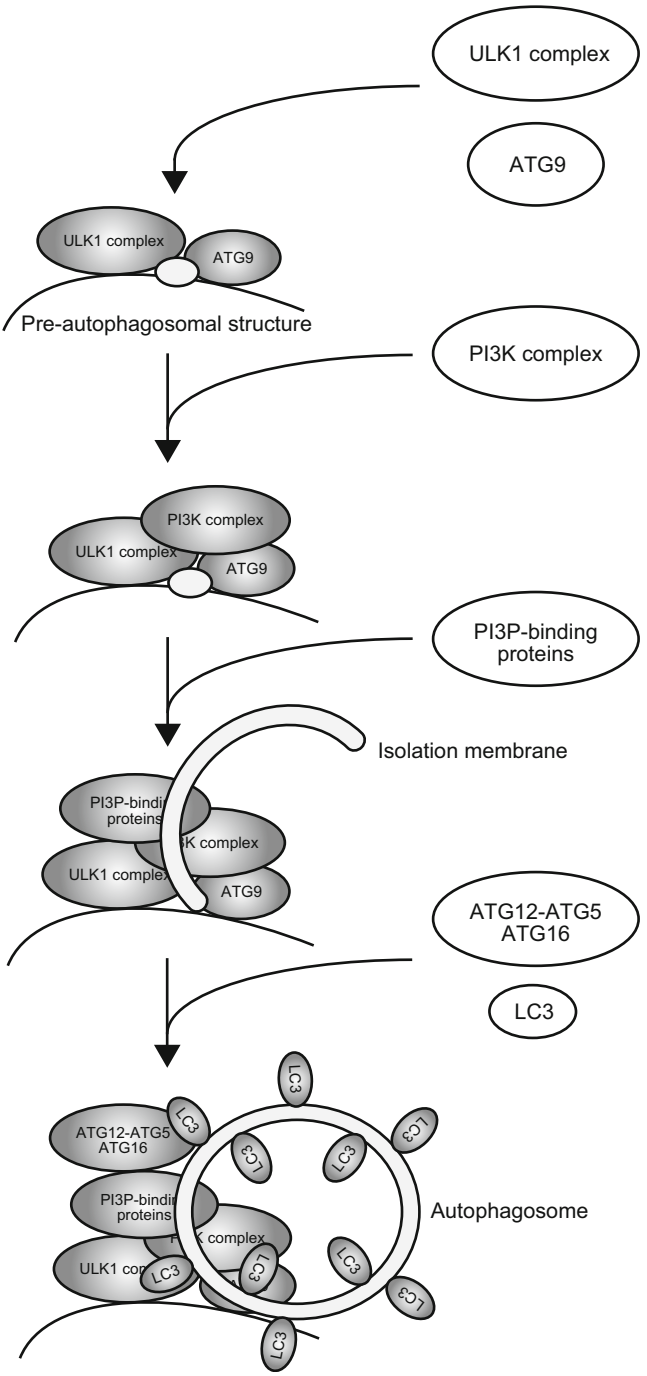

Fig. 1 (continued)

Signaling Pathways of Starvation for Inducing Mitophagy in Yeast

Nitrogen starvation is the most commonly used cellular stress to induce mitophagy in yeast. However, the cells are usually pre-cultured in medium with a non-fermentable carbon source as the sole carbon source before nitrogen starvation to enhance the level of mitophagy. Two mitogen-activated protein (MAP) kinases (Hog1 and Slt2) are involved in mitophagy. The upstream factors of Hog1 signaling, Sln1, Ssk1, and Pbs2, and the upstream factors of Slt2 signaling, Wsc1, Pkc, Bck, and Mck1/2, are also involved in induction of mitophagy (Mao et al. 2011). This suggests that the cell wall osmosensor Sln1 and the stress sensor Wsc1 recognize cellular stress by starvation and activate the Hog1 and Slt2 signaling cascade to induce mitophagy. However, downstream of Hog1 and Slt2 is not well understood.

Starvation inactivates TOR kinase. It was shown that expression levels of a key factor of mitophagy, Atg32, were suppressed by Tor kinase and its downstream histone deacetylase complex (Ume6-Sin3-Rpd3) under normal conditions. Upon starvation or rapamycin treatment, TOR kinase and the downstream complex Ume6-Sin3-Rpd3 were inactivated, and then expression of Atg32 was increased (Aihara et al. 2014). This regulation of Atg32 expression is important to efficiently induce mitophagy responding to starvation. The expressed Atg32 targets the mitochondrial outer membrane and is phosphorylated by starvation. This phosphorylation is essential for mitophagy (see below).

Atg32, a Receptor Protein for Mitophagy

Atg32, which is an outer mitochondrial membrane protein, is required for mitophagy in yeast. Atg32 has a single transmembrane domain and its N-terminus is oriented toward the cytoplasm (Fig. 2). After nitrogen starvation, two serine residues, Ser 114 and 119, on the cytoplasmic region of Atg32 are phosphorylated by casein kinase 2 (CK2) (Aoki et al. 2011; Kanki et al. 2013). Phosphorylated Atg32 then interacts with cytoplasmic Atg11, an adaptor protein for selective autophagy. Finally, Atg11 anchors mitochondria on the PAS for selective degradation. Therefore, the phosphorylation-mediated Atg32–Atg11 interaction is essential for mitochondrial recognition as cargos. However, how phosphorylation of Atg32 by CK2 is regulated under starvation is still unclear.

Atg8, an ubiquitin-like modifier, is conjugated to phosphatidylethanolamine, which is located on the isolation membrane. The interaction between Atg8 and

Fig. 1 Atg proteins in autophagosome formation. The Atg1/ULK1 complex and Atg9 vesicle are initially recruited to the site for autophagosome formation, resulting in the pre-autophagosomal structure (*PAS*). Subsequently, the phosphatidylinositol 3-kinase (*PI3K*) complex localizes at the site and forms phosphatidylinositol 3-phosphate (PI3P). PI3P-binding proteins localize at the site through PI3P-binding. Finally, two ubiquitin-like conjugation systems mediate closure of the isolation membrane, resulting in autophagosomes

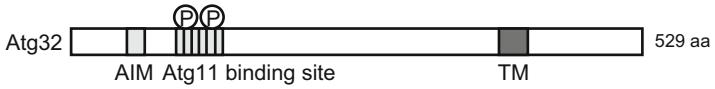

Fig. 2 Functional domains of Atg32 for mitophagy. Atg32, an outer mitochondrial membrane protein, consists of a C-terminal single transmembrane domain and N-terminal cytosolic region. The N-terminal region has two functional domains of the Atg8-family-interacting motif (*AIM*) and Atg11-binding site. Under starvation conditions, phosphorylation at Ser 114 and 119 by casein kinase 2 is required for binding with Atg11

receptor proteins for selective autophagy through the Atg8-interacting motif (AIM)/LC3-interacting region (LIR) is important for selective autophagy. The consensus sequence of AIM (W/Y-X-X-L/I/V) can be found in almost all receptor proteins for selective autophagy from yeast to mammals. Atg32 also possesses an AIM in its cytoplasmic region and interacts with Atg8 (Okamoto et al. 2009). In this case, however, an AIM is not essential for mitophagy because an Atg32 mutant lacking AIM, which cannot interact with Atg8, only has a partial effect on mitophagy (Kondo-Okamoto et al. 2012). Therefore, the Atg32–Atg8 interaction may facilitate engulfment of mitochondria by autophagosomes via tethering mitochondria to the isolation membrane.

Mitophagy in Mammals

Mitochondrial Receptor Proteins for Mitophagy in Mammalian Cells

In mammalian cells, some functional counterparts of Atg32 in yeast, receptor proteins for mitophagy, have been reported by several groups. All of the receptor proteins, FUNDC1, BNIP3, BNIP3L/Nix, and BCL2L13, are outer mitochondrial membrane proteins and have AIM/LIR in their cytoplasmic region (Fig. 3). LC3 is a mammalian homolog of Atg8 and is localized on the isolation membrane. Mitophagy receptor proteins interact with LC3 via LIR and facilitate engulfment of mitochondria by autophagosomes. Similar to Atg32 in yeast, the function of these mitophagy receptors are thought to be regulated by their phosphorylation. These receptor proteins for mitophagy in mammalian cells are summarized below.

FUNDC1

FUNDC1 has three transmembrane domains and exposes its N-terminal region containing LIR to the cytoplasm. Under normal cell culture conditions, Ser 13 and Tyr 18 on FUNDC1 are phosphorylated by CK2 and Src kinase, which inhibit the interaction between FUNDC1 and LC3 (Liu et al. 2012; Chen et al. 2014). Upon mitophagic conditions, such as hypoxia or depolarization of the inner mitochondrial membrane (IMM), dephosphorylation of Ser 13 on FUNDC1 is induced by the phosphoglycerate mutase family member 5 phosphatase. This leads to activation of

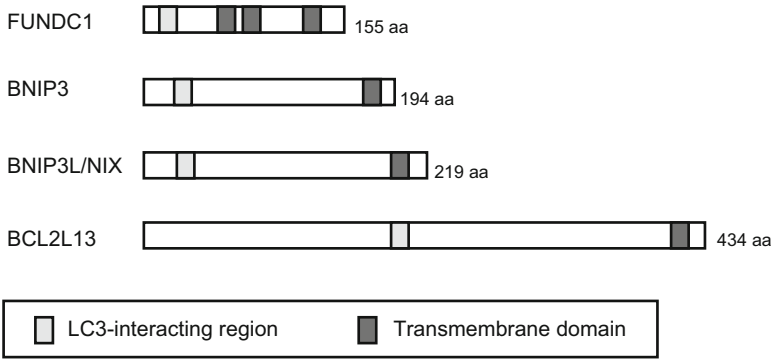

Fig. 3 Mitophagy receptor proteins in mammalian cells. FUNDC1, BNIP3, BNIP3L/NIX, and BCL2L13 are localized at the outer mitochondrial membrane through C-terminal transmembrane domains, and N-terminal regions are oriented toward the cytoplasm. The LC3-interacting region (*LIR*) is located at the N-terminal region of these receptors

an interaction with LC3 proceeding mitophagy. Additionally, Ser 17 on FUNDC1 is phosphorylated by ULK1, and this phosphorylation enhances FUNDC1 interaction with LC3 (Wu et al. 2014).

BNIP3 and BNIP3L

BNIP3 and its homolog BNIP3L/NIX are BH3-only proteins and members of the pro-apoptotic BCL2 family. They are localized on the OMM via their C-terminal transmembrane domains, and their N-terminal regions are exposed to the cytoplasm. Under hypoxic conditions, expression of BNIP3 and BNIP3L/NIX is increased at the transcriptional level by stabilization of hypoxia-inducible factor 1. BNIP3 and BNIP3L/NIX can induce loss of the mitochondrial membrane potential, permeability of the OMM, and cell death through their homodimerization (Kubli et al. 2007; Chen et al. 2010). In addition to cell death, these proteins can also induce macroautophagy and mitophagy. The N-terminal regions of BNIP3 and BNIP3L/NIX contain LIR and can interact with LC3 upon mitophagy. In the case of BNIP3, Ser 17 and 24 adjacent to the LIR are phosphorylated by an unidentified kinase. Phosphorylation of BNIP3 on Ser 17 is required for its interaction with LC3, whereas phosphorylation at Ser 24 only enhances the affinity (Zhu et al. 2013). BNIP3L/NIX-dependent mitophagy is particularly important for elimination of mitochondria during maturation of erythroid cells (Sandoval et al. 2008). In this case, BNIP3L/NIX induces loss of the mitochondrial membrane potential and autophagic elimination of mitochondria. BNIP3 and BNIP3L/NIX are also involved in regulation of bulk autophagy as upstream signal transduction machinery. Under hypoxic conditions, BNIP3 and BNIP3L/NIX expression levels are increased. This can lead to release of Beclin1, an essential component of the autophagy-specific PI3K complex, from complexes with Bcl-XL and Bcl-2 through their competitive binding with Bcl-proteins (Bellot

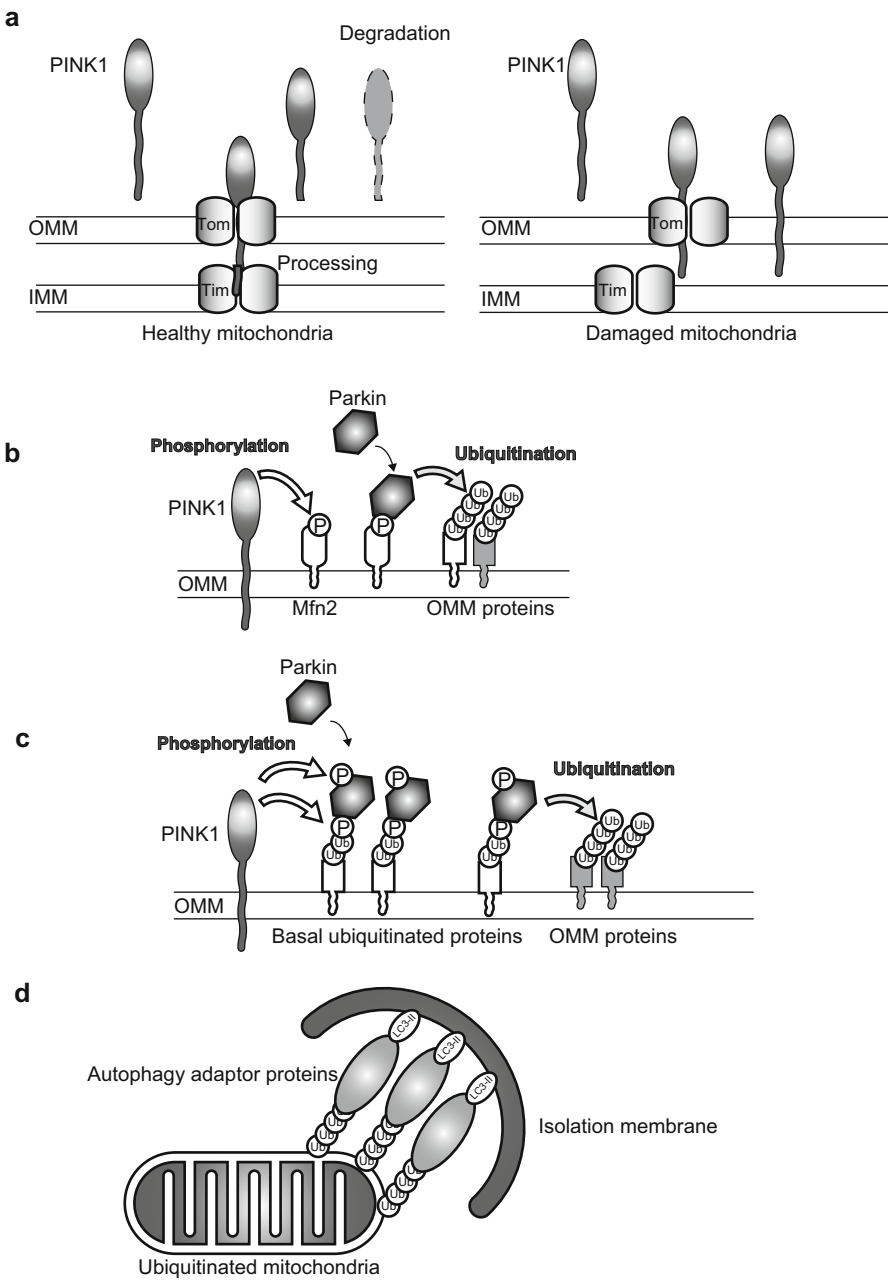

Fig. 4 PINK1-Parkin-mediated mitophagy. (**a**) PINK1 is constitutively targeted to the inner mitochondrial membrane (*IMM*). IMM targeted-PINK1 is degraded in healthy mitochondria, while it is stabilized at the outer mitochondrial membrane (*OMM*) in damaged mitochondria. (**b**) PINK1 phosphorylates mitofusin 2 (*Mfn2*) and Parkin is translocated to the OMM through binding

et al. 2009). Further, BNIP3 and BNIP3L/NIX can bind to Rheb GTPase, a factor for mTOR complex 1 (mTORC1) activation, and enhance autophagic activity by inactivation of mTORC1 activity (Li et al. 2007).

Bcl2L13

Recently, Bcl2-like 13/Bcl-Rambo (BCL2L13) was identified as one of the functional counterparts of Atg32. Similar to Atg32 and the other mitophagy receptor proteins in mammals, BCL2L13 also localizes at the OMM via the C-terminal transmembrane domain and its N-terminal region is exposed to the cytoplasm. The LIR is found in the N-terminal region, and it can interact with LC3 upon mitophagic conditions. Mutation at S272A adjacent to the LIR in BCL2L13 causes a decrease in the binding affinity to LC3. Therefore, the binding between BCL2L13 and LC3 is likely regulated by phosphorylation at Ser 272, resembling the other adaptor proteins for mitophagy (Murakawa et al. 2015).

Autophagy Adaptor Proteins in PINK1 and Parkin-Related Mitophagy

In the last decade, PINK1- and Parkin-mediated mitophagy has been extensively examined (Fig. 4). Serine/threonine kinase PINK1 and the E3 ubiquitin ligase Parkin are encoded by PARK6 and PARK2, respectively, and they are linked to familial Parkinson's disease. PINK1 is constitutively targeted to the IMM via a mitochondrial targeting sequence at its N-terminal region. IMM-targeted PINK1 is rapidly degraded by the combination of presenilin-associated rhomboid-like protein and the proteasome (Jin et al. 2010; Matsuda et al. 2010). Therefore, mitochondrial PINK1 is maintained at a low level in healthy mitochondria (Fig. 4a). However, upon loss of the mitochondrial membrane potential, PINK1 cannot translocate IMM and is accumulated on the OMM (Fig. 4a). OMM-localized PINK1 recruits Parkin from the cytoplasm to mitochondria through its kinase activity dependent pathways. The molecular mechanism for PINK1 recruiting Parkin is not well understood. One possible mechanism is that PINK1 phosphorylates mitofusin 2 (Mfn2) and phosphorylated Mfn2 interacts with Parkin on the OMM (Chen and Dorn 2013) (Fig. 4b). Another possible mechanism is that PINK1 phosphorylates the ubiquitin-like domain of Parkin and ubiquitin. This phosphorylation contributes to recruitment of

Fig. 4 (continued) with phosphorylated Mfn2. (**c**) PINK1 phosphorylates ubiquitin (*Ub*) on the OMM and Parkin is recruited to the OMM through binding with phosphorylated Ub. PINK1 activates ubiquitin ligase activity of Parkin through its phosphorylation and induces further ubiquitination of OMM proteins. (**d**) Autophagy adaptor proteins link ubiquitinated mitochondria to the isolation membrane through binding between autophagy adaptor proteins and LC3-II

Parkin to the OMM (Kane et al. 2014; Koyano et al. 2014) (Fig. 4c). Once Parkin is recruited on the OMM, Parkin ubiquitinates mitochondrial proteins (Fig. 4b, c). Autophagy adaptor proteins, including p62, neighbor of BRCA1 gene 1 (NBR1), optineurin (OPTN), calcium-binding and coiled-coil domain 2 (CALCOCO2/NDP52), and TAX1 binding protein 1 (TAX1BP1), have a ubiquitin-binding domain and LIR and can interact with ubiqutinated proteins and the isolation membrane (Fig. 4d). Therefore, these adaptor proteins function in selective autophagy of ubiquitinated proteins. In the case of PINK1-Parkin-mediated mitophagy, OPTN, NDP52, and TAX1BP1 function as autophagy adaptors to recognize and degrade ubiquitinated mitochondria (Lazarou et al. 2015). Notably, OPTN has several mutations that cause amyotrophic lateral sclerosis (ALS) and primary open angle glaucoma (POAG), and these mutations are involved in mitophagy flux. ALS mutation of OPTN is more effective than POAG (Maruyama et al. 2010; Rezaie et al. 2002). This suggests that in addition to Parkinson's disease, deficiency of mitophagy may contribute to the onset of ALS and POAG.

Regulation of Mitochondrial Morphology in Response to Starvation

Mitochondrial morphology is regulated by fission and fusion (Bereiter-Hahn and Voth 1994). Mitochondrial fission and fusion are maintained by dynamin-like GTPases, including dynamin-related protein 1 (Drp1) (Otera et al. 2013), Mfn1, Mfn2, and optic atrophy 1 (OPA1) (Cipolat et al. 2004; Santel and Fuller 2001). In the case of mitochondrial fission, cytoplasmic Drp1 localizes on the OMM through its modification-dependent recruitment. Drp1 binds to OMM-anchored receptor proteins, including mitochondrial fission factor (Mff) and mitochondrial dynamics proteins of 49 and 51 kDa (MiD49 and MiD51). During mitochondrial fission, the GTP-binding form of Drp1 oligomerizes and wraps around mitochondria at the fission site and then constricts their membrane through GTP hydrolysis (Otera et al. 2013). Therefore, recruitment of Drp1 on the OMM dependent on Mff, MiD49, or MiD51 and GTPase activity is essential for mitochondrial fission. Recruitment of Drp1 to the OMM is also regulated through post-translational phosphorylation at several different sites. Under starvation conditions, protein kinase A is activated by increased levels of cyclic AMP and phosphorylates Drp1 at Ser 637. This results in inhibition of recruitment of Drp1 to the OMM. Phosphorylation at Ser 637 is negatively regulated by calcineurin, calcium, and calmodulin-dependent phosphatase (Gomes et al. 2011). Mitochondrial fusion is activated under conditions of stress, including serum and amino acid deprivation (Tondera et al. 2009). Therefore, mitochondria are elongated upon starvation. The physiological relevance of morphological regulation of mitochondria is protection from unexpected elimination through non-selective macroautophagy upon starvation. Elongated mitochondria are thought to be too large to be engulfed by autophagosomes and mitochondrial fission events prior to enwrapping might be required for mitophagy. However, elongated mitochondria can be degraded by mitophagy in Drp1-deficient cells. Interestingly, in

the course of mitophagy, the mitochondrial portion that is destined for degradation in tubular mitochondria is first recognized by the isolation membrane, and then this portion is budded and separated concomitant with autophagosome formation (Yamashita et al. 2016). This suggests that mitochondria-specific autophagy is not induced at the early stage of starvation, whereas non-selective autophagy is induced, and protection of mitochondria is only from non-selective autophagy.

Furthermore, starvation-induced mitochondrial elongation potentiates mitochondrial ATP synthesis by possessing more cristae, and increasing dimerization and activation of ATP synthase on the IMM. Cells that are deficient in mitochondrial elongation upon starvation consume cytoplasmic ATP to maintain the mitochondrial membrane potential, resulting in cell death (Gomes et al. 2011). These findings suggest that regulation of mitochondria in response to starvation plays an important role in cellular survival.

Signaling Pathway for Starvation-Induced Mitophagy in Mammals

As mentioned above, at the early stage of starvation, mitochondria are enlarged through inhibition of fission and induction of fusion, and as a result, autophagic degradation of mitochondria is inhibited (Gomes et al. 2011). However, mitochondria can be degraded by autophagy under long-term starvation (Hirota et al. 2015). In this case, similar to yeast, two MAP kinase cascades, the extracellular signal-regulated kinase (ERK) 1/2 and p38 pathways, are required for mitophagy. Upstream kinases, rapidly accelerated fibrosarcoma (RAF) and MAPK/ERK kinase (MEK) 1/2 for ERK1/2, mixed-linage protein kinase (MLK) 3 and MAP kinase kinase (MKK) 3/6 for p38, are also involved in starvation-induced mitophagy (Hirota et al. 2015). With regard to the p38 signaling pathway, Hog1, a yeast homolog of p38, is also required for mitophagy. This suggests that the p38 MAP kinase cascade in starvation-induced mitophagy is conserved from yeast to mammals (Mao et al. 2011; Kanki et al. 2013). Furthermore, BNIP3L is required for starvation-induced mitophagy, whereas Parkin is dispensable (Hirota et al. 2015).

Physiological Relevance of Mitophagy

The majority of cellular ATP is produced by oxidative phosphorylation complexes of the IMM. In this process, the mitochondrial membrane potential is formed through the electron transport chain and used as the driving force of ATP synthase in the IMM. Although most of the electrons pass through the oxidative phosphorylation complexes, some electrons occasionally leak out from them constitutively. These electrons eventually cause formation of ROS (mitochondrial ROS, mtROS), and thus mitochondria are constitutively exposed to mtROS and eventually disordered. To protect cells from excess ROS, damaged mitochondria, which produce further mtROS, must be eliminated. Mitophagy-deficient Atg32 or Atg11 knockout yeast

strains cause abnormalities of mitochondrial DNA and show petite colony formation by culturing in stressful conditions, such as starvation (Kurihara et al. 2012). In this case, the ROS scavenger N-acetylcysteine can suppress the phenotype, suggesting that excess mtROS emerges in mitophagy-deficient cells. Therefore, mitophagy adequately eliminates damaged mitochondria to minimize mtROS production in wild-type cells (Kurihara et al. 2012). In mammalian cells, loss of the mitochondrial membrane potential causes PINK1-Parkin-mediated mitophagy, suggesting that damaged mitochondria-specific autophagy is implicated in Parkinson's disease (Narendra et al. 2008). However, further in vivo examination is required to clarify the pathophysiological role of mitophagy.

Perspectives

Mitophagy plays an important role in maintaining mitochondrial quality and quantity. The molecular mechanisms of mitophagy have been extensively examined in yeast and are currently relatively well understood. However, with regard to the regulation of Atg32 activity, how phosphorylation of Atg32 by CK2, a constitutive active kinase, is regulated remains unclear. To clarify regulation of starvation-induced mitophagy in yeast, spatiotemporal analysis for phosphorylation of Atg32 may be required. In mammalian cells, several types of mitophagic processes have been reported in different types of cells and under different mitophagy-inducing conditions. This impairs our understanding of mitophagy in mammalian cells. Additionally, the physiological role of mitophagy in mammals is largely unclear because of experimental limitations of analysis for mitophagy *in vivo*. To determine the physiological and pathophysiological role of mitophagy in mammals, a versatile strategy for detection of mitophagy in animal tissues must be developed.

Policies and Protocols

Policies for Studying Mitophagy: Proposal of the Molecular Basis for Treatment of Mitochondria-Related Diseases

In this chapter, we have described the molecular mechanisms and physiological roles of mitophagy under starvation in yeast and mammalian cells. Mitochondria have energy-producing machinery termed oxidative phosphorylation, and most of the energy used within a cell is produced in mitochondria. However, this pathway is a "double-edged sword," producing reactive oxygen species (mtROS) simultaneously with energy production. Therefore, mitochondria are also known as a harmful component for the host cell. Accordingly, if dysfunction of mitochondria increases within the cell, it causes many types of human diseases, including neurodegenerative diseases and aging-related diseases.

Mitophagy is the only degradation system that can degrade whole mitochondria and can contribute to mitochondrial quality control by eliminating dysfunctional

mitochondria. Therefore, the policy for studying mitophagy should determine the molecular basis of mitochondrial degradation and molecular targets in mitophagy for treatment of mitochondrial dysfunction-related diseases.

Dictionary of Terms

- **Reactive oxygen species** – Reactive oxygen species are reactive molecules derived from molecular oxygen. They are mainly produced as byproducts from mitochondrial electron transport chain complexes and can potentially harm other proteins in cells.
- **Organelle** – Organelles are membranous compartments in eukaryotic cells, including the nucleus, endoplasmic reticulum, Golgi apparatus, lysosomes, peroxisomes, and mitochondria. Each organelle has specific activity for numerous cellular events.
- **Mitochondria** – Mitochondria are double membrane-bound organelles that are present in all cells of the body, except for red blood cells. Mitochondria have many important metabolic functions, including energy production, calcium buffering, regulation of apoptosis, beta-oxidation of fatty acids, and thermogenesis.
- **Mitochondrial disease** – Mitochondrial diseases result from failure of the mitochondria. When mitochondria fail, the level of mitochondria-generated energy is decreased, resulting in cellular damage, followed by cell death. When disordered mitochondria emerge throughout the body, whole organ systems become dysfunctional.
- **Mitochondrial DNA** – Mitochondrial DNA, termed mtDNA, is an approximately 16-kbp circular DNA that is located in the mitochondrial matrix. mtDNA encodes 13 proteins of oxidative phosphorylation complexes, 2 ribosomal RNAs, and 22 transfer RNAs.
- **Oxidative phosphorylation** – Oxidative phosphorylation is a reaction for energy production at the IMM. During oxidative phosphorylation, electrons are transported through four complexes and eventually transferred to oxygen. The pathway forms discontinuous proton concentrations across the IMM, termed the mitochondrial membrane potential. The mitochondrial membrane potential is used as the driving force for the ATP synthase complex, which is the fifth complex of this pathway.

Summary Points

- Mitochondria are multifunctional organelles and important for cell survival.
- Mitochondria can generate most of the energy in cells.
- Mitochondria are constitutively exposed to reactive oxygen species that are derived from their electron transport chain.
- Damaged mitochondria are harmful to host cells.

- Mitophagy is a type of autophagy that can selectively degrade mitochondria and contributes to eliminating damaged mitochondria from cells.
- Mitophagy is induced under starvation in yeast and mammalian cells.
- Most mitophagy receptor proteins are regulated through their phosphorylation upon stress, including starvation.
- Mitophagy may contribute to the onset of human diseases, including Parkinson's disease.
- Molecular analysis of mitophagy has the potential for determining molecular targets for treatment of mitochondrial dysfunction-related diseases.

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Starvation in Fish: Sturgeon and Rainbow Trout as Examples

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Miriam Furne and Ana Sanz

Contents

Abstract

All animals need the input of energy from the environment to stay alive. As a survival mechanism they have developed adaptive processes that allow them to continue living for a certain time when energy input does not occur. Periods of food deprivation are frequent in many species of fish in their natural environment. Climate changes, seasonal variations, competition for food, breeding migrations are common processes that involve starvation in fish. During starvation, adaptive physiological processes affect organs such as liver, brain, skeletal muscle, and digestive and produce changes in the intermediary metabolism of carbohydrates, lipids, and proteins to maintain the homeostasis. This book chapter focuses on

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metabolic changes, alterations in the oxidative state, and changes in digestive enzymatic activities in response to starvation in fish with the main focus on the trout *Oncorhynchus mykiss* and the sturgeon *Acipenser naccarii*.

Keywords

Fish · Sturgeon · Trout · Metabolism · Oxidative stress · Digestive · Starvation · Enzymes · Liver · Muscle

List of Abbreviations

ACoAT	Acetoacetyl CoA thiolase
CAT	Catalase
GDH	Glutamate dehydrogenase
GK	Glycerol kinase
GOT	Aspartate aminotransferase
GPT	Alanine aminotransferase
GPX	Glutathione peroxidase
GR	Glutathione reductase
HK	Hexokinase
HOAD	β-hydroxyacyl CoA dehydrogenase
LDH	Lactate dehydrogenase
PK	Piruvate kinase
ROS	Reactive oxygen species
SOD	Superoxide dismutase
β-OHBDH	β-hydroxybutyrate dehydrogenase

Introduction

All animals need the input of energy from the environment to stay alive. As a survival mechanism they have developed adaptive processes that allow them to continue living for a certain time when energy input does not occur. It is appropriate to name these periods of external limitation of food resources as starvation and not as fasting (McCue 2010).

Periods of food deprivation are frequent in many species of fish in their natural environment. Climate changes, seasonal variations, competition for food, breeding migrations are common processes that involve starvation in fish. To survive these periods with lack of food intake, fish have developed a series of adaptive physiological processes. These processes allow fish to endure starvation periods of several months and are closely related to biotic and abiotic factors. Thus, carnivorous species are better adapted to face periods of food restriction than herbivores and detritivores species which tend to eat continuously. Furthermore, the consequences of starvation are more pronounced in larvae and juvenile stages than in adult fish, probably due to a higher amount of energy reserves in the latter (Gadomski and Petersen 1988). Environmental variations such as temperature may also have an impact on these adaptive mechanisms.

During starvation, adaptive physiological processes affect organs such as liver, brain, skeletal muscle, and digestive and produce changes in the intermediary metabolism of carbohydrates, lipids, and proteins to maintain the homeostasis (McCue 2010; Rossi et al. 2015; Barreto-Curiel et al. 2017).

Short-term starvation promotes rapid mobilization of available reserves. In the first week of starvation, the glycogenolytic pathway increase in liver. Gluconeogenic processes, from the seventh day and onwards, tends to increase glycemia. At the same time, an increase in the ketogenic capacity of the liver occurs in order to produce alternative energetic substrates for its potential use in organs such as the brain. Glycogen depletion is followed by lipid and protein catabolism, as well as a loss of body weight. In addition, many anabolic pathways are diminished, such as the synthesis of glycogen, protein, lipids, and nucleic acid precursors (Rossi et al. 2015).

In the fish brain, after 1 week of starvation, there is a depression of the glycolytic pathway and a greater use of ketone bodies as energetic substrates (Vigliano et al. 2002; Polakof et al. 2012). In addition, it is necessary to emphasize the gluconeogenic capacity of the brain in fish, since in other animals this occurs only in the liver and renal cortex (Lehninger et al. 1993; Polakof et al. 2012).

Muscle metabolism is characterized by a rapid consumption of glycogen, lipid, and protein reserves during starvation (Machado et al. 1988). Lack of food results in a decrease in the synthesis and secretion of enzymes in the digestive system (Gannam 2008; Shan et al. 2016). Recent studies have shown an alteration of the intestinal microbiota with an increase of bacteroidetes and a depletion of betaproteobacteria (Hong and Rhee 2014).

Finally, other notable change in response to fasting in fish is the alteration in oxidative state (Rossi et al. 2015).

All functional changes during fish starvation are controlled by the neuroendocrine system. Neuroactive and hormonal substances (cholecystokinin, neuropeptide Y, galanin and orexin, leptin insulin, glucagon, somatostatin, growth hormone, thyroid hormones, catecholamines, cortisol, corticosterone, and cortisone) have been shown to have important regulatory functions in physiological processes during starvation in fish (Bar 2014; Bond 1996).

This book chapter focuses on metabolic changes, alterations in the oxidative state, and changes in digestive enzymatic activities in response to starvation in fish with the main focus on the trout *Oncorhynchus mykiss* and the sturgeon *Acipenser naccarii*.

Trout is an actinopterigian fish belonging to the Salmonidae family. This family includes 3 subfamilies, 11 genera, and 176 species (Nelson et al. 2004). It is original of the rivers of the west of North America, although introduced in rivers almost all over the world. It lives in clear and relatively cold waters and has a longevity from 4 to 6 years. Sexual maturity is reached when they exceed 10 cm in length, in the second or third year of life. Predatory feeding is mainly composed of zooplankton, aquatic macroinvertebrates, and small fish. The size generally does not exceed 40–50 cm in length and 5 kg in weight.

Sturgeons are bony fishes, actinopterigios of the family Acipenseridos that includes two subfamilies. The genus Acipenser includes 21 species. Sturgeons have been revealed as fish with remarkable peculiarities in many aspects of their biology, in

particular in their physiology and metabolism, with respect to other fish groups. To a large extent, these peculiarities have been attributed to their remarkable phylogenetic antiquity (they are called authentic "living fossils") estimated to having existed for 250 million years. Its habitat is associated with the great fluvial systems of the northern hemisphere, being abundant in the Black Sea, Caspian, and in seas and rivers of North America and Europe. *Acipenser naccarii* is a migrating species that reproduces in fresh water, using the sea for its growth. Sexual maturity reaches the age of 6–8 years. The adult specimens reach a weight greater than 100 kg and up to more than 2 m in length, being the larger females, although there is no evident sexual dimorphism (Domezain Fau 2003). It mainly feeds on benthic invertebrates, although they also consume remains of animals, plants, and seeds (Soriguer et al. 1999). This would explain that nutritional studies in this species have revealed a greater predisposition of the digestive and metabolic machinery for the use of carbohydrates than exists in a strictly carnivorous fish such as rainbow trout (*Onchorynchus mykiss*) (Furne et al. 2008). Likewise, Sanz et al. (1997) have found a better use of feed in this sturgeon species in which the protein/energy ratio is lower than that of a strict carnivore.

Metabolic Modification During Starvation

To maintain the vital processes and survival during periods of starvation, fish reduce their energy expenditures, which in a high percentage are derived from protein synthesis and mobilize their endogenous reserves to obtain the energy.

The utilization of energy reserves during starvation periods appears to differ according to the species of fish. Moreover, intraspecific adjustments to these conditions will depend on different factors, such as age or nutritional state (Navarro and Gutiérrez 1995). The metabolic responses of fish to starvation are also influenced by the feeding habits of the species.

Liver Metabolism During Starvation

Most studies report that glycemia never falls below the basal levels established for most fish species (65–70 mg 100 ml^{-1}) (Echevarría et al. 1997; Rios et al. 2006; Pérez-Jiménez et al. 2007). Plasma glucose levels in sturgeon notably decreased after 2 days of starvation, while in the trout plasmatic glucose showed a more gradual response over time (Furne et al. 2012) (Fig. 1). Plasma glucose levels decline in starved fish (Gillis and Ballantyne 1996; Soengas et al. 1996; Figueiredo-Garutti et al. 2002; Pérez-Jiménez et al. 2007, 2008; Rossi et al. 2015).

The maintenance of plasma glucose levels during food deprivation is attributed to three processes: glycogen mobilization, decreased glucose consumption, and gluconeogenesis.

In both sturgeon and trout, a mobilization of liver glycogen has been observed during the first few days of starvation. Although hepatic glycogen is consumed to cover energy demands in both species, this response has been reported to occur earlier in sturgeon. A

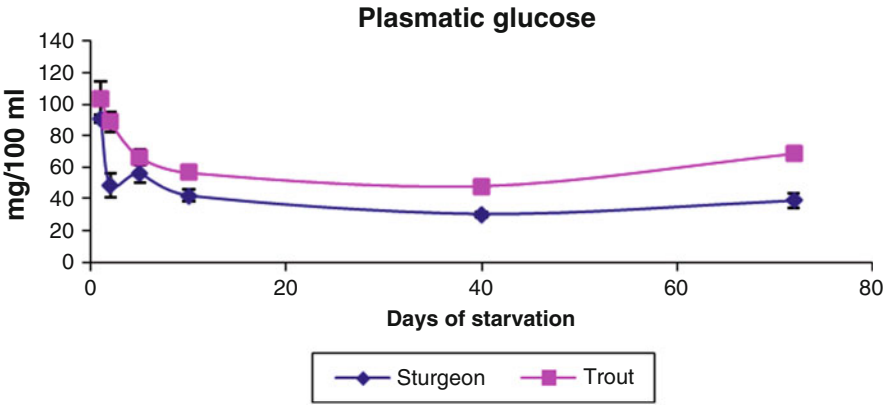

Fig. 1 Plasmatic glucose levels during starvation in sturgeon and trout. Values are mean ± SEM ($n = 5$; number of fish sampled per sampled points) (Data are taken from Furne et al. 2012)

large reduction of liver glycogen has been reported in many starved fish species, at least during the initial stages of starvation (Navarro and Gutiérrez 1995; Metón et al. 2003; Rios et al. 2006; Pérez-Jiménez et al. 2007; Menezes et al. 2015), but in most fish species, glycogen deposits were not completely exhausted, suggesting that a strategy is operating to preserve liver reserves (Collins and Anderson 1997; Rios et al. 2006; Pérez-Jiménez et al. 2007; Pérez-Jiménez 2008).

The reduced activity of hepatic enzymes from the glycolytic and the pentose phosphate pathways, and enhanced or sustained hepatic gluconeogenesis from amino acids and glycerol, are other strategies operating in fish to maintain glycemia during starvation (Polakof et al. 2012).

The decrease of blood glucose in sturgeon in the first few days of food deprivation is accomplished by a peak in the activities of the glycolytic enzymes such as HK and PK (Figs. 2 and 3). Although glycogenolysis occurs in the sturgeon, glucose demands and a minor content of hepatic glycogen would explain the decrease of glycemia. On the other hand, in the trout, a higher gluconeogenic activity in the liver from noncarbohydrate substrates such as amino acids and lactate was found (increased of GPT and LDH activities). Induction of gluconeogenic processes and a decrease of glycolytic enzymes activity (HK and PK) in the trout liver during the starvation would explain the more gradual decline of glycemia in this specie (Furne et al. 2012).

Triglycerides are the main form of energy storage in fish. During starvation, fatty acids derived from triglyceride hydrolysis are preferentially used through β-oxidation as fuels for most fish tissues. Triglycerides may be deposited as perivisceral fat, in liver or in muscle. Liver is the main site for lipid storage in sturgeon, while salmonids like the trout accumulate perivisceral fat. Despite the storage site, a marked reduction in fat storage occurs in starved fish (Grigorakis and Alexis 2005; Rios et al. 2006; Furne et al. 2008; Pérez-Jiménez 2008; Barreto-Curiel et al. 2017) (Fig. 4). Regarding the enzymes involved in fatty acid catabolism, it has been

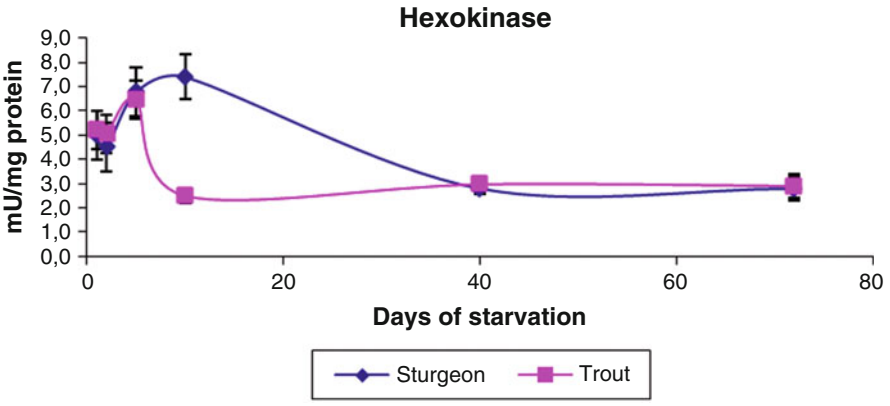

Fig. 2 Hepatic hexokinase activity during starvation in sturgeon and trout. Values are mean ± SEM ($n = 5$; number of fish sampled per sampled points) (Data are taken from Furne et al. 2012)

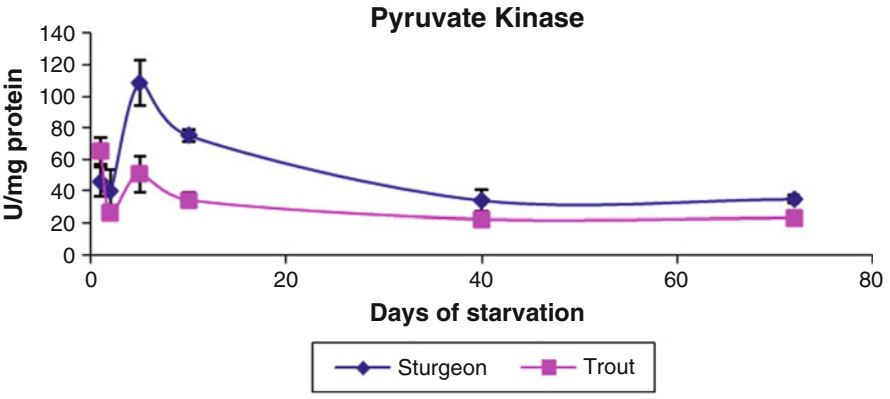

Fig. 3 Hepatic pyruvate kinase activity in the liver during starvation in sturgeon and trout. Values are mean ± SEM ($n = 5$; number of fish sampled per sampled points) (Data are taken from Furne et al. 2012)

reported an increase in hepatic HOAD activity in starved sturgeon but not in trout, which agrees with a higher lipid content storage in the liver in the sturgeon (Fig. 5).

It has been suggested by some authors that ketone bodies do not play an important role as an energy source during starvation in fish (Zammit and Newsholme 1979; Black and Love 1986). However, many studies have already demonstrated β-OHBDHB activity in several teleost species. Furthermore, an increase in plasma levels of ketone bodies and in the activity of enzymes involved in ketone body synthesis has been reported in fish deprived of food (Soengas et al. 1996; Furne et al. 2008). In the sturgeon, the maintenance of triglyceride catabolism, which produces acetyl-coA, encourages the use of this metabolite for the synthesis of ketone bodies in the liver. This fact was supported by high β-OHBDH and ACoAT activities in

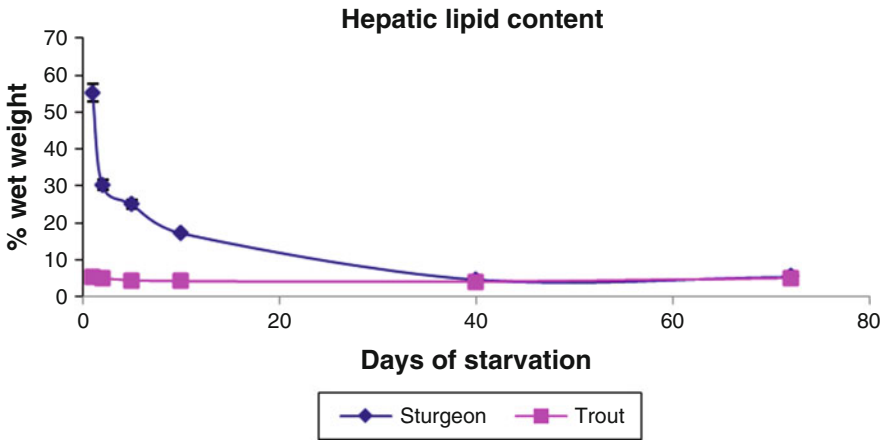

Fig. 4 Lipid content levels in liver during starvation in sturgeon and trout. Values are mean ± SEM ($n = 5$; number of fish sampled per sampled points) (Data are taken from Furne et al. 2012)

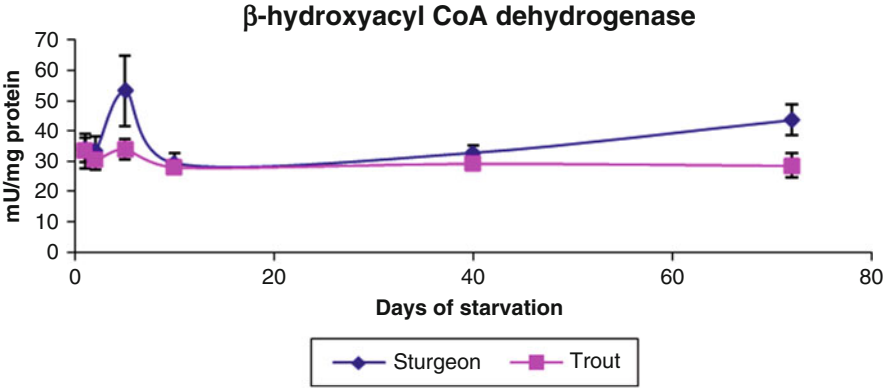

Fig. 5 Hepatic β-hydroxyacyl CoA dehydrogenase activity during starvation in sturgeon and trout. Values are mean ± SEM ($n = 5$; number of fish sampled per sampled points) (Data are taken from Furne et al. 2012)

sturgeon. However, the trout synthesizes ketone bodies in the liver (increased of ACoAT) as a response to the excess of acetyl CoA produced by Krebs cycle from catabolic activity during starvation (Furne et al. 2012).

Muscle Metabolism During Starvation

Muscle is one of the tissues that is considerably affected by starvation in fish (Johansen and Overturf 2006). As muscle glycogen is a short-term reserve, during periods of starvation, lipid stores and muscle protein breakdown provide the bulk of energy.

In a few days of starvation, a mobilization of carbohydrate stores in white muscle was detected as an energy resource in both sturgeon and trout (Furne et al. 2012). Mobilization of carbohydrates during starvation has been reported in Atlantic cod by Black and Love (1986), whereas other species showed no variations in muscle glycogen reserves (Gutierrez et al. 1991; Blasco et al. 1992; Barcellos et al. 2010).

Sturgeon and trout mobilize lipids and proteins from the muscle during starvation. In both species, an increase in lipid catabolism enzymes (HOAD and GK) has been reported and the fish keep protein catabolism enzymes active (GPT, GOT and GDH). In contrast to the sturgeon which showed a decrease in enzymes involved in gluconeogenesis during starvation periods, the trout keeps the gluconeogenic pathways active using lactate as a substrate. Because protein is the main muscle component, they are the main energy source in muscle under prolonged starvation.

Starvation and Antioxidant Defenses

Oxidative stress arises from an imbalance between antioxidant defenses and oxidative processes. The high quantity of ROS present in situations of oxidative stress can provoke tissue damage, affecting biomolecules, which in the case of lipids could be quantified by peroxide formation (Halliwell and Gutteridge 2015).

Food deprivation leads to depletion of antioxidant stores and increased levels of ROS in organs of living organisms. Starvation has been reported to have pro-oxidant effects in mammals as ROS generation is not adequately neutralized by antioxidant systems (Robinson et al. 1997; Domenicali et al. 2001). It is known that a lack of available food can affect the antioxidant defenses in fish but, as opposed to studies on mammals, few publications are available on the consequences of starvation with regard to antioxidant mechanisms in fish. Most available studies on the impact of starvation on antioxidant defenses of fish are focused on liver, since it is the main organ for metabolic control and has a key role in ROS generation.

Oxidative Stress in Red Blood Cells and Liver During Starvation

Sturgeon and trout under prolonged starvation showed an increase in the lipid peroxidation levels in liver and in red blood cells. Studies in fish unanimously show that starvation augments lipid peroxidation. However, with respect to the activity of antioxidant enzymes, studies disagree, particularly depending on the tissue examined. In liver and red blood cells in sturgeon and trout, there was an early decline at 10 days starvation in both species. This decline, triggered by the restriction of enzyme synthesis substrates, was probably responsible of the rise of the lipid peroxidation levels seen (Furne et al. 2009). The enzymes SOD, CAT, GPX, and GR constitute the main enzymatic mechanism of antioxidant defense. The rise in the lipid peroxidation levels, together with a fall in the activity of the main antioxidant enzymes, appears to indicate the incapacity of the liver to meet the stress

situation provoked by prolonged starvation, leading ultimately to the oxidation of biomolecules.

Hepatic GR activity decreased in rainbow trout subjected to 3 weeks of starvation (Blom et al. 2000). Atlantic cod (*Gadus morhua*) showed an increased activity of antioxidant enzymes in liver, while in muscle those enzymes did not vary after 12 weeks of starvation (Guderley et al. 2003). Lipid peroxidation increased due to food deprivation in gilthead seabream (*Sparus aurata*). A decrease of CAT activity was found while SOD, GR, and GPX activities increased. Moreover, it has been detected new isoforms of the SOD enzyme (Pascual et al. 2003). An increase in activity in SOD, CAT, and GPX, and a decreased in GR activity in the liver of Common dentex (Dentex dentex) submitted to 5 weeks of starvation was reported by Morales et al. (2004). Zhang et al. (2008), in lang yellow croaker (*Pseudosciaena croceae*), showed an increase in hepatic SOD and GPX activities during starvation. Several studies have also evaluated the transcriptional responses to starvation of major antioxidant enzyme genes in the liver of some fish species. In rockbream (*Oplegnatus fasciatus*) significant alterations were apparent, whereas mRNA levels of SOD, CAT, GPX, and GST in the liver increased in fish subjected to short-term starvation (Nam et al. 2005). In rainbow trout (*Oncorhynchus mykiss*) starved for 3 weeks, expression levels of GST and GPX were reduced (Salem et al. 2007). Also in the zebrafish (*Danio rerio*), genes involved in neutralizing ROS, such as SOD, GPX, and several other selenium-binding proteins, were downregulated in livers of fish starved for 21 days (Drew et al. 2008). These results indicate that prolonged starvation decreases the capacity of fish liver to ameliorate oxidative stress.

Oxidative Stress in Heart During Starvation

Studies analyzing the antioxidant defenses of the heart in starved fish are scarce and the results contradictory. In Adriatic sturgeon and rainbow trout, a starvation period of 10 weeks did not induce lipid oxidative damage in the heart. The activity of antioxidant enzymes in the heart of starved sturgeon remained unchanged, whereas cardiac CAT and SOD activities were enhanced by starvation in rainbow trout (Furne et al. 2009). These observations might indicate that the heart, being a crucial organ for life, would be in some way protected from stressful circumstances such as starvation. However, in common dentex, 5 weeks of starvation depressed cardiac activities of SOD, GPX, and GR. Although CAT activity increased during these circumstances, it was not sufficient to avoid an increase in lipid peroxidation (Pérez-Jiménez 2008).

Oxidative Stress in White Muscle During Starvation

In white muscle, specific activity of the antioxidant enzymes did not differ between fed and starved cod (Guderley et al. 2003). Also in common dentex, the specific activity of antioxidant enzymes, as well as the levels of lipid peroxidation, remained

unchanged by starvation (Pérez-Jiménez 2008). An increased activity of antioxidant enzymes and no variation in lipid peroxidation in white muscle was found in Adriatic sturgeon and rainbow trout starved for 10 weeks (Furne et al. 2009). Probably, cellular disruption during macromolecular mobilization may increase the sensitivity to damage by ROS, explaining why maintenance of antioxidant defenses would be useful during starvation.

Starvation and Digestive Physiology

The lack of food is a situation undergone and tolerated by many fish species in the natural environment. The metabolic strategy used to supply energy to the organism depends on several factors such as species, physiological state of the fish, and environmental conditions. Thus, some fish use protein as the main energy source, maintaining the hepatic glycogen reserve by means of glyconeogenesis, while other fish use lipids ahead the glycogen reserve. In addition, not all body tissues contribute to such reserves in the same way; some fish have a large energy reserve in the mesenteries of the digestive tract, while other fish have storage in the liver and still others in muscle.

Because the gastrointestinal tract and its associated organs can account for up to 40% of an animal's metabolic rate (Cant et al. 1996), one would have the a priori expectation for the digestive tract toatrophy during periods of food deprivation and flourish during food abundance (Theilacker 1978; Bogé et al. 1981; McLeese and Moon 1989; Diamond and Hammond 1992; Wang et al. 2006). Indeed, fish enduring food deprivation have been observed to decrease their gut length (Rios et al. 2004), intestinal fold and microvilli length (Gas and Noailliac-Depeyre 1976; German et al. 2010), and digestive enzyme activities (Krogdahl and Bakke-McKellep 2005; Chan et al. 2008; Furne et al. 2008; Abolfathi et al. 2012).

In the trout, the digestive somatic index declined during starvation, which could indicate a quicker mobilization of energy reserves from the digestive tract with respect to the overall body mass. Similar findings have been reported by other authors in rainbow trout (Storebakken et al. 1998). Metabolic and digestive results suggest that the trout mobilizes preferentially energy from the perivisceral tissues, while sturgeon mobilizes energy from muscle and liver tissues.

Regarding digestive enzymes, sturgeon and trout showed a decrease in protease, amylase, and lipase activities after 10 weeks of starvation. Sturgeon and trout have shown different eating habits. Trout has higher protease activity than sturgeon and sturgeon has higher amylase activity. Furthermore, sturgeon has greater digestive capacity for carbohydrates than trout (Furne et al. 2005). With respect to starvation, amylase activity in both species decreased more than 50% at 10 days of food deprivation. However, the protease activity did not change until 3 weeks of starvation. The literature shows that alkaline phosphatase, an enzyme located in the microvilli of the intestinal epithelium, gradually diminishes in starved carp, and after 13 month of starvation this activity cannot be detected (Gas and Noailliac-Depeyre 1976). A decline in trypsin activity during prolonged starvation in Atlantic

salmon was reported by Bélanger et al. (2002). On the other hand, a very different effect was noted after a brief starvation, with an increase in digestive enzymatic activities in Nile tilapia (Mommsen et al. 2003). Krogdahl and Bakke-McKellep (2005) detected a decrease in the total activity of enzymes in the intestinal microvellosities in Atlantic salmon after 40 days of starvation and a rapid recuperation after 7 days of re-feeding. A proteolysis of the intestinal mucosa, a progressive degeneration of exocrine pancreatic cells, and a decrease of zymogen activity during 48 h of starvation were observed in tench by Ostaszewska et al. (2006). Studies realized with catfish submitted to 150 days of starvation showed that the fish reduced the surface area of their intestines by 70% and reduced the microvilli surface area by 52% (German et al. 2010).

Fluctuations in food availability are natural in most aquatic systems, and fish show an impressive capacity to withstand prolonged periods of food limitation, particularly in comparison with mammals. Despite the many ways in which food limitation affects the metabolic capacities of fish, perhaps even more striking is their capacity to rebound from these difficulties. In fact, after starvation, fish enter a period of compensatory growth during which they rapidly accumulate reserves and reinstate their metabolic capacities. This compensatory growth occurs despite the previous atrophy of the digestive system. The malleability of the piscine digestive system and its capacity to rise to renew feeding opportunities is impressive. There are several areas in which information about oxidative defense mechanisms is scant, so new investigations concerning the control of oxidative defense mechanisms during renewed tissue growth after starvation should help elucidate how and why fish are so adapted to survive prolonged periods of food limitation.

This book chapter describes the mechanisms that allow fish to enhance prolonged starvation periods. The ability to face prolonged starvation is higher in fish than for example in humans, and the following two facts might contribute to the explanation of this phenomenon. First, fish are ectotherms (animals that do not regulate the body temperature) and maintaining vital processes requires lower energy levels in comparison to endotherms like humans (endotherms require high expenditures of metabolic energy in order to keep a constant body temperature). Second, fish do not need to keep balance since they live in aquatic environment, whereas humans require the performance of the antigravity muscles. Consequently, during food deprivation events, the energy balance in fish is less negative than in humans. The mechanisms of mobilization of energetic reserves occur at a lower rate in fish such that energy reserves last longer and allow prolonged starvation periods.

Policies and Protocols

The animals to which this study refers were sturgeons (*Acipenser naccarii*) and trout (*Oncorhynchus mykiss*) of 1+ year of age. The maintenance and feeding conditions were those of the fish farm. The fish were starved during a total period of 72 days. Five fish for each species were sampled on days 1, 2, 5, 10, 40, and 72 of food deprivation. All the procedures were conducted according to the guidelines of the

Council Directive 609/86/EEC (European Communities 1986) on the use of animals for experimental and scientific purposes. Animals were anesthetized with clove oil. Blood samples were taken from the caudal vessels using heparinized syringes and transferred to heparinized tubes, to be kept on ice until centrifugation. Following blood collection, the heart, liver, and a white-muscle portion, from anterior dorsal region, were quickly removed. Tissue samples were frozen in liquid nitrogen and stored in the laboratory at −80 °C until analyze.

Dictionary of Terms

- **Glycogenolysis** – Process by which glycogen, the primary carbohydrate stored in the liver and muscle cells of animals, is broken down into glucose to provide immediate energy and to maintain blood glucose levels during fasting.
- **Gluconeogenesis** – Metabolic pathway that results in the generation of glucose from non-carbohydrate substrates such as lactate, glycerol, and amino acids
- **Glycemia** – Level of glucose in blood.
- **Ketone bodies** – Three related compounds (acetoacetic acid, acetone, and beta-hydroxybutyric acid) produced during the metabolism of fats, they are used as a source of energy instead of glucose during a period of fasting.
- **Reactive oxygen species (ROS)** – A type of unstable molecule that contains oxygen and that easily reacts with other molecules in a cell.

Summary Points

- The decrease in blood glucose in trout during the first days of starvation is more gradual than in sturgeon. One of the reasons could be a greater gluconeogenic capacity of the liver of the trout in comparison to the sturgeon liver and increased glycogen storage capacity in trout liver.
- Although it seems to be that ketone bodies do not play an important role as an energy source during starvation in fish, the synthesis of ketone bodies in the liver of the sturgeon comes from the catabolism of hepatic triglycerides, whereas trout liver comes from the Krebs cycle.
- In a few days of starvation, a mobilization of carbohydrate, lipid, and protein in the muscle was detected as an energy resource in both sturgeon and trout.
- Sturgeon and trout under prolonged starvation show an increase in the lipid peroxidation and a decrease in antioxidant enzymes in liver and in red blood cells. Conversely, the oxidative state of the sturgeon and trout heart and muscle is less disturbed by the starvation than the liver.
- The trout mobilizes preferentially energy from the perivisceral tissues, while sturgeon mobilizes energy from muscle and liver tissues.
- The starvation motivates a decrease of the amylase activity earlier than that of the protease in both species.

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Activity-Based Anorexia and Food Schedule Induction

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María José Labajos and Ricardo Pellón

Contents

Abstract

The term activity-based anorexia refers to the animal model of anorexia nervosa in humans, by which laboratory rats lose weight rapidly and progressively when submitted to a food access regime of 1 h a day and free access to an activity wheel the remainder of the time. This combination of diet and exercise eventually leads the animals into a process of self-starvation that can end up in their death, a reason by which activity-based anorexia is considered analogous of anorexia nervosa in humans, a disease that combines self-imposed food restriction with an excessive increase in physical activity. The best-studied example of schedule-induced behavior is the excessive ingestion of water in animals that are food deprived and for whom the food episodes occur intermittently. Schedule-induced

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polydipsia is characterized by the consumption of water around the feeding episodes, similarly to what is seen with activity in the phenomenon of activity-based anorexia. The study presented supports the relationship between both phenomena, so that the initial development of excessive drinking facilitates the subsequent development of wheel running. It is suggested that the imposition of very strict feeding episodes could play an important role during the initial stages of the development of anorexia, facilitating the development of hyperactivity that could end up interfering with food ingestion. Prevention for the development of anorexia should focus on a more efficient control of food regimes rather than limiting physical exercise. The adequate scheduling of feeding episodes should reduce hyperactivity, as it follows from its conception as induced behavior.

Keywords

Activity-based anorexia · Animal models · Anorexia nervosa · Excessive behavior · Feeding regime · Hyperactivity · Induction by intermittent food · Laboratory rats · Schedule-induced polydipsia · Wheel running

List of Abbreviations

ABA	Activity-based anorexia
ANOVA	Analysis of variance
DSM	Diagnostic and Statistical Manual of Mental Disorders
FT	Fixed time
ICD	International Classification of Diseases
SIP	Schedule-induced polydipsia
WHO	World Health Organization

Activity-Based Anorexia

The Disorder

In 1988, Epling and Pierce defined the term activity-based anorexia (ABA) in reference to the animal model of anorexia nervosa in humans, a serious psychiatric disorder included in the International Classification of Diseases (ICD-10), published in its tenth edition in 1992 by the World Health Organization (WHO). This manual describes anorexia nervosa as an eating disorder characterized by the existence of a great weight loss induced or maintained by the patient him/herself, which must include the indispensable diagnostic criterion of the said loss of body weight being originated by the patient him/herself through excessive body exercise. In 2016, the WHO published the 11th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-11), which will not be approved until May 2018. However, the ICD-11 has the same descriptive criteria for the anorexia nervosa disorder, characterized by a significantly low body weight accompanied by a persistent pattern of behaviors that prevent recovery of normal weight, including

restricted eating along with behaviors aimed at increasing energy expenditure, such as excessive exercise.

The American Psychiatric Association published in 2013 its fifth version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), which included anorexia nervosa under the feeding and eating disorders section, defining it as a restriction of energy intake that leads to a significantly low body weight in relation to age, sex, developmental stage, and physical health. The restrictive type of anorexia nervosa is described as the loss of weight due to dieting, fasting, and/or excessive exercise.

The Animal Model

Although it is true that animal models used in scientific research may never fully cover the human psychological complexity, they are necessary to study characteristic aspects of clinical disorders, which could otherwise not be addressed in humans for ethical reasons.

In the case of anorexia nervosa, although there are different proposals for animal models (Siegfried et al. 2003; Treasure and Owen 1997), it is worth highlighting the analogous model called ABA, cited at the beginning of this section. This model reproduces the most objective signs of the anorexia nervosa psychiatric illness, such as weight loss, restrictive diet, and excessive exercise (see Gutiérrez 2011).

The beginnings of this research line refer back to Richter (known as "the father of the biological clock" (Boakes 2007) for his pioneering research on biological rhythms in the 1920s), who, while studying the organisms' spontaneous activity in response to various external stimuli, such as food restriction, found that the experimental subjects, in this case rats subjected to a food diet, increased their physical activity considerably and concluded that once the activity rhythms have been established, these tend to persist even after of the elimination of the stimulus that provoked them (Richter 1922).

Hall et al. (1953) and Hall and Hanford (1954) systematically replicated an experiment where when rats were subjected to a food restriction schedule of 23 h a day, their level of activity increased rapidly in a negative way depending on the number of days lapsed under the deprivation regimen. This data contrasted markedly with the relatively constant level of activity found for a control group that had been subjected to the same activity conditions but under an ad libitum feeding schedule.

Routtenberg and Kuznesof (1967) called this combination of diet and exercise self-starvation due to the decline of the experimental subjects who, when subjected to a regime of access to food for only 1 h a day and with access to an activity wheel during the remaining 23 h, could not maintain their own body weights, presenting very high levels of activity without apparent compensation in their food intake.

Cheney and Epling (1968; cited in Pierce and Cheney 2004) replicated the procedure described in the previous paragraphs, drawn by how rats exercised in the activity wheel more and more, losing substantial amounts of body weight, to the

extent that if they were not removed from this experimental situation, they ended up dying from starvation.

Paré and Houser (1973) and Paré (1975) proposed this experimental technique as a new paradigm in order to investigate gastrointestinal pathology, calling it "activity-stress paradigm." These researchers, using experimental rats aged between 60 and 110 days, housed in activity cages and fed only for 1 h a day for 21 days, reported very high mortality rates before the end of the experiment, caused by extensive lesions in glandular portions of the stomach, in comparison with control rats that were also fed for only 1 h a day but housed in standard cages without access to an activity wheel. The experimental rats that died were more active than the experimental rats that survived and ate less than the surviving rats and the control rats. These researchers emphasized that the main cause of the rapid development of the gastrointestinal disease was the level of activity exerted by the experimental rats, instead of the food deprivation to which all the rats were subjected, both the experimental ones and those in the control group.

Epling et al. (1983) proposed that the ABA phenomenon in rodents was a plausible analogue model of human anorexia nervosa, pointing out that the majority of cases of anorexia nervosa, which they estimated to be up to 75%, were actually examples of anorexia due to activity, a psychiatric illness that combines food self-starvation with an excessive increase in physical activity of the patients (Gutiérrez and Pellón 2002).

Epling et al. (1981, 1983) observed that when rats were fed only for 1 h a day, they lost a lot of body weight but were able to adjust to that diet and survive. Yet, when those same animals were exposed to the same food restriction conditions and in addition were provided with an activity wheel on which they were free to exercise on for the rest of the time they were not eating, the rats started a process that inexorably leads to death, due to their excessive level of activity on the wheel, running up to 10,000 laps a day, which is equivalent to about 10 km. Interestingly, these researchers were even more surprised to observe that the rats, although eating a little bit more when their level of exercise on the wheel began to increase, actually later declined food intake, leading to drastic consequences such as the phenomenon of self-starvation.

Explanations

The interaction between physical activity and diet becomes lethal for subjects, because strenuous exercise reduces the value of food reinforcement, thus decreasing intake and increasing the motivational value of physical activity. Therefore, this is an ideal analogous model to human anorexia nervosa. The cultural practices of diet and exercise initiate the anorexic cycle, which, once initiated, is resistant to change. It is a biological-behavioral perspective of the phenomenon, as this type of anorexia could be the result of natural selection that favors those organisms that became active in times of food shortages. Activity-based anorexia could be part of the explanatory basis of the development of anorexia nervosa (see Epling and Pierce 1988).

The above proposals could be considered as granting a reinforcing and motivational value to physical exercise. On the contrary, other authors formulate different explanatory arguments. Kanarek and Collier (1983) reasoned that the ABA phenomenon results from a failure to adapt to food restriction conditions. These researchers were able to demonstrate how rats did not develop ABA when they had more than one daily access to food (specifically, four 15-min accesses), compared to a control group with the standard access of 60 min all together. Based on these results, they proposed that the weight loss was not induced solely by the activity but rather resulted from a failure to adapt to the new regime of food availability.

Likewise, Dwyer and Boakes (1997) highlighted as an explanatory argument for the ABA phenomenon that it is the history of food restriction that modulates the loss of body weight and physical activity on the wheel. To support this hypothesis, they preexposed experimental rats for 2 weeks to a food restriction schedule with 60 min of access every 24 h, before being placed in boxes with available activity wheels. The rats in the control group did not receive this preexposure phase, resulting in that the subjects of the experimental group ran more than the control group rats and that the weight reduction or weight loss was lower in the preexposed rats. This experimental result conforms to the theory that the history of food deprivation or restriction is what modulates the loss of body weight and the level of activity on the wheel, also strengthening the theoretical explanation of the ABA phenomenon as the subjects' failure to adapt to food regime due mainly to the development of excessive food-anticipatory activity.

A third explanatory line of the ABA phenomenon would be based on the intermittent occurrence of food episodes, capable of generating induced behaviors in the intervals between them. Following Epling and Pierce (1992), it can be suggested that the excessive running in the ABA phenomenon could be a behavior induced by the schedule of intermittent food presentation, as the activity wheel in the experimental situation is continuously available. In the next section, we will present evidence that activity can be induced by the intermittent occurrence of biologically relevant events, such as food for a relatively hungry animal, thus, yielding a better conceptualization than the traditional view of a non-induced facultative behavior (Roper 1981; Staddon and Ayres 1975).

Schedule-Induced Behavior

In 1961, J.L. Falk, investigating rats' fluid regulation and exposing these animals to intermittent reinforcement schedules with food and with a bottle of water present in the experimental situation, highlighted the enormous amount of water that rats could drink in such situation. This excessive drinking has been labelled as schedule-induced polydipsia (SIP), characterized by the fact that rats drink a little immediately after the ingestion of each pellet of food, which appears intermittently, resulting in excessive accumulation throughout the experimental session. Because animals are just deprived of food, but not of water, and they do not have to drink in order to get

the food reinforcer, SIP has been considered the prototype of a category of behavior named by Falk (1971) as an adjunctive, different from the operant and other forms of learned behavior. This type of distinction has been frequently discussed, and today the operant vs. adjunctive distinction is not so clear (Killeen and Pellón 2013).

Whatever the nature of the adjunctive (or schedule-induced) behavior, it can be defined as an increase in the frequency of a behavior not explicitly reinforced in the presence of conditions that require intermittently reinforced responses, compared to the frequency of such behavior when intermittent reinforcement is not required (Wallace and Singer 1976). There are three fundamental characteristics to consider a behavior as adjunctive: (1) It must occur at a significantly higher rate, persistently and even excessively, with respect to its baseline. (2) It must occur immediately after the reinforcer. (3) It must produce an occurrence pattern in the form of an inverted U shape, depending on the duration of the interval between reinforcements (see reviews by Pellón 1990; Wetherington 1982). Under this triple criterion, many behaviors have been identified as adjunctive: excessive drinking (Falk 1961), running on the activity wheel (Levitsky and Collier 1968), licking an air current (Mendelson and Chillag 1970), pica, or aggression and attack (Azrin et al. 1966). They have also been demonstrated in different animal species and even replicated in humans (Kachanoff et al. 1973), using food (Porter et al. 1982), games (Wallace et al. 1975), or sweets and candy in children (Granger et al. 1984) as reinforcers.

According to Staddon (1977), three behavior categories can be identified that occur during the period between reinforcers: terminal activities directed toward obtaining the prize or reward (see Skinner 1948); interim activities, which occur at a high frequency just after obtaining the reinforcer and preceding the terminal response with which they are usually incompatible; and facultative behaviors, which are not usually excessive and show an inverse relationship with the frequency of reinforcement. Both interim and terminal behaviors, in contrast with facultative ones, would be schedule-induced behaviors, being by their very nature facilitated by the parameters of the schedule, the level of deprivation of the subject, or the frequency and magnitude of the reinforcer (Pellón 1992). Timberlake and Lucas (1991), for example, considered excessive drinking as an interim behavior, while they catalogued wheel activity as a facultative one. Research such as that of Penney and Schull (1977) demonstrated, also with laboratory rats, the different functionality of excessive drinking and activity wheel as adjunctive behaviors.

Excessive Activity and Induction

In an attempt to demonstrate that the ABA procedure resembles that of the schedule-induced behaviors due to the intermittent availability of the food reinforcer, and considering the resemblance of the level of running exerted by the experimental rats to that of their licking behavior during exposure to a SIP procedure, an experimental study was designed whereby the rats were exposed successively to the procedures, firstly to SIP and secondly to ABA, in order to check the degree of facilitation in the

development of ABA compared to another group of animals that was directly exposed to the ABA procedure without preexposure to SIP.

An Experimental Test

Eight Wistar male rats were subjected to a fixed time (FT) 60-s schedule of administration of a food pellet, whereby a pellet of food was dispensed at said regular intervals without the animals needing to do anything to obtain them. The rats were maintained at 100% of their theoretical growth weight during the 30 sessions of the SIP procedure. As a result of the exposure to the intermittent reinforcement schedule, and given that they had continued access to water during the experimental sessions, the animals developed induced drinking in the intervals between reinforcers (see also Todd et al. 1997).

Once the preexposure to the adjunctive drinking-inducing schedule phase was finished, the animals were kept at 100% theoretical weight and rested for 30 days.

Immediately afterward, the ABA procedure began, for the eight rats that had been preexposed to the SIP procedure and for another eight rats that had not had such experience. In this procedure, access to food was restricted to 1 h a day, and access to an activity wheel was enabled for the remaining 23 h (for a detailed description of the procedure, see Carrera et al. 2014). All rats, at the beginning of the ABA procedure, had a 100% weight with respect to their own theoretical weight. The procedure was in operation for each rat until it reached for 2 consecutive days a loss greater than 75% of its initial weight, at which time the said animal was removed from the experiment (Boakes and Dwyer 1997).

The first rats that had to be withdrawn from the ABA procedure were four belonging to the group preexposed to the SIP procedure, and, in general, the rats that were directly exposed to ABA took longer to develop the phenomenon than the preexposed rats, thus showing a greater resistance to its development. Statistically, there were differences between groups ($p < 0.01$) in the direction of facilitation for the development of ABA by rats that had had previous experience in SIP.

The acquisition of ABA was analyzed by means of an ANOVA with a between-group factor with two levels (SIP/ABA vs. ABA groups) and a within-subject factor with six levels (corresponding to the six acquisition sessions in ABA necessary to reach the withdrawal criterion of the first rat from the activity wheel). The dependent variables were the decrease in the rats' body weight in percentage with respect to the value at the initial day of the procedure, the amount of food ingested in grams, and the level of running exercised on the activity wheel. The obtained results are shown in Fig. 1.

The upper panel of Fig. 1 shows the mean (± standard error) of the percentage of daily body-weight loss of the rats in both groups while the ABA procedure was taking place. It can be observed that the decrease in body weight, although drastic for both groups, was somewhat more marked in the SIP/ABA rats (black triangles), which reached the withdrawal criterion one session earlier than the ABA-only rats (white circles). Taking the data of the first six sessions, during which all the rats from

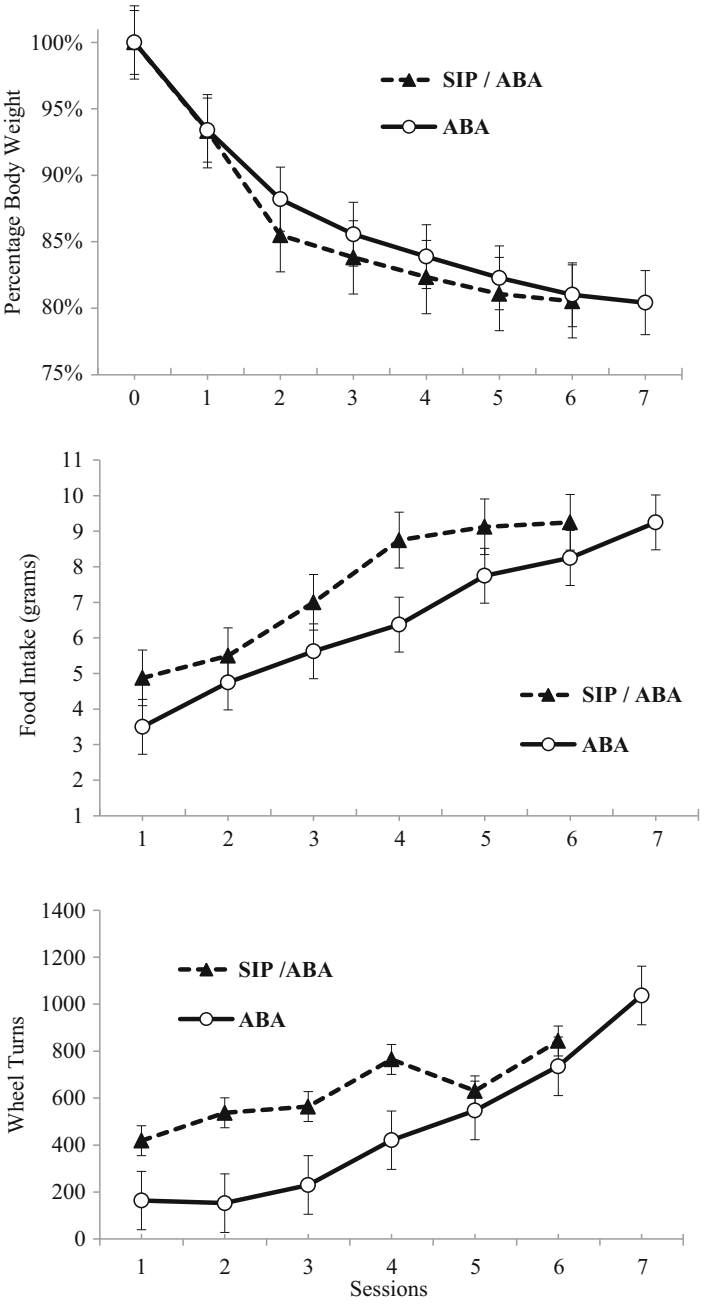

Fig. 1 Development of activity-based anorexia. Percentage of body-weight reduction, grams of food intake, and wheel turns in the two groups of rats that either went directly into activity-based anorexia (ABA) or were previously exposed to an experience of schedule-induced polydipsia (SIP/ABA). For the ABA rats, the first withdrawn animal was in session 7; for the SIP/ABA rats, the first four withdrawn animals were in session 6

both groups were on the activity wheel, the results of the ANOVA were significant for the Group factor [$F(1,84) = 5.93$, $p < 0.01$] and for the Session factor [$F(5,84) = 49.63$, $p < 0.01$], which point toward the progressive development of ABA in both groups with a greater overall weight loss for the SIP/ABA group. Post hoc tests indicated that there were significant differences ($p < 0.01$) in the first session compared to the second, third, fourth, fifth, and sixth sessions. There was no significant effect ($p > 0.05$) for the Group x Session interaction.

In the intermediate panel of Fig. 1, the mean (± standard error) of the amount of food ingested by the rats throughout the daily experimental sessions is observed. In general, a progressive increase in the grams of food ingested as the ABA procedure progressed can be observed, with always a slightly higher consumption for the SIP/ABA group. The ANOVA performed confirmed statistical significance for both the Group factor [$F(1,84) = 36.30$, $p < 0.01$] and the Session factor [$F(5,84) = 43.27$, $p < 0.01$], but not for their interaction ($p > 0.05$). The post hoc comparisons yielded significant differences ($p < 0.01$) in a lower food consumption during days 1 and 2, with respect to days 4, 5, and 6.

The lower panel in Fig. 1 shows the mean (± standard error) of the level of running exercised on the activity wheel during the sessions that the ABA (white circles) and SIP/ABA (black triangles) groups were in the experimental boxes submitted to the ABA procedure. A progressive increase in running can be observed as the experimental sessions lapsed. This level of running was generally somewhat higher for the SIP/ABA group during the first four sessions. The statistical results of the ANOVA for the first six sessions showed significant effects for the Group factor [$F(1,84) = 9.18$, $p < 0.01$] and for the Session factor [$F(5,84) = 3.46$, $p < 0.01$], but not for the interaction between them ($p > 0.05$). In the post hoc analyses, significant differences were found ($p < 0.05$) between the first and sixth sessions.

Figure 2 shows the mean (± standard error) of the distribution of wheel turns performed by the rats during the interval between meals (white circles for the ABA group and black triangles for the SIP/ABA group) during the last criterion day for each rat. The wheel turns performed during the 23 h between meals were recorded in units of 15 min in order to observe the way in which running developed. In general, running maximums can be observed after each meal, approximately when the dark period began (or was about to begin), and then in anticipation of the following meal, showing the SIP/ABA group, in general, greater activity during those periods. The ANOVA performed yielded a main effect of Bin [$F(3,176) = 35.70$, $p < 0.01$] and of Group [$F(1,176) = 12.90$, $p < 0.01$]. For the Group x Bin interaction, there was also statistical significance [$F(3,176) = 14.91$, $p < 0.01$]. The post hoc comparisons were significant ($p < 0.01$) for the activity displayed during the hours just prior to the delivery of food (12:00–17:00 h) with respect to the greater majority of the night hours spent in the activity boxes.

Some Caveats and Conclusions

Rats that were previously exposed to SIP developed ABA faster by means of running more and eating also more than the rats that were not preexposed to SIP. Therefore, to

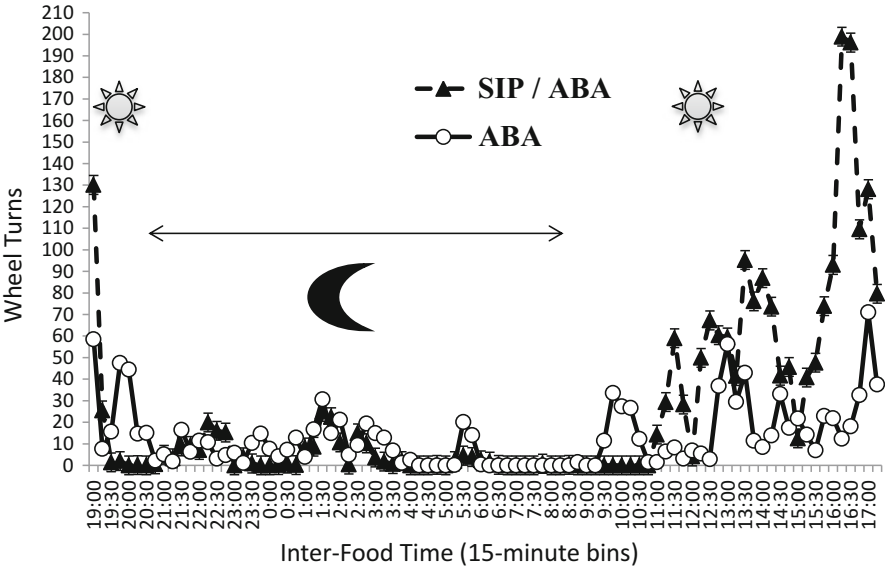

Fig. 2 Hourly distribution of wheel turns in anorexic rats. Wheel turns during the 23 h of running availability of day 6. After this session it was necessary to withdraw the first four rats from the SIP/ABA group

the extent of the results presented here, the critical variable for a higher development of ABA seems to be the running activity developed by the rats, not their failure to adapt to the food regime as both groups ate progressively more as exposure to the ABA procedure advanced and also to a greater extent in the rats that lost more weight.

Indeed, a clinical key aspect of anorexia nervosa is a voluntary reduction of food intake, a symptom that only occurs in the animal model if rats are exposed to the ABA protocol for a long extended time at the risk of dying. Because the procedure is typically stopped when animals have reached a loss of 75% in their weight, with this model we are possibly investigating the conditions that favor the initial development of anorexia rather than the development of the clinical syndrome at full.

Previous literature has shown mutual substitutability between schedule-induced activities, such as when drinking (SIP) and running compete for expression under time constraints imposed by food reinforcement schedules (Roper 1978; Segal 1969; Wetherington and Riley 1986). The running generated under the ABA procedure seems also functionally similar to induced drinking, given its facilitation by prior SIP. Furthermore, the running in ABA occurred predominantly before and after food episodes, which is characteristic of schedule-induced behaviors (Staddon 1977) and contrary to facultative behavior given that it does not seem to be independent of the induction and reinforcement provided by the schedule of food availability.

Thus, a good way to prevent initial risk of developing anorexia nervosa may involve paying attention to feeding regimes, as we know that the frequency of meals is the best determinant of level of induction (e.g., Íbias and Pellón 2011), and by

considering activity as being initiated by the intermittency of feeding, very high and very low feeding schedules will be best options to prevent the development of excessive activity that might lead to the initiation of a phenomenon that can end up in deleterious effects.

Additionally, activity is regarded as reinforcing by itself for caged laboratory rats (e.g., Belke et al. 2017), so that the combination of inducing activity by the intermittency of restricted food and reinforcer activity itself could create a circle for the excessive development of running and the development of losing weight as the defining characteristic of ABA achievement.

Alternatively, the possibility exists that prevention of activity might be a therapeutic treatment of anorexia once developed, but because health recommendations are geared toward prompting exercise and good eating practices, we are more inclined at this point to pay attention to food regimens rather than restricting access to activity, at least as a first approach to prevent development of the disease.

This viewpoint is in line with the original proposal of Epling and Pierce (1992) on the inducing capacity of events that occur intermittently at low frequency, in addition to the contribution of access to activity as a reinforcing event in addition to food, not just in substitution as suggested by Epling and Pierce.

That the marker of running comes from the occurrence of food contradicts in part the proposal of Beneke et al. (1995) who conferred a larger influence to the 24-h circadian rhythm based on light/dark periods. The initial circadian control of activity subsides to the control of the food rhythm in the case of ABA (cf. Fuentes et al. 2015).

Policies and Protocols

In this chapter we have described a study in which it is suggested that the critical role for the initial development of anorexia in an animal model of the disease is the development of excessive activity as a consequence of the intermittent occurrence of feeding episodes. The loss in weight that characterizes the development of anorexia seems to be initiated by the hyperactivity developed by the animals and not as an initial failure to adapt to the feeding regime. This excessive activity finally interferes with eating, thus leading to the deleterious circle of increasing activity and decreasing food intake. It is our recommendation to focus on the strict eating regimes that normally accompany the initial stages of the development of anorexia in humans, suggesting that by setting different meals to appropriate times within a day might be a good health recommendation for potentially attenuating the development of the disease.

Dictionary of Terms

- **Activity-based anorexia** – A laboratory model mostly used with rats that simulates the combination of food dieting and strenuous exercise that characterizes anorexia in humans.

- **Animal model** – The development with nonhuman animals in the laboratory of a model of a disease in humans that looks similar in its appearance and underlying mechanisms.
- **Anorexia nervosa** – A human disease with a high prevalence in young adolescent women (but not only in women) characterized by a dramatic loss in weight normally accompanied by concurrent food dieting and hyperactivity.
- **Schedule-induced polydipsia** – A phenomenon first shown in the laboratory by which moderately hungry rats drink excessive quantities of water when food is scheduled intermittently.
- **Schedule induction** – The occurrence of patterns of behavior only by the delivery of noncontingent relevant events, such as when food is delivered on a time basis to food-deprived rats and it induces drinking, running, grooming, etc.

Summary Points

- This chapter focuses on activity-based anorexia, an animal model of anorexia nervosa in humans.
- Anorexia is characterized by the loss in weight that results from a combination of dieting and exercise.
- It is suggested that excessive exercise is a product of strict intermittent feeding episodes.
- Excessive activity can be induced by intermittent food schedules that also induce other patterns of behavior, notably excessive drinking.
- The previous development of schedule-induced drinking facilitates later development of activity-based anorexia by means of increased wheel-running activity.
- Initial development of activity-based anorexia does not reflect a failure to adapt to food restriction.
- Excessive activity in anorexia seems to be the result of the intermittent scheduling of food episodes.
- Wheel-running activity also seems to be reinforcing for caged animals.
- The way to reduce excessive activity by means of controlling feeding episodes seems to appropriate in place of, or in addition to, simply restricting the possibility of exercising.

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Adaptation of Hepatic, Renal, and Intestinal Gluconeogenesis During Food Deprivation

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Gilles Mithieux, Fabienne Rajas, Amandine Gautier-Stein, and Maud Soty

Contents

Abstract

The maintenance of plasma glucose is a vital necessity in all situations. Multiple adaptations permitting endogenous glucose production from the liver, kidneys, and gut take place during starvation. The mobilization of liver glycogen stores is the more rapid process to tune blood glucose from the moment where glucose

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availability from food starts to lack. Increased lipolysis from the adipose depots plays a crucial role to provide energy to the whole body and especially to allow the liver to carry out gluconeogenesis from lactate and alanine, which is an endergonic process. Proteolysis from skeletal muscles then supplies carbon skeletons to build glucose from amino acids released in blood. Among the exquisite late adaptations of gluconeogenesis taking place after the exhaustion of liver glycogen stores, the progressive replacement of hepatic gluconeogenesis by renal and intestinal gluconeogenesis (from glutamine) is essential, since it permits to maintain plasma glucose and in the same time to preserve energy balance of the body. Gluconeogenesis from glutamine, indeed, is an exergonic process. The late blunting in hepatic GNG paralleling the increase in intestinal GNG in late starvation allows the liver to store glycogen again, which might be a key adaptation for survival.

Keywords

Endogenous glucose production · Liver · Kidney · Intestine · Gluconeogenesis · Glycogen · Glucose-6 phosphatase · Phosphoenolpyruvate carboxykinase · Metabolic acidosis · Insulin · Glucagon

List of Abbreviations

CREB	cAMP-response element binding protein
EGP	Endogenous glucose production
G6Pase	Glucose-6 phosphatase
G6PC	G6Pase catalytic subunit
G6PT	G6Pase transporter subunit
GNG	Gluconeogenesis
PCK1	Phosphoenolpyruvate carboxykinase-cytosolic form

Introduction

The maintenance of plasma glucose concentration around 0.9 mg/L is a vital necessity for all animal classes. Indeed, a few minutes under hypoglycemia is sufficient to rapidly promote deleterious metabolic events in some organs, such as the brain or the gut that require glucose permanently (Delaere et al. 2010). Glucose supply in blood must therefore be continuous. The main source of external glucose under physiological conditions of nutrition derives from food assimilation. However, it is well known that most animal have the capacity to endure long periods of fasting (weeks or even months), which is a condition for survival in the wildlife. Hopefully, natural selection has allowed animals to cope with the eventuality of hypoglycemia during starvation, owing to glucose-producing organs. These are the liver, kidney, and intestine, which are able to synthesize glucose from nonglucidic three carbon compounds and to release it in blood, a process called gluconeogenesis. Specifically, the liver is able to store glucose imported from blood during food assimilation under the form of a

polymeric macromolecule called glycogen. This store can be rapidly mobilized when glucose in blood is lacking, owing to a process called glycogenolysis (Mithieux 1997; Nordlie et al. 1999).

Glucose-6 Phosphatase: Key Enzyme of Endogenous Glucose Production

What makes that the liver, kidneys and intestine are able to produce glucose in blood is the presence of glucose-6 phosphatase (G6Pase). This is the only intracellular enzyme of the body capable of hydrolyzing glucose-6 phosphate that can be either released from glycogen stores in the liver or synthesized via gluconeogenesis in the 3 organs (Mithieux et al. 2004b) (Fig. 1). Both the catalytic subunit of G6Pase (called C6PC) and a glucose-6 phosphate transporter subunit (called G6PT) essential to G6Pase activity are intramembranous proteins, deeply embedded in the endoplasmic reticulum membrane.

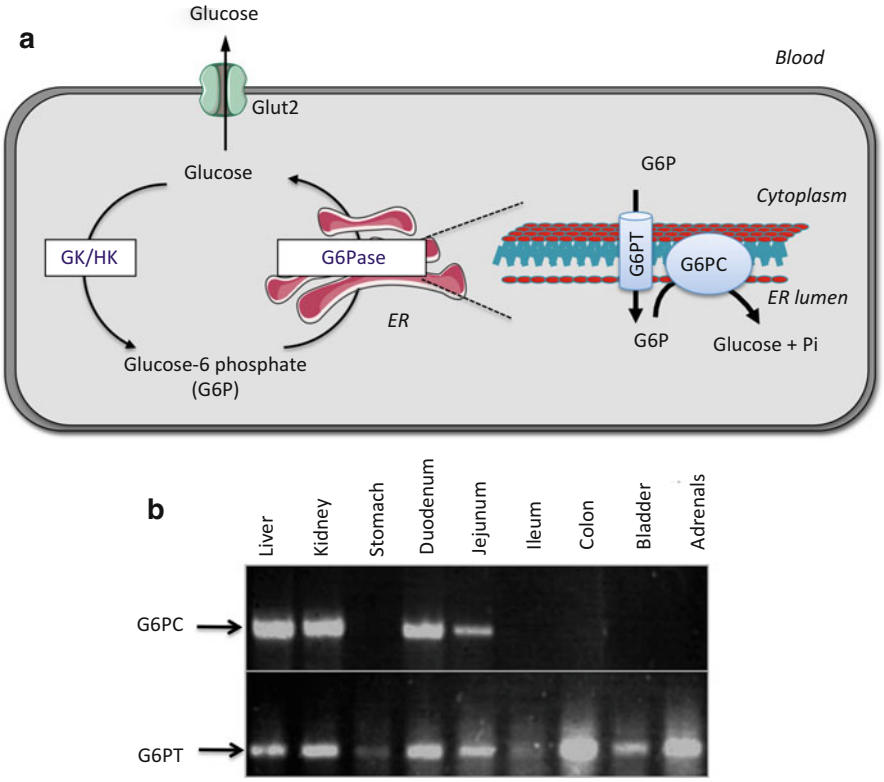

Fig. 1 Glucose-6-phosphatase is the key enzyme in endogenous glucose production. *Panel A*: Position of glucose-6-phosphatase in metabolism and structure within the membrane of the endoplasmic reticulum. *Panel B*: Expression of *g6pc* and *g6pt.* genes in various tissues, studied through the presence of their respective mRNAs

Whether or not they are in physical interaction is still to be deciphered (Soty et al. 2012). The G6PT subunit is ubiquitous, and therefore, it is G6PC expression which confers the capacity on the liver, kidney, and gut to produce glucose in blood (Fig. 1). It is noteworthy that *G6PC* gene expression and G6Pase activity are increased during starvation, when glucose release in blood is needed. This takes place in the three gluconeogenic organs (Mithieux 1997; Rajas et al. 1999).

Adaptations of Endogenous Glucose Production in Postabsorptive Period

Utilization of Hepatic Glycogen Stores

Once the postprandial period is ending, glucose production in blood becomes crucial to avoid the drop in plasma glucose, which would be the consequence of glucose utilization by all organs of the body to feed glycolysis. Endogenous glucose is first produced from the hepatic glycogen stores, possible owing to the activity of a key enzyme: glycogen phosphorylase. Glycogen phosphorylase is controlled by glucagon secreted by pancreatic α-cells in the postabsorptive and fasting states, and insulin secreted by pancreatic β-cells in response to elevations in plasma glucose. Under the action of the glucagon receptor and its second messenger cAMP, the phosphorylation of glycogen phosphorylase by the catalytic subunit of protein kinase A (PKA) activates the enzyme (Saltiel 2001). In the same time, PKA inhibits glycogen synthase, the regulatory enzyme of glycogen synthesis opposing to glycogen phosphorylase. The decrease in plasma insulin (an inhibitory hormone of glycogen phosphorylase and an activating hormone of glycogen synthase) occurring from the end of the postprandial period facilitates the activation of glycogen phosphorylase by glucagon and the release of glucose from hepatic glycogen stores (Fig. 2). Glucose-1 phosphate released from glycogen is converted in glucose-6 phosphate, which is then hydrolyzed in glucose and Pi by G6Pase. Glycogen stores are considered to contribute to endogenous glucose production (EGP) for about 10–12 h in rodents and 20 h in human subjects. After exhaustion of hepatic glycogen, gluconeogenesis must relay glycogenolysis to maintain plasma glucose. It must be recalled that glycogen storage is a specificity of the liver among gluconeogenic organs, since the kidney and the small intestine are unable to carry out glycogen synthesis under physiological conditions.

Posttranslational Activation of Gluconeogenesis

It is noteworthy that the enzymatic activity of two regulatory enzymes of gluconeogenesis, namely G6Pase and fructose-1,6-bisphosphatase, is activated by mechanisms dependent on glucagon and cAMP (Ichai et al. 2001; Rider et al. 2004; Soty et al. 2016). It could be further specified that G6PT, rather than G6PC, is the target of the activation of G6Pase activity by PKA (Soty et al. 2016) and that the mechanism

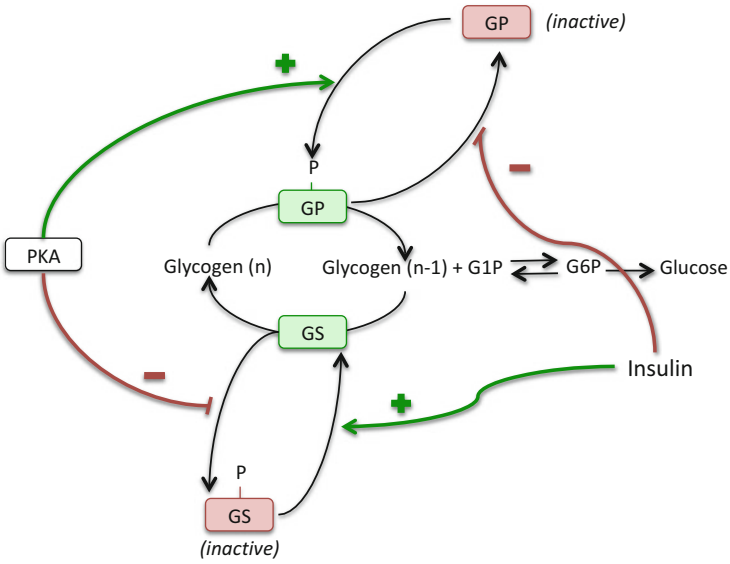

Fig. 2 Control of glycogen synthesis and glycogenolysis by glucagon and insulin. Regulation by phosphorylation of glycogen synthase (*GS*) and glycogen phosphorylase (*GP*) by PKA and insulin

of activation requires biochemical conditions compatible with membrane fluidity (Ichai et al. 2001) (Fig. 3). It is noteworthy that G6Pase activity is also controlled (inhibited) during the fasted to fed transition (Minassian et al. 1995, 1999), via a mechanism involving both hyperglycemia and insulin (Guignot and Mithieux 1999). Under the action of insulin, its target phosphatidylinositol-3 kinase is translocated at the endoplasmic reticulum membrane and suppresses G6Pase activity, via competitive inhibition by its product phosphatidylinositol-(3,4,5) triphosphate (Fig. 3) (Mithieux et al. 1998; Daniele et al. 1999). The drop in plasma insulin and glucose accompanying fasting thus promotes reversal of this inhibition. It must be mentioned that additional regulations of G6Pase activity involve free fatty acids and fatty acyl-CoA species (Mithieux et al. 1993; Mithieux and Zitoun 1996) and α-ketoglutarate in the presence of Mg^{++} (Mithieux et al. 1990; Minassian et al. 1994). Interestingly, the decrease in liver α-ketoglutarate concentration at the beginning of fasting might play a role in the stimulation of hepatic glucose production (Minassian et al. 1994).

Besides, fructose-1,6 bisphosphatase is indirectly activated in response to the drop in fructose-2,6 biphosphate, its key allosteric activator. The latter, indeed, is hydrolyzed by fructose-2,6 bisphosphatase-phosphofructokinase 2, a bifunctional enzyme hydrolyzing or synthetizing fructose-2,6 bisphosphate from fructose-6 phosphate, which phosphatase activity is activated by PKA (Rider et al. 2004) (Fig. 4). Interestingly, the PKA-dependent drop in fructose-2,6-bisphosphate promotes the inhibition of phosphofructokinase 1, the glycolytic enzyme opposing to fructose-1,6-bisphosphatase, thus favoring the substrate flux in the sense of gluconeogenesis (Fig. 4).

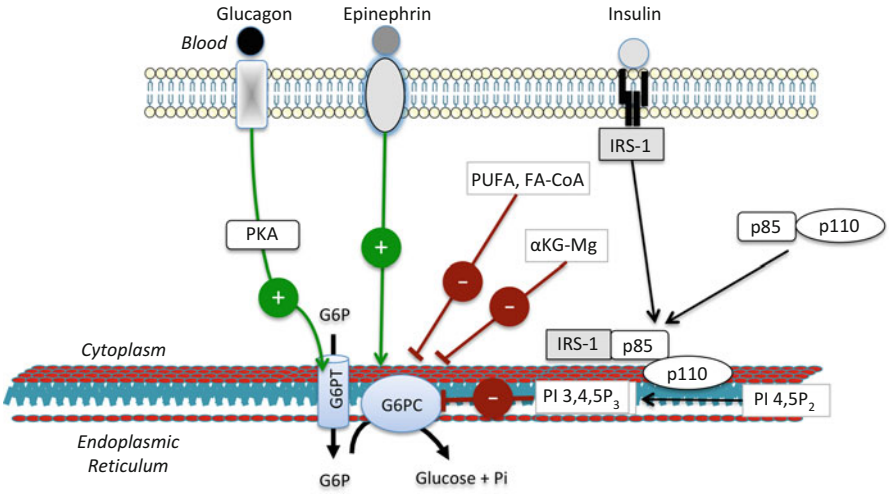

Fig. 3 Regulation of glucose-6-phosphatase activity by hormones and nutrient-derived metabolites. Biochemical regulations of G6Pase activity by glucagon/PKA, epinephrin, polyunsaturated fatty acids (*PUFA*), fatty-acyl-CoAs (*FA-CoA*), alpha-ketoglutarate-magnesium chelates (*aKG-Mg*), and insulin/phosphatidylinositol-3 kinase (p85 refers to the regulatory subunit and p110 to the catalytic subunit). It has been specified that PKA-dependent phosphorylation is targeted on G6PT and not G6PC

Two other important enzymes in gluconeogenesis are pyruvate carboxylase converting pyruvate in oxaloacetate and phosphoenolpyruvate carboxykinase-cytosolic form (PCK1) converting oxaloacetate in phosphoenolpyruvate. No short-term regulation has been demonstrated up to know for PCK1. It is noteworthy that pyruvate carboxylase activity is stimulated by acetyl-CoA, a product of free fatty acid oxidation, which is a process activated by glucagon (McGarry and Foster 1980; Puigserver and Spiegelman 2003). Therefore, pyruvate carboxylase is activated during fasting, once adipose tissue lipolysis has taken place and free fatty acids are elevated in the plasma (Hue 1987) (Fig. 5).

Long-Term Adaptations of Gluconeogenesis During Fasting

Induction of Gluconeogenesis Gene Expression

If fasting continues after the prior postabsorptive period, the induction of gluconeogenesis genes expression takes place to sustain EGP. Glucagon and its second messenger cAMP are key regulators of gluconeogenesis genes expression, together with glucocorticoids, the secretion of which is increased during starvation. This increases the amount of enzymes able to produce glucose in blood. This control relates to *G6pc* gene expression at a transcriptional level, especially involving a crucial transcription factor, named CREB for cAMP-response element binding

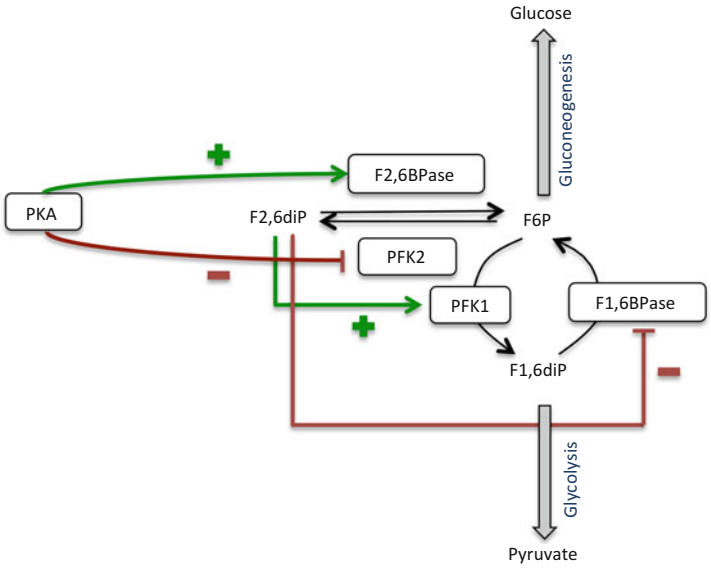

Fig. 4 Control of fructose-1,6-bisphosphatase and phosphofructokinase activities by protein kinase A. Regulation of fructose-1,6 bisphosphatase (*F1,6BPase*) and phosphofructokinase-1 (*PFK1*) by fructose-2,6 diphosphate (*F2,6diP*). The synthesis/degradation of F2,6 diP is controlled by PKA-phosphorylation via the regulation of the bifunctional enzyme (activation of fructose-2,6 bisphosphatase (*F-2,6BPase*), inhibition of phosphofructokinase-2 (*PFK2*)

protein. This factor is phosphorylated by PKA under the influence of cAMP, which increases its binding to the *G6pc* gene regulatory sequences (promoter) and the production of *G6pc* mRNA (and then protein product). Transcriptional regulation by glucagon and glucocorticoids requires functional interaction of phosphorylated CREB with several other transcription factors (Gautier-Stein et al. 2005), including the glucocorticoid receptor (Vander Kooi et al. 2005) (Fig. 6). Interestingly, CREB plays a crucial role in the liver and in the two other gluconeogenic organs as well (Gautier-Stein et al. 2006; Mutel et al. 2011). It must be mentioned that comparable regulations involving cAMP, PKA, and glucocorticoids take place for PCK1, another crucial regulatory enzyme of gluconeogenesis, in the liver and intestine (Hue 1987; Mutel et al. 2011). However, it must be specified that a process dependent on metabolic acidosis accounts for the induction of PCK1 gene expression in the kidney as fasting prolongs (Alleyne and Scullard 1969; Mutel et al. 2011).

Altered Repartition of Endogenous Glucose Production Among Gluconeogenic Organs

At the beginning of postabsorptive period, a time where liver glycogen stores are present and are being mobilized, the liver accounts for at least 80% of EGP in rats (Fig. 7). Renal GNG in this situation accounts for about 15–20% and intestinal GNG

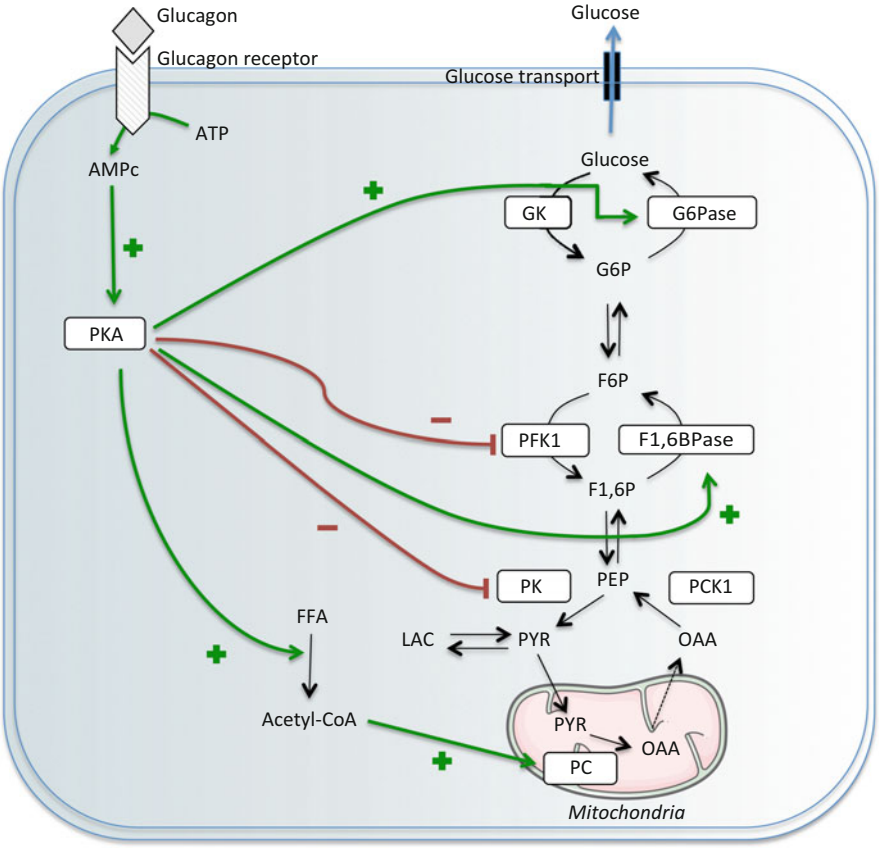

Fig. 5 Regulation by glucagon and protein kinase A of key steps of glycolysis and gluconeogenesis. Glucagon and PKA control multiple steps of glycolysis/GNG. Protein kinase A inhibits pyruvate kinase (*PK*) directly by phosphorylation, which inhibits glycolysis and redirects phosphoenolpyruvate (*PEP*) in GNG. In the same time, PKA activates pyruvate carboxylase (*PC*) indirectly via acetyl-CoA produced from free fatty acids (*FFA*). This increases the amount of oxaloacetate (*OAA*), the substrate of PCK1, and contributes to the stimulation of GNG

is weak, estimated between 5% and 10% of EGP (Croset et al. 2001; Mithieux et al. 2006; Pillot et al. 2009). It must be mentioned that intestinal GNG can be induced under the influence of some macronutrients, such as in animals fed foods enriched in proteins (Mithieux et al. 2005; Duraffourd et al. 2012) or in soluble fibers (De Vadder et al. 2014; De Vadder et al. 2016). However, this is out of the scope of the adaptations to starvation described herein. The repartition is dramatically different at 24 h of fasting, a time at which glycogenolysis has ended because of glycogen store exhaustion. In this case, intestinal and renal GNG increase their participation to EGP to account for about 20–25% and 50–55% of EGP, respectively, in fasting rats (Croset et al. 2001; Pillot et al. 2009) (Fig. 7). It is noteworthy that comparable results were obtained for renal GNG in humans in the postabsorptive state and after

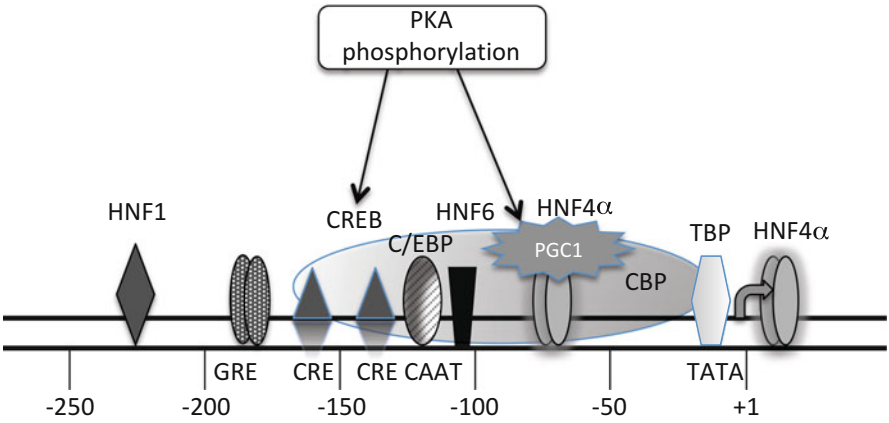

Fig. 6 Regulation by protein kinase A and glucocorticoids of gene transcription of glucose-6 phosphatase catalytic subunit. The phosphorylation of CREB by PKA activates its binding to two CREB response elements (*CRE*) of the *G6pc* promoter and in parallel to the promoter of *Pgc1* (peroxisome proliferator-activated receptor gamma coactivator 1-alpha). Glucocorticoids promote the binding of the glucocorticoid receptor (*GR*) to the GR element of the *G6pc* promoter. Several tissue-specific transcription factors: C/EBP (CAAT-enhancer binding protein), HNF1, HNF4α, and HNF6 (hepatic nuclear factors) also contribute to the stabilization of the cofactors PGC1 and CBP (CREB binding protein), which results in the induction of the transcriptional activity of *G6pc*

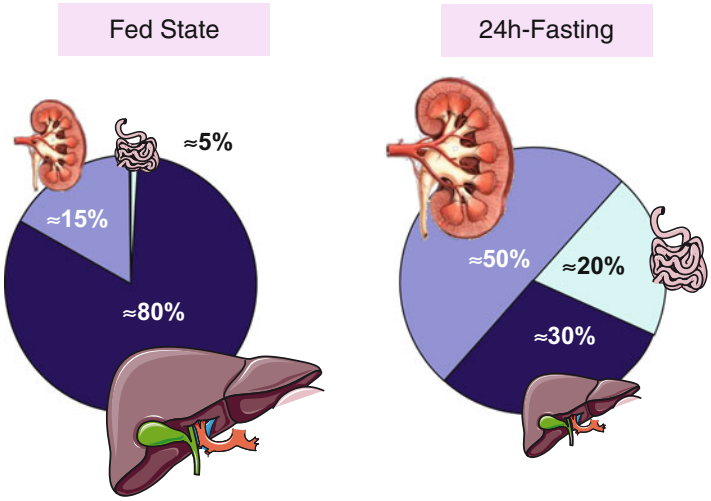

Fig. 7 Repartition of glucose production among gluconeogenic organs in the fed post-absorptive state and in 24 h-fasting state. *Left panel*: the rats were studied at the beginning of the postabsorptive period (food removal 6 h before). *Right panel*: relates to the situation at 24 h of fasting. The results shown here are compiled from Croset et al. (2001), Mithieux et al. (2006), and Pillot et al. (2009)

prolonged fasting, respectively (Owen et al. 1969; Gerich et al. 2001). It is likely that a combination of posttranslational regulations of gluconeogenic enzyme activities and inductions of gluconeogenic gene expression concur to these changes in EGP.

Late Adaptations During Starvation

Surprisingly, if fasting lasts more than two days in rats, after a prior phase of liver glycogen exhaustion up to 48 h of fasting, a rebound in glycogen stores to about 15% of the fed state level takes place at 72 h and 96 h of fasting (Minassian et al. 1994). This is the likely result of the combination of at least three processes taking place after 48 h of fasting. (1) There is a further increase in intestinal GNG between 48 h and 72 h of fasting, a time where IGN may account to about 1/3 of EGP (Mithieux et al. 2004a). (2) There is an intriguing drop in hepatic *G6pc* gene expression, promoting a decrease in G6Pase activity at 72 h of fasting (Minassian et al. 1994; Minassian et al. 1996). In contrast, G6Pase activity further increases in kidney and plateaus in gut after 48 h of fasting (Fig. 8). (3) There is a reset of hepatic α-ketoglutarate at a fed level, i.e., a concentration at which this metabolite is able to inhibit G6Pase activity (Minassian et al. 1994). In agreement with the inhibition of G6Pase activity, an increase in hepatic glucose-6-phosphate occurs at 72 h of fasting, thus favoring the synthesis of glycogen, since glucose-6-phosphate is a key allosteric activator of glycogen synthase (Villar-Palasi and Guinovart 1997). That *G6pc* gene expression resumes fed level at 72 h of fasting could appear surprising, since the gene is controlled by the glucagon/insulin ratio and no change in this ratio occurs between 48 h and 72 h of fasting (Minassian et al. 1996). Interestingly, it has been shown that IGN, via the nervous detection of glucose released in the portal vein, is able to activate a gut-brain-liver neural circuit promoting hepatic insulin sensitivity and inhibiting hepatic glucose production (Pillot et al. 2009; De Vadder et al. 2016). Therefore, intestinal GNG might have a role in this process, being further increased at 72 h of fasting compared with 48 h (Mithieux et al. 2004a).

Benefits for Survival Provided by Induction of Renal and Intestinal Gluconeogenesis During Starvation

Glucose homeostasis being a vital phenomenon (see introduction), it is obvious that the recruitment of renal and intestinal GNG is beneficial during food deprivation. However, there are also multiple other benefits in energy homeostasis provided by the induction of renal and intestinal GNG during food deprivation.

First, glutamine is a major substrate for both renal and intestinal GNG (Croset et al. 2001; Gerich et al. 2001). On the contrary, the liver is unable to metabolize glutamine since, due to its kinetic properties, liver glutaminase cannot use glutamine at physiological concentration (Hartmann and Plauth 1989). Therefore, renal and intestinal GNG allow the body to engage glutamine carbons in gluconeogenesis, a process that the liver is unable to do. One must keep in mind that glutamine released

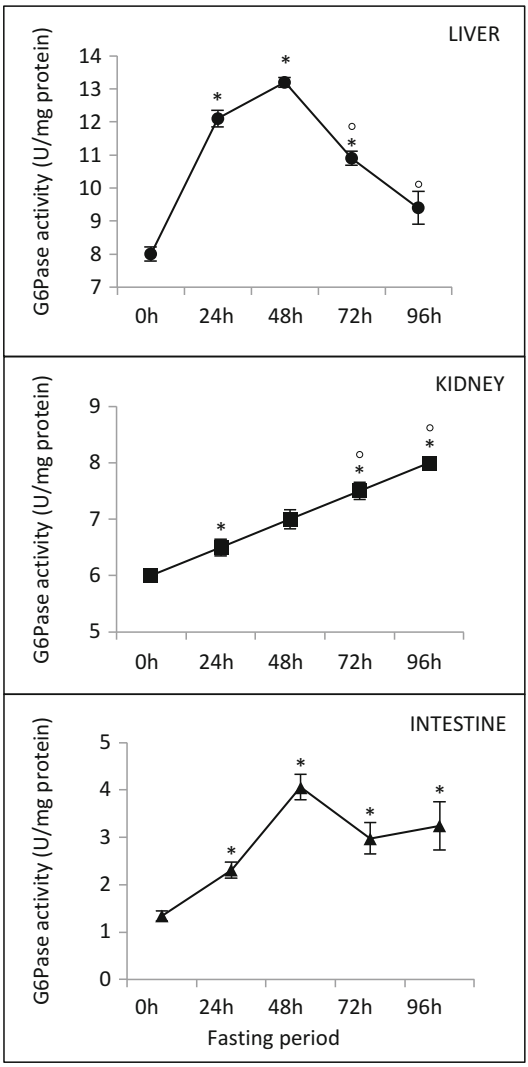

Fig. 8 Time course of hepatic, renal, and intestinal glucose-6-phosphatase activity in starvation. The results are expressed as unit of enzymatic activity/g of tissue. The data shown are compiled from Minassian and Mithieux (1994), Minassian et al. (1994), and Mithieux et al. (2004a). *, different from time 0; °, different from time 48 h

in blood in response to the activation of muscular proteolysis represents a crucial gluconeogenic reservoir during starvation.

Second, in the intestine, glutamate (produced from glutamine by glutaminase) is metabolized by glutamate-pyruvate carboxylase, a two-substrate enzyme utilizing pyruvate as a second substrate and releasing alanine from pyruvate (Windmueller and Spaeth 1974; Watford 1994). Pyruvate is also utilized by lactate dehydrogenase to produce lactate. Alanine and lactate, released in the portal vein, are then captured by the liver, in which they constitute the main gluconeogenic substrates. Therefore, a consequence of the use of glutamine by intestinal GNG is the feeding of hepatic GNG (Croset et al. 2001).

Third, it is a widely accepted dogma that gluconeogenesis, as anabolic process, is endergonic and consumes energy. This is indeed true for hepatic GNG from lactate and alanine. However, glutamine, a five-carbon compound, is partly oxidized in the Krebs cycle and provides energy before three remaining carbons are incorporated into the glucose skeleton. As a consequence, glucose production from glutamine by renal and intestinal GNG is exergonic and produces four ATP per mole of glucose synthesized (Mithieux et al. 2004b). Therefore, a major advantage of the switch from hepatic to intestinal and renal GNG during starvation is that it permits to fulfill the need of glucose homeostasis and in the same time this preserves the energetic balance of the kidney and intestine. It is noteworthy that in the liver and intestine, a comparable benefit may be ascribed to gluconeogenesis from glycerol (+4 ATP per mole of glucose produced). Gluconeogenesis from glycerol becomes substantial following the activation of adipose tissue lipolysis accompanying fasting (Croset et al. 2001; Mithieux et al. 2004b).

At last, the presence of glycogen stores in starvation is likely very useful in the situations requiring an urging availability in blood glucose. This relates for example to situations requiring to run, e.g., to escape a predator, to catch a pray, or to fight, which are under the influence of stress hormones (glucagon, catecholamines). Therefore, the novel repartition of gluconeogenesis permitting the rebound of liver glycogen stores (see above) may be essential for survival in the wild life.

Conclusion

There are multiple metabolic adaptations taking place in animals permitting to cope with food deprivation. The maintenance of plasma glucose constant is certainly a vital necessity within a time scale in the minute range. Increased lipolysis from the adipose depots plays a crucial role to provide energy to the whole body and especially to permit liver gluconeogenesis. The mobilization of liver glycogen stores is the more rapid process to tune blood glucose from the moment where glucose availability from food starts to lack. Among the exquisite adaptations of gluconeogenesis taking place after the exhaustion of liver glycogen stores, the replacement of hepatic GNG by renal and intestinal GNG seems essential, permitting to maintain plasma glucose and in the same time to preserve energy balance of the body. The late mirror evolution of hepatic and intestinal GNG in late starvation, allowing the liver to store glycogen again, is likely a key adaptation for survival.

Dictionary of Terms

- **Gluconeogenesis** – A process of synthesis of glucose from nonglucidic compounds, e.g. amino acids.
- **Glycogen** – A macromolecule that is a polymer of glucose and serves as storage of glucose.

Summary Points

- The maintenance of plasma glucose is a function of vital necessity during starvation.
- Endogenous glucose production allows the body to maintain plasma glucose during food deprivation.
- Glucose-6-phosphatase is the key enzyme of endogenous glucose production, since it is capable of hydrolyzing glucose-6-phosphate.
- Glucose-6-phosphatase is expressed in the liver, kidney, and intestine exclusively, and confers on these organs only the capacity to release glucose in blood.
- Hepatic glycogen stores are first mobilized to cope with short deprivation of food glucose.
- Gluconeogenesis from the liver, kidney, and intestine is then induced to maintain plasma glucose during lasting food deprivation.
- Glucagon and its intracellular relay protein kinase A play a key role in the activation of gluconeogenesis, via biochemical regulations of several gluconeogenic enzymes and inductions of gene expression at a transcriptional level.
- The progressive replacement of liver gluconeogenesis from alanine and lactate (an endergonic process) by renal and intestinal gluconeogenesis from glutamine (an exergonic process) during starvation is beneficial for energy balance.
- The late storage of glycogen in the liver, combining a suppression of hepatic glucose production and sustained intestinal gluconeogenesis, is a notable adaptation of interest for survival.

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Stress Response of Nutrient-Starved Cardiovascular Cells

111

Lakshmi Pulakat and Madhavi P. Gavini

Contents

Abstract

Both starvation and over-nutrition are conditions that exert nutrient stress on cardiovascular cells. Undernutrition results in reduced availability of micro- and macronutrients to all organ systems. However, studies have shown that overweight and obese individuals also have micronutrient deficiencies that contribute to the pathogenesis of cardiovascular disease. In utero nutrient deficiency negatively affects birth weight, total cholesterol, glucose tolerance, triglycerides, risk of coronary heart disease, and obesity. These effects are intergenerational. Some of the key signaling pathways responsible for these effects are discussed in this chapter. mTORC1 functions as a nutrient sensor; both overactivation of mTORC1

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via over-nutrition and chronic suppression of mTORC1 causes cardiovascular stress and CVD. mTORC1 overactivation is well-addressed clinically via the development of inhibitors such as rapamycin. However, chronic suppression via inhibitors leads to suppressed expression of cardiac prosurvival proteins including MCL-1. Activation of the anti-inflammatory arm of the RAS (AT2R) via peptide agonist NP-6A4 restores MCL-1 and protects cardiomyocytes from nutrient stress.

Keywords

Cardiovascular disease · *mTOR* · Rapamycin · MCL-1 · IRS · AKT/PKB · Angiotensin II · AT1R · AT2R · NP-6A4 · ARB

List of Abbreviations

ACS	Acute coronary syndrome
AdipoR1	Adiponectin receptor 1
AMP	Adenosine monophosphate
Ang II	Angiotensin II
AKT/PKB	Protein kinase B
asTORi	Active-site mTOR inhibitor
AT1R/AT2R	Angiotensin II type 1 receptor/ type 2 receptor
ARB	Angiotensin II type 1 receptor blocker
BMI	Body mass index
CI	Cell index
CGP42112A	AT2R peptide agonist
CHF	Congestive heart failure
CRP	C-reactive protein
CVD	Cardiovascular disease
Deptor	DEP-domain-containing mTOR interacting protein
GAP	GTPase activating protein
GDP	Guanosine diphosphate
GFP	Green fluorescent protein
GTP	Guanosine triphosphate
GPCR	G-protein coupled receptor
hCAVSMC	Human coronary artery vascular smooth muscle cells
HL-1	A mouse atrial cardiomyocyte cell line
IL-1alpha	Interleukin 1 alpha
IL-6	Interleukin 6
IRS1/2	Insulin receptor substrate 1/substrate 2
IRS	Insulin receptor substrate
LAMP-1	Lysosomal-associated membrane protein 1
MACE	Major adverse cardiac events
MCL-1	Myeloid cell leukemia 1
miR-26/miR-29	microRNA 26/29
mLST8	Sec13 protein 8 (also known as GbL)
mSIN1	Mammalian stress-activated protein kinase interacting protein

mTORC1 and mTORC2	Mechanistic target of rapamycin complexes 1 and 2
MMP2/9	Matrix metalloproteinase 2/9
NP-6A4	AT2R agonist developed by Novopyxis Inc.
NRK	Normal rat kidney cells
PD123319	AT2R antagonist
PLC	Phospholipase C
PRAS40	Prolinerich AKT substrate 40 kDa
Protor-1	Protein observed with Rictor-1
PTEN	Phosphatase and tensin homolog
PP2A	Protein phosphatase 2A (PP2A)
PP242	A drug that inhibits mTOR
PI3K	Phosphatidyl inositol 3 kinase
RAG GTPases	Sixth subfamily of Ras-related GTPases (consisting of RAGA, RAGB, RAGC, and RAGD) where Ras is a superfamily of small molecular weight GTPases
Raptor	Regulatory-associated protein of mTOR
Rictor	Rapamycin-insensitive companion of mTOR
Rheb	Ras homolog enriched in brain
S6 K1	Ribosomal protein S6 kinase beta-1 also known as p70s6 kinase
TSC	Tuberous sclerosis complex consisting of TSC1 or hamartin and TSC2 or tuberin
TIMP1	Tissue inhibitor of metalloproteinases
TNFα	Tumor necrosis factor-alpha
RAS	Renin angiotensin system
Ras-Raf-MEK-ERK1/2	Ras-mediated growth promoting signaling pathway
Src	Proto-oncogene tyrosine-protein kinase Src
4E–BP	Eukaryotic translation initiation factor 4E–binding protein

Introduction

Despite significant advances in diagnosis and treatment options, cardiovascular disease (CVD) is still the leading cause of death globally (Benjamin et al. 2017). Several factors contribute to the high mortality and increasing prevalence of CVD including increased rates of diabetes, obesity, poor diet, and physical inactivity. As a result, the majority of research efforts are focused on overnutrition and lack of physical activity, as they are the leading contributing factors to CVD. Overnutrition causes obesity and exerts nutrient stress on cardiovascular cells via a pathological increase in a constellation of nutrients such as glucose, cholesterol, and amino acids. These conditions lead to hemodynamic overload, hypertension, insulin resistance, Type 2 diabetes, atherosclerotic plaque formation, narrowing of arteries, and ischemic conditions that result in reduced availability of oxygen and nutrients to cardiovascular cells. These effects are intergenerational and lead to elevated CVD rates (Patti 2013).

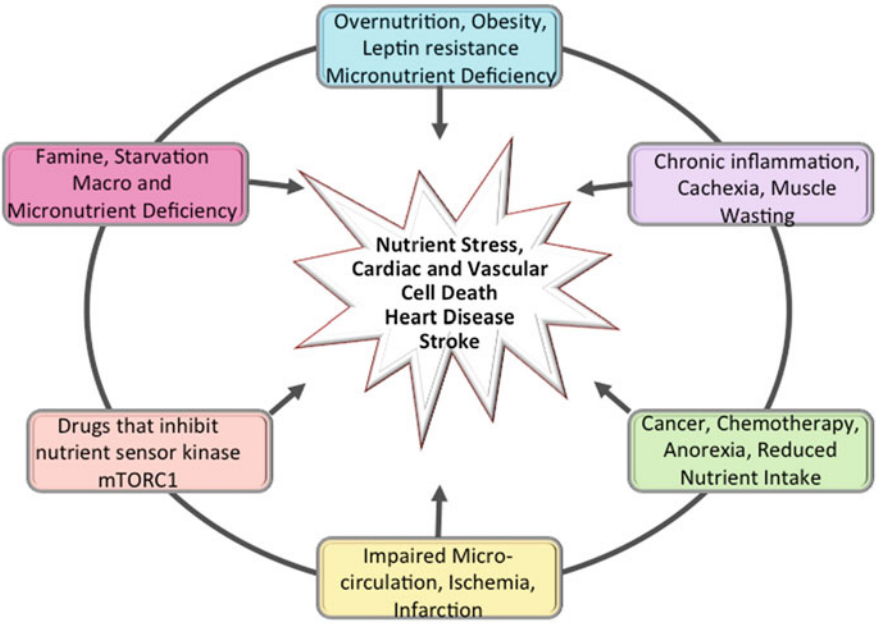

Fig. 1 Conditions that contribute to nutrient stress of cardiovascular cells. Famine, starvation, and micronutrient deficiency caused by drugs (mTORC1 inhibitors, chemotherapeutic agents), obesity, impaired microcirculation, and ischemia induce nutrient stress of cardiovascular cells

Like obesity, starvation and chronic undernutrition can also cause cardiovascular stress and subsequent CVD (Gottdiener et al. 1978; Fisler 1992). Additionally, starvation increases the intergenerational risk factors for developing CVD (Desai et al. 2015). Cachexia also exerts severe nutrient stress on cardiovascular cells. Thus, nutrient stress on cardiovascular cells comes from different underlying conditions (Fig. 1.). The focus of this review is on the mechanisms that contribute to nutrient stress of cardiovascular cells, their effects on cardiovascular cell survival and function, and current approaches to mitigate such stress.

Effect of Famine on CVD

Since it is not possible to study chronic undernutrition in humans via clinical trials, the majority of our knowledge on starvation comes from historical instances of famine exposure in various populations. Despite some limitations in famine research, there are strong population-wide correlations to mortality, fertility, weight gain during pregnancy, birth weight, and subsequent risk of cardiovascular disease (Painter et al. 2006, Lumey et al. 2011).

In studies on prenatal famine exposure in the Dutch famine, gestation stage during famine exposure impacted outcomes (Painter et al. 2006). For example,

Table 1 Effect of in utero exposure to famine on cardiovascular diseases and their predictors. *Pink labeled columns* denote female-specific effects

Characteristic	Early Gestation	Mid-Gestation	Late Gestation
Obesity	More Prevalent	Normal	Normal
Elevated Cholesterol	Elevated	Elevated	Elevated
Elevated Triglycerides	Elevated	Elevated	Elevated
Glucose Tolerance	Reduced (–21%)	More Reduced (–53%)	Reduced (–21%)
Birth Weight	Low	Low	Normal
Coronary Artery Disease (CAD)	High (8.8% vs 3.2%)	Normal	Normal

Lumey et al. stated that while children of mothers who were exposed to famine during their third trimester had normal birth weights, those exposed earlier in pregnancy (during their first or second trimesters) had firstborn offspring with lower birth weights compared to unexposed controls (Stein and Lumey 2000). Importantly, famine exposure effects proved to be intergenerational since mothers born after third-trimester famine exposure had babies with lower birth weights. In addition to low birth weight, the cardiovascular effects of in utero famine exposure are seen primarily in those exposed to famine in early gestation (Lumey et al. 2011). Those individuals had a higher prevalence of coronary heart disease compared to nonexposed individuals (8.8% v 3.2%; odds ratio adjusted for sex 3.0, 95% confidence interval (CI) 1.1 to 8.1) (Table 1) (Roseboom et al. 2000).

A significant risk factor for CVD is type 2 diabetes. Diabetes risk was also affected by famine exposure in utero (Lumey et al. 2011). Glucose tolerance was shown to be impaired in those who were prenatally exposed to famine. This effect was more profound in those exposed to famine mid-gestation with a disposition (−53% [−126 to −3]) compared with controls. Impaired glucose tolerance is an early symptom of type 2 diabetes, which is an independent risk factor for CVD (Table 1).

Elevated triglycerides and total cholesterol, which are other risk factors for CVD, also increased in populations exposed to famine in utero (Stein et al. 2009; Lumey et al. 2011). Studies by Lumey et al. showed that famine exposure at any time in gestation was associated with an increase in the level of total cholesterol and triglycerides in women. While the increase in triglycerides was independent of mid-thigh circumference, it was attenuated by controls for waist circumference and BMI. However, increases in total cholesterol were independent of BMI as well as waist and mid-thigh circumference. Interestingly, obesity was also increased in women exposed to famine during early gestation in the Dutch famine (Ravelli et al. 1999). This effect was also seen in studies that examined populations from other famines including those born during the famine years of the Chinese Great Leap Forward (Luo et al. 2006; Yang et al. 2008). Results from these population studies

show that famine exposure resulted in increases in many of the same independent risk factors for CVD such as obesity. These observations also provide insights into famine survival and subsequent development of CVD. The mechanisms behind this seeming paradox are explained in the section below.

Both starvation and overnutrition are conditions that exert nutrient stress on cardiovascular cells. Undernutrition results in reduced availability of micro- and macronutrients to all organ systems in the affected individual. Micronutrient deficiency is an important underlying reason for cardiovascular pathology in response to starvation (Benjamin et al. 2017). However, obesity results in impaired microcirculation, which can restrict nutrient flow and availability to key organs (de Jongh et al. 2004). Although increased dietary consumption (carbohydrate, sugar, protein, lipids) is linked to overnutrition and obesity, studies have shown that overweight and obese individuals still have micronutrient deficiencies (Via 2012). Michael Via highlights that obese individuals often have significant deficiencies in vitamin D, chromium, biotin, and thiamine (Via 2012) and their absence contributes to the progression of type 2 diabetes. Supplementation with the micronutrients results in positive clinical trial outcomes. This was corroborated in a recent study by Saponaro et al., which used data from 261 heart failure patients and demonstrated that they were deficient in vitamin D (Saponaro et al. 2017). This has been corroborated by several meta-analyses of epidemiological studies (Grandi et al. 2010; Pilz et al. 2016, Wei et al. 2016; Saponaro et al. 2017).

Other important micronutrient deficiencies include reduced vitamin A and iron (Syrkis and Machtey 1973; Zhao et al. 2015; Wei et al. 2016). A recent study on school-age children in Chongqing, China, has highlighted the association of vitamin A insufficiency with obesity (Wei et al. 2016). Iron deficiency (hypoferremia) is also associated with obesity. A recent quantitative meta-analysis was performed using 26 cross-sectional and case-control studies comprising 13,393 overweight and obese individuals and 26,621 normal weight participants. Results from that study demonstrate that overweight and obese individuals have hypoferremia (Zhao et al. 2015). Additionally, it is suggested that hypoferremia is associated with myocardial infarction (Syrkis and Machtey 1973). These analyses show that micronutrient deficiencies that arise as a result of either obesity or undernutrition can induce similar nutrient stress on the cardiovascular system (Fig.2). In the next section, the maladaptive cardiovascular signaling due to nutrient stress as a way of elucidating the molecular mechanisms responsible for cardiac dysfunction is discussed.

Nutrient Stress Associated Maladaptive Cardiovascular Signaling

The mTORC1 and the critical need to achieve a fine balance of mTOR signaling in cardiovascular cells: The mechanistic target of rapamycin (mTOR) serves as a regulator of an evolutionarily conserved mechanism that is critical for the survival of cardiovascular cells (Mazelin et al. 2016). mTORC1 functions as a nutrient sensor. During the initial stage of starvation and conditions of calorie restriction without malnutrition, mTORC1 activity is suppressed (Wullschleger et al. 2006). mTOR is a hub for the signaling activated by amino acids, glucose, insulin, and

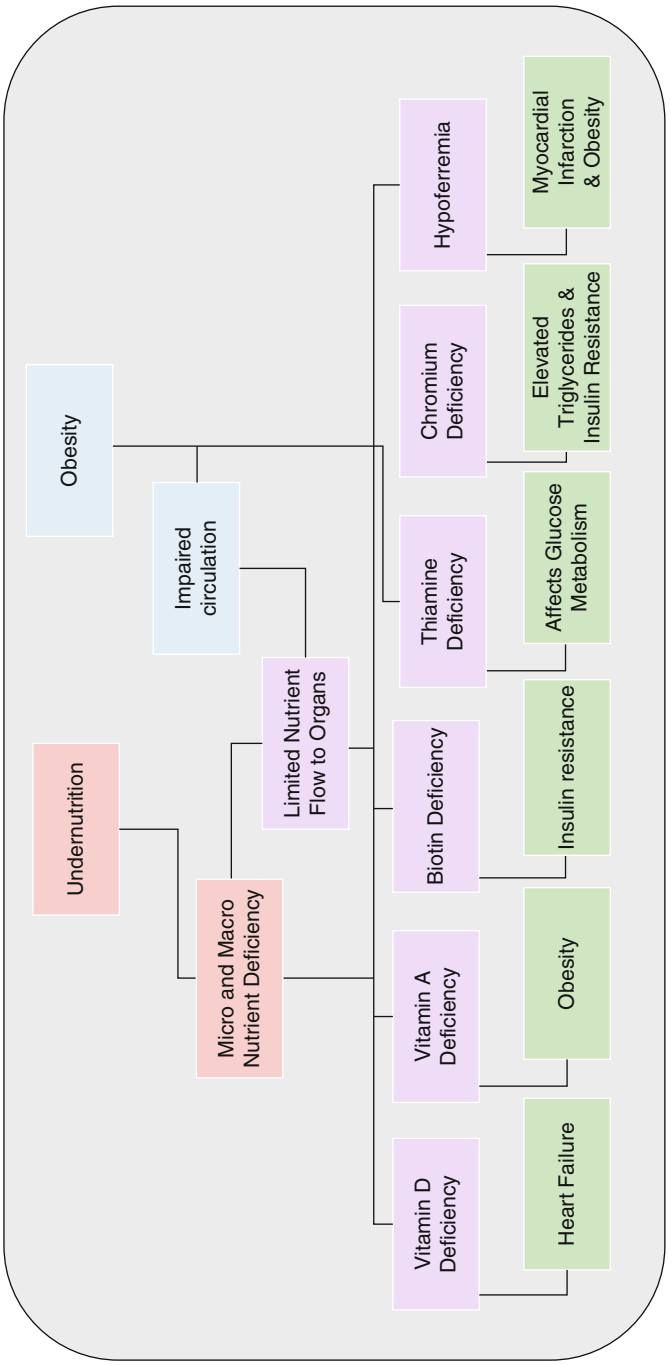

Fig. 2 Nutrient deficiencies common to obesity and undernutrition and their effects on CVD and its predictors. Undernutrition results primarily in the lack of availability of macro- and micronutrients critical for cell survival and cause CVD. Obesity causes impaired circulation and changes in signaling pathways that lead to ischemia and restrict availability of nutrients to cardiovascular cells

many growth factors and cytokines. There are two distinct complexes that activate differential signaling mechanisms via complexes formed with mTOR. These are mTOR complex 1 (mTORC1) and complex 2 (mTORC2). The mTORC1 consists of several protein units including mTOR, regulatory-associated protein of mTOR (Raptor), mammalian lethal with Sec13 protein 8 (mLST8, also known as GbL), prolinerich AKT substrate 40 kDa (PRAS40), and DEP-domain-containing mTOR interacting protein (Deptor). The mTORC2 consists of mTOR, rapamycin-insensitive companion of mTOR (Rictor), mammalian stress-activated protein kinase interacting protein (mSIN1), protein observed with Rictor-1 (Protor-1), mLST8, and Deptor (Laplante and Sabatini 2012). These complexes and the signaling mechanisms that regulate them are under extensive investigation (see: Wullschleger et al. 2006; Shende et al. 2011; Morita et al. 2013; Hancer et al. 2014; Romero et al. 2016). Here, the specific role of mTOR in the functions of cardiovascular cells is discussed. The critical roles of mTOR by itself and mTORC1 in cardiac function are highlighted by the following observations:

1. Early postnatal inactivation of cardiac mTOR in mice resulted in the animals exhibiting dilated cardiomyopathy, cardiomyocyte growth defects, apoptosis and fibrosis, and death (Mazelin et al. 2016).
2. In mice, conditional deletion of Raptor gene in the heart results in impaired adaptive hypertrophy, reduced mitochondrial content and mitochondrial respiration, and cardiomyocyte apoptosis and heart failure (Shende et al. 2011; Morita et al. 2013).
3. Active-site mTOR inhibitor (asTORi) PP242 suppresses mRNAs encoding mitochondria-related proteins (Morita et al. 2013).
4. Chronic treatment of healthy rats with low dose rapamycin, the inhibitor of mTORC1, results in cardiac fibrosis (Luck et al. 2017).
5. Chronic treatment of diabetic rats with rapamycin results in increased cardiac microRNA miR-29 expression, suppressed myeloid cell leukemia 1(MCL-1) and cardiomyocyte disorganization (Arnold et al. 2014). The critical role of MCL-1 in the heart is evident from the observation that the deletion of MCL-1 in the heart causes impaired autophagy, mitochondrial dysfunction, and is lethal to the heart (Thomas et al. 2013).

The mTORC1 is the only mTOR complex that is sensitive to amino acids (Zoncu et al. 2011). The sensitivity of mTORC1 to amino acids is mediated via the RAG GTPases (Fig. 3). Previous studies have indicated that mTORC1 inhibition results in induction of autophagy, an evolutionarily conserved process to catabolize cytoplasmic proteins and organelles. The different types of autophagy have been discussed extensively in other reviews and will not be covered here (Mizushima 2007, Xie and Klionsky 2007). However, it was also reported that during starvation, mTORC1 eventually becomes reactivated. In normal rat kidney (NRK) cells, nutrient deprivation caused fusing of multiple lysosomes with the autophagosome (Yu et al. 2010). They showed that at the end of 4 hours essentially all lysosomes were consumed into fewer and larger lysosomal-associated membrane protein 1 (LAMP1)-stained

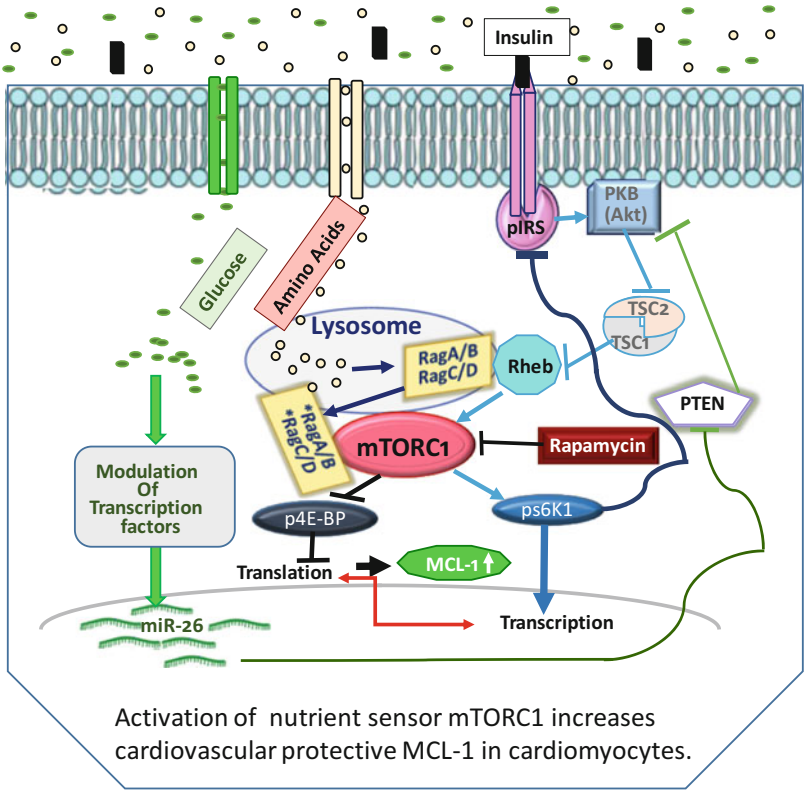

Fig. 3 Molecular mechanisms involved in the tight regulation of mTORC1. Glucose; Amino Acids; Mechanisms by which nutrients (glucose and amino acids) and growth factors (such as insulin) activate mTORC1 are shown. The RAG GTPases form heterodimers consisting of RagA or RagB bound to RagC or RagD and are localized to lysosomes. Amino acids that enter the cell activate these RAG heterodimers by promoting GTP loading of RagA/B and GDP loading of RagC/D(Yuan et al. 2013). These active Rag heterodimers directly interact with mTORC1 by binding to Raptor and recruit mTORC1 to the surface of lysosomes where it interacts with and be activated by the small GTPaseRheb (Shende et al. 2011). Rheb is regulated by growth factors such as insulin (Garami et al. 2003; Hancer et al. 2014). Insulin binds to insulin receptor and causes tyrosine phosphorylation of insulin receptor substrates (IRS1, IRS2) (Saucedo et al. 2003). IRSs, in turn will activate protein kinase B (also known as Akt). Akt phosphorylates TSC2 (tuberin) and causes it to dissociate from TSC1 (hamartin). Dissociation of TSC complex disables it from inactivating Rheb. Rheb is active in its GTP bound form. Since Rheb's ability to stay in GTP bound form depends on the inactivation of TSC complex by growth factor (insulin)-induced Akt activation, this step is a converging point for mTORC1 activation by both amino acids and growth factors (Yuan et al. 2013). Glucose activates mTORC1 by increasing microRNA miR-21 expression. miR-21 suppresses PTEN, an inhibitor of Akt. mTORC1 activates s6 K1 that initiates the feedback loop to inhibit IRS activation and thus prevent insulin signaling and induce de novo transcription. mTORC1 also inhibits 4E–BP (eukaryotic translation initiation factor 4E–binding protein) by phosphorylating it and promotes de novo translation. These effects may underlie increase in MCL-1 expression that is essential for cardioprotection during ischemia and nutrient stress

autolysosomes. However, after 12 h of starvation, lysosome size and number had largely recovered (Yuan et al. 2013).

This effect is seen across species; the authors also observed similar changes in other in vitro studies using cell lines derived from fish, amphibians, birds, and other mammals. These results were corroborated through in vivo studies following starvation in the lysosomes of the fat body of *Drosophila melanogaster* expressing LAMP1-green fluorescent protein (GFP) (Yu et al. 2010). Consistent with previous observations, mTORC1 signaling was inhibited during autophagy initiation in these models. However, prolonged starvation reactivated mTORC1 as evidenced by phosphorylation of mTORC1 substrates p70s6 kinase (s6 K1) and eukaryotic translation initiation factor 4E–binding protein (4E–BP). This reactivation of mTOR was autophagy-dependent and required the degradation of autolysosomal products. Reactivation of mTORC1 attenuated autophagy. Moreover, mTORC1 reactivation resulted in proto-lysosomal tubules and vesicles that extrude from autolysosomes and matured into functional lysosomes. Thus autophagy-mTORC1 signaling cycle is tightly and differentially regulated during the length of starvation. Given the critical role of mTORC1 signaling in the survival of cardiovascular cells, this reactivation of mTORC1 by autophagy may play a role in regulating excessive autophagy-induced cardiovascular damage. The hypothesis that reactivation of mTORC1 by autophagy can render resistance to cell death is further supported by the observation that mTORC1 activation decreases autophagy in aging (Romero et al. 2016).

While starvation and amino acid deprivation induce inhibition of mTORC1, increases in amino acids and nutrients and growth factors from overnutrition lead to overactivation of mTORC1 (Pulakat et al. 2011; Gul et al. 2012a,b). In the context of overnutrition, postprandial increases in blood glucose and the subsequent secretion of insulin by pancreatic beta cells regulate mTORC1. Insulin binds to membrane receptors to activate class I phosphatidyl inositol 3 kinase (PI3K) that, in turn, activates PKB/Akt and puts in motion the mechanisms involved in activation of mTORC1 as described earlier. High glucose activates mTORC1 by increasing microRNA miR-26 expression that inhibits expression of phosphatase and tensin homolog (PTEN) (Dey et al. 2015) (Fig. 3). However, activation of s6 K1 by mTORC1 puts in motion a negative regulatory feedback loop for Akt. Activated s6 K1 causes serine phosphorylation of IRSs that results in inhibition of IRS signaling and thus attenuates PI3K signaling (Fig. 3). Thus, mTORC1 activation prevents insulin signaling and causes insulin resistance. In fact, Hanceret al., reported that both insulin and metabolic stress induced by treatments with anisomycin, thapsigargin, or tunicamycin caused multisite serine/threonine phosphorylation of the insulin receptor substrate 1 (IRS1). These treatments also inhibited IRS1 tyrosine phosphorylation and normal insulin signaling (Hancer et al. 2014)).

Type 2 diabetes is an independent predictor of CVD and both hyperinsulinemia and hyperglycemia are toxic for cardiovascular cells. Hyperinsulinemia in the presence of insulin resistance results in overactivation of IGF receptor to promote cardiac and vascular hypertrophy. Additionally, hyperglycemia and impaired glucose tolerance induces cardiovascular glucotoxicity. These conditions lead to diastolic dysfunction with preserved ejection fraction, fibrosis, atherosclerotic plaque formation, and coronary artery disease. These structural damages to the heart and vascular system lead to

ischemia and nutrient deprivation to cardiovascular cells, causing cardiomyopathy. Therefore, scientists have hypothesized that inhibition of mTORC1 in conditions of overnutrition could be beneficial. In vivo studies using mTORC1 inhibitors such as rapamycin to suppress mTORC1 activation have shown mixed results in terms of cardiovascular protection. Short-term (up to 4 weeks or 6 weeks) mTORC1 inhibition is reported to be cardioprotective in obese and diabetic murine models (Das et al. 2014, 2015; Luck et al. 2017). However, long-term (12 weeks) rapamycin treatment caused exacerbation of type 2 diabetes and no improvement in cardiac functions in an obese diabetic murine model (Luck et al. 2017). Thus both overactivation of mTORC1 via overnutrition and chronic suppression of overactive mTORC1 in obesity via inhibitors cause cardiovascular stress and CVD.

Leptin, Angiotensin, and Cachexia-Associated Nutrient Stress on Cardiovascular Cells

Several chronic diseases including cancer, chronic infections, diabetes, and cardiovascular diseases can lead to muscle wasting syndrome or cachexia. In chronic inflammatory conditions such as cancer, serum leptin levels are significantly lower in patients despite correction for body fat (Mantovani et al. 2000). Leptin secretion is regulated by insulin, glucocorticoids, and catecholamines (Engineer and Garcia 2012). However, while leptin deficiency is associated with hyperphagia, overnutrition, and obesity, this reduction in serum leptin was not associated with greater appetite or lower energy expenditure in cancer patients. This indicates that for cancer patients, normal leptin signaling is disrupted. This reduction in leptin was concurrent with increases in inflammatory cytokines interleukin-1alpha (IL-1alpha), Interleukin 6 (IL-6), and TNFα (Mantovani et al. 2000). Increased leptin levels are associated with fibrosis and neointimal hyperplasia (Shan et al. 2008). It was observed that administering exogenous leptin at levels comparable to those found in obese humans promoted neointimal vascular smooth muscle hyperplasia in a murine femoral artery wire injury model. Moreover, hyperleptinemia (high levels of leptin) suppressed the efficacy of mTORC1 inhibitors in attenuating neointima. Researchers have suggested that hyperleptinemia in diabetics could be a reason for the increased restenosis in these patients even when they are treated with mTORC1 inhibitors (Shan et al. 2008). Leptin is also implicated in vascular extracellular remodeling. Zhang et al. reported that in 3D co-culture vessel models that mimic true blood vessels, treatment with leptin stimulated secretion of collagen types II/IV and TIMP1 (tissue inhibitor of metalloproteinases). It also increased matrix metalloproteinases MMP2/9 activity. These effects were suppressed by administration of adiponectin that acted via adiponectin receptor 1 (AdipoR1) and activation of AMP kinase (Zhang et al. 2014). These observations highlight the unique role of leptin in regulating blood vessel pathology.

In 2008, Evans et al. proposed the diagnosis of cachexia to be based on at least 5% weight loss in 12 months or less in the presence of underlying illness, plus 3 of the 5 following criteria: (1) Decreased muscle strength, (2) Fatigue, (3) Anorexia, (4) Low-

fat free mass index, and (5) Abnormal biochemistry (increased inflammatory markers (CRP, IL-6), anemia, and low serum albumin) (Evans et al. 2008). Nutritional support did not reverse this condition. These patients suffer from muscle atrophy, fatigue, weakness, and even loss of appetite. They have reduced protein synthesis and increased degradation of myofibrillar proteins. Since the heart is a muscle, this condition leads to cardiac cachexia. Chemotherapy is also an important contributor to cardiac cachexia. Weight loss, reduced muscle mass, and poor cardiovascular function are key indicators of poor quality of life and high mortality in cachexia patients. There are no effective treatments for cachexia. Studies in mice have shown that TNF-α can induce cachexia and cause myotube atrophy in vitro via activation of E3 ubiquitin ligases (Oliff et al. 1987; Li et al. 2005). However, circulating levels of TNF-α showed no correlation with weight loss and anorexia in cancer cachexia patients. Moreover, use of anti-TNF-α antibody (infliximab) in a clinical trial in cancer patients with cachexia was preemptively ended because it did not prevent or palliate cancer-associated weight loss. In fact, the patients developed greater fatigue and worse global quality of life scores (Jatoi et al. 2010). IL-6 is an inflammatory molecule that also showed an association with cachexia in tumor bearing mice. Anti-IL-6-antibody could mitigate cachexia in tumor bearing mice (Strassmann et al. 1992). However, a clinical trial using anti-IL-6 antibodies in lung cancer patients suffering from weight loss showed that though the treatment could mitigate anorexia, anemia, and fatigue, it was not effective in improving body weight (Bayliss et al. 2011).

Activation of the renin-angiotensin-system (RAS) is an important contributor to chronic inflammation that causes nutrient stress. The primary effector of RAS is angiotensin II (Ang II) that activates inflammatory pathways leading to hypertension, heart failure, and stroke via the angiotensin II type 1 receptor (AT1R). AT1R is a G-protein coupled receptor (GPCR) that activates Gi-adenylyl cyclase and Gq-phospholipase C pathways in addition to G-protein independent and β-arrestin-mediated Ras-Raf-MEK-ERK1/2 and Akt-mTORC1 pathways (Fig. 3). AT1R over-activation causes production of reactive oxygen species (ROS) and mitochondrial dysfunction that promote endothelial dysfunction, vasoconstriction, hypertension, insulin resistance, cardiac hypertrophy and fibrosis, and heart failure and stroke (Pulakat et al. 2011; Gul et al. 2012). In addition to these effects, in 1996 it was discovered that AT1R activation by Ang II causes muscle wasting (Fig. 3) (Brink et al. 1996; Yoshida and Delafontaine 2015).

AT2 Receptor (AT2R) and Protection of Cardiovascular Cells from Nutrient Stress

Angiotensin II binds and activates two different GPCRs, the AT1R and the AT2R, that have opposing actions (Fig. 4). While the AT1R-mediated signaling constitutes the inflammatory actions of RAS, the AT2R-mediated signaling results in protective and anti-inflammatory actions of RAS. Ang II acting through the AT2R induces vasodilation via the kinin/nitric oxide dependent mechanisms (Fig.4). Like the AT1R, the AT2R is also a seven transmembrane domain receptor, but it shares

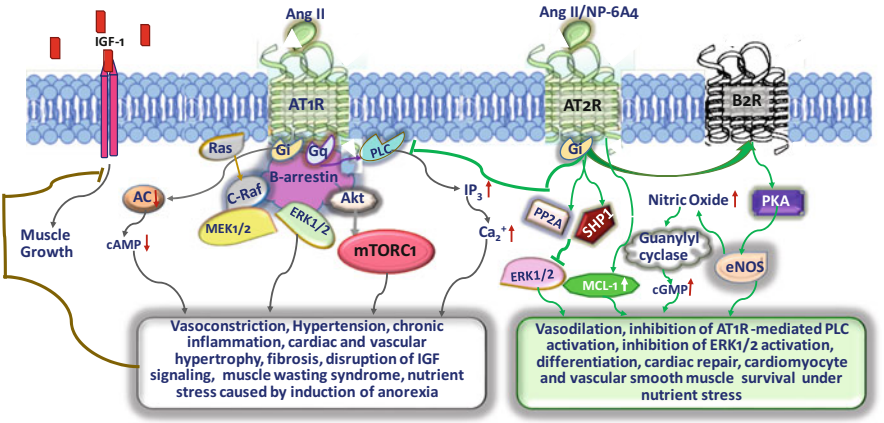

Fig. 4 Cross-talk between angiotensin II receptors AT1R and AT2R in cardiovascular cells. Signaling mechanisms activated by Ang II binding to AT1R and AT2R are shown. AT1R-mediated activation of mTORC1 could have both beneficial and detrimental effects. While mTORC1 activation is useful in maintaining cardioprotective MCL-1 expression and adaptive hypertrophy, overactivation of mTORC1 also causes insulin resistance in cardiovascular cells in the context of AT1R-mediated hypertension and chronic inflammation. Moreover, AT1R activation is implicated in muscle wasting syndrome via inhibition of IGF signaling. AT2R activation by Ang II inhibits AT1R-mediated vasoconstriction via inducing nitric oxide-cGMP axis. Moreover, NP-6A4-mediated AT2R activation improves MCL-1 expression

only 34% sequence identity with the AT1R. AT2R inhibits AT1R-mediated PLC activation (Kumar et al. 2002). Moreover, AT2R-mediated activation of protein phosphatase 2A (PP2A) and Src homology region 2 domain containing phosphatase (SHP-1) I can inhibit extracellular signal-regulated kinases 1 and 2 (ERK1/2) that is activated by the AT1R-Ras-RAf-MEK1/2 pathway (Fig. 4). Importantly, the gene that codes for AT2R, the *Agtr2*, is an X-linked gene. Studies in murine models have shown that higher expression of AT2R in female vasculature confers increased protection from hypertension and cardiovascular and renal injury (Sampson et al. 2008; Hilliard et al. 2012). AT2R is also known to localize to mitochondria and improve mitochondrial respiration. A nonpeptide AT2R agonist compound-21 is reported to reduce infarct size after myocardial infarction (Ludwig et al. 2012). Heterologous expression of *Agtr2* gene is reported to render cardioprotection from ischemic injury in murine models (Qi et al. 2012). Thus increasing the expression of *Agtr2* is a critical step in drug development to mitigate CVD, and *Agtr2* has remained as a drug target for this purpose for nearly three decades.

Myocardial infarction and ischemic heart disease are conditions that induce severe nutrient stress to cardiovascular cells. These stresses cause apoptosis, necrosis, or autophagy of these cells. NP-6A4, a new peptide agonist of the AT2R (from Novopyxis Inc., Cambridge), was more effective than β-blockers (atenolol, metoprolol, carvedilol, and nebivolol) and losartan, a widely used angiotensin receptor blocker (ARB), in improving the survival and viability of nutrient-stressed cultures

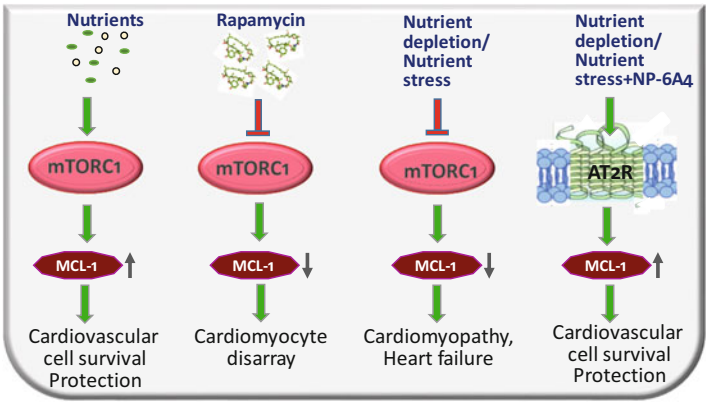

Fig. 5 Regulation of cardiovascular protective MCL-1 by nutrients, rapamycin, and NP-6A4. Nutrient stress and rapamycin treatment suppress cardioprotective MCL-1 expression. Interestingly, activation of AT2R by NP-6A4, a new AT2R agonist by Novopyxis Inc., increases MCL-1 expression in cardiovascular cells subjected to nutrient stress

of mouse cardiomyocyte HL-1 cells and human coronary artery vascular smooth muscle cells (hCAVSMCs) (Mahmood and Pulakat 2015). Moreover, β-blockers significantly suppressed and NP-6A4 increased the expression of myeloid cell leukemia 1 (MCL-1), an essential protein for cardiovascular cell survival and implicated in cell adhesion. CGP42112A, the other AT2R peptide agonist, was not as effective as NP-6A4 in mitigating the nutrient stress. Thus, NP-6A4 seems to activate a protective AT2R-MCL-1 axis in nutrient-stressed mouse and human cardiovascular cells (mouse HL-1 cells and primary cultures of hCAVSMCs). Moreover, mTORC1 inhibition by inhibitors such as rapamycin or nutrient stress suppress MCL-1 whereas NP-6A4 acting through the AT2R increases MCL-1in conditions of nutrient stress despite the fact that AT2R induces differentiation of cells rather than growth (Fig. 5) (Mahmood and Pulakat 2015). Both the AT2R and MCL-1 have important roles in mitochondrial function and the NP-6A4-AT2R-MCL-1 signaling is specifically useful to mitigate nutrient stress. These observations imply that NP-6A4 treatment could mitigate undernutrition and overnutrition induced nutrient stress of cardiovascular cells.

Policies and Protocols

Famine Research Limitations: There are some clear limitations to these observational studies. For example, the effect of various factors including seasonality, stress, famine frequency, and duration are independent variables whose effects are difficult to distinguish from those of undernutrition. To date, no study has been able to assess the contribution of these confounding factors (war time stress, exposure to extreme

temperatures, and ingestion of potentially toxic food substitutes) to famine outcomes (Lumey et al. 2011; Moore et al. 1997; Waterland et al. 2010).

Impaired Glucose Tolerance Protocol In Dutch Famine Cohort: A cohort of 94 normoglycemic men and women from the Dutch famine birth cohort were shown to have impaired glucose tolerance compared to control subjects. The disposition index (a product of insulin sensitivity and first-phase insulin response to glucose) was derived as a measure of activity of beta cells adjusted for insulin resistance. People exposed to prenatal famine showed a difference in an intravenous glucose tolerance test (Kg value −21% [95% CI −41 to −4]) (Lumey et al. 2011).

RTCA analysis of CI and NP-6A4: The cell index (CI) of HL-1 cells as determined by the Xcelligence Real-Time Cell Analyzer (RTCA) showed that the CI of HL-1 cells was the highest when treated with NP-6A4 and this effect was completely blocked by PD123319, an AT2R antagonist (Mahmood and Pulakat 2015).

Dictionary of Terms

- **The Dutch famine** – Is one of the most well studied periods of famine (1944–1945) that lasted approximately six months (Trienekens 2000; Lumey et al. 2011).
- **Cachexia or wasting syndrome** – Is associated with diseases such as cancer, AIDS, congestive heart failure, rheumatoid arthritis, and tuberculosis.
- **Hypoferremia** – Is a deficiency of iron in the blood.
- **Leptin** – activates specific leptin receptors in the hypothalamus and changes the expression of several hypothalamic neuropeptides that regulate energy intake and expenditure.
- **Neointimal hyperplasia** – Is a condition in which the vascular smooth muscle cells (primarily in the tunica intima) proliferate and migrate. This results in the arterial walls thickening and therefore reducing the space in the arterial lumen.
- **Dilated cardiomyopathy (DCM)** – Is a common cause of heart failure and occurs when the heart cannot pump blood effectively due to enlargement.
- **β-adrenergic receptor blockers (β-blockers)** – Are the standard of care for myocardial infarction and ischemic heart disease.

Summary Points

- Like obesity, starvation and chronic undernutrition can cause also cardiovascular stress and subsequent CVD.
- In utero famine exposure negatively affects birth weight, total cholesterol, glucose tolerance, triglycerides, risk of coronary heart disease, and obesity and these effects are intergenerational.

- Micronutrient deficiency occurs in conditions of starvation, but is also seen in obesity and contributes to the pathogenesis of cardiovascular disease in both populations.
- mTORC1 functions as a nutrient sensor; during the initial stage of starvation, mTORC1 activity is suppressed.
- Chronic treatment of healthy rats with low-dose mTORC1 inhibitor (rapamycin) results in cardiac fibrosis.
- Chronic treatment of diabetic rats with rapamycin results in increased cardiac microRNA miR-29 expression, suppressed myeloid cell leukemia 1(MCL-1), and cardiomyocyte disorganization.
- Both overactivation of mTORC1 via overnutrition and chronic suppression of mTORC1 causes cardiovascular stress and CVD.
- Activation of the renin-angiotensin-system (RAS) is an important contributor to chronic inflammation that causes nutrient stress.
- The primary effector of RAS is angiotensin II (Ang II) that activates inflammatory pathways leading to hypertension, heart failure, and stroke via AT1R.
- Ang II acting through the AT2R activates the anti-inflammatory arm of RAS and induces vasodilation.
- Increasing the expression of *Agtr2* is a critical step in drug development to mitigate CVD and *Agtr2* has remained as a drug target for this purpose for nearly three decades.
- NP-6A4, a new peptide agonist of the AT2R (from Novopyxis Inc., Cambridge), was more effective than β-blockers and losartan (an ARB) in improving the survival and viability of nutrient-stressed cultures of mouse cardiomyocyte HL-1 cells and human coronary artery vascular smooth muscle cells (hCAVSMCs) via activation of a protective AT2R-MCL-1 axis.
- mTORC1 inhibition by inhibitors such as rapamycin or nutrient stress suppresses MCL-1, whereas NP-6A4 acting through the AT2R increases MCL-1 in conditions of nutrient stress.
- The NP-6A4-AT2R-MCL-1 signaling is specifically useful to mitigate nutrient stress.

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Cancer Cells and Effects of Glucose Starvation

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Abstract

As the main energy source for the human body, glucose metabolism plays multiple roles in the physiology of cancer cells. In the environment of hypoxia and low sugar, cancer cells transform the normal glucose metabolism to aerobic glycolysis autonomously, regulated by different molecules. Under conditions of glucose deprivation, cancer cells suffer from the inhibition of growth, the arrest of cell cycle, apoptosis, and autophagy, regulated by respective associated proteins and pathways. It is possible that glucose deprivation alone or in combination with pharmacological therapy would be effective in the treatment of cancer "addicted" to glycolysis. However, several experiments have demonstrated that cancer cells may develop tolerance to glucose deprivation. In this review, we discuss these issues in order to provide a clear understanding of effects of glucose starvation on cancer therapy.

Keywords

Cancer cells · Warburg effect · Oncogene · Pathway · Apoptosis · Autophagy · Tolerance to glucose deprivation

List of Abbreviations

AMP	Adenosine monophosphate
AMPK	Adenosine 5′-monophosphate (AMP)-activated protein kinase
ATG14	Autophagy-related 14
ATP	Adenosine triphosphate
BCL-2	B-cell lymphoma-2
GTP	Guanosine triphosphate
HIF1α	Hypoxia-inducible factor 1 alpha subunit
KRAS	KRAS proto-oncogene, GTPase
LC3	Autophagy marker light chain 3
MAX	MYC-associated factor X
MCL-1	Myeloid cell leukemia 1
mTORC1	Mechanistic target of rapamycin complexes 1 and 2
MYC	MYC proto-oncogene, bHLH transcription factor
NADPH	Triphosphopyridine nucleotide
PAQR3	Progestin and adipoQ receptor family member 3
PI3K/AKT	Phosphatidylinositol 3 kinase (PI3K)/protein kinase B(AKT)
PKA	Protein kinase A
PtdIns3P	Phosphatidylinositol 3-phosphate
ROS-PTP-TK	Reactive oxygen species – protein tyrosine phosphatases – tyrosine kinases
TNFα	Tumor necrosis factor-alpha
ULK1	unc-51-like autophagy activating kinase 1
UPR	Unfolded protein response

Introduction

Living cells require energy and nutrients for their proliferation, differentiation, and movement. Three principal nutrients that provide cells with energy are carbohydrates, especially glucose, lipids, and proteins (Li et al. 2016). Carbohydrate metabolism includes anaerobic glycolysis, aerobic oxidation, pentose phosphate pathway (PPP), glycogenesis, glycogenolysis, gluconeogenesis, and metabolism of other hexoses. Compared to normal cells, cancer cells require a high rate of glucose uptake to meet the energy demand to support rapid tumor progression; however, the extent of adaptive angiogenesis and blood supply are insufficient at the early stages of tumorigenesis (Ferreira et al. 2012). To survive in the hypoxic microenvironment, tumor cells utilize glycolysis instead of mitochondrial oxidative phosphorylation. Besides, tumor cells are used to adopt the less efficient way of getting ATP from substrate-level phosphorylation reactions of glycolysis even under oxygen-rich conditions (Potter et al. 2016). Another situation that cancer cells face is low glucose levels. Cancer cells must exhibit adaptive responses to glucose starvation to facilitate proliferation, migration, and progression (Xing et al. 2015). In this review, we describe how cancer cells undergo metabolic switching so as to develop tolerance to glucose deprivation and provide insights into the therapeutic approaches to target adaptive responses to glucose deprivation.

Reprogramming of Glucose Metabolism: Metabolic Change or Genetic Change, Which Occurs First?

Metabolic reprogramming has been deemed as a necessary process for the growth and proliferation of cancer cells. The switch in glucose metabolism is one of the most important metabolic alterations that occur in cancer cells. Related studies date back to the 1920s when Otto Warburg, a German scientist, pioneered investigations on cancer cell metabolism.

The Warburg Effect

The Warburg effect is a hallmark of cancer cell metabolism (Pavlova et al. 2016). Glucose metabolism in cancer cells is markedly different from that in normal cells, in that glucose is converted to lactic acid even under oxygen conditions that are normally sufficient to facilitate mitochondrial oxidative phosphorylation(aerobic glycolysis) (Fig. 1). This process involves the conversion of glucose to pyruvic acid, which is, then, converted to lactic acid. This entire metabolic reaction generates only 2 ATP molecules per molecule of glucose, while the mitochondrial tricarboxylic acid (TCA) cycle coupled with oxidative phosphorylation (OXPHOS) generates about 30 or 32 ATP molecules per glucose molecule. However, aerobic glycolysis is a mechanism that

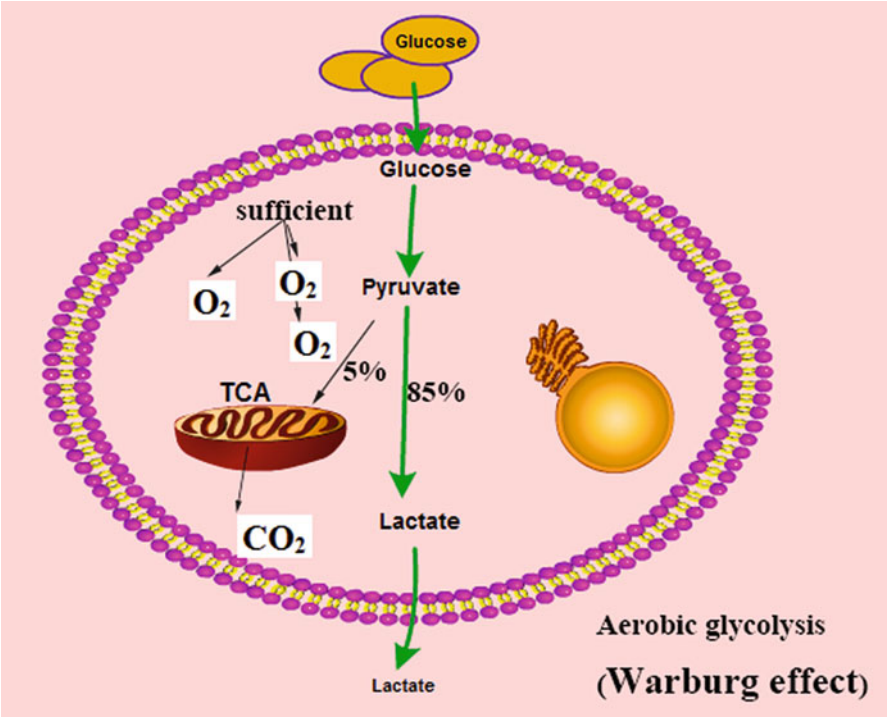

Fig. 1 The schematic diagram of Warburg effect. Glucose is converted to lactic acid even under oxygen conditions that are normally sufficient to facilitate mitochondrial oxidative phosphorylation (aerobic glycolysis)

supports the biosynthetic demands of uncontrolled cancer cell proliferation (Potter et al. 2016). The mechanism of this phenomenon has not been fully clarified yet; some studies hypothesize that it is a consequence of mitochondrial dysfunction or damage (Vander et al. 2009). Other studies focus on mutations in oncogenes and/or loss of tumor suppressor genes that are involved in tumor-linked metabolic changes.

Oncogenes, Tumor Suppressors, and Pathways

Given the large number of recent research studies on reprogramming of glucose metabolism in cancer, *MYC*, *HIF*, *KRAS*, *P53*, and AMP-activated protein kinase (AMPK) pathways, PI3K/AKT pathway, and other signaling cascades are involved.

MYC Family

Mutation in *MYC* or its overexpression is common in tumors. The MYC family consists of well-known proto-oncogenic transcription factors including MYC (CMYC), NMYC (MYCN), and LMYC (MYCL). MYC competes by mass action

to form a heterodimer with another bHLH-LZ (helix–loop–helix leucine zipper) protein, MAX, in order to bind to the E-box region in the promoters of the genes that regulate cell growth, energy production (glycolysis, glutaminolysis), cell cycle, apoptosis, anabolism, and DNA replication (Kress et al. 2015). It has been reported that cells with overexpression of *MYC* consumed more glucose than did cells with normal *MYC* expression levels (McCarthy 2015). The expression of glucose transporter 1 (GLUT1), the lactate dehydrogenase A (LDHA), and pyruvate kinase M2 (PKM2) proteins induced by the MYC-MAX heterodimer in glucose metabolism supports tumor survival in a hypoxic microenvironment.

HIF Family

Hypoxia in the tumor microenvironment usually results in the activation of the hypoxia-inducible factors (HIFs). The HIF family comprises three members, HIF1, HIF2, and HIF3, which activate transcription in a way similar to that of MYC with respect to glucose transport and glycolysis and, thus, help cells tolerate hypoxic conditions (Dang et al. 2008). All of them consist of an α subunit whose expression is oxygen-dependent and a β subunit which is constitutively expressed. The prolyl hydroxylase domain (PHD) fails to modify HIFα under conditions of insufficient oxygen, which then forms a stable heterodimer with HIFβ, and can, thereby, activate related transcription. Otherwise, HIFs function together with MYC to confer metabolic advantages that help tumor cells survive under conditions of low oxygen (Dang et al. 2008).

KRAS

There are four GTP-binding proteins in the p21ras family, namely, HRAS, KRAS, NRAS, and RRAS. Metabolic alterations due to mutations in *KRAS* during tumorigenesis are widely observed. *KRAS* encodes p21ras GTP-binding proteins that activate signaling pathways related to the proliferation of cells. $KRAS^{G12V}$ mutation and *KRAS* overexpression lead to significant metabolic alterations by inhibiting the function of the complex I of the mitochondrial-electron transport chain, increasing radical oxygen species (ROS) generation, and elevating glycolytic activity (Asati et al. 2017).

P53

The inactivation of tumor suppressor genes in addition to the activation of proto-oncogenes is associated with the switch in glucose metabolism in cancer cells. Tumor-associated *P53* mutants lose the function of inhibiting the activity of glucose-6-phosphate dehydrogenase and, thereby, increase glucose influx in the pentose phosphate pathway (Jiang et al. 2011).

AMPK

The AMP-activated protein kinase (AMPK) consists of an α subunit of which function is a catalytic kinase and β and γ subunits that are regulatory in function. Under conditions of low intracellular ATP and high intracellular AMP concentrations, AMPK is activated by AMP. In order to restore energy homeostasis, catabolic pathways are activated but anabolic pathways are suppressed (Ha et al. 2015).

PI3K/Akt/mTOR Pathway

Increased activation of the PI3K/AKT/mTOR pathway is common in tumors and helps tumor cells in meeting their energy requirements for cellular functions and biosynthesis (DeBerardinis et al. 2008). AKT is activated by PI3K and plays a vital role in enhancing glucose uptake by increasing the expression and membrane localization of the glucose transporter 1 (GLUT1) and in promoting glycolysis in cancer. The mTOR pathway that lies downstream of the PI3K/Akt signaling pathway activates protein translation and glycolysis by increasing the hypoxia-inducible factor 1 (HIF1α) protein expression level (Levy et al. 2016).

To Die or to Survive Under Low-Glucose Conditions

In the process of cancer progression, both maladaptive angiogenesis and insufficient blood supply result in significantly low levels of glucose. Although the cancer cells counteract this situation by upregulating the expression of glucose transporters, glucose-metabolizing enzymes as well as pyruvate dehydrogenase kinase (Andersen et al. 2013), some malignant cells are not able to survive glucose starvation and, hence, undergo growth inhibition, apoptosis, or necrosis and finally die (Fig. 2). It was affirmed by some studies that cancer cells subjected to glucose deprivation were more susceptible to cell death than their normal counterparts (El et al. 2011). Meanwhile the process by which cells degrade unnecessary or dysfunctional constituents, termed autophagy, could help in the survival of cells that would otherwise die. Cancer cells probably obtain energy and synthesize the building blocks of biomolecules via the lysosomal pathway of autophagy (Table 1).

Growth Inhibition and Cell Cycle Arrest

It has been shown that glucose deprivation induces cell cycle arrest and inhibition of cell growth in a wide range of cancer cells such as cells derived from hepatic cell cancer (HCC), MDA-MB-231cell line, and so on. Anabela C. Ferretti observed that the number of HCC cells in G0/G1 stage was remarkably increased under conditions of low glucose in about 36 h (Ferretti et al. 2016). In the original study, Yuji Tanaka pointed out that AMPK participated in the KDM2A-dependent (a modular protein containing JmjC and CXXC-zinc finger domains) reduction of rRNA (ribosomal RNA) transcription by de-methylating H3K36me2 in the rDNA (ribosomal DNA) promoter and controlled cell proliferation in MCF-7 cells and MDA-MB-231 cells under limited glucose availability (1 to 2 mM 2-deoxy-D-glucose treatment) (Tanaka et al. 2015).

Cell Death: Apoptosis or Necrosis

Typically, the extrinsic pathway and the intrinsic pathway (also called the mitochondrial pathway) are the two mechanisms for the activation of apoptosis (Fig. 3). The TNF ligand family members, such as tumor necrosis factor α (TNFα), CD95L/

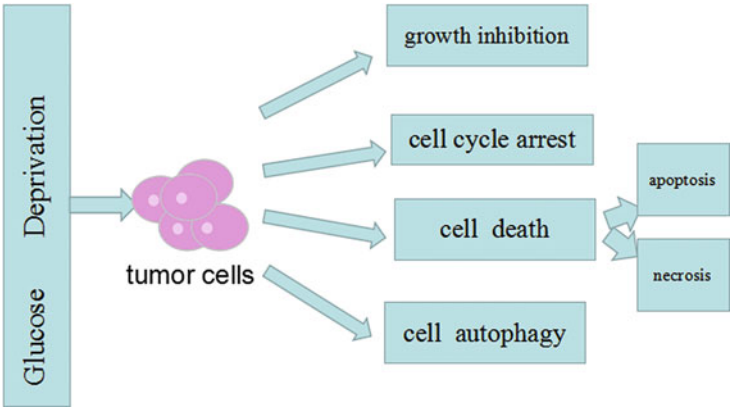

Fig. 2 The fate of cancer cells under low-glucose conditions. Tumor cells are not able to survive from glucose starvation and, hence, undergo growth inhibition, cell cycle arrest, apoptosis, or necrosis and finally die. However, cancer cells may get a chance for survival because of autophagy

FasL, and TNF-related apoptosis-inducing ligand (TRAIL), activate and recruit the Fas-associated protein with death domain (FADD), which is an adaptor protein, to the cytoplasmic death domain of the trimerized or clustered receptor. They bind to their respective receptors on the surface of the cell and undergo oligomerization so as to recruit procaspase-8 to form the death-inducing signaling complex (DISC) and eventually induce cell death by directly activating the effector caspases, caspase-3 and caspase-7. It has been reported that tumor cells subjected to glucose starvation (treatment with 2-deoxy-D-glucose) were susceptible to death induced by activation of FasL receptors. However, there was a report that glucose deprivation inhibited TRAIL-induced death (MacFarlane et al. 2012). Death by apoptosis and necrosis occurs simultaneously in glucose-deprived cells. Anabela C. Ferretti found that both apoptosis and necrosis are augmented almost 2-fold in HCC cells subjected to glucose deprivation. The activation of AMPK by AICAR (the AMPK activator) contributes to the induction of apoptosis in HCC cells after glucose deprivation. The occurrence of apoptosis in glucose-deprived cells decreases observably when the expression of AMPK is silenced. Also, PKA plays a dual role in the HCC cells after glucose deprivation. The activation of the cAMP/PKA axis induces apoptosis initially, following which PKA negatively controls AMPK-induced apoptosis by phosphorylating AMPKα (at Ser173) (Ferretti et al. 2016). R. Palorini wrote that there exists a UPR-dependent (unfolded protein Response) mechanism of death induced by glucose starvation in MDA-MB-231cells (K-RASG13D-transformed cells): the reduction of protein glycosylation, the accumulation of unfolded proteins, and the activation of c-Jun NH2-terminal kinase (JNK) through the IRE1-XBP1 UPR branch (Palorini et al. 2013). A. Garufi found that glucose deprivation induces cell death, mainly ascribed to the activation of c-Jun NH2-terminal kinase when the expression of HIPK2 (Homeodomain-interacting protein kinase 2) is not silenced (Garufi et al. 2013).

Table 1 The fate of cancer cells induced by inhibition of glucose metabolism

Treatment	Fate	Molecules implicated	Cell/tumor type	References
Glucose deprivation	Growth inhibition, G0/G1 arrest, apoptotic and necrotic death	AMPK, PKA	HCC (HepG2/C3A)	Ferretti et al. 2016
Glucose deprivation	Growth inhibition, cell apoptotic death, autophagy	PKA	NIH3T3, MDA-MB-231, and MIA PaCa-2 cells	Palorini et al. 2016
Glucose deprivation	Growth inhibition, cell apoptotic death, autophagy	ER, LC3	NCI-H460 and A549	Parker et al. 2016
Glucose deprivation	Growth inhibition, cell apoptotic death	PI3K/ AKT/ mTOR	A549, HI299, PC3, DU145, and U87-MG	Huang et al. 2015
Glucose deprivation	Growth inhibition	AMPK	MCF-7, MDA-MB-231	Tanaka et al. 2015
Glucose deprivation	Autophagy	LC3, mTOR	NRVMs	Roberts et al. 2014
Glucose deprivation	Growth inhibition, cell apoptotic death	BCL-2	SGC7901, AGS, and MGC803	Wang et al. 2014
Glucose deprivation	Growth inhibition, cell apoptotic death	ER, c-JNK	NIH3T3, MDA-MB-231	Palorini et al. 2013
Glucose deprivation	Growth inhibition, cell apoptotic death, cell autophagy	JNK, AKT, LC3	HIPK2+/+ and siHIPK2 cells	Garufi et al. 2013
Glucose deprivation	Growth inhibition, cell apoptotic death	PTEN, TKs, ROS	GBM cell lines (LN18, LN229, T98, and U87-MG)	Graham et al. 2012
Glucose deprivation	Growth inhibition		MCF-7	Wyld et al. 2002

Glucose-deprived cancer cells are more sensitive to the apoptotic pathway associated with the BCL-2 family. The BCL-2 family consists of antiapoptotic proteins (proteins containing BH1–4 domains) and proapoptotic proteins (proteins containing BH1–3 and BH3 domains). The proteins of Bak and Bax (BH1–3 proteins) play chief roles as apoptotic activators by permeabilizing the mitochondrial membrane, facilitating the release of cytochrome c and apoptosome formation, and eventually lead to cell death. The mechanisms of death in cells exposed to low glucose levels involve the downregulation expression of antiapoptotic proteins such as BCL-2 and MCL-1 (Bhola et al. 2016). N El Mjiyad reviewed that the BH3-only proteins (Bim, PUMA, Nuxa, and Bad) contribute to the induction of apoptosis. Nicholas A. Graham demonstrated that glucose deprivation initiates the ROS-PTP-TK positive feedback amplification loop that involves the generation of reactive oxygen species (ROS) by NADPH oxidase in mitochondria, inhibition of protein tyrosine phosphatase activity by its oxidation, and increased tyrosine kinase signaling and ultimately results in ROS-mediated cell death (Graham et al. 2012).

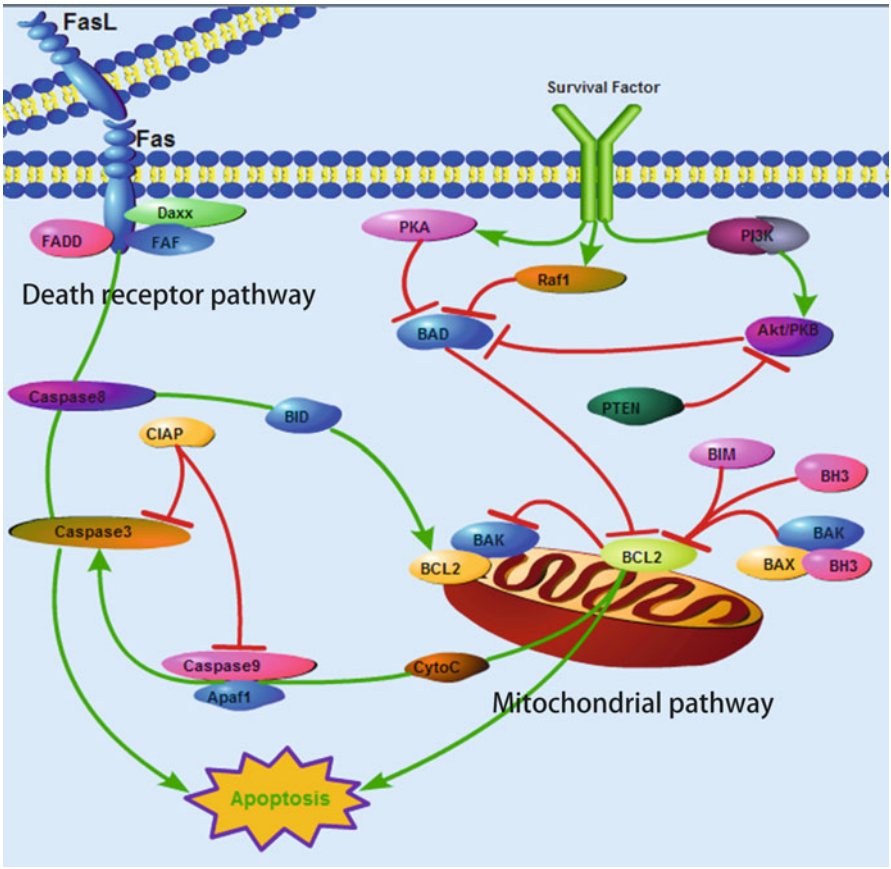

Fig. 3 The two forms of apoptosis in cancer cells. Apoptosis (type IPCD) is one form of programmed cell death (PCD) that can be activated through two dominating signaling pathways, consisting of extrinsic (death receptor pathway) and intrinsic (mitochondrial pathway) pathways. The procaspase-3 is activated at the converge point of two pathways and the target proteins (PARP proteins) of cleaved caspase-3 are activated afterward

Cell Autophagy

Autophagy is the process of self-degradation and recycling of cellular constituents involving double-membraned vesicles and lysosomes; this process also occurs when cancer cells face energy or nutrient deprivation. In order to adapt to conditions of glucose starvation, cancer cells utilize autophagy to recycle cytosolic components and organelles for use in biosynthesis. AMPK, which acts as a sensor of energy, is the positive regulator, while the mechanistic (mammalian) target of rapamycin (mTOR) complex 1 (mTORC1) is the negative regulator of autophagy (Fig. 4). The serine/threonine kinases Atg1/ULK play a pivotal role in the formation of the pre-autophagosome; they are activated when UlK1 (a mammalian homolog of yeast

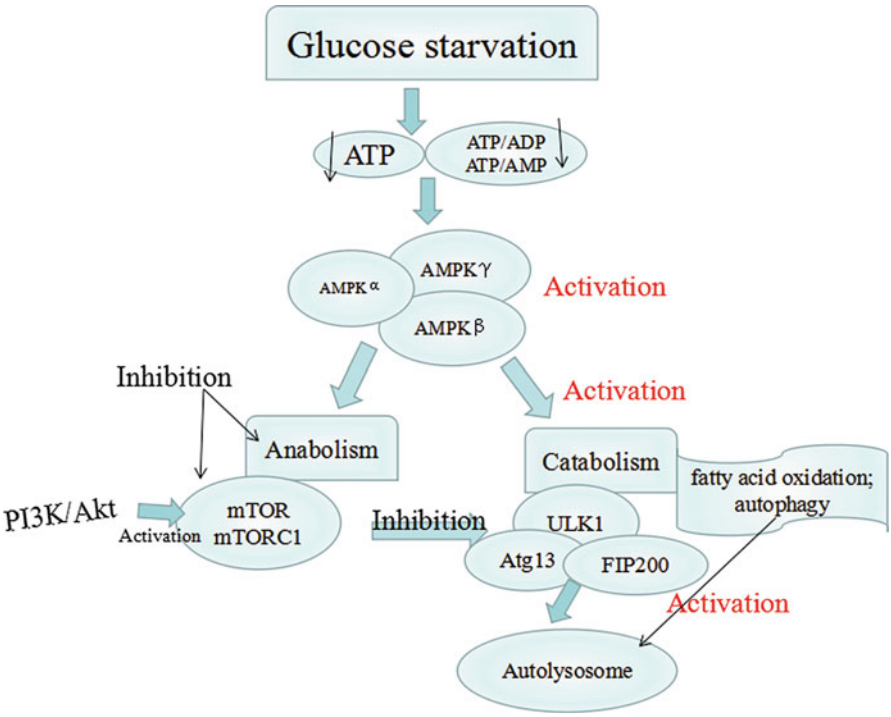

Fig. 4 Inhibition of glucose metabolism promotes catabolism and autophagy regulated by AMPK pathway. Glucose deprivation leads to loss of ATP and decreases the ratio of ATP/ADP and ATP/AMP. ATP depletion can be sensed by AMPK: this kinase, then activated, induces the inhibition of mTOR and mTORC1, leading to autophagy

Atg1, as an mTOR substrate) forms a complex with Atg13 and FIP200 and inactivated when ULK1 and Atg13 are phosphorylated by mTORC1. It has been established that tumor cells accelerate autophagy by inhibiting the activity of mTORC1 through the increased binding of hexokinase-II (HK-II) mediated by TOS motif. Cell death upon glucose deprivation is enhanced with the knockdown of HK-II (Roberts et al. 2014). AMPK directly activates the kinase ULK1, which is essential for autophagy. Moreover, upon glucose starvation, the localization of GAPDH in the nucleus increases due to the phosphorylation of Ser122 via AMPK. Inside the nucleus, GAPDH (glyceraldehyde-3-phosphate dehydrogenase) acts as a transcription factor and interacts directly with Sirtuin 1 (Sirt1, correlating well with the autophagy level in different cell systems) and activates the expression of Sirt1 (Chang et al. 2015). Rui Huang reported that LC3, which is a key initiator of autophagy, is activated in the nucleus when the nuclear deacetylase, Sirt1, deacetylates LC3 (Huang et al. 2015b). AMPK directly phosphorylates T32 of PAQR3 (a 7-transmembrane protein in the Golgi apparatus), which activates the ATG14-linked class III PI3K, resulting in the increased production of PtdIns3P as

well as autophagosome formation under conditions of low glucose (Xu et al. 2016). Otherwise, the activation of PKA attenuates UPR activation, leading to autophagy, and helps cells survive during glucose starvation.

Tolerance to Glucose Deprivation

In view of the above review, glucose starvation may be a good clinical approach for the treatment of cancer. Glucose starvation alone or in combination with chemotherapy can be considered as an effective therapeutic approach. However, some cancer cells that have switched to a high rate of glycolysis develop resistance to conditions of low glucose over a long period of time, leading to their survival and aggressiveness through the activation of a series of signaling pathways and adaptive metabolic responses (Fig. 5) (Palorini et al. 2016; Wyld et al. 2002). Chen Huang deemed that lactate might help cancer cells develop resistance to glucose deprivation (Huang et al. 2015).

Alternative Energy Sources and Related Pathways

Cells switch to other alternative energy source under conditions of low glucose. Glucose starvation, glutamine metabolism, fatty acid oxidation, and other metabolic pathways are counteractive approaches. Some researchers have shown that in order to survive, cancer cells metabolize glutamine upon glucose deprivation (Le et al. 2012). Both molecules increased by glutamine oxidation that recruits steps from the TCA cycle, and elevated glutamine metabolism for glutathione synthesis leads to cell survival in the absence of glucose (Palorini et al. 2016). In our previous research study, it was found that the metabolism of nonessential amino acids inhibited apoptosis of gastric cancer cells and mitochondria DNA copy number induced by glucose starvation (Wang et al. 2014). In response to glucose deprivation, cancer cells exhibit impaired fatty acid synthesis but promoted fatty acid oxidation by AMPK (Monica et al. 2005). The loss of phosphorylation of AMPKα at Ser173 sensitized cancer cells to cell death induced by glucose deprivation (Ferretti et al. 2016). βIII-Tubulin is a marker of resistance to chemotherapy in non-small cell lung cancer (NSCLC) and pancreatic cancer and protects cells from endoplasmic reticulum (ER) stress. It was found that the interaction between βIII-tubulin and GRP78 was increased, then a complex between glucose-regulated protein 78 (GRP78) and Akt was formed, and eventually the reliance of cancer cells on glycolytic metabolism was decreased by utilizing other nutrients (Parker et al. 2016). Cells undergo malignant transformation without sufficient glucose. Glucose deprivation in human tumors can lead to the acquisition of mutations in KRAS, which is an important epithelial-mesenchymal transition (EMT) pathway regulator (Yun et al. 2009).

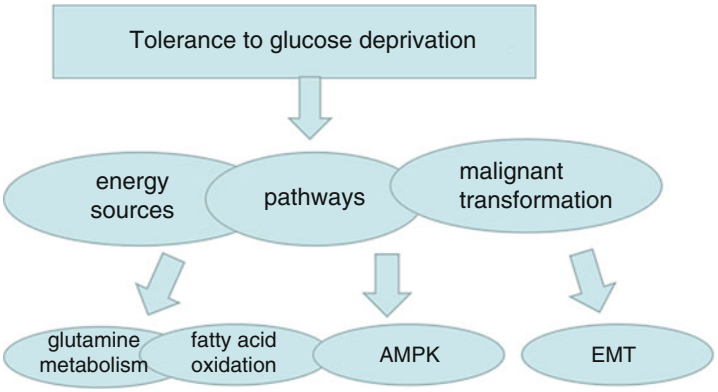

Fig. 5 Cancer cells acquire adaptive responses to glucose starvation. Tumor cells that have switched to a high rate of glycolysis develop resistance to conditions of low glucose over a long period of time

Conclusions and Future Perspectives

Compared to their normal counterparts, cancer cells usually obtain energy through reprogramming of glucose metabolism and survive in harsh environments. Tumors switch to glycolysis for energy production in response to hypoxic conditions even in oxygen-rich conditions. This adaptive response to conditions of low oxygen requires changes in cancer cells including activation of oncogenes, inactivation of tumor suppressor genes, and alterations of crucial signaling pathways. The production of lactate during glycolysis also increases the malignancy of cancer cells. On the other hand, the challenge to cancer cell survival is nutrient starvation, especially of glucose, during early tumorigenesis. The growth of cancers is impeded under conditions of low glucose or presence of 2-D glucose analogue. In some related flow cycling experiments, it was found that cancer cells enter cell cycle arrest; however, most of them cannot survive under conditions of low glucose and eventually die by apoptosis, necrosis, or autophagy. Further, low glucose sensitizes tumor cells to radiotherapy and chemotherapy. These findings suggest that the pathways in cancer metabolism could be specifically targeted for therapy. However, the emerging phenomenon that cancer cells develop tolerance to glucose deprivation poses a challenge to this possibility. The main reason for this tolerance is that cancer cells derive energy alternatively through autophagy. To further support the applicability of therapeutic strategies based on glucose starvation, some researchers have showed that "anti-glycolytic" anticancer agents together with other drugs have achieved the desired results (El Mjiyad et al. 2011; Simons et al. 2009). Further research should focus on glucose starvation and other treatments in order to realize the clinical applications of cancer therapy targeting glucose metabolism as soon as possible.

Policies and Protocols

Studying glucose metabolism in cancer cells involved two aspects: experiment model and test method.

Experiment Model

Researchers take a different treatment scheme with cancer cells in control groups and experimental groups. In control groups, cancer cells are cultivated in normal complete culture medium, while cancer cells of experimental groups are maintained in normal growth media containing no glucose supplemented with 10% serum. 2-Deoxy-D-glucose (2-DG) is applied to enhance cancer therapy in preclinical studies and can be added into the complete cell culture medium in vitro experiments.

Test Method

Researchers usually pay attention to some alterations of cells subjected to glucose starvation. In order to confirm the damage of cell viability under the condition of low glucose, cell viability assay is carried out such as cell counting kit-8 (CCK8) and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay. To measure low-glucose-induced apoptosis and cell cycle arrest, flow cytometry is a kind of detection means. JC-1 (CBIC2(3)) staining, mitochondrial DNA copy number detection, and ATP assay are used to observe the function of mitochondria. Researchers measure the expression changes of related gene by Q-PCR and associated protein by Western blot. These genes and proteins involved in glucose metabolism belong to the different pathways such as PI3K/Akt/mTOR pathway and MAPK pathway.

Dictionary of Terms

- **bHLH-LZ protein (helix–loop–helix leucine zipper)** – This is a type of protein with a secondary structure which consists of a zipper-like structure consisting of leucine, formed when the two structural domains come together.
- **Chemotherapy resistance/tolerance** – Some cells do not die when treated with drugs or other similar conditions which are usually lethal to cells; this is because of gene mutations.
- **Mutation** – Mutation is an abnormal change in the sequence of genes, at single or multiple sites such as point mutation, loss-of-function mutations, insertion mutations, and duplication mutations.
- **Oncogenes** – Some genes, usually expressed at low levels in normal cells but expressed at very high levels in cancer cells, play a vital role in the regulation of proliferation, migration, and so on.

- **Pathways** – There are some proteins owing the personal function, existing the complicated interaction, and eventually acquiring the response of cells in aspect of proliferation, migration, and so on.

Summary Points

- Glucose is the main energy source for cancer cells, participating in the activities of cell proliferation, differentiation, and movement.
- In the tumor microenvironment, cancer cells usually exhibit the Warburg effect in order to survive and reprogram glucose metabolism.
- The reprogramming of glucose metabolism involves the cross talk between pathways, including the expression alternation of oncogenes and tumor suppressor genes.
- When cancer cells are exposed to glucose deprivation, the fate of the cells varies depending on the cell types, duration of starvation, supply of other energy sources, and so on.
- The condition of low glucose can not be tolerated by most cancer cells and it can induce growth inhibition, cell cycle arrest, and apoptosis in cancer cells.
- Activation of the extrinsic and the intrinsic pathways participates in the induction of cellular apoptosis in cancer cells exposed to the low glucose. Procaspase-8 is activated by membrane-associated protein complexes (Fas-L/TNF) in the extrinsic pathway, while procaspase-9 is activated by mitochondria-associated protein in the intrinsic pathway. Then procaspase-3 is activated at the converge point of two pathways and the target proteins (PARP proteins) of cleaved caspase-3 are activated afterward.
- Autophagy could be accelerated in cancer cells under cellular stressors, like nutrient starvation (glucose starvation). The moderate activation of autophagy may enable cancer cells to survive from the low-glucose environment.
- AMPK – as an energy sensor – plays a vital role in the process of glucose metabolism.
- The reprogramming of glucose metabolism and activation or inactivation of certain pathways allow cancer cells to survive and develop resistance to "anti-glycolytic" drugs.
- Therapeutic approaches targeting glucose metabolism together with other conventional methods of treatment may prove successful in cancer treatment.

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Lipid Response to Amino Acid Starvation in Fat Cells: Role of FGF21

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Abstract

Adaptation to food shortage requires temporal homeostatic adaptive responses to a condition of energy deficiency. Mammals have developed a wide range of mechanisms to detect and respond to episodes of malnutrition and starvation, including the capacity to adjust fuel oxidation in function of nutrient availability. Nutrient deprivation or starvation often correlates with amino acid deficiency. This chapter will outline the changes in the metabolic patterns and molecular mechanisms driving these adaptive responses at the whole body level, and particularly in white and brown adipose tissue.

Keywords

Starvation · Aminoacidemia · Essential amino acids · Protein restriction · ATF4 · FGF21 · White adipose tissue · Brown adipose tissue · Lipolysis · Thermogenesis · Energy expenditure

List of Abbreviations

AAR	Amino-acid response
ATF4	Activating transcription factor 4
BAT	Brown adipose tissue
DIO	Diet induced obesity
DIO2	Iodothyronine deiodinase 2
eIF2	Eukaryotic initiation factor 2
ERK	Extracellular regulated kinase
FA	Fatty acids
FASN	Fatty acid synthase
FGF	Fibroblast growth factor
FGFR	Fibroblast growth factor receptor
FRS2	Fibroblast growth factor receptor substrate 2
GCN2	General control nonderepressible 2
GLUT1	Glucose transporter 1
HCD	High carbohydrate diet
HSL	Hormone sensitive lipase
KD	Ketogenic diet
KLB	Beta klotho
LPD	Low protein diet
mTOR	Mammalian target of rapamicine
NRF2	Nuclear respiratory factor
PERK	Protein kinase R-like endoplasmic reticulum kinase
PGC1	PPAR gamma coactivator 1
PPAR	Peroxisome proliferator activated receptor
SLC6A19	Solute carrier family 6 member 19
SREBP	Steroid response element binding protein
TSC1	Tuberous sclerosis complex
UCP1	Uncoupling protein 1
UTR	Untranslated region
WAT	White adipose tissue

Introduction

Adaptation to food shortage requires temporal homeostatic adaptive responses to a condition of energy deficiency. Mammals have developed a wide range of mechanisms to detect and respond to episodes of malnutrition and starvation, including the capacity to adjust fuel oxidation in function of nutrient availability. Nutrient deprivation or starvation often correlates with amino acid deficiency.

Amino acids are required mainly for the synthesis of proteins and other biomolecules, and they also serve as signaling molecules and an energy source. Higher organisms are unable to synthesize all amino acids in sufficient amounts to meet cellular needs. Nine amino acids are deemed essential (valine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, histidine, and tryptophan), while others become essential in certain circumstances (some illness) or during specific stages of life (pregnancy, childhood, etc.). All essential amino acids must be supplied by diet. As there is no dispensable amino acid store in mammals, the rest must be synthesized or released by protein degradation when needed.

In mammals, the blood concentration of amino acids is affected by diet. Protein malnutrition or an imbalance in dietary intake of amino acids strongly affects aminoacidemia. This is the main problem of diets in which amino acid sources are limited to plant-derived products (legumes, grains or corn), which are deficient in some amino acids. In addition, pathological situations that cause stress, such as trauma, thermal burning, sepsis, and fever, can lead to a negative nitrogen balance. Overall, the concentration of each amino acid in cells results from the balance between inputs, namely de novo synthesis, protein breakdown and diet, and outputs, which comprise protein synthesis and amino acid degradation (Fig. 1).

This chapter will outline the changes in the metabolic patterns and molecular mechanisms driving these adaptive responses at the whole body level, and particularly in white and brown adipose tissue (WAT and BAT, respectively). It will firstly summarize the mechanism for sensing the amino acid circulating levels and secondly the role of the hormone-like protein Fibroblast Growth Factor (FGF) 21 on the metabolic response to amino acids imbalance.

Amino Acid Homeostasis: Amino Acid Response (AAR)

The maintenance of amino acid homeostasis depends on the cell capacity to sense amino acid availability. While the mTOR signaling pathway monitors amino acid sufficiency and promotes protein translation and cell growth, among other processes, the depletion of amino acids is detected by the GCN2/ATF4 pathway, which in turn triggers the amino acid response (AAR) (Fig. 2).

Amino acid starvation initiates a signal transduction cascade that starts with the activation of the general control nonderepressible 2 (GCN2) kinase, the phosphorylation of eukaryotic initiation factor 2 (eIF2), and increased synthesis of activating transcription factor (ATF) 4 (Kilberg et al. 2009). GCN2 kinase is a direct sensor of amino acid supply, performing this function by binding deacylated tRNAs (Qiu et al. 2001). When levels of an indispensable amino acid fall below a given threshold, its

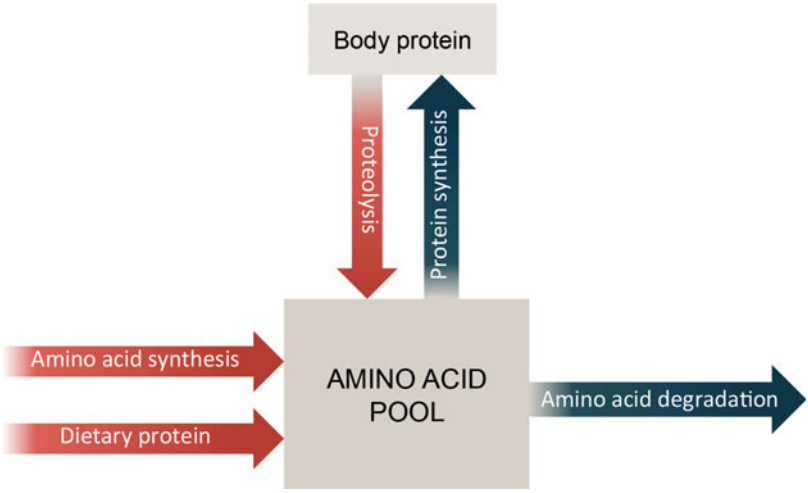

Fig. 1 Amino acid pool is a balance between the dairy inputs and outputs. The circulating amino acid levels are the result of inputs: the synthesis of amino acids, dietary protein intake, and protein degradation or proteolysis, and outputs: protein synthesis and amino acid degradation

tRNA becomes deacylated (i.e., uncharged). When activated by uncharged tRNAs, GCN2 increases eIF2α phosphorylation (Anthony et al. 2004; Hao et al. 2005), which results in the slowing or stalling of the initiation step of mRNA translation. Hence, phospho-eIF2α reduces general protein synthesis rates. Paradoxically, the delay in the re-initiation rate promotes an increase in the translation of discrete mRNAs that contain upstream open reading frames in the 5′-UTR region, including that coding for ATF4. Once induced, ATF4 directly or indirectly triggers the transcription of a subset of specific target genes in order to adapt to dietary stress (Shan et al. 2009).

The dietary content of amino acids alters metabolic pathways beyond protein homeostasis, since there is a link between amino acid intake and lipid metabolism. The synthesis of fatty acids (FAs) in the liver is diminished by GCN2-dependent inhibition of fatty acid synthase (FASN) activity and by the repression of lipogenic gene expression in liver. Leucine-deprived mice show an increase in the mobilization of lipid stores (Guo and Cavener 2007). In addition, increased expression of β-oxidation genes and decreased expression of lipogenic genes and activity of FASN in WAT and increased expression of uncoupling protein 1 (UCP1) in BAT have been observed (Cheng et al. 2010).

Metabolic Response to Protein Imbalance: The Key Role of FGF21

The fibroblast growth factor (FGF) 21 is the link between an imbalance in amino acid intake and adaptive metabolic response, and it serves to restore metabolic

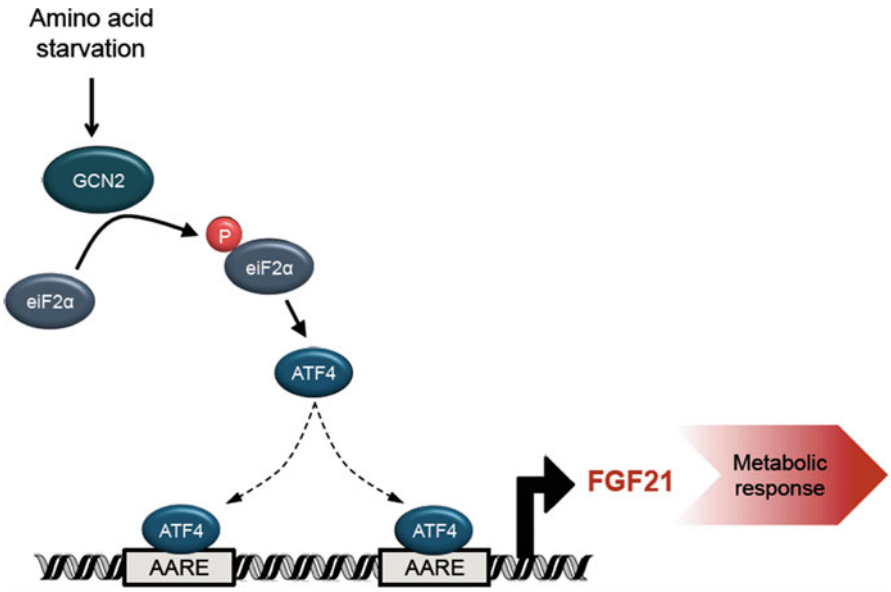

Fig. 2 The GCN2 pathway in the response to amino acids starvation. GCN2 kinase is a direct sensor of amino acid supply via binding deacylated tRNAs. When levels of an indispensable amino acid decrease enough, its tRNA becomes deacylated. When activated by uncharged tRNAs, GCN2 increases eiF2α phosphorylation. Hence, phospho-eiF2α reduces general protein synthesis rates. Paradoxically the translation of the transcription factor ATF4 and the expression of its target genes are increased

homeostasis. FGF21 is a member of the FGF family. It is predominantly produced by the liver but also by other tissues such as WAT and BAT, skeletal muscle, and pancreatic β cells (Domouzoglou and Maratos-Flier 2011). FGF21 expression in liver is under tight control by peroxisome proliferator-activated receptor alpha (PPARα). FGF21 is induced in the liver during fasting and its expression triggers a metabolic state that mimics long-term fasting. Thus, FGF21 is critical for the induction of hepatic FA oxidation (FAO), ketogenesis, and gluconeogenesis – all metabolic processes that are critical for the metabolic adaptation to starvation (Reitman 2007).

FGF21 acts as a hormone-like peptide and its signaling pathway requires FGF21 binding to a fibroblast growth factor receptor (FGFR). FGFRs are tyrosine kinase receptors, and seven isoforms have been described (1b, 1c, 2b, 2c, 3b, 3c, and 4). FGFR1c has been defined as the main mediator of FGF21 response in vivo (Yang et al. 2012) through an obligate dimerization with the co-receptor β-klotho (KLB) (Ding et al. 2012). The co-expression of these two receptors determines the sensitivity of a tissue or organ to FGF21 signaling. Regarding the signal transduction pathway, the binding of FGF21 to the FGFR-KLB dimer stimulates the phosphorylation of FGFR substrate 2α (FRS2α) and the activation of extracellular signal-regulated kinase 1/2 (ERK1/2) and Akt (Fisher and Maratos-Flier 2016) (Fig. 3).

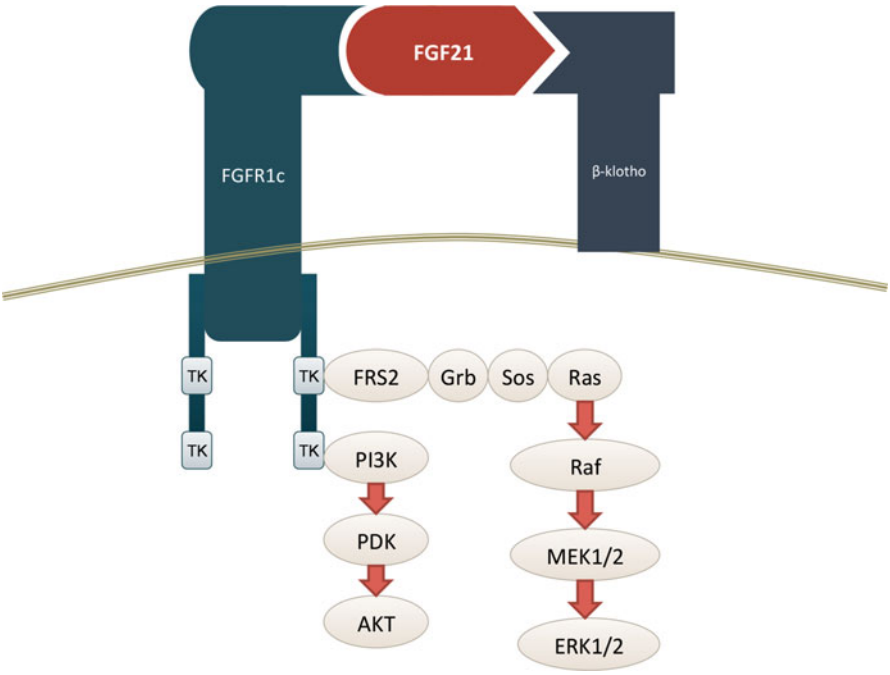

Fig. 3 The FGF21 receptor complex and signaling cascade. The binding of FGF21 to the FGFR-KLB dimer causes the tyrosine autophosphorylation of FGFR. This action facilitates the binding and activation of FGFR substrate 2α (*FRS2α*), which transduces the signal to MAPK signaling cascades via the recruitment of several adaptor molecules. The activation of extracellular signal-regulated kinase 1/2 (*ERK1/2*) and PI3K is critical to induce transcription of early response genes

The similarities between the metabolic response to essential amino acid deprivation and to the administration of recombinant FGF21 together with the induction of FGF21 under amino acid deprivation (De Sousa-Coelho et al. 2012), and the repression of the transcription and maturation of sterol regulatory element binding protein (SREBP) 1c induced by FGF21 in HepG2 cells (Zhang et al. 2011), suggest that FGF21 is a key mediator between amino acid deprivation and lipid metabolism in liver, WAT, and BAT.

Analysis of the response of FGF21-deficient mice to deprivation of the essential amino acid leucine reveals a huge increase in FGF21 expression in liver of wild-type animals, along with a repression of lipogenic genes after 7 days of deprivation. In this condition, these mice develop liver steatosis as a result of the unrepressed expression of lipogenic genes. Under leucine deprivation, the expression of lipogenic genes in WAT is also repressed, and the phosphorylation of hormone-sensitive lipase (HSL) increases. The absence of leucine also triggers an increase in the expression of UCP1 and type 2 deiodinase (Dio2) in BAT. All these effects in WAT and BAT are impaired in FGF21-deficient mice (De Sousa-Coelho et al. 2013).

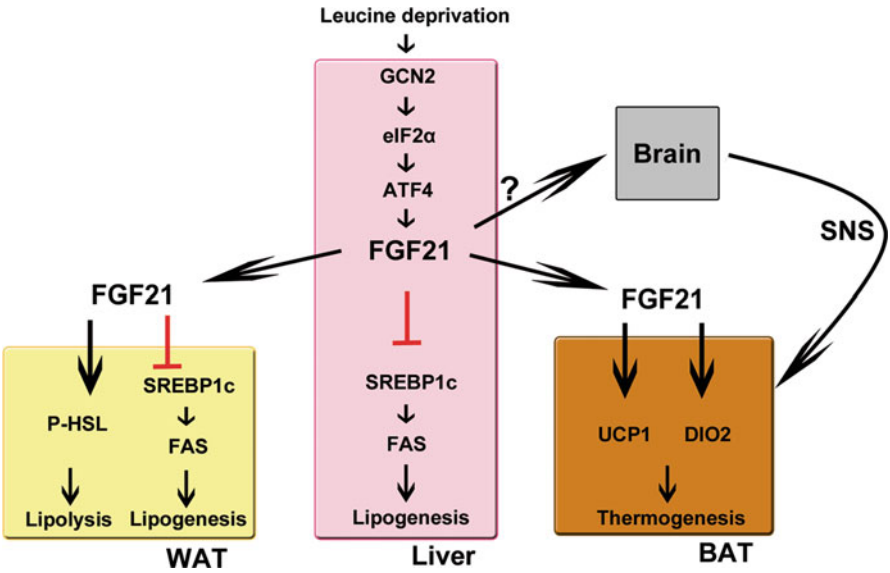

Fig. 4 Model of the FGF21 regulatory pathway under leucine deprivation. This research was originally published in Journal of Lipid Research. De Sousa-Coelho et al. 2013. Copyright © the American Society for Biochemistry and Molecular Biology

These results demonstrate the involvement of FGF21 in the regulation of lipid metabolism during amino acid starvation. These observations thus reinforce the role of this growth factor as an endocrine factor, coordinating energy homeostasis under a variety of nutritional conditions (Fig. 4).

Along the same lines, methionine-deprived mice show a phenotype comparable to that of leucine deprivation. The metabolic response to methionine deficiency includes resistance to diet-induced obesity (DIO), improved glucose homeostasis, increased FA activation and oxidation in liver, increased lipolysis in WAT, and increased Ucp1 expression in BAT (Ables et al. 2012; Lees et al. 2014; Stone et al. 2014). All these effects are coupled to an increase in circulating FGF21 levels. In fact, it has recently been shown that FGF21 is a critical mediator of the effects of dietary methionine restriction on energy expenditure, WAT remodeling, and increased insulin sensitivity, but not of the effects of this diet on hepatic gene expression (Wanders et al. 2017).

The metabolic response to protein limitation is similar to that observed under leucine or methionine restriction (Laeger et al. 2014). Serum levels of FGF21 increase in both rodents and humans subjected to low protein diets (LPDs), regardless of overall caloric intake (Morrison and Laeger 2015; Ozaki et al. 2015). LPDs are accompanied by weight loss and an increase in both food intake and energy expenditure (Laeger et al. 2014; Ozaki et al. 2015). Remarkably, neither food intake nor energy expenditure of Fgf21-deficient mice is altered by the administration of such diets (Laeger et al. 2014). Moreover, a LPD has been reported to induce thermogenic markers in BAT of obese rats (Pezeshki et al. 2016).

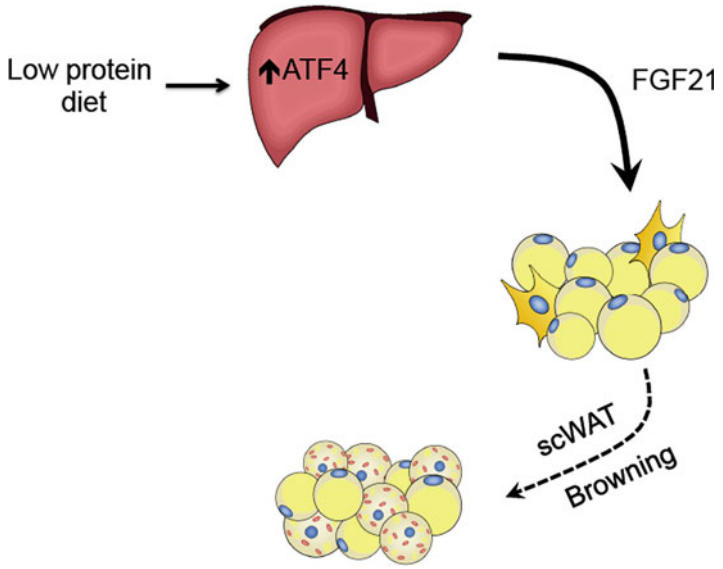

Fig. 5 Model of the FGF21 mediated response to a LPD in scWAT. In mice, a LPD induces a huge increase in liver FGF21 expression and in serum levels, which correlates with enhanced ATF4 protein levels. Also, this diet caused an FGF21-dependent browning of scWAT. This research was originally published in Molecular Nutrition and Food Research. Pérez-Martí et al. (2017); In press. Copyright © 2017, Wiley-VCH Verlag GMbH & Co. GaA

Analysis of the metabolic response of wild-type and FGF21 liver-specific knockout mice (L*Fgf21*KO) to a LPD (up to 5% of energy as protein) showed that a decrease in dietary protein content induces a huge increase in FGF21 serum levels, significant weight loss, and an increase in the expression of UCP1 in the subcutaneous WAT (scWAT) of wild-type mice. Remarkably, no effects were observed in L*Fgf21*KO mice, thereby indicating that the absence of FGF21 blunts or completely blocks the response to a LPD in this mouse model. These observations thus reveal that FGF21 is likely to be involved in the metabolic Response to Protein-restricted diets (Pérez-Martí et al. 2017) (Fig. 5).

Moreover, ketogenic diets (KDs), which are widely known to induce FGF21 expression, are usually low in carbohydrates and proteins but rich in fat. The protein content of KDs underlies the increased levels of circulating FGF21, since protein supplementation but not carbohydrate supplementation blunts this induction (Bielohuby et al. 2011). This effect could also explain the induction of FGF21 observed in high carbohydrate diets (HCDs), which are characterized by low protein content.

Finally, in humans, the analysis of protein intake through nutritional questionnaires and the determination of the serum levels of FGF21 in individuals randomly selected from two nodes of the PREDIMED (*Prevención con Dieta Mediterránea*) trial show like in the animal model, an inverse correlation between circulating FGF21 levels and protein intake (Pérez-Martí et al. 2017) (Fig. 6).

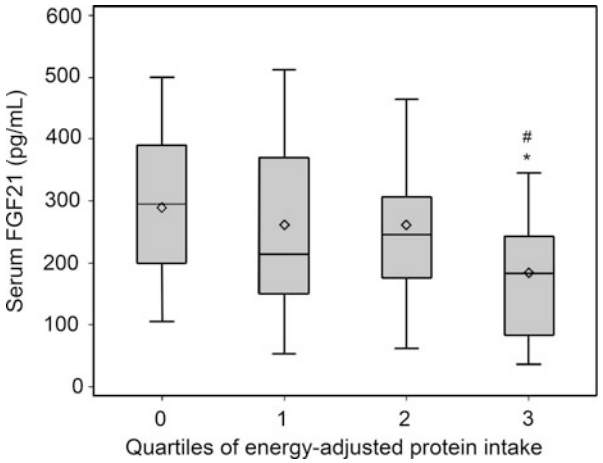

Fig. 6 Circulating FGF21 levels correlate negatively with protein intake in humans. Plasma FGF21 concentration divided into quartiles of protein intake adjusted for the calorie intake of 78 participants in the PREDIMED trial. Error bars represent the mean ± SEM. * $p < 0.05$ from 1st quartile; # $p < 0.05$ from the 2nd quartile. This research was originally published in Molecular Nutrition and Food Research. Pérez-Martí et al. (2017); In press. Copyright © 2017, Wiley-VCH Verlag GMbH & Co. KGaA

Adipose Tissue as the Main Target of the Effects of FGF21

Adipose tissue is the main target tissue of FGF21 and the major mediator of its beneficial effects. While the physiological effects of FGF21 during fasting remain elusive, most data on its signaling in WAT derive from studies in which it was pharmacologically administered or overexpressed in obese mice. Nevertheless, depending on its source, FGF21 shows paradoxical actions on WAT. Fgf21-over-expressing mice show induced lipolysis (Inagaki et al. 2007; Li et al. 2009). In contrast, FGF21-knockout mice present enhanced lipolysis in late fasting (Hotta et al. 2009). In addition, while FGF21 suppresses lipolysis in mouse and human adipocytes (Arner et al. 2008), this process is induced by peroxisome proliferator activating receptor gamma (PPARγ) in WAT upon feeding, thus stimulating adipogenesis (Dutchak et al. 2012; Muise et al. 2008). Regarding glucose metabolism, FGF21 induces glucose uptake in 3T3L1 adipocytes by increasing glucose transporter 1 (GLUT1), independently of insulin action (Kharitonenkov et al. 2005). Moreover, later studies reported increased glucose uptake in both WAT and BAT of lean mice infused with FGF21 and fed a chow diet (Camporez et al. 2013). In summary, in WAT, FGF21 induces genes involved in glucose uptake, lipogenesis, and lipolysis, depending on the metabolic state of the adipocytes. These apparently contradictory observations may be explained by compensatory effects of genetic modifications in mice, a different nutritional status, and different FGF21 concentrations reached between pharmacological administration and physiological secretion. WAT is not only a FGF21 target tissue but also a mediator of the effects of this growth factor. In this regard, the glucose- and insulin-sensitizing effects of FGF21

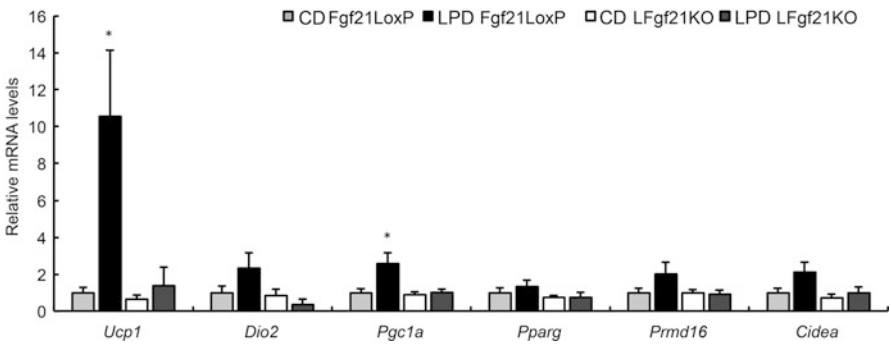

Fig. 7 Hepatic FGF21 is required for inducing thermogenic gene expression during a LPD. Ucp1, Dio2, Pgc1α, Pparγ, Prmd16, and Cidea expression was measured by qRT-PCR in mouse scWAT. Error bars represent the mean ± SEM. * $p < 0.05$ versus Fgf21LoxP mice fed a CD ($n = 7$–9/group). This research was originally published in Molecular Nutrition and Food Research. Pérez-Martí et al. 2017; In press. Copyright © 2017, Wiley-VCH Verlag GMbH & Co. KGaA

require the production and secretion of adiponectin from WAT. Accordingly, FGF21 stimulates this mechanism in rodents, and adiponectin-knockout mice fail to reproduce the sensitizing effects of FGF21 (Lin et al. 2013). Similarly, FGF21 also reduces the levels of sphingolipid ceramides. These lipids have been associated with insulin resistance caused by lipotoxicity. By inducing adiponectin secretion, FGF21 diminishes the accumulation of ceramides in obese animals (Holland et al. 2013). Overall, despite some contradictory effects of FGF21 in adipose fat depots, adipose tissue is considered indispensable for the physiological and pharmacological effects of this growth factor. Finally, FGF21 induces the expression of Ucp1, thus producing the so-called WAT browning in an autocrine, paracrine or endocrine fashion (De Sousa-Coelho et al. 2013; Fisher et al. 2012; Pérez-Martí et al. 2017). Browning occurs in multilocular beige adipocytes in specific susceptible WAT depots, such as inguinal and perirenal tissue, through an increase in the expression of genes involved in thermogenesis, and it confers a brown fat-like phenotype to white adipocytes (Fig. 7).

BAT is a FGF21 target tissue since it expresses FGFR1 and KLB; however, this tissue is also a source of FGF21. In BAT, FGF21 stimulates glucose uptake and thermogenesis through the induction of UCP1 in the interscapular depot in an autocrine and paracrine fashion (Hondares et al. 2011). Upon exposure to cold, FGF21 expression is increased in BAT and other cold-sensitive fat depots in the β-adrenergic/ATF2-dependent pathway (Chartoumpekis et al. 2011; Fisher et al. 2012; Hondares et al. 2011). In this regard, Fgf21-deficient mice respond poorly to cold exposure and show greater shivering.

The mechanisms underlying the action of FGF21 on BAT/WAT are still not well understood. Part of the FGF21-induced activation of the thermogenic program is driven by PPARγ coactivator 1 alpha (PGC1α), as FGF21 increases the protein levels of this coactivator. Similarly, Pgc1a-knockout mice show an impaired response to FGF21 (Fisher et al. 2012). Furthermore, hepatic FGF21-mediated

thermogenesis has also been described in response to maternal milk consumption in neonatal pups (Hondares et al. 2010) and also in situations of metabolic stress, for example, upon amino acid restriction (De Sousa-Coelho et al. 2013). These observations suggest that the hepatic FGF21-mediated increase in thermogenic capacity is an adaptive response to metabolic stress. It has been proposed that the effect of FGF21 on energy expenditure and weight loss may be due to an increased thermogenic capacity of BAT and WAT (browning). However, recent experiments in Ucp1-null mice and interscapular BAT-excised mice show that when FGF21 is administered pharmacologically, UCP1 is not required for the improvement of the glucose, cholesterol, and free FA profile. However, the increment in metabolic rate associated with the administration of FGF21 is diminished in these mice (Bernardo et al. 2015; Samms et al. 2015; Véniant et al. 2015). These data suggest that the metabolic benefits of FGF21 are partly UCP1-independent.

Molecular Mechanisms for the FGF21 Induction in Response to Protein Intake

FGF21 is induced by amino acid deprivation both in HepG2 cultured cells and mouse liver, but not in WAT or BAT, and FGF21 is a target gene for the transcription factor ATF4. These results add FGF21 gene induction to the transcriptional program triggered by increased levels of ATF4 in response to amino acid starvation and offer a new mechanism for the induction of FGF21 expression under nutrient deprivation (De Sousa-Coelho et al. 2012).

GCN2, a kinase that acts as a sensor of amino acid supply (Qiu et al. 2001), and PPARα are indispensable for the induction of FGF21 in response to protein restriction. In this regard, the respective knockout mice present blunted induction of FGF21 when fed a LPD. To date, there is no evidence of PPARα activation in response to a LPD, thus suggesting that PPARα plays a role in the constitutive expression of FGF21. In contrast, GCN2-dependent phosphorylation of eIF2α increases in response to a LPD, resulting in greater ATF4 protein levels (Anthony et al. 2004; Guo and Cavener 2007). Therefore, the GCN2/eIF2α/ATF4 cascade emerges as the main signaling pathway in the induction of FGF21 by protein restriction. As has been mentioned before, ATF4 directly or indirectly triggers the transcription of a subset of specific target genes, including FGF21, to modulate many cellular processes in order to adapt to amino acid deficiency (De Sousa-Coelho et al. 2012; Shan et al. 2009). However, it is worth mentioning that LPD administration to Pparα-KO and Gcn2-KO mice still induces FGF21 expression (Laeger et al. 2014). This observation suggests that additional signaling pathways are involved in triggering the increase in FGF21 expression in response to protein restriction. GCN2-independent mechanisms that induce hepatic FGF21 in response to methionine-restricted diets have recently been described (Wanders et al. 2016). These results point to a noncanonical PERK/nuclear respiratory factor 2 (NRF2) pathway in liver as an alternative mechanism used to sense and respond to methionine restriction when GCN2 is absent. Along the same lines, during long-term dietary protein restriction, the absence of GCN2 is compensated upstream of ATF4 in

order to maintain FGF21 induction (Laeger et al. 2016). In summary, amino acid-deficient diets diminish aminoacidemia and trigger the AAR, thereby resulting in elevated FGF21 levels. The contribution of each single amino acid to the modulation of FGF21 and how a deficiency in specific types of dietary protein alters FGF21 expression require further study.

Hepatic mTORC1 activity is also related to FGF21 expression. The mTOR-signaling pathway monitors amino acid sufficiency and promotes protein translation and cell growth, among other processes. In this case, liver-specific Tsc1 knockout mice, which present mTORC1 hyperactivity in the liver, show increased expression of FGF21 and depleted levels of glutamine (Cornu et al. 2014). Moreover, when these animals are treated with rapamycin (mTORC1 inhibitor) or glutamine, the increase in FGF21 is blunted. Finally, in human hepatic tumors, mTORC1 activation also correlates with FGF21 levels (Cornu et al. 2014). It has been proposed that the mechanism underling the increase in FGF21 expression occurs through PGC1α; however, additional mechanisms could be involved and it is feasible that depleted glutamine levels trigger the AAR.

Alternative Situations that Cause a Negative Nitrogen Balance and Change FGF21 Expression

In addition, pathological conditions caused by various forms of stress, such as trauma, thermal burning, sepsis, and fever, can lead to a negative nitrogen balance. In this context, several scenarios that alter aminoacidemia also lead to an increase in FGF21 expression. The absence of slc6a19 (neutral amino acid transporter) causes a lack of systemic neutral amino acids, resulting in an increase in FGF21 transcription (Jiang et al. 2015). In mice, treatment with the antileukemic agent asparaginase depletes circulating asparagine and glutamine levels, thereby promoting FGF21 expression (Wilson et al. 2015), and skeletal muscle-specific knockout mice for glucocorticoid receptor (GR) show reduced alanine flux from skeletal muscle during fasting, resulting in an increase in FGF21 plasma levels (Shimizu et al. 2015). Hence, it is likely that many other situations that reduce amino acid availability also lead to an induction of FGF21 expression.

Concluding Remarks

To summarize, here we define the molecular Mechanisms by which amino acid starvation or LPDs exert their metabolic effects through the induction of hepatic FGF21 expression and increased thermogenic gene expression in different fat depots. Furthermore, given the data collected from humans, manipulation of dietary protein content emerges as an approach to modulate circulating FGF21 levels and thus an alternative to its pharmacological administration.

Policies and Protocols

Gene Expression Analysis

This chapter described how a particular dietary pattern changes the mRNA expression profile of some metabolic regulatory genes. Nowadays, gene expression is analyzed by quantitative PCR (qPCR). Briefly, tissues or organs to be analyzed are extracted from the animals and immediately frozen at −80 °C. Total RNA from different tissues are extracted using phenolic derivatives such as TriReagent solution. To perform the gene expression assays, the RNA is converted to single-stranded DNA (cDNA) that can be used as a template to amplify by PCR, specific regions from the genes of interest. The qPCR is based on a fluorescence reporter molecule – the most usual are TaqMan® probe or SYBR® Green dye – that monitor the amount of target amplicon. In this case, the fluorescence is directly proportional to the initial quantity of the target template (cDNA) and it is reflections of the amount of target mRNA. The calculi of the expression levels must be normalized with housekeeping genes (it is highly recommended to use more than one) and is expressed as a fold induction, a comparison between the number of cycles of amplification needed to detect the fluorescence (Ct) with the gene of interest in control animals and in treated ones after the normalization by the Ct of the housekeeping genes in both conditions.

Protein Analysis

Western Blot and ELISA are two experimental approaches to the study of protein levels. In both cases, total protein extracts need to be obtained. The protein analyses can be done from cell cultures, tissues, and organs or from plasma/serum/blood/urine. In the case of organs and tissues, those must be homogenized before the purification. The protein extraction from cells requires the lysis of the cells prior purification. In both cases, this first step is done with a detergent – the most used are NP40, SDS, or Triton. Later, the proteins are solubilized and purified from the cell debris. The proteins from liquid samples are already in solution and the only critical step could be the need to concentrate the sample.

Finally and after total protein quantification, the next step is the detection of a specific protein. Western Blot and ELISA are both based on an antibody-antigen reaction. In the case of Western Blot, the proteins are resolved by electrophoresis in a SDS-polyacrylamide gel, transferred to a PVDF or nylon membrane and detected in the membrane using a specific antibody against the target protein. This primary antibody could be linked to a detection method (fluorescence molecule or an enzyme that catalyzes a reaction easy to quantify), but in most of the cases it is necessary to add a second antibody that recognizes the first one and that is linked to the detection method. In the case of ELISA, the binding between antigen (target protein) and antibody takes place in a well plate and the detection method is usually a colorimetric method, but the proof of concept is the same than in Western Blot.

Generation of Tissue Specific Knockout Mice

The goal of this protocol is the generation of a genetically modified mouse in which the function of a particular gene has been eliminated in one specific tissue. To generate tissue-specific knockout mice, two previously genetically modified mice need to be crossed. In the first animal, the genetic modification consists in the introduction of a tag (a DNA sequence called loxP, for instance) in the gene which function will be altered. The genetic modification of the second mice is the introduction of the gene that encodes for an enzyme called Cre recombinase. The Cre recombinase gene is introduced in mice targeted to be expressed only in a particular tissue. In mice resulting from the crossing between the previous two, the Cre recombinase recognizes the loxP sequence and as a result of its enzymatic activity the region flanked by loxP sequences is deleted. This deletion means the loss of function of the target gene only in the tissues where the Cre recombinase is expressed. **The animal experimental policies** stablish that: all the animal protocols must be approved by the corresponding Animal Ethics Committee of the Institution and/or Country where the experiments will be made.

Dictionary of Terms

- **Essential amino acids** – Amino acids that cannot be synthesized by higher organisms and consequently must be supplied by diet.
- **Lipolysis** – Mobilization of fatty acids from fat depots.
- **Thermogenesis** – Heat production in brown adipose tissue by disconnection of ATP synthesis from the electron transport chain.
- **Signal transduction** – Transmission of an input signal through a cell by a series of molecular events mainly cascades of protein phosphorylation.
- **Specific knockout mice** – A genetically engineered mouse in which the function of a particular gene has been abolished only in a specific tissue.
- **Browning** – The appearance of cells with a brown fat-like aspect in specific susceptible white adipose tissue depots.

Summary Points

- This chapter reviews the changes in the metabolic patterns induced during amino acids starvation and the molecular mechanisms driving these changes at the whole body and particularly in both white and brown adipose tissue.
- The dietary amino acids content alters metabolic pathways beyond protein homeostasis since there is a link between amino acids intake and lipid metabolism.
- The synthesis of fatty acids in liver is diminished by a repression of the lipogenic genes expression and inhibition of the activity of fatty acid synthase.
- Leucine deprivation causes an increased mobilization of lipid stores

- Leucine deprivation or protein restriction causes a change in the gene expression pattern that includes: increased expression of β-oxidation genes and decreased expression of lipogenic genes in white adipose tissue, and increased expression of uncoupling protein 1 (UCP1) in brown adipose tissue.
- Amino acid starvation initiates a signal transduction cascade starting with the activation of general control nonderepressible 2 (GCN2) kinase.
- Paradoxically, with the overall protein synthesis stopped, the synthesis of activating transcription factor 4 (ATF4) is increased.
- Activating transcription factor 4 (ATF4) directly or indirectly induces the transcription of a subset of specific target genes, including fibroblast growth factor 21 (FGF21), to modulate many cellular processes to adapt to amino acid deficiency or protein restriction.
- Fibroblast growth factor 21 (FGF21) plays a relevant role in the regulation of lipid metabolism during amino acid starvation and protein restriction coordinating energy homeostasis.
- Amino acid starvation or a protein-restricted diet induce a fibroblast growth factor 21 (FGF21)-dependent weight loss and increased uncoupling protein 1 (UCP1) expression in the subcutaneous white adipose tissue.

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Fasting Influences Conditioned Memory for Food Preference Through the Orexin System: Hypothesis Gained from Studies in the Rat

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Barbara Ferry and Patricia Duchamp-Viret

Contents

Abstract

A large variety of behaviors that are essential for animal survival depend on the processing and perception of surrounding smells present in the natural environment. In particular, food-search behavior, which is conditioned by hunger, is directly driven by the perception of odors associated with food, and feeding status modulates olfactory sensitivity. The orexigenic hypothalamic peptide orexin A, one of the main central and peripheral hormones that triggers food intake, has been shown to increase olfactory sensitivity in various experimental conditions including the conditioned odor aversion learning paradigm. Conditioned odor aversion is an associative task that corresponds to the association between an olfactory conditioned stimulus and a delayed gastric malaise. Previous studies have shown that this association is formed only if the delay separating the

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conditioned stimulus presentation from the malaise is short, suggesting that the memory trace of the odor is relatively unstable. To test the selective impact of the orexin system in olfactory sensitivity, a recent study compared the effects of fasting and of central infusion of orexin A during the acquisition of conditioned odor aversion. Results showed that the increased olfactory sensitivity induced by fasting or by orexin infusion was accompanied by enhanced conditioned odor aversion learning performances. In reference to the duration of action of orexin, the present work details the results obtained during the successive conditioned odor aversion extinction tests and suggests a hypothesis concerning the role of the orexin component of fasting on the memory processes underlying the odor-malaise association during conditioned odor aversion. Moreover, referring to previous data in the literature, we suggest a functional circuit model where fasting modulates olfactory memory processes through direct and/or indirect activation of particular orexin brain targets including the olfactory bulb, the locus coeruleus, and the amygdala.

Keywords
Associative learning · Olfactory memory · Orexin · Feeding · Rat

List of Abbreviations

aCSF	Artificial cerebrospinal fluid
BLA	Basolateral amygdala
COA	Conditioned odor aversion
CS	Conditioned stimulus
i.p.	Intraperitoneal
Icv	Intracerebroventricular
ISI	Interstimulus interval
LC	Locus coeruleus
LH	Lateral hypothalamus
NA	Norepinephrine
nM	Nanomolar
OB	Olfactory bulb
OX	Orexin
SEM	Standard error of the mean
US	Unconditioned stimulus
µl	Microliter

Introduction

A large variety of behaviors that are essential for animal survival depend on the sensory processing and perception of odor cues present in the natural environment. Food-search behavior, which is conditioned by hunger, is directly driven by the perception of odors associated with food items (Le Magnen 1959). In turn, several studies have demonstrated that nutritional status influences odor processing. For

example, the olfactory bulb activity was shown to be directly modulated according to hunger and satiation status (Apelbaum et al. 2005; Pager 1974; Pager et al. 1972; Pager 1978; Royet et al. 1983). Such a modulation supports results showing that fasting enhanced odor detection in rats, whereas satiety reduced detection of odors in general (Aimé et al. 2007), or of one odorant specifically associated with the food type involved in the satiation (O'Doherty et al. 2000; Mulligan et al. 2002). The literature suggests that the CNS regulates food-search behavior by modulating the detection threshold of the food odorant items through centrifugal innervations (Doucette et al. 2007; Doucette and Restrepo 2008; Fletcher and Chen 2010), and a large body of data indicate that the hypothalamus plays an important role in this process. Firstly, anatomic characterization of the lateral hypothalamus has shown the existence of a functional loop between hypothalamic orexin system (OX) and structures involved in the first levels of odor processing (i.e., olfactory mucosa, bulb and piriform cortex) (Peyron et al. 1998; Nambu et al. 1999; Caillol et al. 2003; Shibata et al. 2008; Hahn and Swanson 2010; Sakurai 2005; Swanson et al. 2005). Secondly, the crucial role of the hypothalamus, and in particular of orexigenic (appetite-stimulating) and anorexigenic (appetite-inhibiting) neurochemicals, in appetite regulation and energy balance has long been established (see Rodgers et al. 2002 for review). Thirdly, among the multitude of neurochemicals found in the hypothalamus, OX peptides (orexin A (OX A) and orexin B (OX B)) have been shown to be strongly involved in the regulation not only of feeding and energy metabolism (see Willie et al. 2001 for review) but also of olfactory sensitivity.

In particular, we have shown that intracerbroventricular (icv) infusion of OX A increased olfactory detection performance in the same way as physiologically induced fasting in the rat (Aimé et al. 2007; Julliard et al. 2007). Fourthly, the two classes of receptors for OX A and for OX B are expressed in olfactory bulb (OB) neurons (Caillol et al. 2003) and OX A impacts mitral cell activity (Hardy et al. 2005). In addition, central infusion of OX A increased OB Fos responses to food odor in both fasted and satiated animals (Prud'Homme et al. 2009). All these results indicate that orexins, mainly OX A and secondarily OX B, are involved in the control of feeding behavior by modulating olfactory sensitivity.

It is, however, very unlikely that olfactory sensitivity is completely dissociated from olfactory memory. Rusiniak et al. (1982) and Slotnick et al. (1997) showed that the more intense the odor, the stronger the memory of its association with a reinforcement. Interestingly, other than its projection on the primary olfactory centers, the hypothalamic OX neurons project to various structures involved in olfactory associative learning (see Rodgers et al. 2002 for review). Moreover, OX system was shown to be involved in the memory processes underlying various kinds of learning (Telegdy and Adamik 2002; Jaeger et al. 2002; Di Sebastiano et al. 2010; Mair and Hembrook 2008) and in particular in conditioned flavor aversion paradigms (Touzani and Sclafani 2002). Thus, OX system may be involved in odor memory formation, directly, by modulating olfactory sensitivity, and indirectly, by activating particular hypothalamus target regions.

In a natural environment, the relevance of the odor coming from a food source encountered by an animal during food-search is a crucial key, determining approach

and ingestion of the food. Whether the odor of the food is new to the animal or has previously acquired a hedonic valence during a first intake experience will condition either attraction or avoidance. Acquisition of hedonic valence by a food item has been shown to result from conditioned learning during which the sensory stimuli characterizing a particular food (odor and taste) become associated with the positive (energy input) or negative (gastric malaise, poisoning) consequences of the ingestion of the food, so that processing the odor and taste stimuli will cue the appropriate agonistic or antagonistic responses (Rescorla 1988; Mackintosh 1991; Holland 1990). These kinds of association have been experimentally studied for years (Nigrosh et al. 1975; Slotnick 1984; Slotnick and Katz 1974), and conditioned food aversion paradigms, such as conditioned taste or odor/taste-potentiated odor aversion learning, have provided fundamental insights into the mechanisms and CNS structures involved in food-reward/food-poisoning associations (see Miranda 2012 for review).

Conditioned Odor Aversion Learning Paradigm and Orexin

One such paradigm, conditioned odor aversion (COA), is a trace conditioning paradigm of avoidance of a tasteless odorized solution (conditioned stimulus (CS)) the ingestion of which precedes gastric malaise (unconditioned stimulus (US)). COA is a robust long-lasting learned association that can be obtained with only a single CS–US pairing (Hankins et al. 1973; Lorden et al. 1970; Taukulis 1974). In contrast to the well-known conditioned taste aversion paradigm, COA is normally obtained only if the interval between CS and US is shorter than 30 min (Andrews and Braveman 1975; Bouton et al. 1986; Garcia et al. 1966), suggesting that the memory trace of the odor, which must be maintained during the interstimulus interval (ISI) in order to be associated with the US, is subject to rapid decay. Our team's investigations of the neurobiological substrate involved in COA showed that the basolateral nucleus of the amygdala (BLA) plays a critical role in the acquisition of odor-US associations by modulating the odor memory trace during the ISI (Ferry et al. 1995; Ferry and Di Scala 1997, 2000).

Taking these data together with the fact that (i) OX neurons project to the amygdala and (ii) hypothalamic OX neurons are activated by cues associated with consummatory rewards such as food (Harris et al. 2005) suggests that the OX system may play a role in the learning and memory processes linked to higher cognitive aspects of feeding.

In order to test this hypothesis, a series of studies have been aimed at evaluating the effect of manipulation of OX A level in the brain system during COA learning. Using icv microinjection technique, we have been able to show that fasting induced by 24-h food-deprivation as well as preacquisition central OX A infusion potentiated the memory processes underlying the association between the olfactory CS and the delayed US in the rat, thus rendering the association between the odor CS and the US possible even though the ISI delay is longer than 35 min. Moreover, this effect was

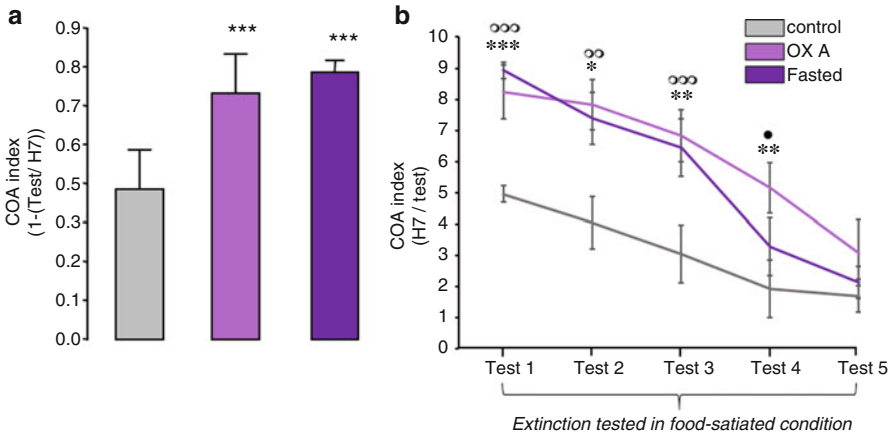

Fig. 1 Effects of icv infusion of orexin A (10 µg/3 µl, OX A group), icv infusion of artificial CSF (3 µl, aCSF group) and food-deprivation (Fasted group) during COA acquisition on performance measured during the test (48 h after acquisition) and extinction (5 consecutive testing days) when the ISI was about 35 min. (**a**) Bars represent mean COA index of the form "1-(water intake measured during the test/water intake measured the day before acquisition H7" (±S.E.M.) calculated for the various groups during the test measured in satiated condition. An index close to 1.0 represents a strong COA. ***: $p < 0.001$ compared to the control group. (**b**) Curves represent the COA index of the form "water intake measured the day before the acquisition H7/water intake measured during the test" (±S.E.M.) for each group from testing days 1 to 5. The Fasted group was food-deprived only during acquisition. All animals were tested while food satiated. *, **, and ***: $P < 0.05$, $P < 0.01$ and $P < 0.001$ between OX A and control group. ○○ and ○○○: $P < 0.01$ and 0.001 between Fasted and control group. ●: $P < 0.05$ between OX A and Fasted group

independent from the fasting-induced enhancement of stress level (Ferry and Duchamp-Viret 2014; Fig. 1).

Referring to the fact that COA is a trace conditioning that results from several processes that follow one another over time, these data have shed some light on the processes that have been influenced by the activation of OX system. During acquisition, CS and US processing are followed by association of the two stimuli. Then, the CS-US association is consolidated and finally retrieved during the test when the CS is presented for the second time. Some studies have shown that behavioral effects of icv OX A infusion, such as feeding and drinking behavior (Sakurai et al. 1998; Edwards et al. 1999, Kunii et al. 1999) or olfactory hypersensitivity (Julliard et al. 2007), persist for at least 3 h. Therefore, the effects of fasting and OX A infusion obtained on COA resulted from changes in the processes of acquisition and/or consolidation taking place 2 days after, when COA was tested in satiated condition.

Effect of Fasting and OX A Infusion on Acquisition of the Task

As discussed earlier, the acquisition of COA reflects the association between the memory trace of the olfactory CS and the delayed visceral US (see Bures and

Buresova 1990; Roldan and Bures 1994). Several studies have shown that, when ingested, a tasteless olfactory stimulus acquires a strong aversive value, even with CS–US intervals equivalent to those generally used for tastes (Slotnick et al. 1997; Rusiniak et al. 1982; Bouton et al. 1986); however, COA can only be acquired if the interstimulus interval (ISI) is kept under 30 min, suggesting that the CS trace is subject to gradual decay over time (Miranda et al. 2007; Ferry et al. 2006; Inui et al. 2006). Moreover, Rusiniak et al. (1982) and Slotnick et al. (1997) have shown that the effectiveness of an olfactory CS in inducing strong COA when paired with a delayed illness is directly related to the intensity of the CS used during acquisition. Thus, in the light of previous studies showing 24-h fasting and icv OX A infusion increase olfactory sensitivity (Julliard et al. 2007), it is possible that the potentiation of COA observed in the study of Ferry and Duchamp-Viret (2014) resulted from the enhanced perception of the CS that lengthened its memory trace duration thus rendering possible its association to toxicosis occurring with a delay longer than 30 min (Ferry et al. 1995, 1996).

Effect of Fasting and OX A on Consolidation of the Task

Previous works have proposed that consolidation involves two types of process: synaptic consolidation, accomplished within the first minutes to hours after the CS-US association has been acquired; and system consolidation, involving reorganization of the brain circuits encoding the memory, which takes weeks, months, or even years to be accomplished (Dudai 1996; Dudai and Morris 2000). Considering the duration of OX A action when infused icv, it may be suggested that the enhanced COA observed in our study was mediated by enhanced synaptic consolidation of the memory processes underlying COA in OX A containing structures such as hippocampus, amygdala, piriform cortex, or entorhinal cortex that have been previously involved in such learning (Marcus et al. 2001; Campbell et al. 2017; Caillol et al. 2003; Ferry et al. 1996, 2006; Ferry and Di Scala 1997; Chapuis et al. 2009).

Together, these data show that physiological as well as OX A-induced fasting status affected COA through changes in memory processes occurring during the acquisition of a CS-US association and/or during the synaptic consolidation of this association.

Hypothesis on the Neurobiological Substrate Mediating Fasting and OX A Effects on COA Learning and Memory

The neurobiological substrate through which OX may influence the memory processes underlying food conditioned learning remains to be elucidated; however, some reports open up a number of possible hypotheses involving hypothalamic projection targets.

As mentioned before, olfactory sensitivity cannot be dissociated from olfactory memory, and some data suggest that the indirect effects of fasting and OX A on the

olfactory memory trace formation through increased olfactory sensitivity may involve the OB. Firstly, in addition to its well-documented role in detection and discrimination, the OB is involved in memory processes underlying various kinds of olfactory learning in adult rats (see Mandairon and Linster 2009 for review). Moreover, the OB receives direct OX innervation from the lateral hypothalamus (LH) (De Lecea et al. 1998; Peyron et al. 1998; Sakurai et al. 1998, Shibata et al. 2008), and an increase in OB electrophysiological response induced by fasting and OX has been described (Pager et al. 1972; Gervais and Pager 1982; Apelbaum and Chaput 2003; Apelbaum et al. 2005 and Hardy et al. 2005). In addition, Prud'Homme et al. (2009) found that OX antagonist treatment blocked the enhancement of OB Fos responses to a food odor. Secondly, some evidence suggests that the OX neurons terminating in the locus coeruleus (LC) may provide a second indirect pathway for orexinergic modulation of olfactory processing: direct OX fibers innervate the LC (Horvath et al. 1999) and activation of OX receptors in the LC increases cell firing of intrinsic noradrenergic (NA) neurons (Hagan et al. 1999; Trivedi et al. 1998). In addition, the LC projects over 40% of its neurons directly into the OB (McLean et al. 1989), and this large noradrenergic input has been shown to modulate OB excitability, olfactory perception, and olfactory learning and memory abilities (see Devore and Linster 2012 for review).

Taken altogether, these data suggest that the fasting-induced increase in olfactory sensitivity described in the literature probably involves the OXsystem in the OB. Moreover, and in the light of the work by Escanilla et al. (2012), it may be suggested that this increased olfactory sensitivity resulted directly from OX system activation in OB (Hardy et al. 2005) and/or indirectly through the effect of LC-OX system activation on norepinephrine (NA) release in the OB; activation of both systems may simultaneously influence the strength of olfactory memory through enhanced olfactory processing (stimulus detection and discrimination).

Among all the other possible ways the OX A may have influenced COA, anatomical and behavioral data suggest that the amygdala could be an important part of the circuit involved in the memory processes underlying this learning (Ardeshiri et al. 2017; Campbell et al. 2017; Sears et al. 2013; Petrovich 2013).

Orexinergic innervation of the extended amygdala (including the basolateral amygdala (BLA)) was clearly described by Schmitt et al. (2012). OX A administered into the LH significantly elevated cFos-immunoreactivity in the amygdala (Mullett et al. 2000) and fasting increased OX mRNA levels in the amygdala (Lu et al. 2000). OX applied in acute rat brain slices activated neurons in the amygdala (Bisetti et al. 2006). Moreover, the amygdala receives OB and visceral inputs (Saper and Loewy 1980; Inui et al. 2006) and may be a nodal point at which olfactory and neuroendocrine stimuli are integrated to modulate feeding behavior (King 2006).

Otherwise, a large amount of data indicate that the amygdala, and more precisely the BLA, is involved in the processes underlying the formation of the olfactory memory trace and its maintenance across the ISI during COA (Ferry et al. 1995; Ferry and Di Scala 1997, 2000). Although a direct effect of starvation-induced OX release in the amygdala on COA cannot be excluded, to our knowledge, the

involvement of the OX system in the amygdala has never been demonstrated in learning and memory.

On the other hand, some data indicate that the enhancing effect of starvation on COA memory processes could be indirectly mediated by activation of the NA system in the amygdala. The LC projects strongly onto the amygdala (Fallon et al. 1978), and the BLA β-adrenergic system is involved in the memory processes underlying the association between odor and delayed US during COA (Miranda et al. 2007).

Given the direct action of LH orexinergic neurons on the LC (Horvath et al. 1999), activation of the LC-amygdala NA system during processing of the new odor CS may be potentiated by fasting-induced OX release. Possibly, the strength of the olfactory memory trace, and/or its association to the US, was influenced by activation of this pathway in the Fasted and OX A groups.

Taken together, all the above-mentioned data suggest hypothalamic OX release during fasting enhances learning performance through a direct or indirect influence on a circuit involving different structures such as the LC, amygdala, and OB, which receive LH-OX projections (Ferry 2014). In reference to previous models in which the multiple feedback and feedforward interactions between olfactory and non-olfactory areas contribute to complex processes (Cleland and Linster 2005; Sullivan et al. 2000; Linster and Cleland 2002; Yuan et al. 2003; Aston-Jones and Cohen 2005; Miranda 2012), we propose that the activation of the LH-OX systems induced by fasting reinforces the role of each structure in the circuit by enhancing the neural processes underlying attention and olfactory memory through direct and/or indirect influences (Fig. 2).

Of course the list of structures included in this model is not exhaustive, and involvement of other feedback and feedforward interactions between these structures and others (e.g., piriform cortex, entorhinal cortex, orbitofrontal cortex, hippocampus) will have to be considered in order to achieve a more realistic idea of the circuit actually involved in food conditioned learning (see Ferry et al. 2006; Chapuis et al. 2009; Wilson and Sullivan 2011; Sahay et al. 2011, Chapuis et al. 2013).

Conclusion

Feeding behavior is part of a complex integrated adaptive system, governed by the brain, in which the processing of metabolic signals reflecting the animal's nutritional state (gastrointestinal distention, blood glucose, feeding peptides such as OX) and of olfactory signals indicative of food determines the appropriate response to a food source. However, the differentiation between palatable and unpalatable items that conditions ingestive behavior often depends on previous experience during which the odor of the food acquired a hedonic valence after feeding, through CS-US associative learning, where the CS is represented by the taste and odor of food and the US is represented by the positive (nutritional) or negative (intoxication) post-ingestion value. By showing that OX A system influenced the memory processes underlying the CS-US association during COA, the present study introduces a new mechanism by which the LH-OX system may influence the processes that enable

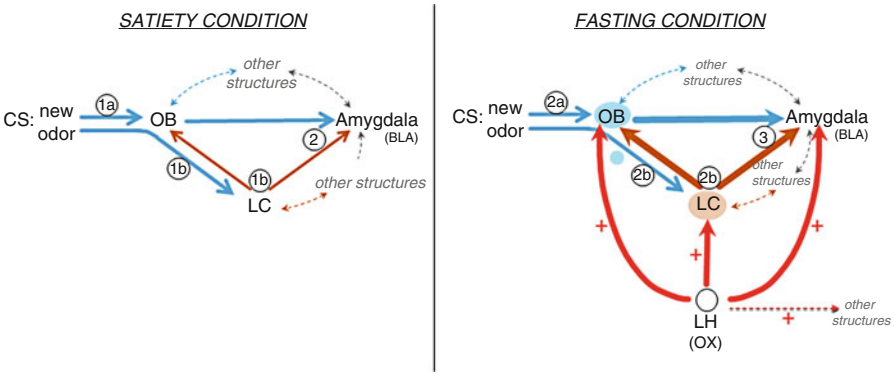

Fig. 2 Representation of a hypothetic model according to which OX A terminating in regions such as the OB, LC, and amygdala may constitute a pathway for orexinergic modulation of the olfactory memory trace formation underlying COA. The left panel represents the sequence of events that may take place during presentation of a new olfactory CS *in the satiated condition*. (1a) Olfactory CS induces activation of the OB. (1b) The novelty of the olfactory CS induces LC activation, which results in NE release in the OB and BLA. (2) The olfactory information is transmitted to the BLA where the odor trace is formed pending its association with the US. This sequence of events results in normal COA. The right panel represents the sequence of events that may take place during presentation of a new olfactory CS *in the fasting condition*. (1) Fasting induces release of OX A in the OB, BLA, and LC, preparing the system to respond to any food-odor event. (2a) Olfactory CS induces activation of the OB, potentiated by activation of the OX A system and LC-mediated NE system in the OB. In the fasting condition, CS leads to improved olfactory detection and processing. (2b) The novelty of the olfactory CS induces LC activation, resulting in enhanced NE release in the OB and BLA. (3) The enhanced olfactory information is transmitted to the BLA. Combined with OX A system activation, LC-mediated NE release in the BLA and potentiated OB activation, the olfactory memory trace strengthened or lengthened, and can thus be associated to a delayed US. This sequence of events results in enhanced COA

animals to learn to select food available in the environment and to adapt their behavior to previous experience through a modulation of complex neural circuit activity. Finally, the OX system represents a critical link between peripheral energy balance and central nervous system mechanisms that coordinate olfactory processing and memory, especially in the physiological state of fasting.

Policies and Protocols

Results described in this study were obtained using a conditioned odor aversion (COA) task in the rat. For this, naïve male adult Long-Evans rats were implanted 2 mm above the left lateral cerebral ventricle with a stainless steel guide cannula (12 mm long; 23 gauge) following a surgical procedure described in the handbook of Best Practices in Neurostereotaxic Neurosurgery (Ferry 2014). After 10 days of postsurgery recovery period, rats were habituated to have access to water once a day during 15 min during 7 days. Twenty-four hours before COA acquisition, animals were food deprived. On the conditioning day (Day 8), animals received a

microinjection of 3 μl of a solution of orexin A (1 nM). Twenty minutes after microinfusion, animals had access to the olfactory CS during 15 min and received a single i.p. injection of 0.15 M Lithium Chloride. On Days 9 and 10, rats received water during 15 min according the water deprivation schedule. From Days 11 to 15, COA was assessed by presenting the olfactory CS during each daily sessions. All testing sessions were conducted under a food-satiated condition. All experimental sessions were carried out during the light portion of the cycle between 11:00 a.m. and 1:00 p.m. All procedures involving animals and their care conformed to the institutional guidelines, which comply with international laws and policies (directive 2010/63/European Community). Permission references were 69–387517 for BF and 69387 0202 for PV. After completion of the last behavioral testing, the brain of all implanted rats was subject to histological analysis in order to confirm correct location of the canula track placement.

Dictionary of Terms

- Fasting - Physiological state induced by giving an exact amount of food only once a day in order to keep the animals at 90% of their initial weigh.
- **Conditioned odor aversion learning** – Results from an association between an olfactory stimulus (usually odorized water intake) and an aversive unconditioned stimulus (usually a gastric malaise induced by an intraperitoneal injection of lithium chloride). Conditioned odor aversion can only be acquired if the time interval between the presentation of the olfactory stimulus from the gastric malaise is kept under 30 min, suggesting that the memory trace of the olfactory stimulus is subject to gradual decay over time.
- **Food conditioned learning** – Results from three successive processes. The acquisition during which the conditioned stimulus is associated with the unconditioned stimulus; the consolidation during which the conditioned stimulus – unconditioned stimulus association in memorized and the retrieval during which the presentation of the conditioned stimulus induces the recovery of the previously acquired association and elicits an aversive response.
- Unconditioned stimulus - Stimulus that elicits an unconditioned response. Presented in association with a conditioned stimulus, it can be positive (a food reward) or aversive (a gastric malaise, an electric footshock. . .).
- **Microinfusion** – This technique is used for a reversible and discrete manipulation of a particular structure in the brain in order to test its implication in a specific learning or/and memory process.

Summary Points

- Fasting enhanced odor detection in rats, whereas satiety reduced detection of odors.
- Central nervous system regulates food-search behavior by modulating the detection threshold of the food odorant items through centrifugal innervations.

- Feeding status modulates olfactory sensitivity through central orexin system activation.
- Fasting as well as central orexin system activation enhances olfactory memory during conditioned odor aversion learning.
- Our data suggest that fasting modulates olfactory memory through direct and/or indirect activation of particular orexin brain targets.

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Magnesium Deficiency, Sphingolipids, and Telomerase: Relevance to Atherogenesis, Cardiovascular Diseases, and Aging

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Abstract

An attempt is made, herein, to reconcile, and integrate, the various phenomena associated with magnesium deficiency and etiology of various cardiovascular disorders, including atherogenesis and systemic inflammation, particularly as it pertains to the aging population. A number of human and ongoing experimental studies are reviewed, which appear to indicate that magnesium deficiency is overlooked by the medical and civilian communities, and is, actually, of world-wide, epidemic proportions and growing. We show, through various human and animal studies, the roles of a dysfunctional microcirculation in development of systemic inflammation and atherogenesis and their relationships to generation of sphingolipids, platelet-activating factor, and reactive oxygen species. We also review the significance of measuring serum, plasma, and whole blood levels of ionized Mg in human subjects and patients suspected of Mg-deficient states. This review also brings the reader's attention to the critical importance of telomeres and telomerase in the aging process and Mg deficiency. Various molecular pathways which underlie the cardiovascular pathophysiological changes in Mg deficiency are presented and discussed. Finally, we discuss the utility of supplementing drinking waters with Mg^{2+} to improve the longevity of the aged and the quality of life.

Keywords

Microcirculation · Ceramide · Vascular smooth muscle · Inflammation · Reactive oxygen species · Reactive nitrogen species · Platelet-activating factor · Vasospasm · Ischemic heart disease · Hypertension · Atherosclerosis · Coronary artery disease

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List of Abbreviations

4-HNE	4-Hydroxy-2-nonenal
8-OH-dG	8-Hydroxydeoxyguanosine
Ca^{2+}	Free ionic calcium
CS	Ceramide synthase
CV	Cardiovascular
CVS	Cardiovascular system
DAG	Diacylglycerol
H_2O_2	Hydrogen peroxide
IHD	Ischemic heart disease
IL	Interleukin
LDL	Low-density lipoprotein
MgD	Magnesium deficiency
NF-kB	Nuclear factor-kappa B
NMRS	Nuclear magnetic resonance spectroscopy
N-SMAse	Neutral sphingomyelinase
PAF	Platelet-activating factor
PC	Phosphatidylcholine
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SCD	Sudden cardiac death
SM	Sphingomyelin
SMAses	Sphingomyelinases
SMS	Sphingomyelin synthase
SPT	Serine palmitoyl-CoA transferase
TNF-alpha	Tumor necrosis factor-alpha
VSM	Vascular smooth muscle

Introduction

Over the past 100-plus years, a great deal of scientific evidence has accumulated, from numerous sources, that multiple, diverse control mechanisms, including physiological, biochemical, nutritional, and epigenetic factors, are responsible for the normal functions of the cardiovascular system (CVS) and in the aging process. These diverse homeostatic factors maintain the patency of blood vessels, cardiac output, and the fluidity of the blood. Numerous pathophysiological processes in peripheral blood vessels and the chambers of the heart can result in alterations of vascular wall geometry, disturbances in oxygenation, and the nutritional status of the blood vessels and myocardium as well as the tissues they perfuse.

Although multiple theories and hypotheses have been generated to account for the hypertrophy of resistance blood vessels (i.e., arterioles, metarterioles, and small arteries) in the etiology of the aging process, atherogenesis, inflammation, essential hypertension, and cardiac failure, there is no agreement as to the precise (controlling) mechanisms.

Disturbances in diet are known to promote lipid deposition and inflammatory responses and accelerate the growth and transformation of smooth muscle cells in the vascular walls, promoting cardiac dysfunctions (Kumar et al. 2015). Interestingly, autopsies of young children (e.g., as early as 5–6 years of age), who have died as a consequence of accidents, have often demonstrated early signs of atherogenesis (i.e., fatty streaks) on aortic and carotid arterial blood vessel walls (Seelig 1980). By the time humans reach 70–80 years of age, many blood vessels (particularly arteries in the heart and brain) become more rigid with increased levels of calcium (Ca^{2+}) and fibrin deposits, vast numbers of lipid deposits with plaques on the inner walls, inflammatory lesions, and concomitant rises in arterial blood pressure and progressive lowering of cardiac output. Although numerous studies have been brought forth, over the past 100 years, precise reasons for these pathophysiological alterations remain nebulous (Majno and Joris 2004; Kumar et al. 2015).

Several epidemiological studies in North America, the UK, and Europe have shown that people consuming Western-type diets are low in magnesium (Mg) content (i.e., <30–50% of the RDA for Mg) (Altura and Altura 1984b, 1985, 1990, 1995b, 2007; Ford and Mokdad 2003; Mosfegh et al. 2009). Many of these Western-type diets, in the USA, demonstrate that 60–80% of Americans are consuming only 185–235 mg of Mg/day. Low Mg content of drinking water, found in areas of soft-water and Mg-poor soil, is associated with high incidences of ischemic heart disease (IHD), coronary vasospasm, hypertension, and sudden cardiac death (SCD) (Crawford and Crawford 1967; Marier 1978; Chipperfield and Chipperfield 1979; Turlapaty and Altura 1980; Altura et al. 1984, 1992b, c; Altura and Altura 1985, 1995b, c, d, e, 1996a, 1997b, 2007; Leary 1986; Marx and Neutra 1997). The myocardial level of Mg has consistently been observed to be lower in subjects dying from IHD and SCD in soft-water areas than those subjects living in hard-water areas (Crawford and Crawford 1967; Altura 1979; Turlapaty and Altura 1980; Leary 1986; Altura and Altura 1995a, 2007; Marx and Neutra 1997). Both animal and human studies have shown an inverse relationship between dietary intake of Mg and atherosclerosis (Turlapaty and Altura 1980; Altura and Altura 1995b, 2007; BT Altura et al. 1990a; Song et al. 2005; King et al. 2009).

Mg plays an essential role in more than 500 enzymatic reactions in the body and is required for all energy-generating reactions and oxidative phosphorylation (Altura and Altura 1984c; Altura et al. 2016a; de Baaj et al. 2015; Hartwig 2001). Mg is a natural Ca^{2+} channel blocker on myocardial and vascular smooth muscle (VSM) (Altura et al. 1981, 1997a, 1998; Turlapaty and Altura 1978; Altura and Altura 1974, 1981a, b; Zhang et al. 1992; Yang et al. 2000a). Mg can be termed a natural statin in that it lowers blood cholesterol, LDL, and triglycerides as well as acts like a peripheral vasodilator agent (Altura and Altura 1974, 1995b; Turlapaty and Altura 1980; Altura et al. 2009; Nishio et al. 1988). Hypermagnesemic diets have been shown to ameliorate hypertension and atherogenesis (Altura and Altura 1984b, c, 1985, 1994, 1995e, 1997b; BT Altura et al. 1990a; dean 2017; Ravin et al. 2001; Altura et al. 2003a; Maier 2012; Saris et al. 2000).

Using sensitive, newly designed Mg^{2+}-selective electrodes, it has been shown that patients with hypertension, IHD, cardiac failure, diabetes, gestational diabetes, renal-induced vascular damage, atherosclerosis, vascular-related headaches, and strokes

exhibit significant depletion of serum/plasma and whole blood-ionized, but not total, Mg levels (Altura 1994; Altura and Altura 1991, 1994, 1995a, b; BT Altura and Altura 1992, 1994b; Handwerker et al. 1993; Markell et al. 1993a, b; Resnick et al. 1993, 1997; Altura et al. 1992c; Mauskop et al. 1995a, b; BT Altura et al. 1992b, c, 1994a, b, c, d, 1997b, c; Yang et al. 2000a, b, c; Bardicef et al. 1995). In this context, dietary deficiency of Mg has been demonstrated, in rats, rabbits, and people to provoke vascular remodeling concomitant with hypertension, inflammation, and atherosclerosis (i.e., arteriolar wall hypertrophy and alterations in the matrices) (Altura and Altura 1981c, 1984b, 1995a, b, d, e, 1996; Altura et al. 1984, 1992b, c, 1993b, 1994a, b, c, 1996, 2003b, 2016a; BT Altura et al. 1990, 2016a, 2017a; King et al. 2009). Moreover, a few years ago, dietary deficiency of Mg^{2+} had been demonstrated to cause an acceleration of the aging process by unknown mechanisms (Killilea and Ames 2008).

Aging is now agreed to be critical in the etiology of metabolic decline in most human subjects as they close in on their 65th–70th birthdays. Many subjects at these ages show signs of metabolic decline, atherosclerosis, high blood pressure, renal failure, liver failure, endothelial cell dysfunctions, CV diseases, and often type 2 diabetes mellitus, which eventually contribute to IHD, congestive heart failure, and SCD by their 75th–85th birthdays. It is important, here, to point out, to the reader, that all of these attributes/characteristics have been associated with the presence of Mg-deficient states in both humans and experimental animals (Altura and Altura 1980, 1983; Altura 1979; Altura and Altura 1981c, 1984c, 1992a, 1995a, b, 1996a, b, 1997a, b, 2016a, c, e; Markell et al. 1993a, b; Wu et al. 1991, 1994; BT Altura et al. 1994, 1997a, b; Handwerker et al. 1993; Rayssiguier et al. 1993; Resnick et al. 1993, 1997; Scott et al. 1996; Schecter et al. 2000; Djurhuus et al. 1999; Maier 2012; Mazur et al. 2007; de Baaj et al. 2015). The aging process is also associated with an increase in the levels of proinflammatory cytokines in tissues and cells as well as oxidation of tissues, cells, and cell membranes (both external and internal) (for recent reviews, see Malavolta and Mocchegiani 2016). Due to the presence of increasing serum and tissue levels of proinflammatory cytokines with increased years, it has been stated that aging is an inflammatory disease (for recent reviews, see Malavolta and Mocchegiani 2016). It is, thus, of considerable interest to note, here, that recent findings in Mg-deficient animals and tissues, as well as cells and membranes of these animals, exhibit elevated levels of the proinflammatory cytokines and chemokines such as IL-1a, IL-1b, IL-6, TNF-alpha, and diverse macrophage factors, among others (Malpeuch-Brugere et al. 2000; Altura et al. 2003a, b, 2012, 2014, 2016a, b, d, 2017a; King et al. 2005; Mazur et al. 2007; Weglicki 2012; Shah et al. 2014). Of these cytokines and chemokines, TNF-alpha is known to be associated negatively with telomerase activities in several cell types in the aging process (Matsutomi et al. 2003; Wong and Collins 2003; Dong et al. 2005; Fleisig and Wong 2012; Lin et al. 2012; Hockemeyer and Collins 2015). In view of such a constellation of findings and reports, our laboratories, approximately 30 years ago, wondered whether there might be a central metabolic pathway(s) that might be activated or set into motion by Mg deficiency, which could play a major role(s) in the aging process and lead to declines in CV functions resulting in atherogenesis, systemic inflammatory diseases, cardiac failure, IHD, and SCD.

Using proton nuclear magnetic resonance spectroscopy (^{1}H-NMRS) and phosphorus-31 nuclear magnetic resonance spectroscopy (^{31}P-NMRS), it has been reported (almost 20 years ago) that low-Mg^{2+} environments ($[Mg^{2+}]_0$) resulted in causing sustained changes in membrane phospholipids and several second messengers (including DAG), as well as the activation of apoptotic pathways, membrane oxidation, membrane lipid peroxidation, and truncation of membrane fatty acids (Morrill et al. 1997, 1998; Altura et al. 2009). Decreases in $[Mg^{2+}]_0$ resulted in a fall in membrane sphingomyelin (SM) and phosphatidylcholine (PC) (Morrill et al. 1997); intracellular ceramide formation and activation of neutral sphingomyelinase (N-SMAse) were found to be inversely proportional to Mg^{2+} (Morrill et al. 1998). Ceramides, either released or generated as a consequence of neutral and acid sphingomyelinases (SMAses) acting on SM or synthesized de novo, are now known to play important, critical roles in fundamental life processes such as angiogenesis, cell proliferation, immunoinflammatory responses, atherogenesis, programmed cell death, and the aging process (Dbalbo et al. 1998; Andrieu-Abadie et al. 2001; Birbes et al. 2001; Altura et al. 2002, 2016a; Hannun and Obeid 2017; Merrill 2002; Crivello et al. 2005; Panjarian et al. 2008; Hage-Sleiman et al. 2013). Recent reports indicate that Mg deficiency (MgD) can induce upregulation of all major enzymes needed for cellular synthesis of ceramides in intact cardiac tissues and VSM cells (Morrill et al. 1997, 1998; Altura et al. 2009, 2010a, b, 2012, 2014, 2016a; Shah et al. 2011, 2014). The de novo synthesis of ceramide is brought about via the action of serine palmitoyl-CoA transferase (SPT), 3-ketosphinganine reductase, ceramide synthase (CS), dihydroceramide desaturase, and SM synthase (SMS) (Merrill 2002; Hanada 2003; Hannun and Obeid 2017). Variation of $[Mg^{2+}]_0$ was shown to control the cellular levels of SM, PC, DAG, sphingosine, and ceramide in all types of CV tissues and cells by our group. It is now known that low $[Mg^{2+}]_0$ can produce cellular synthesis and release of ceramide in CV tissues and cells via upregulation of SPT 1 and SPT 2, CS, and SMS synthase as well as via what has been termed "a salvage pathway" (Altura et al. 2010a, b, 2012, 2014; Shah et al. 2011, 2014). Since ceramides play key roles in programmed cell death (e.g., apoptosis, necroptosis, ferroptosis) in cardiac and VSM cells, these findings are probably quite important in the aging process, particularly as programmed cell death is now known to be a sine qua non of senescence (for reviews, see D'Antona 2016; Malavolta and Mocchegiani 2016). In the normal aging process, telomeres are critical in regulating genome integrity and hence chromosomal integrity (Matsutomi et al. 2003; Wong and Collins 2003; Dong et al. 2005; Fleisig and Wong 2012; Tollervey and Lunyak 2012; Lin et al. 2012).

Below, recent findings are reviewed which lead one to believe that unrecognized MgD is a major underlying, unrecognized reason for why and how dietary deficiency of Mg is causing diverse CV diseases, atherosclerosis, IHD, cardiac failure, and premature aging which, unless recognized and treated, will result in premature death of millions of people.

Dysfunction of the Microcirculation in Developing Systemic Inflammation and Atherogenesis During Mg-Deficient States: Relation to Sphingolipids

Twenty years ago, Ross and colleagues advanced the hypothesis that atherosclerosis is an inflammatory disease brought about by injury to the endothelial surfaces of blood vessels in the macro- and microcirculations (see Ross 1999, for summary of hypothesis). Ross's hypothesis stated that different forms of injury will result in numerous dysfunctions in the homeostatic properties of the endothelium, e.g., an increase in the adhesiveness of leukocytes and/or platelets, alterations in the procoagulant properties, formation/release of cytokines/chemokines, and release of multiple growth factors. Much of Ross's injury hypothesis is actually derived from prescient lectures Rudolph Virchow enunciated in 1858 (Virchow 1858). Inflammation is usually defined as a response of microcirculatory blood vessels and the tissues they perfuse to infections and damaged tissues, which bring cells and host-defense molecules directly from the circulation to all the diverse sites where they are required to eliminate/degrade the offending agent(s) and initiate repair processes (for reviews, see Majno and Joris 2004; Kumar et al. 2015). The major mediators of these defense mechanisms include white blood cells, phagocytic leukocytes, antibodies, chemokines, and complement proteins. Most of these cells and protein molecules are recruited when needed from the circulating blood itself. The inflammatory process brings these cells and molecules to the damaged or necrotic tissues. The absence of the inflammatory process would allow infections to continue unchecked, prevent wounds from healing, and result in festering sores/wounds. A typical inflammatory process develops in a sequential manner: recognition of the offending agent(s) by host cells and molecules, recruitment of leukocytes and plasma proteins (e.g., antibodies, complement fractions), activation of the leukocytes and plasma proteins to destroy and eliminate all offending substances, control and termination of the reaction(s), and finally repair of the damaged tissue(s). During the normal inflammatory process, leukocytes migrate across the venous capillary walls through the endothelium due to increases in permeability and move to the site(s) of injury via chemotaxis. The normal mediators for this process to take place are adhesion molecules, Ca^{2+}, cytokines and chemokines, and more recently sphingolipids like ceramide. Interestingly, all of these mediators are needed for atherogenesis and are released in increasing concentrations in MgD (Altura and Altura 1995e, 2007, 2016b, Altura et al. 2003a, b, 2010b, 2012, 2014, 2016a, b, c; Maier 2012; King et al. 2005; Shah et al. 2011, 2014; Weglicki 2012).

If, however, the inflammatory response is not curtailed, or effectively neutralized, the inflammatory response will go on and stimulate migration and proliferation of VSM cells and protein components to initiate and form an intermediate lesion (i.e., the beginning of the atherosclerotic process). Moreover, if these processes go on unabated, the arterial walls will thicken and initially dilate to compensate, to a point, and then undergo a remodeling process in which the normal contractile VSM cells are transformed into new

noncontractile, secretory phenotypes (Ross 1999; Majno and Joris 2004; Kumar et al. 2015). At every stage of this process, macrophages, monocytes, and T lymphocytes will be attracted to the arterial-endothelial walls. Activation of these cells results in the release of hydrolytic enzymes, cytokines, chemokines, Ca^{2+}, and numerous growth factors as stated above. These factors will sustain and perpetuate the atherogenic process forming, eventually, fibrous tissue and further enlargement of the lesion, which will overlie a core of lipid resulting in plaques and necrotic tissue. MgD states have been shown by our group, and others, to result in a release of numerous enzymes (seen in the atherogenic process), cytokines, chemokines, and growth factors involved in the early stages of the atherosclerotic process exhibiting lesions and plaques on the inner endothelial surfaces (BT Altura et al. 1990; Mazur et al. 2007; Altura et al. 2012, 2014; Maier 2012; Weglicki 2012). In developing atherosclerosis, each plaque has a cap that retains cholesterol, and inflammations inside the cap can dissolve the fibers, but, suddenly, the cap ruptures, spilling cholesterol into the insides of the arteries which can promptly cause clots that can completely block the flow of blood into the microcirculation of the surrounding tissues causing permanent tissue damage and necrosis. It is now known that ceramides and other sphingolipids (e.g., sphingosine and sphingosine-1-phosphate) are found in MgD-induced atherogenic lesions in the arterial walls of rabbits (Altura and Altura 1995a, 2016, unpublished findings). Moreover, using high-magnification, in situ video microscopy (up to 6,500x normal) of the splanchnic, cerebral, and skeletal muscle microvasculatures, it has been demonstrated that ceramides and other sphingolipids cause alterations in postcapillary venular permeability, loss of venular tone, vasospasm, and transudation of leukocytes, monocytes, and T lymphocytes across these microvascular walls into the surrounding perivascular tissue spaces, representing inflammatory reactions (Altura et al. 2002, 2016a, unpublished findings). It has also been noted that similar pathophysiological events have been shown in splanchnic and skeletal muscle microvasculatures in MgD rabbits exhibiting atherosclerotic lesions (Altura et al. 2016a, unpublished findings). Moreover, it has been reported that MgD leads to an upregulation of N-SMAse and acid SMAse, resulting in increased serum and tissue levels of ceramide (Morrill et al. 1998; Altura et al. 2012, 2014; Shah et al. 2014, unpublished findings). We believe such findings, collectively, in rats and rabbits are more than coincidental and must perforce be considered, if found in humans, to be the forerunners of systemic inflammatory responses and initiation of a progressive decline in CV functions leading to atherosclerosis, hypertension, coronary artery diseases, IHD, and SCD. It follows, in our opinion, that this sequela of events takes place in aging people as they reach their 65th–70th years. The aging process, in people, is associated with an increase in the levels of proinflammatory substances in tissues and cells, similar to those we found in the MgD animals. Are these changes found in MgD states correlated to telomere and telomerase activities?

Telomeres, Telomerases, DNA Damage/Fragmentation, Oxidative Stress, Cell Death, and Mg

Telomeric DNAs are highly conserved molecules among all eukaryocytes (Wong and Collins 2003; Fleisig and Wong 2012; Lin et al. 2012). Telomeres are found at the ends of the chromosomes, consisting of short nucleotide repeats and specialized proteins, which are regulated by telomerases (Wong and Collins 2003; Fleisig and Wong 2012; Lin et al. 2012). Chromosomes devoid of telomeres or low telomerase activity usually undergo apoptosis or necroptosis, or both (Matsutomi et al. 2003; Wong and Collins 2003; Dong et al. 2005). Thus, cells with normal telomerase activities are thought to survive periods of oxidative and peroxidative stress (e.g., production of reactive oxygen and nitrogen species) and curtail apoptosis, necroptosis, and the aging process (Matsutomi et al. 2003; Wong and Collins 2003; Dong et al. 2005; Fleisig and Wong 2012; Lin et al. 2012; Hockemeyer and Collins 2015). It should be noted, here, that normal amounts of telomerases in all cell types are required to promote efficient cell cycle kinetics and normal cell growth (Matsutomi et al. 2003; Dong et al. 2005; Hockemeyer and Collins 2015).

MgD is well-known to promote disturbances in cell cycle kinetics (Rubin 1982; Walker 1986; Vernon 1988) via reactive oxygen (ROS) and reactive nitrogen species (RNS), most likely acting to downregulate telomerases) (Shah et al. 2014). A number of studies have recently reported distinct relationships between certain dietary markers and telomere length (for review, see Lin et al. 2012). It has recently been shown that MgD states, both in vivo and in vitro, promote oxidative stress and membrane lipid peroxidation, DNA damage/fragmentation, elevated cellular levels of Ca^{2+}, dysfunction of cardiac bioenergetics, upregulation and accumulation of the tumor suppressor protein p53, upregulation of multiple enzymes which regulate sphingolipid metabolism, accumulation of ceramides, cell death regulation (i.e., apoptosis, necroptosis, ferroptosis), and downregulation of telomerases (Altura et al. 2009, 2010a, b, 2012, 2014, 2016a, b, 2017a, b; Shah et al. 2011, 2014). Recent studies of short-term MgD, and with single VSM cells, in primary culture exposed to low Mg^{2+}, seem to indicate that ceramide and p53 act in concert to downregulate telomerase (Shah et al. 2014). In addition, it has been shown that increased MgD in experimental animals results in increasing levels of multiple cytokines (i.e., IL-1a, IL-1b, IL-2, Il-4, IL-6, IL-10, IL-12, and IL-16, among others) as well as diverse chemokines in blood, all four chambers of the heart, peripheral blood vessels (e.g., aorta, carotid arteries, coronary arteries), and cerebral blood vessels (e.g., cerebral artery, middle cerebral arteries) (Altura et al. 2012, 2014, unpublished findings). Clearly, these increasing levels of cytokines and chemokines, with increasing MgD, could be considered forerunners of the aging process, particularly as it is known that MgD causes concomitant increases in blood pressure, coronary and cerebral arterial and arteriolar vasospasm, vasospasms of umbilical-placental blood vessels, decreases in microcirculatory blood flows, increased levels of Ca^{2+} in both VSM cells and cardiac myocytes, decreased cardiac output, and decreased cardiac contractility (1981, 1984; Altura and Altura 1971, 1974, 1978, 1981a, 1984b, 1987, 1996, 2007;

Altura and Altura 1982; Turlapaty and Altura 1980; Altura et al. 1984, 1987, 1992, 1996, 1997c, 2003a, b, 2016a; Wu et al. 1994; Yang et al. 2000a, b, c; Shah et al. 2014). Measurement of the levels of the five major enzymes responsible for hydrolysis of SM and de novo synthesis and release of ceramide (reviewed above) was found in these CV tissues to be inversely proportional to the concentrations of Mg^{2+} in the blood (Altura et al. 2010a, b, 2012, 2014, 2016a; Shah et al. 2011, 2014). In this context, it must be pointed out that the aging populations in both North America and Europe demonstrate decreasing dietary intakes of Mg/day, often decreasing below 150 mg of Mg/day. Could these rising levels of cytokines, chemokines, and ceramide, observed in MgD states, be causally related to the decline in CV functions and onset of CV diseases in the aging process?

Recently, it has been demonstrated in animals, subjected to mild MgD for 21 days, that telomerase levels are downregulated and coupled to fragmentation and oxidation of DNA as well as to increased levels of the tumor suppressor gene, p53 (Altura et al. 2010; Shah et al. 2014). Regression analyses indicated extremely high degrees of correlation (i.e., $r > 0.94$; $P < 0.001$). Such data supports the idea that MgD could lead to multiple mutations in the genomes of multiple cell types found in the initiation of systemic inflammatory lesions, atherogenesis, congestive heart failure, IHD, and SCD. P53 is known to play an important role not only as a tumor suppressor but in promoting survival of numerous cell types under stresses as seen in developing atherosclerotic plaques and alterations in the phenotypes of vascular smooth muscle cells (Dbalbo et al. 1998; McDonald And Owens 2007; Mercer et al. 2007; Meek 2009). Previous studies from our group (Altura and Altura 2007; Altura et al. 2012, 2014, 2016; Shah et al. 2014), when viewed in the light of these findings, would lend additional support to the hypothesis that mutations and transformations of VSM cells, endothelial cells, and cardiac myocytes caused by MgD, fragmentation of DNA, and oxidation of DNA (all observed in atherogenesis, hypertension, strokes, and IHD) may play major roles in the aging process, thus leading to multiple CV changes, including inflammations of the vascular and cardiac walls (see above), high blood pressure (due to formed element changes, release of ceramides, release of cytokines and chemokines, excess arterial wall lipid depositions and peroxidation, etc.), cardiac dysfunctions, and eventual cardiac failure. We believe this constellation of pathophysiological effects of MgD is consistent with "genotoxic effects" of MgD we suggested several years ago.

Genotoxic Effects of MgD: Effects on NF-kB and Proto-Oncogenes

Approximately two decades ago, using experimental animals (i.e., rats, dogs, and subhuman primates), it was reported that short-term MgD, by itself, produced in intact CV tissues and VSM cells (in primary culture) what could be considered genotoxic effects of low dietary Mg intake, as internal and external cellular membranes became oxidized with DNA fragmentation, and the cells demonstrated lipid peroxidation with formation of ROS (e.g., H_2O_2, hydroxyl radicals, ferrylmyoglobin, and superoxide, among others) and RNS species (e.g., NO, peroxynitrite), activation

of nuclear factor-kB (NF-kB), and proto-oncogenes, along with alterations in the nucleus and nucleolus of the CV cells (Wu et al. 1994; Altura and Altura 1996a; Morrill et al. 1997, 1998; Altura et al. 1993a, b, 2001, 2003a, b, 2010, 2012; Shah et al. 2011). In these studies, it was found that the major enzymes needed to produce many of these ROS and RNS substances, namely, NADPH oxidases, nitric oxide synthases, and xanthine oxidase, were all upregulated in CV tissues and cells from animals exposed to MgD diets. More recently, it was found that CV tissues and cells, excised from rats placed on 21 days of MgD diets, exhibited five- to tenfold increases in both 8-hydroxydeoxyguanosine (a marker of specific DNA oxidation) and 4-hydroxy-2-nonenal (4-HNE) in VSM cells and cardiac myocytes (Shah et al. 2014; Altura et al. 2016a), the latter being a well-known inducer of hydrogen peroxide and DNA methylation (Mikhed et al. 2015; Luczaj et al. 2017). Such data supports the idea that the progressive downregulation of telomerases, noted above, when coupled to these cellular and molecular findings and the upregulation of p53 (Altura et al. 2010) provide impetus for the hypothesis that MgD could lead to multiple mutations in the genomes of multiple cell types and thus be a prelude for premature aging. Previous findings on atherogenesis in MgD (BT Altura et al. 1990), when viewed in light of the latter, would lend support to the concept that mutations and transformations of VSM, endothelial, and cardiac myocyte cells caused by formation of ceramides, ROS, and RNS most likely play major roles in systemic inflammatory lesions, atherosclerosis, aging, IHD, and SCD (Altura et al. 2016a).

The findings of upregulation of NF-kB and several proto-oncogenes (i.e., c-Fos and c-Jun) in MgD states (Altura et al. 2003a, b, 2012, 2014; Shah et al. 2014) are very important for our hypothesis as these molecules are two major regulators of growth, differentiation, cell migration, and programmed cell death (e.g., apoptosis, necroptosis, ferroptosis) (for reviews, see Kumar et al. 2015; Moriwaki and Chan 2016; Serasanambati and Chilakapti 2016). NF-kB is a transcription factor and a pleiotrophic regulator of numerous genes involved in hypertension, inflammatory responses, atherogenesis, and aging (for reviews, see Barnes and Karin 1997; Hayden and Ghosh 2011). Both NF-kB and proto-oncogenes are thought to be critical in numerous vascular disease processes such as inflammation, cell transformations, and cell migration. However, it has not been clear as to how or what factor(s) initiates these molecules to be activated under diverse circumstances. Experimental findings, based on intact animals and primary cell cultures, have shown that either inhibition of NF-kB activation or inhibition of ceramide release and its de novo formation prevent a whole sequelae of events which would normally lead to downregulation of telomerases, oxidation, peroxidation, formation of 8-OH-dG, formation of 4-HNE, apoptosis, and activation of p53 (Altura et al. 2010, 2012, 2014, 2016a, 2017a). Although it has not, as yet, been determined whether inhibition of ceramide formation/release or inhibition of NF-kB activation will prevent MgD-induced atherogenesis or systemic inflammatory conditions, in intact animals, preliminary studies on intact rabbits appear to yield promising results so far. Recent in vivo and in vitro investigations, in our laboratories, seem to indicate that another important factor in MgD, aging, and vascular diseases, may have been overlooked until now.

Potential Roles of Platelet-Activating Factor (PAF)

A little more than 20 years ago, using ^{1}H-NMRS, on single VSM cells and excised canine and rat aortic, coronary, and cerebral arterial vessels, it was reported that a fairly rapid synthesis of PAF was observed when the cells and tissues were exposed to low $[Mg^{2+}]_0$ (unpublished findings, 1995). In addition, the spectra suggested that Mg^{2+}-deficient environments led to very rapid formation of PAF and PAF-like lipids (Morrill et al. 1997). PAF is manufactured from a specific subclass of phosphatidylcholines which contain an ether, instead of an ester, bond at the *sn-1* position of the glycerol backbone. This phospholipid is a minor part of low-density lipoprotein (LDL), which we and others have found to be elevated in blood and tissues of magnesium-deficient animals and human subjects who demonstrate lowered levels of serum-ionized Mg (Rayssiguier et al. 1993; BT Altura et al. 1990; Altura et al. 2009, unpublished findings). LDL is a major component in blood cells, such as leukocytes, and, along with ox-LDL, is a major pro-atherogenic substance (Ross 1999). A polyunsaturated fatty acid such as arachidonate is found in the *sn-2* position in the subclass of phosphatidylcholine molecules, and hydrolysis of this precursor phospholipid forms arachidonate for eicosanoid synthesis (Fruwirth et al. 2007). Acetylation of the other reaction product, viz., alkyl-lysophosphatidylcholine, along with acetyl-CoA finalizes the synthesis of PAF, both enzymes being tightly regulated (Fruwirth et al. 2007). It is important to note, here, that because the precursors of PAF contain polysaturated fatty acids, they are very susceptible to oxidative attack, often resulting in a number of structural products that resemble PAF (i.e., PAF-like lipids) (Fruwirth et al. 2007). It is now known that lowering $[Mg^{2+}]_0$ in primary cells results in changes in phosphatidylcholine levels and production of several PAF-like molecules (Morrill et al. 1997), most of which can act directly on G1-coupled PAF receptors (Fruwirth et al. 2007; Zimmerman et al. 2002). Using VSM cells in primary culture, Altura and colleagues recently found that the rapid rises in PAF and PAF-like lipids noted on lowering $[Mg^{2+}]_0$ could either be inhibited or ameliorated when specific PAF inhibitors were employed along with the cultured VSM cells (Altura et al. 2016a). It was also noted that formation of ceramides along with PAF formation, as well as activation of NF-kB, proto-oncogenes (i.e., c-Fos, c-Jun), and p53, in concert with oxidation and peroxidation of the VSM membranes was greatly attenuated (Altura et al. 2016a). DNA damage/fragmentation was also attenuated by incubation of the MgD cells with specific PAF-receptor blockers (unpublished findings). These new findings, collectively, point to an important role of PAF and PAF-like lipids in the sequelae of events noted in MgD states. We believe these new findings may also have direct bearing on the decline in functions of the CV system in aging, thus probably being a major factor in the systemic inflammatory and atherogenic states normally observed in aged individuals.

Microvascular Remodeling, DNA Damage, Epigenetics, and Telomerase

From the above, it should become clear that the microcirculation, during the aging process, must undergo numerous structural remodeling modifications. For example, close examination of the various bodily microvasculatures demonstrates the following: stiffening of arteries and arterioles, decreases in peripheral blood flows, alterations in vascular permeability of the microvessels (particularly the postcapillary venules), alterations in reactivity of the microvessels to circulating and released vasoactive substances (including sphingolipids and PAF), vasodilation and vasoconstriction of arterioles and metarterioles, modulation of cellular cytoskeletal properties of the arterial and arteriolar walls, proliferation of various cells in the arterial and arteriolar walls, migration, and finally death of many of the VSM cells or their transformation into secretory and foamlike cells (Majno and Joris 2004; Staiculescu et al. 2014; Kumar et al. 2015). Extensive ongoing, in vivo and in vitro studies, using diverse VSM and endothelial cells, indicate that progressive MgD results in progressive vasospasm of arterial and arteriolar vessels via a number of molecular signaling pathways, including activation of mitogen-activated protein kinases, activation of tyrosine protein kinases, activation of P-I-3 kinases, membrane entry and intracellular release of Ca^{2+}, formation/release of diverse vasoactive ROS and RNS molecules (see below), and activation of diverse PKC isozymes (Altura 1981, 1984, 1987; Altura and Altura 1971, 1974, 1978, 1984a, b, 1995d, e, 1996b, 2007; Altura et al. 1987, 1992c, 1993b, 1997c, 2001, 2003a, b, 2014; BT Altura and Altura 1984c, 1987; Li et al. 2007; Morrill et al. 1997, 1998; Shah et al. 2011; Turlapaty and Altura 1978, 1980a; Turlapaty et al. 1988; Yang et al. 2000a, b, c; Zhang et al. 1992, 1993; Zheng et al. 2000, 2011). The reader should consult these latter references for how MgD activates these pathways in VSM and endothelial cells.

It should be recalled, here, that atherosclerotic plaques in the vascular walls of hypertensive human subjects demonstrate considerable DNA damage, activation of DNA repair pathways, increased expression of proto-oncogenes, activation of NF-kB, increased expression of p53, increased expression of PAF, oxidation of DNA, lipid peroxidation, apoptosis, and necroptosis (Lee and Blair 2001; Fruwirth et al. 2007; McDonald and Owens, 2007; Jackson and Bartek 2009; Gray et al. 2015; Razani and Raines 2015). In this context, we have found all of these pathobiological alterations in MgD experimental animals (see above). We do not believe this is a "mere" coincidence. Experimentally, MgD results in accelerated atherosclerotic plaques in rabbit and mouse arterial walls (BT Altura et al. 1990a; Ravin et al. 2001), which have been associated with increased levels of p53 (Altura et al. 2010b) and PAF (Fruwirth et al. 2007; Montrucchio et al. 2000; Altura et al. 2016a) in the thickened atherosclerotic plaques along with decreased levels of telomerase (Altura et al. 2016a, unpublished findings). We, thus, hypothesize that MgD states must perforce play key roles in the generation of atherosclerotic plaques, coronary arterial diseases, hypertension, and IHD via the upregulation of N-SMAses, acid SMAses,

ceramide synthase, PAF generation, and ceramide production as well as down-regulation of telomerases, particularly as the majority of individuals who consume Western-type diets have 45–75% shortfalls in daily Mg intake. All of the foregoing events could be expected to be accelerated in the aging population, particularly in old age and in nursing homes where the daily intakes of Mg often approach deficits near 80–90% (Seelig 1980; Barbagallo et al. 2009).

Various bodily modifications take place daily and over various periods of time via epigenetics (Mikhed et al. 2015; Chatterjee and Eccles 2015). Epigenetic modifications are chromatin-based modifications that alter expression of genes without altering the DNA sequence itself (Tollervey and Lunyak 2012; Ghavifekr et al. 2013; Chatterjee and Eccles 2015; Mikhed et al. 2015). The most common epigenetic modifications include methylation of DNA, translational modification of histone protein, and RNA-induced mechanisms (i.e., micro-RNA and long noncoding RNA) (Tollervey and Lunyak 2012; Ghavifekr et al. 2013; Mikhed et al. 2015). These biochemical and molecular events can modulate the tertiary structure and, therefore, the accessibility of promoter DNAs or encoding transcription factors and numerous regulatory molecules that, in the end, finally alter transcription (Tollervey and Lunyak 2012; Ghavifekr et al. 2013; Chatterjee and Eccles 2013; Mikhed et al. 2015). In these ways, epigenetic pathways become pivotal in the regulation of VSM, endothelial, and cardiac myocyte gene expressions. Alterations in gene expression, for example, would be expected to change a normal contractile VSM cell into a cell that becomes a synthetic, secretory machine for numerous factors needed for atherogenesis (i.e., cytokines, chemokines, growth factors, etc.). The normal contractile VSM cell would no longer function to control microvascular blood flows and their proper distribution into the coronary, cerebral, and peripheral microvascular beds (see above). Very recently, our laboratories have found supporting data for this hypothesis in MgD animals; VSM and cardiac muscle cells were found to demonstrate DNA modifications, including methylation, modification of histone proteins, and alterations in noncoding RNA (Altura et al. unpublished findings). We are now convinced that MgD states can alter transcription and determination of key proteins and lipids. Currently, our laboratories are setting up studies to determine whether aging patients exhibit similar alterations. But, what can be done to prevent or ameliorate such epigenetic modifications of tissues and cells?

Bioavailable Mg Intake and Prevention of Cardiovascular Diseases

Last, but not least, the foregoing results, collectively, bolster the idea, expressed previously, that water intake (e.g., from tap waters, well waters, bottled waters, beverages using tap/well/spring waters, or desalinated waters) in humans should contain at least 25–40 mg of Mg/liter (Altura et al. 2009, 2010a, b, 2012, 2014; Shah et al. 2011, 2014). The latter inclusion in our diets should go a long way toward the

prevention of CV diseases and ameliorate the aging process of bodily tissues and cells in humans worldwide. In 2009, the World Health Organization recommended guidelines of 25–40 mg/l of Mg^{2+} to be included in drinking waters for human consumption (Altura and Altura 2009).

Policies and Protocols

From the foregoing, it is becoming clear that there is a growing, worldwide epidemic of MgD, particularly among the aging. It is also becoming clear that most all CV diseases are likely due, at least in part, to MgD. We believe, due to the rapid advances in molecular biology and technology, the continued study of MgD states will yield invaluable information on how to increase life expectancy, awareness of better nutrition, as well as the quality of life. Continued investigation of the diverse molecular pathways which underlie the specific mechanisms of how MgD causes inflammation and atherogenesis should perforce produce exciting new ventures into the fields of molecular genetics and epigenetics. It is also apparent that drinking waters supplemented with Mg^{2+} will prove beneficial to the elderly, particularly to people living in nursing homes and to people living in nutritionally deprived countries, an area which should be focused on in future studies of MgD.

Summary Points

- Cardiovascular disorders, including atherogenesis and systemic inflammation, are particularly important to the aging population.
- Magnesium deficiency is overlooked by the medical and civilian communities and is, actually, of worldwide epidemic proportions and growing.

Other Points

Supplementing drinking waters with Mg^{2+} may improve the longevity of the aged and the quality of life.

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Part XII

International Aspects, Policy, Management, Case Study, and Resources

Diet and Kwashiorkor in the Democratic Republic of Congo

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Hallgeir Kismul, Mala Ali Mapatano, and Jean Pierre Banea

Contents

Abstract

Despite holding a wealth in natural resources, the population of the Democratic Republic of Congo (DRC) is among the poorest in the world. Food insecurity and

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malnutrition is widespread. While severe child malnutrition in the form of kwashiorkor has become rare in most other developing countries, thousands for children in the DRC are suffering from this disease. Since its early description kwashiorkor among children has been related the consumption of a monotonous diet low in protein. It has also been postulated that kwashiorkor is caused by an excess in free radicals and that protective pathways are compromised in kwashiorkor with these pathways requiring micronutrients in the form of antioxidants. Kwashiorkor typically occurs in areas with severe food insecurity, and in the DRC food insecurity is especially prevalent in the rural areas. The national prevalence of children with edema in the DRC has been reported to be as high as 4% with the highest proportion of children with edema living in the rural areas. The diet in most areas of the DRC is monotonous. Cassava is the major and it is severed with different side dishes including cassava leaves. Animal products are consumed occasionally and wild food constitutes a safety net during periods of shortage. The relation between diet and kwashiorkor in children in the DRC has been investigated in historical longitudinal study. The diet in the study area is low in diversity and the major staples include cassava and maize. Children who did not develop kwashiorkor had consumed food items that were rich in β-carotene while those who did develop kwashiorkor had not.

Keywords
Underdevelopment · Civil war · Poverty · Agriculture · Food insecurity · Diet · Malnutrition · Marasmus · Kwashiorkor · Antioxidants · β-Carotene

List of Abbreviations

DHS	Demographic and health survey
DRC	Democratic republic of Congo
IPC	Integrated food security phase classification
RUTF	Ready-to-use therapeutic food

Introduction

The Democratic Republic of Congo (DRC), a former Belgian colony, is located in central Africa. It has a population of 69 million (The World Bank 2013) and is thereby the fourth most populated country in Africa. It is divided into 26 provinces including the city province of Kinshasa (see Fig. 1 map). The DRC holds a wealth of natural resources including a diversity of mineral and forest resources (The World Bank 2013). It is classified as the 5th most biodiverse country on the earth (Termote et al. 2012). The country has an environment that is favorable to agriculture that in many areas allows for two harvests per year (Rossi et al. 2006). Despite its wealth in natural resources, DRC's population is among the poorest in the world. Based on its poor scores with regards to income and health, it is by Human Development Index ranked as the 176, out of 188, poorest countries in the world (UNDP 2015). There is a rural–urban gap in poverty disfavoring rural areas where eight out of ten

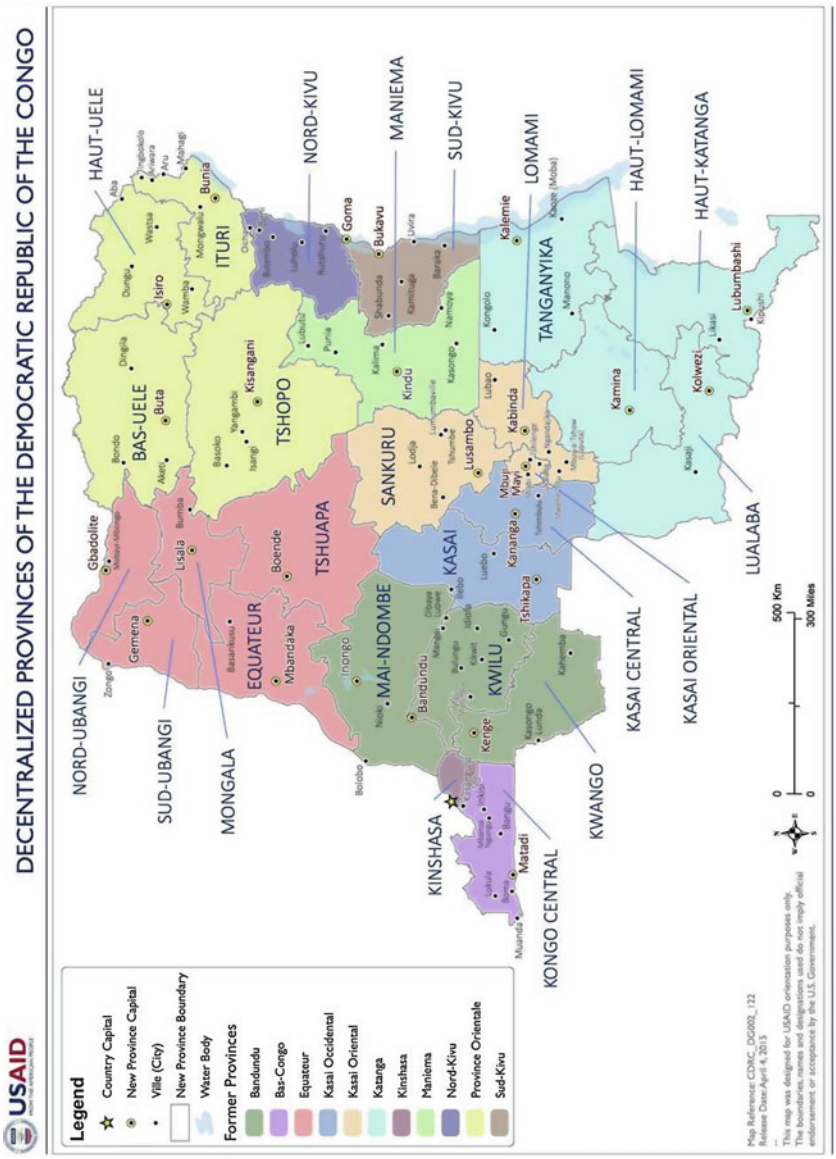

Fig. 1 Map of the Democratic Republic of Congo (Source: USAID 2015, http://www.ocnus.net/artman2/publish/Africa_8/Kabila%20-%20Plan%20B%20for%20the%20Congo.shtml)

households are living below the poverty line of 1.25 dollars a day, while in urban areas it is less than seven out of ten (The World Bank 2013). Agriculture makes up about 70% of people's livelihood and the agricultural sector is dominated by subsistence agriculture (Ministre du Plan et Révolution de la Modernité de la RDC 2015). Since 1997 and until now, the political situation in the country has been characterized by civil wars. The death toll of the civil war, 1998–2004, has been estimated to 3.9 million (Coghlan et al. 2006). The conflicts have restricted the country's ability to promote development and food production and thus improve food security (Weijs et al. 2012; Kandala et al. 2011). Hence, DRC is still strongly dependent on foreign aid (République Démocratique du Congo 2014).

Whereas many other countries in sub-Saharan Africa have been able to reduce the number of people suffering from malnutrition, prevalence of malnutrition in the forms of stunting, wasting and underweight in the DRC is among the highest in sub-Saharan Africa (République Démocratique du Congo 2014). Many children are also suffering from severe malnutrition.

including marasmus and kwashiorkor. In other parts of the world, kwashiorkor has become very rare, but in the DRC thousands of children are still suffering from this disease.

What is Kwashiorkor?

The earliest description of kwashiorkor can be dated back to biblical times. The Jamaican physician Cecilia Williams, working in what is now Ghana, was in an article from 1933 (republished in 1983) the first to describe kwashiorkor in Africa. In a Lancet article from 1935, she gave the disease its African name kwashiorkor (Williams 1935). The name was given after the notion in the Ga language of the coastal Ghana, *kwa ni oshi korkor,* and was translated by Williams as the disease that the deposed child gets when the next is born. Williams suggested that the disease was associated with a monotonous diet, and given that maize was the main source of supplementary food, she suggested that some amino acid or protein deficiency could not be excluded as causing the disease.

Kwashiorkor along with marasmus and marasmic kwashiorkor are classified as syndromes of clinical severe childhood malnutrition. Kwashiorkor and marasmic kwashiorkor are identified by edema (swelling of body tissues as a result of fluid accumulation), whereas marasmus is defined as a nonedematous form of extreme wasting (Figs. 2 and 3). Edema is the most important and defining feature of kwashiorkor (Heikens and Manary 2009) (Fig. 4). There are significant variations of edema severity (Epomedicine 2017). It is classified as mild when it occurs in feet only, moderate when it occurs in legs and feet and lower arms, and severe when it is present on face and arms. Children with kwashiorkor may also develop skin lesions common on the extremities (Heilskov et al. 2014) (Fig. 5). Hair abnormalities are striking when the disease process is of a long duration. Abnormalities include straight hair, dyspigmentation, and thin hair. Fatty liver resulting in enlargement of

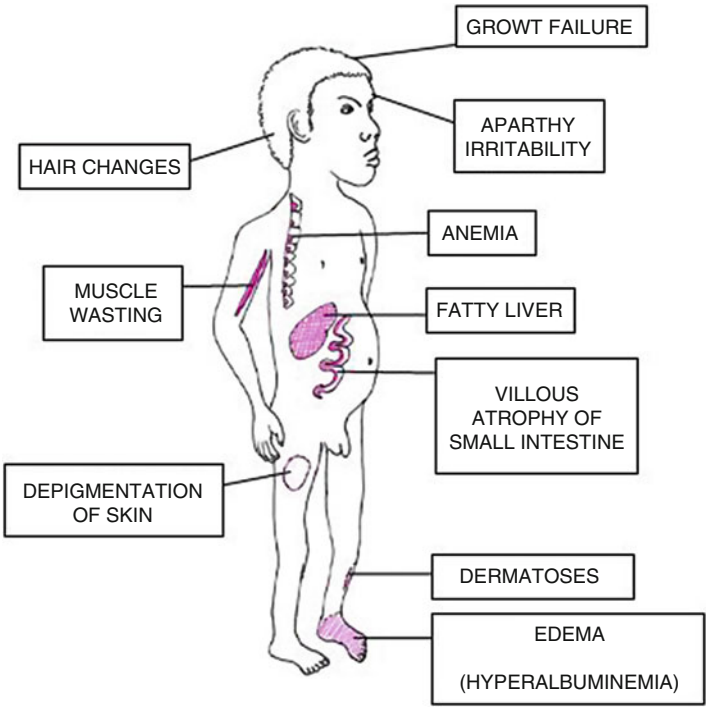

Fig. 2 Signs of kwashiorkor drawing by Hallgeir Kismul

the liver is also commonly reported as a feature of kwashiorkor (Frenk et al. 1958; Truswell and Miller 1993). The disease has been associated with a high risk of death (Briend et al. 1987; Talbert et al. 2012).

The Global Burden of Kwashiorkor

Kwashiorkor is prevalent in several parts of sub-Saharan Africa and is also found in communities in Asia and in South and Central America. Very few cases have been reported from high-income countries. The disease is still an important public health problem especially in rural areas where children consume a diet low in dietary diversity, often consisting of two major staples.

Kwashiorkor is a transitional condition and typically those who suffer from the disease recover after a few weeks of case management or die from the disease (Williams 1935). This makes it difficult to capture the magnitude of kwashiorkor in cross-sectional surveys that report prevalence of malnutrition (CMAM Forum 2015). A report attempting to describe the burden of kwashiorkor in low-income countries concluded that prevalence of kwashiorkor in most countries was less than

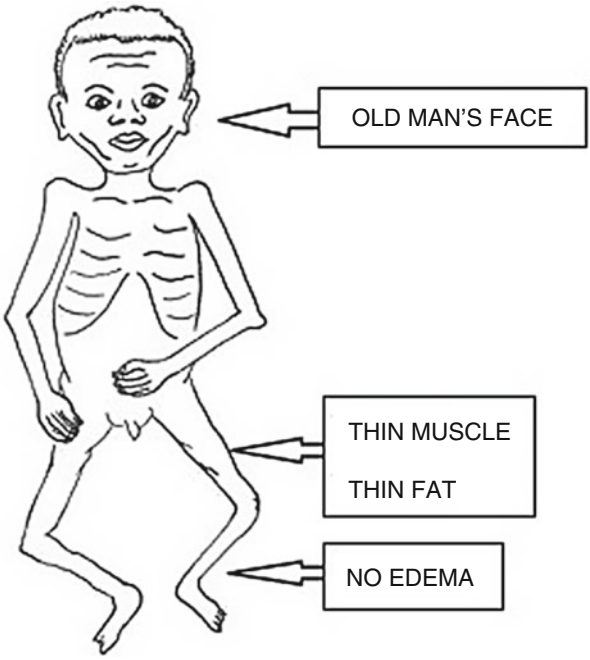

Fig. 3 Signs of marasmus drawing by Hallgeir Kismul

Fig. 4 Child from the Democratic Republic of Congo with kwashiorkor showing pitting oedema photo with permission from Branly Mbunga Kilola

Fig. 5 Child from the Democratic Republic of Congo with kwashiorkor showing skin lesions, photo with permission from Branly Mbunga Kilola

1% (CMAM Forum 2015). Then again some countries reported rates between 1% and 2% including Yemen, Zimbabwe, and the DRC.

The Etiology of Kwashiorkor

The development of kwashiorkor has been linked to diet and metabolism. Until the 1980s Kwashiorkor was with marasmus classified as a form of protein-energy malnutrition. Kwashiorkor was seen as a result of children consuming a diet high in energy but low in protein. This consumption pattern leads to pathological abnormalities such as edema and fatty liver (Whitehead and Alleyne 1972). The hypothesis was challenged and studies found that the diet of children who developed kwashiorkor was not characterized by a low intake of protein when compared with the diet of children who developed marasmus (Gopalan 1992). In the early 1980s, it was postulated that kwashiorkor was caused by an excess of free.

radicals. According to this suggestion, protective pathways were compromised in kwashiorkor. These pathways require the supply of micronutrients in the form of antioxidants, which could be deficient in kwashiorkor, thus causing a loss of the protective pathways (Golden and Ramdath 1987). Some population studies have challenged the free radical hypothesis. As an example a randomized control trial of children aged 1–4 years assessed the efficiency of providing antioxidants to prevent kwashiorkor (Ciliberto et al. 2005). This study failed to show that the supplementation of antioxidants prevented the children from developing kwashiorkor. Still other studies have shown that there is apparently a lack of micronutrition in the diet of children who develop kwashiorkor (Kismul et al. 2014). A child with kwashiorkor typically lives in environments where the diet is restricted to cooked but starchy foods including cassava and maize. The diet characteristically lacks vegetables and fruits. These are natural sources of micronutrients and dietary antioxidants.

The literature has described relations between breastfeeding and presence of kwashiorkor. Apparently children who have developed kwashiorkor are less likely to have been breastfed in comparison with those who have not developed kwashiorkor (Rytter et al. 2015). Prolonged breastfeeding and supplementation with solid food have been associated with a substantial reduction of clinical malnutrition in the form of kwashiorkor and marasmus (Cousens et al. 1993).

Although there is an apparent relation between diet and the development of kwashiorkor, there still exist many uncertainties as to the cause of kwashiorkor. A number of hypotheses are put forward and are being tested to better understand the etiology of kwashiorkor. As an example abnormal gut microbiota has been suggested as a factor contributing to the development of the syndrome. The hypothesis has been tested in animal and population studies (Subramanian et al. 2014). This literature suggests that there is a link between the consumption of a nutrient-poor diet, abnormal microbiota, and the development of kwashiorkor (Smith et al. 2013).

Moreover, abnormal gut microbiota has been related to environmental enteric dysfunction (Crane et al. 2015).

Food Insecurity in the DRC

Food security is considered an underlying factor with implications for children's and adults nutritional status and is manifest at the household level. Food insecurity is a major problem in the DRC, and according to the recent Integrated Food Security Phase Classification (IPC) of 2016, over 5 million of the country's population are in acute food security and livelihood crisis (IPC 2017). In the DRC, malnutrition is most widespread in the rural areas (The World Bank 2013; Kandala et al. 2011). There are several factors that constraint food production in the rural areas. The majority of the rural population depends on rainfed subsistence farming cultivating land less than 2 acres (WFP 2014). The farmers use traditional farming techniques and all operations are carried out manually. Poor access to seeds both improved varieties and traditional is considered a major obstacle to agricultural production (WFP 2014). Furthermore, other agricultural inputs including fertilizers and pesticides are more or less unavailable. Poor infrastructure also puts constraints on the possibility of developing agriculture especially cash cropping. The infrastructure, roads and waterways connecting local communities, agricultural land, and markets, has been described as "in an advanced stage of deterioration" (Mathys and Remancus 2010).

The IPC report subdivides the country with regard to food insecurity in 4 regions (IPC 2017):

1. The regions severely affected by armed conflicts, typically in the Eastern DRC.
2. The regions susceptible to climatic and natural changes including floods, El Nino, outbreaks, etc., in the Eastern and central provinces of the country.
3. The regions under the pressure of internal displaced populations and refugees, these are provinces along the border.
4. The regions under chronic food insecurity comprising northern and central provinces. They are characterized by extreme poverty and ill access to social services.

People living in the capital Kinshasa are in comparison with people residing in other provinces food secure. The most food insecure provinces are typically landlocked provinces that are extremely difficult to reach. In such areas, manufactured goods including food are sold at very high price, whereas cash cropping is difficult to develop. In the most food insecure provinces, people rely upon artisanal mining. In these provinces, there is a tendency that people neglect food production and these provinces have to import much of its food. Provinces affected by war including South Kivu are also food insecure. The land in South Kivu is fertile, but shortage of land and landlessness are problems closely related to food insecurity. Several food insecure provinces face problems due to influx of internal as well as refugees from neighboring countries (Mathys and Remancus 2010).

Malnutrition in the DRC

The Demographic and Health Survey (DHS) from 2013/14 provides data on the prevalence of malnutrition in children in 2013 in the DRC (République Démocratique du Congo 2014). According to the survey, 43% of the children below 5 years suffered from stunting, 8% from wasting, and 23% from underweight. The DRC thereby had among the highest proportions of children suffering from stunting in the world.

While the most recent DHS report from 2013/14 (République Démocratique du Congo 2007) does not contain data on kwashiorkor, this information is included in the DHS from 2007 (République Démocratique du Congo 2007). This report informs that 4.1% of the children below 5 years had edema with the largest proportion of children with edema in the age category 9–11 months. There were no gender differences in the occurrence of edema. On.

the other hand the report demonstrates a rural urban gap and most of the children with edema lived in the rural areas. There were also important regional differences as to occurrence of edema. In particular Orientale and South Kivu provinces had high prevalence of children with edema. The former province is depending on artisanal mining and the latter province has over the last decades been affected by civil war. It is also reported that children with edema were most likely to live in the poorest households.

Diet in the DRC

The diet in the DRC depends on regional context and there are also important differences between diet in rural and urban areas (Bervoets and Lassance 1959; Termote et al. 2012). Cassava root is the main staple food crop in most parts of the country. In the northern regions, cassava is served with cassava leaves as a side dish. In addition cassava is provided with different vegetables, legumes, and fish. In the western part of the country, cassava is typically consumed with vegetables and fish. In the south, maize is the major staple food and is consumed with vegetables and legumes. Cassava is also a major staple in the eastern part of the country, but food such as sweet potatoes and beans are commonly consumed. In the urban areas, cereals play a more important role in the diet than in the rural parts of the country. Animal products such as meat and egg are only eaten occasionally, whereas dairy products are rarely consumed. Wild edible plant and bush meat are components of food production especially in the rural areas and wild food constitutes a safety net during periods of food shortage. The most import wild plants consumed include wild yam, wild nuts, wild leafy vegetables, and various wild fruits. In addition bush meat and fish are part of the diet in areas located to the vicinity of forests and rivers. However, even in areas characterized by being very rich in biodiversity, wild edible plants are insufficiently consumed to increase nutrition security or dietary adequacy (Termote et al. 2012).

Typically the Congolese consume one to two meals per day. The major meal usually consists of a stiff porridge made from cassava or maize flour. The porridge is often served with a stew made of cassava leaves, spinach, and okra. Typically only a minority enrich the porridge with vegetable, fruits, fish, and meat. Meat is rarely consumed. If there are any left-overs, these are consumed for breakfast the next day. In between meals, people drink tea and palm wine and eat various fruits. For the households that do not produce their own fruits and vegetables, the cost of purchasing such food items at the market is often too high.

For the meals, water is collected from deep tube wells or from streams in the forests. During meals, household members are served the same food, but split into groups; women and young children in one group, older children in another and men in the third.

Infant Feeding

Breastfeeding is universally accepted and practiced in the DRC and 99% of infants 0–5 months of age have been breastfed (République Démocratique du Congo 2014). Normally breastfeeding continues up to 2 years and 67% of toddlers 18–23 months are breasted. Early complementary feeding is common and more than half (60%) of the infants in the DRC experienced mixed feeding by 0–2 months (République Démocratique du Congo 2007). In addition to water, early complementary food consists of a gruel basically made from porridge and cassava-leaf stew. Women's workload is a major obstacle to women practicing exclusively breastfeeding (Burns et al. 2016; Babakazo et al. 2015). In the rural areas women are required to cultivate, gather food, and collect firewood. While the mothers work in the field, their young children are left with relatives and neighbors who provide them with food. At the age of 7 months, the children are provided with solid food mostly in the form of porridge. As the children grow older, their diet does not change substantially. The children continue to eat porridge and other family food as they grow older.

Diet and Kwashiorkor in Northwest DRC: The Bwamanda Study

Bwamanda is one among the 516 health zones in the DRC and is located in the South-Ubangi province in the northwest part of the DRC. Health services are provided by a central hospital and 10 associated health centers. These health facilities serve a population of approximately 200,000. The hospital provides essential health services including some degree of nutritional rehabilitation services. The Ngbaka is the dominant ethnic group in the area and are predominantly subsistence farmers. The major crops cultivated in the area are cassava and maize. Besides crop production, the farmers grow vegetables such as sweet potatoes, taro, green leafy vegetable, and fruits such as papaya and African pear. The Ngbaka are also involved in gathering wild vegetables and fruits. A number of water bodies and small rivers

support local fishing. The natural resources in the area are widely dispersed restraining local people's access to wild food.

Agriculture production is constrained by a number of factors. Farming techniques are traditional and very labor intensive. All operations are done by hand, farmers do not have access to draught animals and fertilizers are unavailable. Some farmers therefore face labor shortage during critical agricultural periods. Farmers can reduce this problem of seasonal bottlenecks by participating in reciprocal farm workgroups involving relatives and neighbors. In Bwamanda farmers who take part in such groups are usually in better positions to diversify their food production. As in other parts of the DRC, a number of distal factors constraints the development of local food production and major obstacle to production is access to agricultural inputs, particularly seeds. Furthermore, poor infrastructure restricts the farmers' ability to expand into cash crop production.

The low farm production diversity contributes to little diversity in the diet. The Ngbaka normally eat two meals per day. The major meal is served in the evening and it consists of *ka.*

a stiff porridge made from cassava and maize flour. The dish is served with a side dish: a stew made of cassava leaves and palm oil. Sometimes the stew is enriched with fish and groundnuts. The leftovers are eaten for breakfast the next day. In between the meals, people eat fruits and drink tea and palm wine. Infants are mainly breastfed up to the age of 7 months at which point solid food is introduced. Early complementary food consists of a gruel made from *ka* and cassava-leaf stew.

A secondary analysis of a historical material from Bwamanda was undertaken, with the field study carried out between 1989 and 1991 (Kismul et al. 2014). The Bwamanda study is a dynamic population study. Three-monthly contacts were organized making up 15 months of follow-up with totally 6 contacts. During the first round, 4328 children below the age of 6 years were enrolled and at the end of the study 5567 children had been included. The children were examined for kwashiorkor and pitting edema on feet and ankles were used as the major criteria for determining the presence of the disease. Marasmus was assessed by inspection of abnormal visibility of skeletal structures and by absence or near-absence of palpable gluteus muscle without presence of edema. Children's nutritional status was also assessed through anthropometric assessment and children's weight and length/height was measured. As a part of the study, a nonquantitative 24-h recall was carried out. The recall contained the 41 commonly most consumed food items in the area. The respondents, the children's caretakers in most cases the biological mother, provided "yes" or "no" answers to the question if the children had consumed the listed items. First, in the statistical analysis the percentage of food consumed before children developed kwashiorkor was tabulated. Second, causal factors for kwashiorkor including diet and infectious diseases were analyzed. Table 1 describes the food items eaten by children during the survey round before children developed kwashiorkor. Maize, cassava roots, and cassava leaves were the major food consumed by the children. Kwashiorkor has been associated with a low intake of protein rich food. Then again the table shows that children who developed kwashiorkor and marasmus as well as children who did not develop these diseases had eaten food items that are

Table 1 Consumption of different food items in the survey round preceding the development of kwashiorkor (n = 37) and non-development of kwashiorkor (n = 8108) and development of marasmus (n = 374) in children between 6 and 50 months of age.(Kismul et al. 2014).

Food items	Children with Kwashiorkor % (95 CI)	Children without kwashiorkor % (95 CI)	Children with marasmus % (95 CI)	Food items	Children with kwashiorkor % (95 CI)	Children without kwashiorkor % (95 CI)	Children with marasmus % (95 CI)
African pears	0.0(0.0, 8.2)	0.0(0.0,1.1)	0.0(0.0,1.3)	Okra	2.3 (0.4,12.1)	4.5 (4.2,4.8)	5.2 (3.2,8.4)
Amaranth	7.0(2.4,18.6)	1.8(1.7,2.1)	3.1(1.6,5.8)	Pam oil	86.0 (72.0,93.4)	88.8 (88.3,89.3)	80.3 (75.4,84.5)
Aubergine	0.0 (0.0,8.2)	0.8 (0.7,0.9)	0.0(0.0, 1.3)	Papaya	2.3 (0.4,12.1)	15.5(15.0,16.1)[a]	11.8(8.5,16.0)[a]
Avocado	0.0 (0.0,8.2)	0.2(0.1,0.2)	0.0(0.0, 1.3)	Pineapple	4.7(1.3,15.5)	1.4 (1.2,1.6)	1.7 (0.7,4.0)
Banana	9.3(3.7,21.6)	19.1(18.5,19.7)	16.3 (12.5,21.0)	Powder milk	0.0 (0.0,8.2)	0.0(0.0,0.1)	0.0(0.0, 1.3)
Beans	31.1(30.4,31.8)	0.4 (0.3,0.5)	0.0(0.0, 1.3)	Rice	2.3 (0.4,12.1)	0.6 (0.5,0.8)	0.0(0.0, 1.3)
Breadfruit	0.0(0.0,8.2)	1.1 (0.9,1.2)	1.7 (0.7,4.0)	Sesame	0.0 (0.0,8.2)	0.1 (0.1,0.2)	0.3(0.1,0.9)
Cassava leaves	76.7(62.3, 86.8)	79.2 (78.6,79.9)	70.7 (65.2, 75.6)	Shrimp	0.0 (0.0,8.2)	0.1 (0.1,0.2)	0.0(0.0, 1.3)
Caterpillars	2.0(1.8,2.2)	2.0(1.8,2.2)	1.0(1.4,3.0)	Snails	2.3 (0.4,12.1)	1.4(1.3,1.6)	1.4(0.5,3.5)
Cassava roots	77.7 (62,3,86.8)	72.6(71.9,73.3)	72.3 (66.9,77.2)	Soya	4.7(1.3,15.5)	5.2 (4.9,5.5)	5.9 (3.7,9.2)
Chili pepper	4.7(1.3, 15.5)	8.9 (8.4,9.3)	4.5 (2.6,7.5)	Spinach	2.3 (0.4,12.1)	2.8(2.6,3.1)	2.1(1.0,4.5)
Egg	4.7(1.3,15.5)	0.7 (0.5,0.8)	1.0 (0.4,3.0)	Squash	0.0 (0.0,8.2)	4.9 (4.6,5.2)	5.2 (3.2,8.4)
Fish	18.6 (9.7,32.6)	31.1(30.4,31.8)	25.3(20.6,30.6)	Sugar cane	0.0 (0.0,8.2)	0.7 (0.6,0.9)	0.3 (0.1, 1.9)
Fruit (other)	0.0 (0.0,8.2)	1.8 (16,2.0)	1.0 (0.4,3.0)	Sweet potato	0.0 (0.0,8.2)	6.8 (6.4,7.2)	4.5 (2.6,7.5)
Ground nuts	18.6 (9.7,32.6)	28.6 (27.9,29.3)	23.9(19.3,29.1)	Termites	0.0 (0.0,8.2)	0.3 (0.3,0.4)	0.0(0.0, 1.3)
Maize	97.7 (87.9,99.6)	93.5(93.1,93.8)	91,7 (88.0,94.4)	Tomatoes	0.0 (0.0,8.2)	1.3 (1.1,1.5)	0.0(0.0, 1.3)
Mango	0.0 (0.0,8.2)	0.9 (0.7,1.0)	0.7 (0.2,2.5)	Wheat	0.0 (0.0,8.2)	0.6 (0.5,0.7)	0.3(0.1,1.9)
Meat	0.0 (0.0,8.2)	4.7 (4.4,5.0)	5.5 (3.4,8.8)	Other vegetables	2.3 (0.4,12.1)	15.1(14.6,15.7)[a]	17.6(13.7,22.5)[b]
Milk	0.0 (0.0,8.2)	0.1(0.1,0.1)	0.0(0.0,1.3)	Yam	2.3 (0.4,12.1)	1.3 (1.1,1.4)	0.7 (0.2,2.5)
Mushroom	0.0 (0.0,8.2)	2.3(2.1,2.5)	1.7 (0.7,4.0)				

[a] $p < 0.05$

[b] $p < 0.01$ with values estimated using 2-sample test for equality of proportions with continuity corrections as implemented in prop test in *R*

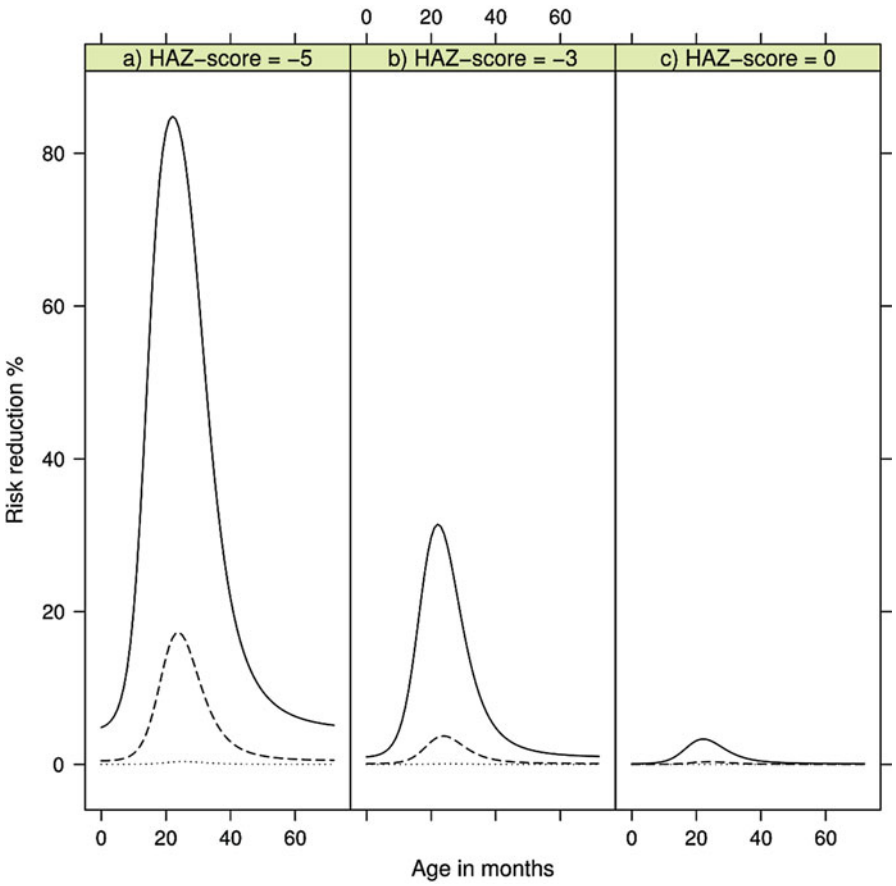

Fig. 6 Risk reduction for developing kwashiorkor showing reduction of consuming β-carotene rich products according to age in months. The dotted line is risk reduction after 2 months, dashed line after 4 months, and solid line after 6 months, (**a**) shows risk reduction for a child with a height-for-age Z-score (HAZ) of minus five, (**b**) for a child with HAZ of minus three, and (**c**) a child with HAZ of zero. HAZ-scores are based on the WHO-2006 Child Growth Standards (Kismul et al. 2014)

rich in proteins. These food items included caterpillar, egg, meat, and egg. In terms of consumption of these food items, there were no significant differences between children with kwashiorkor and children with marasmus and children without any of these diseases. On the other hand a significant higher proportion of children who did not develop kwashiorkor had consumed papaya, sweet potatoes, and "other vegetables," with the latter term comprising taro leaves, wild vegetables, and fruits. These food items are characterized by their high content of β-carotene. The statistical analysis demonstrated that the risk of developing kwashiorkor in children who were chronically malnourished increased the longer the children did not consume food that contained β-carotene Fig. 6 illustrates how the risk of developing kwashiorkor is reduced by eating food that is rich in β-carotene.

To summarize the Ngbaka live in environment characterized by food insecurity and in particular farmers who are not able to overcome labor constraints are susceptible to food and nutrition insecurity. In addition distal factors limit farmers' ability to develop a more diverse food production. The diet in the area is monotonous and cassava and maize are the major food items consumed by young children. In this food and nutritional unsecure environment, children are at high risk of developing different forms of malnutrition. The consumption of a monotonous diet that does not include vegetable and fruits increase the risk of chronically malnourished children to develop severe forms of malnutrition such as kwashiorkor.

Conclusion

Kwashiorkor in Africa has been related to diet since its first description. Kwashiorkor occurs in situations characterized by food insecurity and famine. Furthermore, kwashiorkor has been related to the consumption of a monotonous diet often characterized by the absence of.

consumption of fruit and vegetables. Thousands of children in the DRC are living in this type of environment and they are at high risk of developing kwashiorkor. Nutritional programs in the DRC need in particular to focus on rural areas with particular attention to provinces with high prevalence of malnutrition. It is especially important to assist ongoing efforts that aim at stimulating the consumption of a varied diet through improved agricultural and horticultural production.

Policies and Protocols

Protocol

Children with kwashiorkor can be treated with ready-to-use therapeutic food (RUTF), a home-based treatment carried out within a model for community-based management of acute malnutrition (Trehan and Manary 2015). RUTF is a high-energy, lipid-based spread. The powder ingredients are embedded in lipid-rich paste. The result is an energy dense food that resists microbial contamination (Briend 2001). Treatment with RUTF is provided for children with uncomplicated kwashiorkor (Manary et al. 2009), as it is also applied by PRONANUT, the national nutrition program which further recommends association with an antibiotic, and, where needed, vitamin A and an antimalarial (Manary et al. 2009). These are children who are treated in their early stage of illness. Children who are treated later in the stage of the illness may have a number of complications. These children should receive individual treatment. For these children, extended

nutritional supplementation as well as enrolment in nutritional program can be insufficient.

Policies

The strategy to reduce severe child malnutrition in the form of kwashiorkor should build on an approach that acknowledges how underlying, immediate, and basic factors determine the development of kwashiorkor. It is important to tackle factors at all three levels and recognize that addressing factors at only one level will have restricted effect on the development of kwashiorkor.

The development of kwashiorkor in the DRC occurs in situations characterized by severe poverty and food insecurity. To reduce severe malnutrition, there is a need for significant changes at the macro level. Policies that aim at reducing malnutrition should therefore be directly related to policies that put emphasis on economic growth and poverty reduction.

At the immediate level, it is important to address the problem of food insecurity. It is essential to target children living poor food insecure households in rural areas. These children are especially at high risk of developing kwashiorkor. People living in rural areas of the DRC are predominantly semisubsistence farmers. Food security policies should attend to causes leading to low production and little diversity in food production among small-scale farmers. Policies should especially improve better access to agricultural extension and agricultural inputs such as seeds, fertilizers, and credit. In terms of diversity in agricultural production, it is important to promote the cultivation of crops and fruits that are rich in β-carotene. Furthermore, improvements in food production and consumption rely on infrastructure development and farmers in particular need better access to markets for sale of their agricultural produce. Strategies that aim at reducing malnutrition through strengthening small-scale agricultural production should in particular target households with few resources in form of land, labor, and capital.

At the basic level, policies should promote nutrition-specific interventions. Such interventions could be effective ways of reducing the incidence of kwashiorkor. The diet in many areas of the DRC is to a large extent based on cassava and maize. Children are therefore at high risk of developing vitamin A deficiency. Vitamin A supplementation might be an effective way of reducing malnutrition including severe malnutrition.

Dictionary of Terms

- **Clinical severe childhood malnutrition** – A term used for the three clinical syndromes of clinical severe malnutrition including marasmus, kwashiorkor, and marasmic kwashiorkor.
- **Complementary feeding** – A process that start when breast milk no longer is enough to meet the nutritional needs of the infant other foods and liquids are needed, along with breast milk.

- **Diet** – A term that is commonly defined as the food that a person eats every day. It can be portrayed with regards to chemical composition in terms of nutrient content or described as food and food groups.
- **Free radical** – Is an atom or groups of atoms with an unpaired number of electrons. It can be formed when oxygen interacts with certain molecules.
- **Kwashiorkor** – A form of severe malnutrition where the presence of edema is the most significant feature.
- **Marasmus** – Nonedematous severe child malnutrition and characterized by extreme wasting
- **Edema** – Swelling of body tissues as a result of fluid accumulation.

Summary Points

- Kwashiorkor typically occurs among children in areas that are characterized by severe food and nutrition insecurity.
- Kwashiorkor has since its early description among children in Africa been related to the consumption of a monotonous diet.
- Earlier kwashiorkor was considered as a result of children consuming a diet high in energy but low in protein.
- Scholars have postulated that kwashiorkor is caused by an excess of free radicals. According to this suggestion, protective pathways are compromised in kwashiorkor. The pathways require the supply of micronutrients in the form of antioxidants.
- Food insecurity is a major problem in the DRC with food insecurity and child malnutrition being most widespread in the rural areas.
- Cassava root is the main staple food crop in most parts of the DRC and it is often eaten with a side dish such as stew made of cassava leaves.
- Animal products such as meat and egg are only eaten occasionally, whereas dairy products are rarely consumed.
- A longitudinal study examining the relation between diet and kwashiorkor has been carried out using material from Bwamanda an area in northwest Congo.
- The study demonstrated that the risk of developing kwashiorkor in children who were chronically malnourished increased the longer the children did not consume food that contained β-carotene.

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Double Burden of Underweight and Overweight: The Example of Bangladesh

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Mohammad Enamul Hoque

Contents

Abstract

Many low- to middle-income countries (LMICs) in the world are presently facing an epidemiological paradox, namely, a double burden of underweight and overweight, with the prevalence of both underweight and overweight simultaneously. Such double burden of malnutrition in many of these countries is a result of the rapid increase in the prevalence of an overweight burden while an underweight burden continues to persist. Considering the increasing overweight and persistent underweight found in many developing countries, a nutritional transition is going on in the LMICs, especially in countries of the Indian subcontinent including Bangladesh.

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A rising trend of prevalence of overweight both in urban and rural areas among the population in Bangladesh was observed. The prevalence of overweight exceeded that of underweight in 2014. A higher average annual rate of reduction of overweight was found among the wealthy, highly educated, and urban women, while a higher average annual rate of increase was found among the poor, uneducated, and rural women. In addition, a large variation between rural and urban areas was observed in the prevalence of overweight among children and adults in Bangladesh. The overweight children and adults were mainly from urban areas.

The presence of a double burden of underweight and overweight in adult males and females was observed in Bangladesh. However, the prevalence of overweight has increased significantly during the last three decades both in rural and urban areas. Reasons for the increase in the overweight prevalence may be attributable to current economic development in the country. Future research is needed to determine the driving force of the higher growth rates of overweight and to work out the reasons for the rapid increase of the overweight burden among the poor, uneducated, and rural people. As the burden of overweight is predicted to increase in the future, it is also important to estimate the future economic burden attributable to the overweight burden in Bangladesh.

Keywords

Nutritional transition · Underweight · Overweight · Low- to middle-income countries · Bangladesh

List of Abbreviations

AARR	Average annual rate of reduction
ARRI	Average annual rate of increase
BMI	Body mass index
CVD	Cardiovascular disease
DALY	Disability-adjusted life years
GDP	Gross domestic product
HNPSDP	Health, Nutrition and Population Sector Development Program
LMICs	Low- to middle-income countries
NGOs	Non-governmental organizations
OOP	Out of pocket
THCE	Total health-care expenditure

Double Burden of Underweight and Overweight in Bangladesh

Introduction

In the past, overweight and obesity has generally been considered as a public health problem of developed countries. However, several studies suggested that this public health problem is now spreading in the low- to middle-income countries

(LMICs) undergoing rapid economic transition, demographic changes, and urbanization (Popkin 2001, 2004; Monteiro et al. 2002, 2004). While overweight and obesity prevalence is rapidly increasing, the prevalence of underweight persists in many of these countries. This leads to a phenomenon referred to as a double burden of malnutrition, that is, coexistence of underweight and overweight in a population (Popkin et al. 2012; Kapoor and Anand 2002; Popkin 1994; Ke-You and Da-Wei 2001; Corsi et al. 2011; do Ha et al. 2011). Considering the increasing overweight and persistent underweight found in many LMICs, a paradox in the nutritional transition has emerged. Such nutritional transition is a consequence of changes in pattern of health and socioeconomic factors (Popkin 2004; Monteiro et al. 2002), increased consumption of energy-dense food (Popkin 1994; do Ha et al. 2011), higher dependence on technology-based activities (Popkin 1994, 2004; do Ha et al. 2011), and growth of urbanization (Popkin et al. 2012; Kapoor and Anand 2002; Popkin 1994; do Ha et al. 2011). The changes in food habits and dietary patterns along with changes in physical activity patterns are responsible for nutritional transition – from nutritional deficiency to over sufficiency disorders. The consequence of such nutritional transition is the increasing epidemic of an overweight coupled with underweight burden.

Like many LMICs in the world, Bangladesh was the habitat of leaner and underweight people, and thus for decades the major public health concern of Bangladesh was reducing underweight burden. With an urban population of around 35% (Worldometers 2015), continuous economic development, rapid industrialization, and market globalization along with higher incomes and wealth inequity among rural and urban people have made a significant impact on epidemiological and nutritional transition in Bangladesh. In addition, in the recent decades, several changes such as increased consumption of food outside the home, taking more energy-dense food, and drinking a lot of sugar-sweetened beverages have occurred in Bangladesh. Several recent studies have mentioned that the prevalence of overweight is increasing in Bangladesh (Balarajan and Villamor 2009; Shafique et al. 2007; Hoque et al. 2015), which is now facing a double burden of underweight and overweight problems.

Trend of Underweight

Globally, the underweight prevalence is decreasing (Corsi et al. 2011; Ahmed et al. 2012a; Black et al. 2008). In the developing regions, the proportion of children under 5 years who are underweight declined from 29% to 18% during 1990 and 2010 (UN 2012). However, despite such improvement, the global maternal and child underweight burden is still alarmingly high, with an estimated 112 million children under 5 years being underweight (Ahmed et al. 2012b). Among Asian countries, although the underweight prevalence is decreasing, it is still high. The underweight rate ranges between 20% and 50% in countries such as China, India, Indonesia, Thailand, and Vietnam (Stevens et al. 2012). Although historically women in Bangladesh have experienced chronic nutritional deficiency, national data indicates the decline of

underweight from 50.3% to 18.6% during 1996 (NIPORT 1996) and 2014 (NIPORT 2014). The prevalence of underweight among women aged 15–49 years decreased by 32% during the period 1996 (NIPORT 1996) and 2014 (NIPORT 2014). In 2014, approximately 21% of rural women and 12% of urban women were classified as underweight (NIPORT 2014).

The prevalence of underweight (weight-for-age Z-score < -2) among children under 5 years of age in 1996 (NIPORT 1996) was more than 55%. Though this prevalence decreased to 47% in 2000, little change was observed until 2007 (NIPORT 2007) when the prevalence began to decrease again and dropped to 32.6% in 2014 (NIPORT 2014). From 1996 (NIPORT, 1196) to 2014 (NIPORT 2014), the prevalence of underweight among children aged less than 5 years decreased by 24%. However, in 2014 the prevalence of underweight (32.6%) among children under 5 years of age was still high in Bangladesh, with over one-third underweight (NIPORT 2014).

Trend of Overweight and Obesity

The prevalence of overweight and obesity is increasing globally. The prevalence of overweight increased globally between 1980 and 2013 from 28.8% to 36.9% in men and from 29.8% to 38% in women (Ng et al. 2014). Kelly et al. (2008) predicted that in 2030, globally an estimated 2.2 billion adults will be overweight and further one billion will be obese. The prevalence of overweight has increased significantly in the last decades, with substantial variation in levels and trends across countries and within regions.

The prevalence of overweight and obesity in Bangladesh is especially increasing among women (Shafique et al. 2007; Stevens et al. 2012). In 1996–1997, the prevalence of overweight and obesity in reproductive-age women in Bangladesh was 2.7% (NIPORT 2007). In 2014 (NIPORT 2014), the rate of overweight and obesity among this group of women reached 39.2%, with an average yearly increment of 2.02%. At the national level, the mean body mass index (BMI) of women increased to 3.5 kg/m^2 points, from 18.8 kg/m^2 in 1996 (NIPORT 1996) to 22.3 kg/m^2 in 2014 (NIPORT 2014). During 1996 and 2014, the mean BMI increased among women both in rural (3.1 kg/m^2) and urban (3.3 kg/m^2) areas of Bangladesh. Over the last 18 years, mean BMI among the rural and urban women of Bangladesh increased by 0.17 kg/m^2 and 0.18 kg/m^2 per year, respectively.

A recent study (Hoque et al. 2015) provided information on existing double burden of underweight and overweight among both genders in Bangladesh, though underweight dominates in both cases. The study also suggested that there are important gender differences in the prevalence of underweight and overweight among the adult population of the country. Adults living in urban areas were more likely to be overweight compared with adults living in rural areas. On the other hand, adults living in rural areas were more likely to be underweight compared with adults living in urban areas.

Consequences of Double Burden of Underweight and Overweight

The existence of both underweight and overweight concurrently in a developing country adds pressure to the health system of this country, as resources are already limited in general. This has an impact on both public health and the economy of a country.

Public Health Burden

Both underweight and overweight are important risk factors for mortality and morbidity of various diseases. Underweight is associated with increased comorbidities, including osteoporosis, diabetes, infertility, and asthma (Sairenchi et al. 2008; Zheng et al. 2011). Underweight is also associated with increased risk of death in Asian populations (Ni Mhurchu et al. 2004). Maternal underweight also has adverse effects on pregnancy outcome. On the other hand, overweight and obesity are risk factors for cardiovascular disease (CVD), cancer, type 2 diabetes, and hypertension (Ni Mhurchu et al. 2004; WHO 2009). It is estimated that overweight and obesity alone are responsible for 3 million deaths every year worldwide (Mendez et al. 2005). In 2010, worldwide overweight and obesity were responsible for 3.4 million deaths and caused 3.8% of disability-adjusted life years (DALYs).

Economic Burden

The increasing burden of overweight and obesity coupled with the existence of the underweight problem creates an increasing threat to the overall health status of a country's citizen and on its health-care system. The cost associated with this double burden is expected to be immense and to have an impact on individuals, families, and the country. This health disorder negatively affects the health-related quality of life of individuals (Muennig et al. 2006). Households may incur expenditure related to treatment for diseases occurring from being underweight or overweight. This may also be associated with the reduction of economic growth of the country as this burden adversely affects various factors of economic growth – labor supply, productivity, investment, and education.

Several studies using data from Asian countries, other than those of the Indian subcontinent, have been conducted to measure the economic burden of overweight; and they have identified the total economic toll of overweight to be between 1.5% (Pitayatienanan et al. 2014) and 9.9% (Ko 2008) of a country's total health-care expenditure (THCE). In the case of countries of the Indian subcontinent, the economic burden seems to be immense as well. Like many developing countries in the world, out-of-pocket (OOP) payments are the major source of financing health care in countries of the Indian subcontinent. Households have to finance large and unpredictable costs, both medical and nonmedical, from household budget. Such

dependence on OOP payment for health services may lead to a catastrophic burden for households and may also have an impact on reducing economic growth through less investment by households in economic activities and education of children.

Nutritional Transition

With the global tsunami of overweight and obesity (Monteiro et al. 2002), the wave is also splashing in Bangladesh which is home to some of the leanest population in the world. Overweight and obesity has become a serious and growing problem of the country over the past decades. The overall prevalence of overweight and obesity is lower among urban children and adolescents than would be expected of an affluent society. This lower prevalence may be due to the existence of slums in these urban areas, and thus the urban poor and urban affluent seem to have very different obesity trajectories. The health status of children and adolescents in urban slums is very poor, so it is obvious that when combining these urban slum children with the other urban children, the total pooled prevalence of overweight among urban children and adolescents is reduced, substantially.

In case of Bangladesh, the prevalence of underweight women decreased from 50.1% in 1996 (NIPORT 1996) to 18.6% in 2014 (NIPORT 2014), whereas the prevalence of overweight women increased from 2.92% in 1996 (NIPORT 1996) to 39.2% in 2014 (NIPORT 2014). Prevalence of undernutrition of women decreased more than 60%, whereas prevalence of overweight women increased by more than 1000%. This has resulted in a shift from a greater prevalence of underweight to a greater prevalence of overweight. The prevalence of overweight increased in the last three decades, and in 2014 the prevalence of overweight superseded that of underweight. It is apparent that a nutritional transition is happening in Bangladesh. The reasons for such nutritional transition are multidimensional and are described below.

Economic Development of Bangladesh and Health Trend

The nutritional transition in a country occurs in consort with the economic growth of that country (Mendez et al. 2005). In the last decades, Bangladesh has gone through significant socioeconomic developments. According to World Bank data (World Bank 2017), the gross domestic product (GDP) has increased consistently in Bangladesh at around 6% per year for the last couple of decades and has increased from 53.31 billion US$ in 2000 to 221.41 billion US$ in 2016. The personnel remittance received in the country has increased from 778 million US$ in 1990 to 15 billion US$ in 2015 (UNDP 2014). The percentage of population below the national poverty line fell from 56.6% in 1991 to 31.5% in 2010 (World Bank 2017). During 1990 to 2016, the life expectancy at birth increased from 60 years to 72.2 years (World Bank 2017). Over the same period, the mortality rate of children under 5 years decreased from 143.7 per 1000 to 34 per 1000 (World Bank 2017). In addition, the secondary school enrolment rate increased from 20% in 1990 to 64% in 2016. The mobile cellular subscription increased from 6% in 2005 to 78% in 2016 (World Bank 2017). As a result, between 1990 and 2015, Bangladesh's Human Development Index increased from 0.386 to 0.579 (UNDP 2014), an increase of 50%. The trend in

Bangladesh's socioeconomic development is reflected with nutritional transition in the country as the overweight prevalence is increasing and underweight prevalence is decreasing.

Lifestyle Change

Bangladesh is one of the most densely populated countries in the world. In the recent decades, urbanization has risen very fast, with around 3.5% urban population growths annually (Monteiro et al. 2002). In urban areas there is a lack of parks, playgrounds, and even community walkways. All these have impact on physical activity and sedentary behavior, leading to higher ownership of vehicles and increased use of cars. Moreover, use of modern amenities (mobile telephones, electricity), increased time spent viewing television, and playing computer games have impacts on the increasing prevalence of the overweight burden. Nationally the rate of television watching has increased from 20.5% in 2004 (NIPORT 2004) to 50.9% in 2014 (NIPORT 2014).

The increasing wealth, due to increasing GDP, leads people with higher disposal incomes and thus more ready cash at hand than before. Furthermore, the globalization of the food market, availability of canned food in modern supermarkets, and changes in food distribution channels have contributed to changes in dietary habits within families. Increasingly, individuals eat outside the home or buy readily available takeaway food which characteristically refined carbohydrates, meat product, and energy-dense food with added sugar. As the food supply chain in Bangladesh changes, people are becoming accustomed to regularly having breakfast at local restaurants. Readymade cooking ingredients have become widely available, and women do not need to spend a lot of time collecting and preparing these ingredients. A recent survey found that rural women spent 17.7 h per day on average in noneconomic activities (BBS 2010). In contrast, the women growth rate in labor force is higher than men (8.7% vs. 1.4%) in 2010 (BBS 2010). However, this higher increasing participation of women in labor force may be due to an expanding garment sector in recent decades, as most of the garment workers are young women. Though the salary in the garment sector is very low, these young women have cash ready to be used to consume basic food to protect them from undernutrition. All these factors contribute to the increasing prevalence of overweight in the country. In addition, due to higher access to technology, people have moved from labor-intensive activities to capital-intensive activities, such as using tractors in agricultural production. Due to the growing trend in manufacturing, especially the garment sector, people are involved more in less energy-intensive services than before.

There are many such trends impacting on the overweight burden in Bangladesh. It was observed that in 2014 (NIPORT 2014) the prevalence of overweight among urban women was as high as 53.2% and among the richest group the prevalence of overweight was as high as 64.7%. Such high rate of overweight prevalence is comparable to other high-income countries like Canada and Singapore and is even higher than many high-income Asian countries like South Korea and Japan (Monteiro et al. 2004).

Nutritional Transition

Risk Factors for Nutritional Transition

Several risk factors are estimated to be involved with the overweight and underweight burden. The following discussion relates to the effect of these risk factors on underweight and overweight.

Education Level

The risk of underweight was lower for a higher level of education, and the risk of being overweight was higher for a higher level of education compared to people with no education. The women with higher level of education in general belong to well-off family. They are also involved in highly paid job. So they have ready cash to eat away from home, meeting friends and colleagues in fast-food restaurant, thus consuming energy-dense food. At the same time, most of these women are involved in desk-based job, which may also have impact on gaining weight.

In contrast, between 1996 and 2011, the total average annual rate of increase (AARI) of overweight was much higher among women with no education compared to women who completed secondary education or higher educational level (15.43% vs. 4.39%). On the other hand, the average annual rate of reduction (AARR) of underweight was higher among women who completed secondary or have higher educational level compared to the women with no education (6.71% vs. 3.84%). Thus, though the AARR of underweight is higher among the higher educated women, the AARI of overweight is much higher among the women with no education. This indicates a shift of overweight problem over the years among women with no education.

Age Category

A recent study (Hoque et al. 2014) on children and adolescent from countries of Indian subcontinent indicates that whereas only 2% of under 5-year-old children are overweight, 15% of children aged 10–18 years old were overweight in these countries. This may be due to the following biological reason: girls, after 12 years of age, experience pubertal growth spurt. In general, girls of this age in Bangladesh are confined to involve in sports and several physical activities. The study shows that individuals at 50 years old and above had higher risk of overweight and underweight compared to individuals who are 19–34 years old. However, the risk of overweight among individuals at 50 years old and above is higher than the risk of underweight.

Socioeconomic Status

Household socioeconomic status, as represented by household asset ownership, was found to be associated with the nutritional status of family members. Individuals from higher socioeconomic status families had significantly higher rate of overweight burden compared with individuals from lower socioeconomic status families. This may be due to higher food-purchasing capacity of the wealthy household. It can be argued that food-purchasing capacity and food-

consuming behaviors are not always positively associated. Several studies have proved that food purchasing and food intake patterns are related to the household wealth.

Rural-Urban Difference

A large variation in the prevalence of overweight and obesity between rural and urban residence exists in the country. The study findings showed that urban living is associated with overweight as the individuals from urban areas had higher odds of overweight compared with individuals from rural areas. This may be due to the fact that the urban women have higher baseline prevalence of overweight compared to their rural counterpart. The following are the reasons between the association of urban stay and higher overweight prevalence: increased level of sedentary behavior; lack of physical activity due to lack of parks, walkway, and playgrounds; higher use of modern utilities; and consuming more energy-dense food as most of the fast-food restaurants are located in urban areas.

Gender Difference

The higher annual average rate of increase of overweight among the rural and low socioeconomic group, especially among women in the group, is a reflection of the socioeconomic development of Bangladesh. This indicates that the rural women are quickly catching up to their urban counterpart who had higher baseline overweight prevalence. However, this increasing trend of overweight burden among the rural and poor people indicates a rising inequality burden of overweight in the country. Gender inequality exists in the prevalence of underweight and overweight as women were found to be more overweight and less underweight than men. Urban men had lower risk of overweight and higher risk of underweight compared with their female counterpart. This might be due to the tendency of women in Bangladesh to stay at home and to be involved in less physical activity than men.

Emerging Burden of Noncommunicable Disease and Economic Burden

It is obvious that a nutritional transition in Bangladesh is underway as the burden of overweight is increasing and the burden of underweight is decreasing. However, the underweight burden is still very high and thus ensures the existence of a double burden of underweight and overweight. The rate of increase of overweight prevalence was higher than the rate of decrease of underweight prevalence in both urban and rural areas. This increasing trend of an overweight burden also leads to rising burden of noncommunicable diseases attributable to overweight. In 2010, around 30% of the total population in Bangladesh was at young age (10–24 years), and as this young population grows up, there is a possibility that the noncommunicable disease burden will increase. Regarding economic burden, the cost associated with this burden will be immense and will create a significant challenge for the country in terms of financial burden.

Policy Response Addressing the Nutritional Transition

Though both underweight and overweight health burdens coexist in Bangladesh and there is a nutritional transition from an underweight burden to an overweight burden, the overweight burden is a comparatively new issue in Bangladesh as poor nutritional status is still a big public health concern. Most of the policies related to nutritional issues are mainly focused on reducing the undernutrition problem as the country has been facing this burden for a long time. For instance, the National Health Policy (GoB 2011) and National Plan of Action for Nutrition (GoB 1997) both branded and concentrated on undernutrition and identified only undernutrition as an issue, but not overweight. It seems that Bangladesh's health policy still does not recognize the importance of preventive public health interventions to control the emerging disease burden associated with overweight and to address the nutritional transition. This omission may be due to lack of experience and the ability to handle the emerging overweight burden and lack of national policy guidelines to deal with the disease burden and focus on undernutrition issue. However, a vital document "The Country Investment Plan: A Road Map towards Investment in Agriculture, Food Security and Nutrition" identified both under- and overnutrition as important issues. In addition, the current Health, Nutrition and Population Sector Development Program (HNPSDP 2012) identified overweight as an obstacle for economic development of the country. However, the operation plan of executing HNPSDP is inclined to prevent noncommunicable disease-related risk factors more than the overweight burden only.

Conclusion

Future research is needed to get a complete picture of the health and economic burden of underweight and overweight in Bangladesh and to address the nutritional shift. The consequences of the transition from an underweight burden to an overweight burden and the coexistence of both underweight and overweight burden, and the future implication for the health and economic sector of the country need to be analyzed and understood. As the public health burden and concern about overweight is comparatively a new area in Bangladesh, policies addressing this health burden are rare. Policy and research gap analysis is needed to address the nutritional transition in Bangladesh, addressing both the underweight and overweight burden, and to recommend key strategies to curb the increasing burden of overweight. Several sporadic efforts have taken place to curb or control the overweight burden. In addition to the government initiative, several Non-Government Organizations (NGOs) are conducting various programs to address Bangladesh's nutritional problem. However, these NGOs are targeting the undernutrition issues, especially for mothers and children. Such involvement of the NGOs in one specific area of nutrition is understandable as most of the health policies of the country are directed towards controlling the underweight burden.

Policies and Protocols

This chapter describes the double burden of underweight and overweight in Bangladesh and currently undergoing nutritional transition from underweight to overweight in Bangladesh. To address this problem, several policies should be adopted as mentioned below:

- Bangladesh's health policy should recognize the importance of preventive public health interventions to control the emerging disease burden associated with overweight and to address the nutritional transition.
- It is needed to conduct operation research involving the community into policy action through citizen engagement to identify strategies for simultaneously controlling the increasing trend of overweight prevalence among Bangladeshi people while maintaining the decreasing trend of underweight prevalence.
- A policy and research gap analysis is needed to address the nutritional transition in Bangladesh, addressing both the underweight and overweight burden, and to recommend key strategies to curb the increasing burden of overweight.

Dictionary of Terms

- **BMI** – an index of weight for height that is commonly used to classify individual nutritional status
- **Overweight** – BMI $\geq$ 25 kg/m^2
- **Obese** – BMI $\geq$ 30 kg/m^2
- **Underweight** – BMI $\leq$ 18.5 kg/m^2
- **z-scores** – cutoff value of <-2 and $>$ +2 z-scores are commonly used for assessing nutritional status of the children. A z-score cutoff point of <-2SD is classified as low weight-for-height (wasting), low height-for-age (stunting), and low weight-for-age (underweight). The cutoff point of $>$ + 2 SD classified high weight-for-weight as overweight in children

Summary Points

- A clear existence of a double burden of underweight and overweight among both genders, though underweight dominates in both cases, was observed. A quarter of the adult population were underweight at national level, and almost one in seven of the adult population is overweight.
- A rising trend of overweight both in urban and rural areas among the population was observed.
- A higher average annual rate of reduction of overweight was found among the wealthy, highly educated, and urban women, while a higher average annual rate of increase was found among the poor, uneducated, and rural women.

- There are important gender differences in the prevalence of underweight and overweight among the adult population of the country. High socioeconomic status, higher educational attainment, and urban residency increased and reduced the risk of women being overweight or underweight, respectively, compared with men.
- Adults living in urban areas were more likely to be overweight compared with adults living in rural areas. A large variation between rural and urban areas was observed in the prevalence of overweight among children and adults.

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Malnutrition and Intestinal Parasites: Mexico Perspectives

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Contents

Abstract

Mexico is the most populated country in Latin America and the second largest in extension after Brazil. According to the population census (2010), more than

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80% of its inhabitants are under the age of 50 years. Throughout the years, Mexico has been plunged into poverty and this calamity is expanding overtime. In addition, income inequality has been a constant which keeps the country under economic and health-related hardships. Among other factors, poverty contributes to the onset of malnutrition and intestinal infectious diseases which prevail in the southeast of the country where the majority of poverty and marginalization is concentrated. In 1999, the prevalence of underheight was ~70%, mainly affecting indigenous children. For almost half a century, malnutrition has led to the proliferation of certain diseases such as intestinal amebiasis and ascariasis, positioning them among the twenty most common causes of morbidity in Mexico. In 2015 for example, the incidence rates of both diseases were 211.04 and 39.81 cases for every 100,000 inhabitants, respectively. In a similar trend, intestinal infectious diseases and helminthiasis were positioned within the ten leading causes of death in the southern and central part of the country. From 1998 to 2014, the cysticercosis and ascariasis claimed the most lives with 2575 and 448 cases, respectively. Other medical relevant protozoa include *Cryptosporidium parvum*, which contributes to malnutrition in children, and *Giardia lamblia* whose seroprevalence in Mexico has been determined to be as high as 55.3%. Recommendations by the WHO are to provide antiparasitic drugs, improve the access to drinking water, and implement health education with the purpose of eliminating these neglected diseases. To complete these actions, it is necessary to provide additional investment into the nation's health sector which will foster and promote the well-being of Mexico's children.

Keywords

Ascaris lumbricoides · *Entamoeba histolytica* · Helminthiasis · Infectious and parasitic diseases · Intestinal parasites · Malnutrition · Mexico · Mild Protein-energy malnutrition · Neglected diseases · Overweight · Protozoa · Stunting

List of Abbreviations

DALY	Disability adjusted life years
ENSANUT	Encuesta Nacional de Salud y Nutrición (Mexico National Survey of Health and Nutrition)
EPG	Eggs per gram of feces
H/A	Height for age index
ICD	International classification of diseases
INEGI	Instituto Nacional de Estadística y Geografía
W/A	Weight for age index
W/H	Weight for height index
WHO	World Health Organization
YLL	Years of life lost

Introduction

For almost half a century, intestinal infectious diseases have affected the peasant and indigenous Mexican population. These diseases are some of the main causes of morbidity and mortality over the last few decades. A similar situation has been observed with mild protein-energy malnutrition. Fortunately by 2014, this condition left the top 20 principal causes of morbidity. It is important to mention that in the country there is great disparity of nutritional status. The northern states present the highest prevalence of overweight and obesity, while the southern states present major malnutrition problems. Regions where populations are affected by malnutrition are more prone to have a greater number of cases of intestinal infectious diseases, in comparison with the northern part of Mexico (Monarrez-Espino et al. 2011; Quihui et al. 2006).

Mexico's socioeconomic factors have played an extremely important role in these health disparities. For example, the income inequality that Mexicans perceive is more accentuated in the southern part of the country, in comparison with the northern. Even though, this context does not allow reducing the high mortality rates in regards of infectious intestinal diseases.

In the current chapter we present an overview of the most important intestinal parasitic diseases affecting the Mexican population, including studies of the mortality burden in different regions, i.e., states of Mexico, where data is available. We introduce a perspective about the state of the infections within the social environment where they have developed. In the same way, the work will enable us to view and assess action strategies in order to correct or reduce long-term trends of these diseases in Mexico.

The Scenario of Intestinal Parasitic Disease in Mexico

The results of the National Population Census performed in 2010 revealed that the population of Mexico is predominantly young with 53.4 million inhabitants between the ages of 0–24 years, followed by those 25–49 years and finally those from 50–65 years or more (38. 4 and 20.4 million, respectively) (INEGI 2016). Almost half of the Mexican and Latin-American inhabitants are classified as living in poverty, besides the fact that in these regions there is high income inequality (Hotez et al. 2008).

According to official figures, from 2008 to 2014, the poverty level has increased in Mexico, passing from 44.5% to 46.2%. In regard to social deprivation, the most notable were: social security access (58.5%), followed by food access (23.4%), access to basic housing services (21.2%), educational lagging (18.7%), and access to health services (18.2%) (INEGI 2016).

Additionally, the World Bank pointed out that the slow economic growth and income inequality among Mexico's inhabitants have increased poverty (Walton and

Lopez-Acevedo 2005). For example, in almost half a century, the Gini coefficient, which evaluates the distribution of income within a nation, only improved 18% going from 0.53 (in 1963) to 0.435 (in 2010) (Narro Robles and Zepeda Tena 2012). During 2014, the value of this indicator was of 0.438 (INEGI 2016), revealing that inequality is still valid.

All of these above mentioned factors, along with ethnicity, age, sex, and ecological conditions provide breeding ground for neglected tropical diseases (Hotez et al. 2008). In Mexico, the most frequent tropical diseases are intestinal parasitosis caused by helminths and protozoa. Among helminths, the most prevalent etiological agents are: *Ascaris lumbricoides* (roundworms), *Trichuris trichiura* (whipworms), *Necator americanus*, and *Ancylostoma duodenale* (hookworms). Some of the effects caused by helminthiasis are: anemia, vitamin A deficiency, weakness, malnutrition, and intestinal obstruction (Stephenson et al. 2000; WHO 2011, 2015). During 2002, trichuriasis was the most prevalent in Latin-America, followed by ascariasis and ancylostomiasis with a total of 100, 84, and 50 million people infected. Most of these people were located in Brazil, Mexico, and Guatemala (de Silva et al. 2003). Furthermore, cysticercosis, fasciolasis, and paragonymiasis constitute the most important infections caused by plathyhelminths in Latin-America. In this part of the world, approximately 400,000 persons were affected by cysticercosis (Hotez et al. 2008).

To respond to the upcoming challenges that helminthiasis poses in high-risk populations, the WHO has suggested the prophylactic use of certain drugs such as albendazole, levamisole, mebendazole, and pyrantel (WHO 2002). It has been observed that in cases of ascariasis, children who were treated with the aforementioned drugs improved their nutritional status, gained weight and height, and their skin fold got thicker. In infections caused by hookworms or trichuriasis, treatment is helpful for preventing anemia brought on by iron deficiency; meanwhile in pregnant women it is useful for preventing maternal morbidity (Savioli et al. 2004). It is important to point out that, if a nation invests in the wellbeing of its inhabitants, the health status of people affected by these parasites could improve and this would represent a beneficial impact on both individual incomes and national incomes promoting sustainable wealth (Jamison et al. 2015). Thus, some actions that should be taken in Mexico, in order to fight against neglected diseases, are the establishment of a universal national health service based on family medicine and a reduction in the economic and social inequality gap which still exists in the country (Narro Robles and Zepeda Tena 2012).

Malnutrition

The impact of infection by intestinal parasites on the nutritional status of the host has been extensively documented. Anorexia is one of the negative effects along with weight loss (Stephenson et al. 2000). Furthermore, when a person is malnourished, the immune system deteriorates, becoming increasingly susceptibile to different intestinal infectious agents (Papier et al. 2014). All of this establishes a vicious circle between malnutrition and such types of infectious agents (Rodríguez et al. 2011).

According to official numbers, for more than a decade, mild protein-energy malnutrition has been positioned within the first 20 causes of disease (2000–2013). It should be noted that malnutrition has mainly affected infants between one to 4 years of age. Starting in 2014, mild protein-energy malnutrition was no longer considered an important cause of morbidity given that the country experienced an abrupt transition toward obesity with the number of new cases almost tripling (3.5×10^5) in comparison to the new cases of mild protein-energy malnutrition of the previous year (1.1×10^5). Alarmingly, in 2015, new cases of obesity increased more than 30% in comparison with 2014. In both years the most affected population included adults between the ages of 25–44 years of age (DGE 2014) (Fig. 1).

Surveys of the Mexico's national health have revealed that the nutritional status of the population is polarized. During 2006, the highest prevalence of obesity among

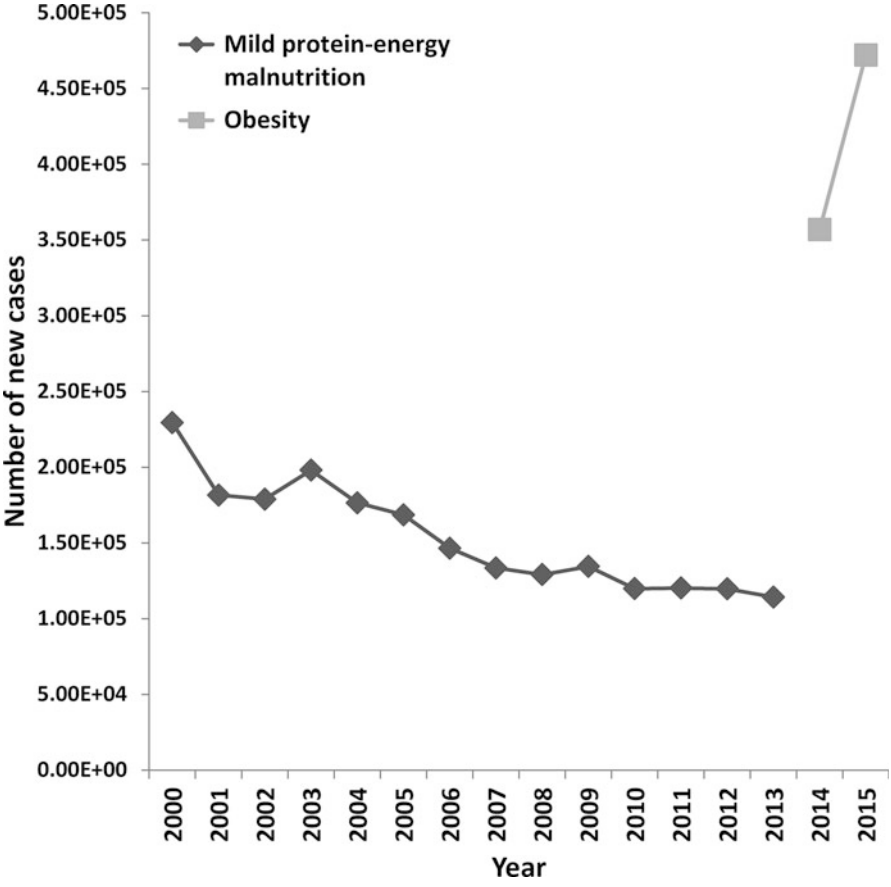

Fig. 1 Mild protein-energy malnutrition (ICD diagnosis code E44.1) and obesity (ICD diagnosis code E66) new cases in Mexico. A timeline shows a decrease in the number of cases of mild protein-energy malnutrition; however, a suddenly onset of obesity appears since 2014 and this trend continues at present

adults was detected in the northern region (34.7%), followed by the southern region (27.3%), and in 2012 the trend was similar in both regions (Barquera et al. 2009; Barquera et al. 2013). In contrast, malnutrition increased in the southern part of the country, mainly among indigenous children. In 1999, prevalence of underheight and underweight were of 69.8 and 49.8%, respectively, whereas in the northern part of the country these were 36.1 and 22.6%, respectively. The northern of Mexico presented the highest prevalence of overweight/obesity among indigenous children (14.2%), in comparison with the southern region (5.6%) (Chavez Zuniga et al. 2003).

Several authors have pointed out that chronic malnutrition has persisted in Mexico for more than 24 years, affecting indigenous children who live in rural zones of the southern part of the country (Rivera-Dommarco and Cuevas-Nasu 2013; Rivera et al. 2003). The focalization of malnutrition has also been pointed out by a study which demonstrated the prevalence of stunting in more than half of 2666 indigenous children younger than 5 years of age (54.1%), living in poverty and marginalization conditions, and under an atmosphere imbued with political conflicts (Sanchez-Perez et al. 2007). Unfortunately the nutritional status of these children has not changed. A recent study performed on children around the age of two, and living in rural and marginalized zones from the central and southern regions of Mexico, revealed that almost half of these children (43.4%) presented stunting associated with retarded speech development (Carrasco Quintero et al. 2016).

Parasitic Diseases in Mexico

The nutritional status of the Mexican population, linked to socioeconomic profile, reveals differential risk between the northern, central, and southern regions of the country, with people living in the southern region being subject to a higher risk of acquiring a neglected disease such as infectious and parasitic diseases covered here. For more than three decades (1984–2015), intestinal infections (ICD diagnosis codes A04, A08-A09, except A08.0); intestinal amebiasis (ICD diagnosis codes A06.0-A06.3, A06.9); helminthiasis caused by trematodes, nematodes, cestodes, and filarial parasites (ICD diagnosis codes B65-B67, B70-B76, B78, B79, B81-B83); and ascariasis (ICD diagnosis code B77) have been positioned within the top 20 causes of transmissible diseases affecting the Mexican population (DGE 2014; Ximenez et al. 2009) (Fig. 2).

Although in Mexico intestinal amebiasis is one the most important parasitic diseases by protozoa, its incidence have diminished the last few years. For example, while 1.6×10^6 new cases were reported in 1998, there were only 2.5×10^5 new cases in 2015. The most affected groups were preschool and grade-school students. The states of Nayarit, Guerrero, and Yucatan (the first two from the central and the last from the southern part of the country) presented incidence rates higher than the national rate (211.04 per 100,000 inhabitants) during 2015: 546.17, 523.52, and 516.15, respectively (Table 1; Fig. 3a). Since 2008, ascariasis has also experienced a similar trend and it has even disappeared from the top 20

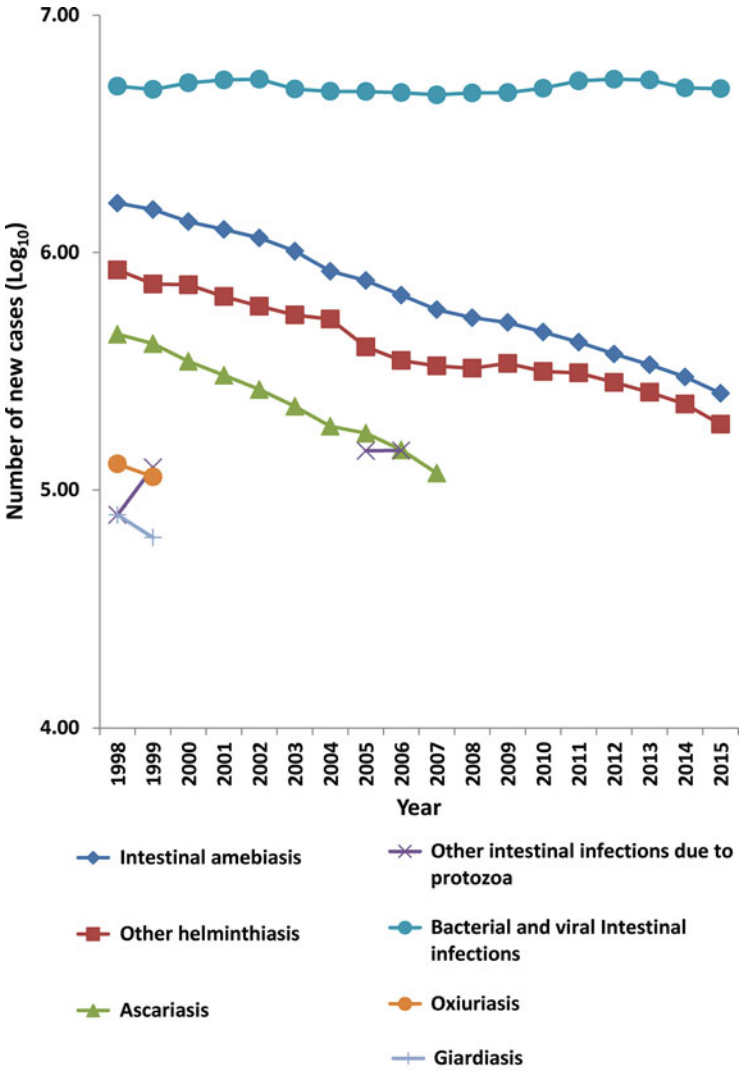

Fig. 2 New cases of parasitic diseases in Mexico (1998–2015) (DGE 2014). A timeline depicts that bacteria and virus are the top etiology agents of intestinal infections; amoebas are the main protozoa affecting the Mexican population, followed by helminths

cases of morbidity in Mexico. This helminthiasis has also affected preschool and grade-school students as the incidence rates which exceeded the national value (39.81) during 2015 were observed mainly in the southern part of the country (DGE 2014) (Table 1; Fig. 3b).

Since 1990, the YLL rate and the DALY rate caused by diarrheal diseases have also shown a parallel decrease in morbidity rates. Nevertheless, these are still concentrated in the southern part of the country. During 2013, the states of Chiapas,

Table 1 Morbimortality of the most prevalent intestinal infectious diseases in Mexico. The incidence rates, and cumulative deaths, caused by the main intestinal infectious diseases in the most affected Mexican states

	Incidence rate (per 100,000 inhabitants; 2015)		Number of deaths (1998–2014)	
State	Intestinal Amebiasis	Ascariasis	Intestinal infectious diseases	Helminthiasis
National rate	211.04	39.81	–	–
Aguascalientes	394.2	7.77	631	61
Campeche	449.84	11.90	307	16
Chiapas	490.82	92.37	9462	245
Colima	362.84	51.97	253	18
Guerrero	523.52	255.2	2585	117
Hidalgo	419.79	71.85	926	117
Mexico	213.64	5.06	8723	224
Mexico city	133.97	4.08	5808	366
Morelos	240.74	10.94	1000	48
Nayarit	546.17	64.47	605	32
Oaxaca	475.41	55.75	5727	187
Puebla	280.47	50.65	4981	455
Quintana Roo	285.05	117.6	444	32
San Luis Potosi	216.56	27.6	1812	104
Sinaloa	314.99	18.50	1007	59
Tabasco	426.7	144.89	996	63
Tamaulipas	129.57	46.03	834	46
Tlaxcala	314.63	4.38	653	42
Veracruz	224.8	93.64	4358	427
Yucatan	516.15	285.54	1751	50
Zacatecas	282.92	6.66	1005	97

Guerrero, and Oaxaca registered YLL rates caused by diarrheal diseases of 494.5, 349.7, and 326.8 respectively, greater than the national rate (172.6) (Gomez-Dantes et al. 2016).

Mortality records caused by this kind of transmissible diseases showed a similar trend. For more than a decade (1998–2014), parasitic infectious diseases caused a total of 310,996 deaths (from a total of 9.1 million during such period) and for this reason they figured among the first 10 causes of mortality in the country, after diseases of the circulatory system, endocrine diseases, nutritional and metabolic diseases, tumors, external causes of morbimortality, diseases of the digestive system and respiratory diseases (INEGI 2016) (Fig. 4).

According to information provided by the National Institute of Statistics and Geography (INEGI by its acronym in Spanish), the largest quantity of deaths due to intestinal infectious diseases were registered in Chiapas, State of Mexico, Mexico City, Oaxaca, and Puebla. On the other hand, deaths caused by helminthiasis were

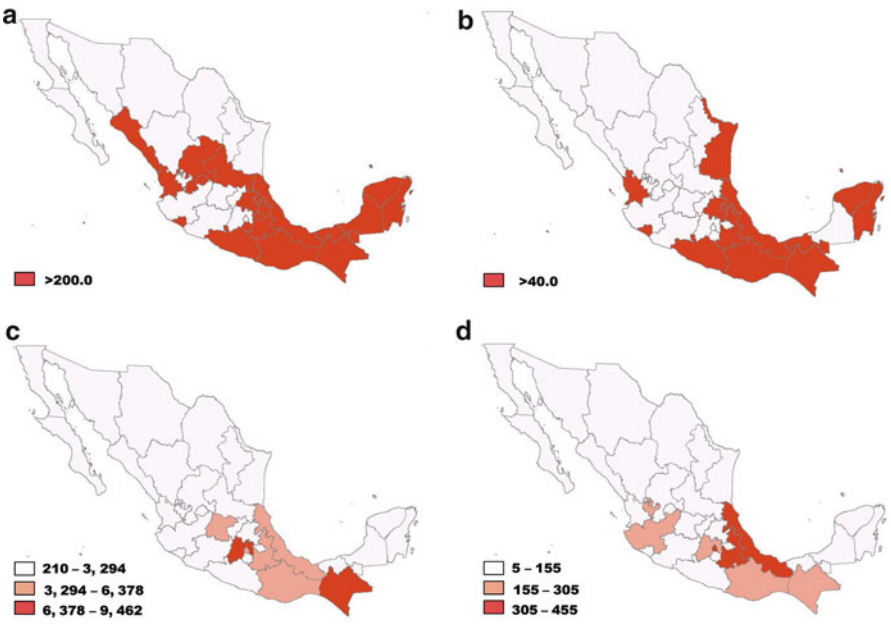

Fig. 3 Distribution of the main intestinal infectious diseases in Mexico. States above national incidence rate (per 100,000 inhabitants) due to: A, intestinal amebiasis; B, Ascariasis. Cumulative deaths (1998–2014) due to: C, intestinal infectious diseases; D, helmithiasis (INEGI 2016) (Maps of figure courtesy Ca. Dr. Jorge Antonio Paz Tenorio, a full-time professor at the Facultad de Ingeniería, Universidad de Ciencias y Artes de Chiapas, using the online free Mapa digital de Mexico software (version 6.1)

observed primarily in Puebla, Veracruz, Mexico City, Chiapas, and Jalisco (Table 1; Figs. 3c, d).

From 1998 to 2014, deaths caused by intestinal infectious illnesses (ICD diagnosis codes A00-A09) and helminthiasis (ICD diagnosis codes B65-B83) added 71, 655, and 3730 cases, respectively and for this reason they were positioned among the top 10 causes of death in Mexico (second and seventh place, respectively). The largest number of deaths caused by both illnesses was registered during 1998 with 6680 and 480 cases, respectively. During 2014, these numbers decreased to 3449 and 137 deaths, respectively (Fig. 5).

In Mexico the access to drugs for use against amoebas has reduced mortality rates caused by this protozoan. Amebiasis are among the top 20 causes of morbidity (Ximenez et al. 2009). Similarly, the reduction of deaths caused by helminths can be attributed to the availability of antihelminthic drugs and to the implementation of other actions suggested by the WHO as: environmental hygiene improvement, access to safe drinking water, and the urge to count on health education (Crompton et al. 2003). For example, during 2006 the Mexican government implemented the "Programa Oportunidades" (Opportunities Program) for disadvantaged and

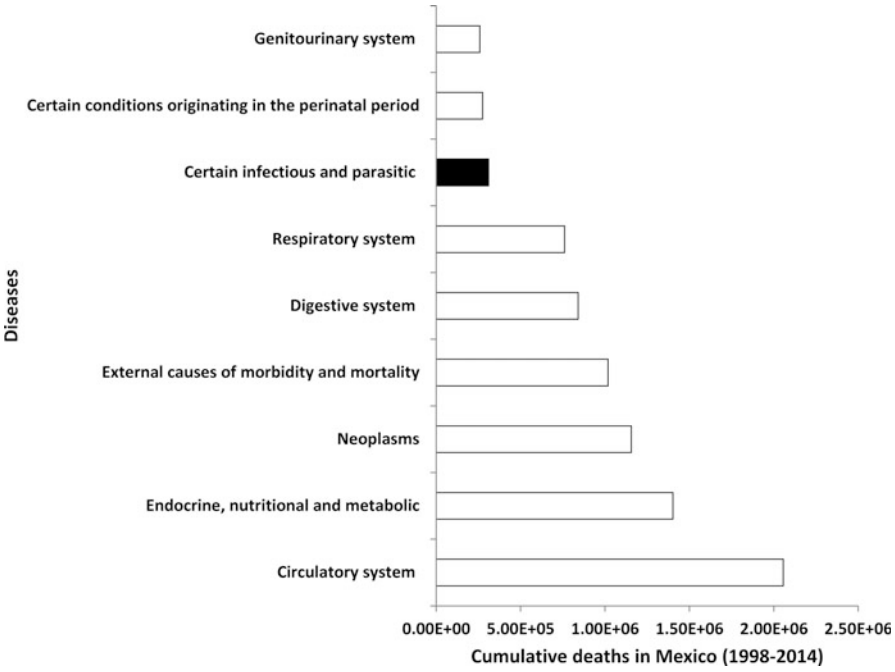

Fig. 4 The main causative agents of death in Mexico, 1998–2014 (INEGI 2016). The infectious and parasitic diseases are within 10 leading causes of death in Mexico. Data show cumulative deaths from 1998–2014

marginalized families. Families received economic support under the condition of family assistance, regular medical checkups, and commitment to take their children to school (Levy 2007). This approach proved to be successful in fighting against parasitic infectious diseases.

A study conducted with the assistance of indigenous students from the northern part of Mexico showed that after an action strategy which lasted 20 weeks and included treatment with oral nitazoxanide, improvement of the physical infrastructure, and implementation of an educational program targeted at children, parents, and school personnel resulted in the reduction of *G. lamblia* from 51.7 to 10.4% and *A. lumbricoides* from 37.5 to 0% (Monarrez-Espino et al. 2011). Another study performed in a rural zone of Colima, Mexico (Mexico's central-west) revealed that from 280 participants, 86 individuals presented infection by *A. lumbricoides*. Adults were the most affected (58%; >18 years of age), followed by children (35%; 2–12 years) and teenagers (7%; >12–18 years). Infection by this parasite may be associated with houses lacking of drainage and dirt floors; nevertheless after the administration of nitazoxanide, prevalence caused by this helminth diminished to 13% (Galvan-Ramirez et al. 2007).

Deaths caused by helminthiasis due to cysticercosis in Mexico ranked first (2575 cases), followed by unspecified intestinal parasitosis and ascariasis (639 and 448 cases, respectively) (Table 2).

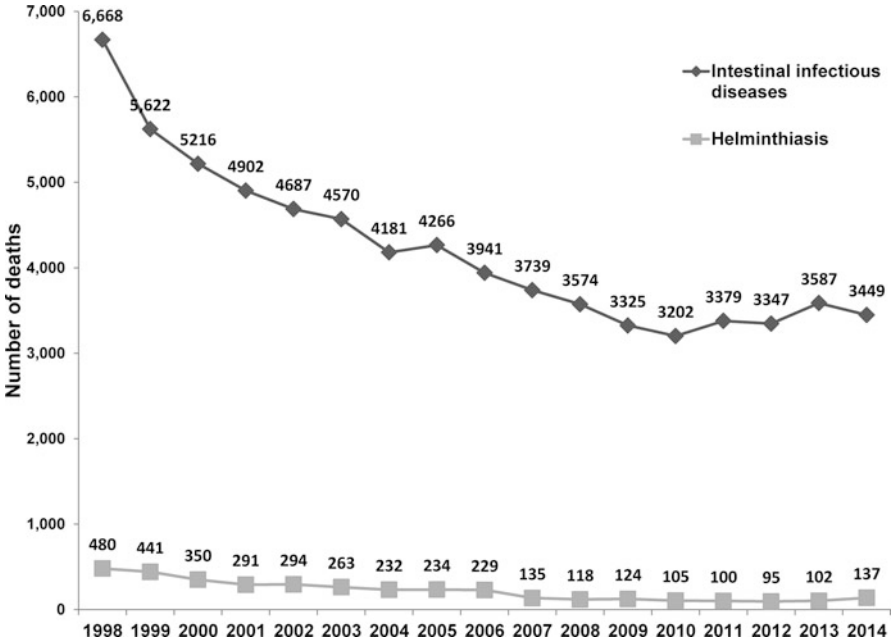

Fig. 5 Deaths by intestinal infectious diseases and helmintiasis in Mexico, 1998–2014 (INEGI 2016). A timeline showing a decrease in the number of deaths due to intestinal infectious diseases and helminthiasis in Mexico

Cysticercosis is caused by the *Taenia solium* cestode (pork tapeworm), a parasite which possesses a biological cycle involving swine and humans. After eggs are consumed through the fecal-oral pathway, the oncosphere hatches out and penetrates the intestine, invading the intestinal wall. Then, it migrates toward the striated muscle, liver, and brain. Once it migrates to these organs it causes severe consequences, such as neurocysticercosis (García et al. 2003).

Fleury et al. (2010) stated that the prevalence of neurocysticercosis reported by the "Instituto Nacional de Neurología y Neurocirugía de Mexico" (National Institute on Neurology and Neurosurgery) did not differ significantly between 1994 and 2000 (2.4% versus 2.5%, respectively). This suggests that, in Mexico, this parasitosis remains of great importance and requires major actions for its eradication.

A study of helminthiasis that was carried out between 1997 and 2000 revealed a greater prevalence of these parasites (53%) in rural communities of Oaxaca (southeast), in comparison with those founded at Sinaloa (northeast), with 33% (Quihui et al. 2006). This trend may be due to the fact that, as it has already been mentioned, in the southern part of the country and in Guatemala it is possible to find a great number of rural zones, primarily inhabited by poor and marginalized indigenous communities and for this reason they present the highest rates of helminthiasis in America (Hotez et al. 2008).

Table 2 Deaths by helminthiasis in Mexico, 1998–2014 (INEGI 2016). Leading causes of death in Mexico, due to helminthiasis

Helminthiasis	Number of deaths
Cysticercosis	2575
Unspecified intestinal parasitosis	639
Ascariasis	448
Echinococcosis	16
Strongyloidiasis	10
Unspecified helminthiasis	11
Filariasis	8
Anchylostomiasis	5
Onchocercosis	5
Enterobiasis	3
Trichinosis	3
Trichuriasis	2
Taeniasis	2
Other trematode infections	1
Fascioliasis	1
Necatoriasis	1
Total	3, 730

Parasitic Diseases Associated with Malnutrition

A recent study carried out in the southeast of Mexico with 250 children of less than 5 years of age (belonging to the zoque and tzotzil indigenous groups) revealed a prevalence of intestinal parasites of 38.8%. *Ascaris lumbricoides* was the most common helminth (33.6%). In more than one-third of the participants, the presence of this parasite was associated with malnutrition (Gutierrez-Jimenez et al. 2013). Another survey done with 243 six-year-old children, living in a rural zone of Colima, Mexico (central-east) showed that more than half of the participants exhibited low ferritin levels (60.9%). Infection with *Trichuris trichiura* in these children was associated with both low ferritin levels and stunting (Gutierrez-Rodriguez et al. 2007).

Fasciolasis is another pathogenic helminthiasis but has only caused one decease in Mexico the last 16 years (INEGI 2016). However, we must not forget a report warning us about an endemic zone of this parasitosis in the municipality of Atlixco, Puebla (central Mexico), characterized by certain ecological elements that are suitable for this kind of helminthiasis as: altitude, presence of snails from the *Galba*/*Fossaria* genus, and infected cattle. In this region the prevalence of disease caused by this parasite ranged from 2.9% to 13.3% and was associated with the consumption of raw vegetables and coinfection with protozoa and helminths. Fortunately the treatment with nitazoxanide showed to be effective (Zumaquero-Rios et al. 2013).

Regarding parasitic protozoa, there are several studies documenting the negative impact of these organisms over the nutritional status of certain populations. *Cryptosporidium parvum* and *C. hominis* appear to have contributed to child malnutrition (Valenzuela et al. 2014). In the north of Mexico (i.e., Hermosillo, Sonora), the

prevalence of *C. parvum* in students between 6 and 13 years of age was of 24%. These children displayed a risk of suffering from malnutrition nearly three times higher than noninfected children (Quihui-Cota et al. 2015). Another work conducted among preschoolers from Guadalajara, Mexico (central-western part of the country) identified *C. parvum* as one of the most frequent protozoans (5.1%) and as the etiology agent of acute diarrhea, followed by *Giardia lamblia*, *Blastocystis hominis*, and *Entamoeba histolytica* (1.2, 0.9, and 0.3%, respectively) (Larrosa-Haro et al. 2010).

In regards to *G. lamblia*, a study revealed that in Mexico the seroprevalence of this parasite was 55.3%, showing the highest prevalence (72.9%) among adults (40–59 years) in comparison with younger age groups (5–9 years of age; 34%), and mainly affecting men (Cedillo-Rivera et al. 2009). In Merida, Yucatan (southeast Mexico) *G. lamblia* cysts appeared in the feces of 33/429 children (7.6%), only slightly fewer cases than those infected with *B. hominis* (N = 45). These parasites were the most predominant. This work reported that six *G. lamblia* isolates corresponded to B genotype, which may be associated with persistent diarrhea (Torres-Romero et al. 2014). Another study that involved children from the northern part of the country reported that infection by *G. lamblia* compromises the absorption of vitamin A in the intestine, causing the mobilization of retinol which is stored in the liver for deficit compensation (Astiazaran-Garcia et al. 2010). As a matter of fact, the National Health Survey (2012) revealed that deficit of vitamin A is still considered to be a public health problem among Mexican children, especially in the southern part of the country (20.3%). Factors that reduce levels of vitamin A are: poor consumption of foods enriched with retinol and presence of intestinal parasites such as *G. lamblia* (Villalpando et al. 2015).

Another protozoan infection affecting Mexican children is amebiasis caused by *Entamoeba histolytica*. Fortunately, as it has already been documented, morbimortality by this parasitic disease has decreased over the time. A recent study conducted in a rural locality of Morelos, Mexico (central part of the country) with 309 children, revealed that 23.3% of these children were mainly infected with *B. hominis* and *G. lamblia* (25, 19.4%, respectively), whereas 9.7% were infected with *E. histolytica*/*dispar*. The ones presenting infection by *E. histolytica* did not show apparent health problems or nutritional deficiencies (Rojas et al. 2016).

Conclusions

In Mexico, infectious diseases caused by intestinal parasites such as protozoa and helminths are still a major public health problem. Even though the actions taken for their eradication are not complex, these diseases have remained prevalent for more than three decades. Although morbimortality rates from these diseases reveal a significant decrease over time, they must be still considered as one the most important infectious diseases, particularly in the southeast part of the country. These infectious diseases may cause several negative effects among inhabitants, causing morbidity and impacting the economy of the nation. A number of studies

have demonstrated the benefits of implementing recommendations made by WHO with the purpose of fighting against neglected diseases. Nonetheless, it is necessary to invest in well-being and it is necessary to do it through the application of programs directed to such "red flags." The fact remains that, inequality is still a challenge to overcome, along with other factors such as corruption, which negatively affects life expectancy and access to health services in this country (Idrovo 2005).

Policies and Protocols

Protocol

Measuring the Nutritional Status of the Mexican Population

This chapter addresses the nutritional changes experienced among the Mexican population. With the purpose of evaluating the nutritional status it was necessary to access the ENSANUT survey (ENSANUT 2015). In these terms, weight is determined through a scale (accuracy of 100 g), size is determined through a stadimeter (accuracy of 1 mm), and length is determined through an infantometer (in child under the age of 2 years of age). For children under the age of five, the nutritional assessment was determined through the "Z scoring" value of the anthropometric indices W/A, H/A, and W/H. Its interpretation, based on the scoring or "Z" value, is performed in accordance to the criteria provided by the world health organization (WHO): underweight, stunting, and wasting, when the W/A, H/A, and W/H (respectively) are of less than −2 DE in relation to the median of the reference population. If the "Z" value of the P/T indicator is superior to 2 DE, it is considered as overweight. For children over 5 years of age, we must calculate the Body Mass Index (BMI) in accordance to the International Obesity Task Force. For persons over 20 years of age, the BMI will be interpreted in terms of the WHO: underweight (<18.5 kg m^{-2}), healthy weight (18.5–24.9 kg m^{-2}), overweight (25 to 29.9 kg m^{-2}), and obesity (>30 kg m^{-2}) (Rivera-Dommarco and Cuevas-Nasu 2013).

Analysis for Intestinal Parasites

The current chapter deals with information about the incidence of intestinal worms which mainly affect Mexican children. These parasites are identified, through optical microscopy, using the zinc sulfate flotation technique which allow the identification of protozoa (cysts, trophozoites) and helminthes (eggs, larvae). This requires 15–20 g of fresh feces, conserved in 5–10% of formaldehyde (Garcia et al. 2005). The helminth parasitic load was determined using a quantitative protocol known as the Kato Katz technique. The interpretation is as follows: for *A. lumbricoides* finding <5000 epg is considered light infection, moderate infection includes 5000 to $<50{,}000$ epg, and severe infection are those individuals with $>50{,}000$ epg. Other examples include: *T. trichiura,* light (<1000 epg), moderate (1000 to $<10{,}000$ epg), and severe ($>10{,}000$ epg) while for *N. americanus,* light (<400 epg), moderate (400 to <3000 epg), severe (3000 to $<10{,}000$ epg), and very severe ($>10{,}000$ epg) (WHO 1987).

Policies

Policies to Improve Access to Health and Food in Mexico

In this chapter we described how malnutrition in Mexico is mainly localized in the southeast where a great portion of indigenous people live in conditions of poverty and marginalization. On the other hand, in the north part of the country we may observe overweight and obesity. In 1997, the Mexican government implemented the program called "Progresa" with the purpose of fighting malnutrition. "Progresa" was focused to indigenous children from rural areas. Through the program, their families received fortified foods, health services, and financial support conditioned to regular attendance to medical check-ups and taking their children to school (Rivera et al. 2004). In 2002, the program was extended to all states of the country under the name of "Oportunidades" (Leroy et al. 2008). In 2014, the program changed again its name to "Prospera" with the main goal of fostering productivity, promoting better family income, and obtaining financial and labor inclusion.

Dictionary of Terms

- **Stunting** – Children with low stature with respect to age.
- **Incidence** – Number of new cases of a disease in a period of time.
- **Mild protein-energy malnutrition** – Weight loss below 2 but less than 3 standard deviations with respect to the threshold mean value for a reference population.
- **Morbidity** – Number of persons affected by a disease in a space and determined time.
- **Mortality** – Number of death caused by a disease in a space and determined time.
- **Seroprevalence** – Number of persons with antibodies against a disease, in a space and determined time.
- **Retinol** – Synonymous of A vitamin with biological actions on retina, bone tissue, and skin.

Summary Points

- This chapter focuses on malnutrition and the main intestinal parasites that affect Mexican population.
- The prevalence of the mild protein-energy malnutrition among Mexican population has decreased until 2013; however, the sudden onset of obesity affects this population at present.
- The obesity affects mainly people from northern Mexico, whereas malnutrition is observed among indigenous infantile population from southeast.
- The intestinal amebiasis and helminthiasis are the main intestinal parasites affects Mexican population from center and south of the country.

- The income inequality, poverty, and marginalization are the main factors that favor malnutrition and intestinal parasites in Mexico.
- However, we argue that in effort to fight against malnutrition and intestinal parasites, it is necessary to have a universal national health service as well as reduce the social and economic inequality in Mexico.

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Management Approaches for Desalination and Water Supplies for Drought

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Abstract

The act of ensuring freshwater is considered the most essential and basic need for humanity. Although the planet is water-rich in some terms, the freshwater sources available for human consumption and beneficial uses are very limited. Excess population growth, industrial development coupled with improving living standards have caused an unprecedented need for freshwater supplies all over the world. Regions once rich in water resources are struggling to meet the ever increasing demands in recent years. In addition, climate change and unsustainable water resource management practices have led to situation called "drought" in many regions. Water supplies in drought conditions can be addressed by taking two major approaches related to management and technology development. The management approaches include demand mitigation and supply enhancement. Demand mitigation can be done by implementing water conservation practices and by enforcing a mechanism to influence user-responsible behavior through higher water fares and other billing routes. Supply enhancement can be achieved by utilizing the methods available for water reclamation, reuse, and recycle including rain harvesting. This chapter provides a critical insight of the causes for drought and the issues caused by persistent drought conditions followed by discussion of management approaches required to maintain adequate water resources in these regions.

Keywords

Drought · Water scarcity · Water reuse · Recycle · Ground water · Sustainability · Energy · Pollution · Water supplies · Desalination · Aquifer storage · Dams · Rain harvesting · Public education · Outreach

Introduction

Water is an essential natural source which is often considered to be available abundantly and free of cost (Yarlagadda et al. 2011). However, due to various reasons related to population growth and increasing living standards all over the world and climate change, these water sources are dwindling at a faster rate than they can be naturally replenished. Most of the world population still depends on groundwater which is available in finite reserves and other populations along the coastal areas depend on the seawater as a source of water supply.

Water is one of the most abundant resources of the earth which covers three-quarters of the earth's surface. About 97% of this volume is saline, and only 3% is fresh water, suitable for humans, plants, and animals. Of this fraction, nearly 2.5% is blocked in polar ice caps, glaciers, and atmosphere, leaving about 0.5% of water accessible to human needs (Gude and Nirmalakhandan 2009). In addition, many communities around the world face water supply challenges due to increasing demand, persistent drought, resource depletion and impairment of surface and of groundwater, and dependence on single sources of supply. Many

of these communities have access to brackish groundwater and seawater, which make the suitable for desalination. While other approaches such as water reuse and recycling help address the water shortage problem, treatment technologies are not affordable.

Causes for Water Scarcity

Causes of water scarcity are related to drought, overuse, anthropogenic pollution, physical distance, and political and social stress.

Water Scarcity Due to Drought

Drought can be occasional, temporary, and perpetual which can have detrimental effects on the society and local water sources (Iglesias et al. 2007). Occasional droughts can be recovered from excess precipitation. Temporary drought usually occurs on annual basis, which may also be recovered in successive years. Perpetual droughts are related to those areas which decertified over long period of time due to climate change, overuse, and poor management practices. It has been noted that some regions of the world have experienced historical droughts in recent years. For example, the California's drought has increased over the past decades due to climate change and global hydrological patterns (Gude 2016). Australia has experienced so-called millennium drought.

Although considered a water-rich country, United States has some disparities in its water source availability. In addition, populations in many regions have increased between 100% and 400% within the four decades. For example, the population of Texas has doubled in this period, while the populations in Florida, Arizona, and Nevada have increased by 175%, 275% and 450%, respectively (U.S. Census 2010; NRC 2012). The southwest region of the country is arid and drought-prone, while other parts of the country are reasonably well sourced. It is estimated that an additional 60.56 billion m^3 per year of water may be required by 2020 for municipal and light industrial uses in USA (Cohen 2007). In California alone, combined agricultural, urban, and environmental demands already exceed average supplies by 1.23 109 m^3 (326 billion gallons) per year, while 50% of the nation's future population growth is forecast to occur in CA, FL, and TX regions already experiencing water shortages.

One of the highly populated and driest continents in the world is Australia (Australian Government National Water Commission 2005). Its water supply sources are continuously being jeopardized due to climate change patterns of severe droughts followed by forest fires and floods. The continent experienced worst drought for many years since early 2000s (Hall et al. 2011). Similar to California in USA, 10–15 years of low rainfall (El Saliby et al. 2009) known as "millennium drought" (most dry period in more than 100 years) has been the cause for the urgent rise of desalination and water reclamation plants in Australia.

Water Scarcity Due to Overuse

Population growth, urbanization, and economic development in many regions of the world have caused overuse of water (Kumar and Singh 2001). Population growth alone stimulates the need for additional water resources for meeting the basic necessities of water for food production and hygiene and commodities. Some other causes for overuse are related to industrial processing of water due to high living standards which give rise to use of electronic devices and tourism where luxurious use of freshwater is a necessity. In some regions of the world, there is a situation called "Artificial shortage of surface water" which is caused by the excess withdrawals than the actual need (Slavikova et al. 2017). This happens due to poor water demand management. As the excess withdrawal becomes a norm, this leads to a situation where the availability of surface water declines, additional costs may be incurred to provide these excess volumes.

Water Scarcity Due to Anthropogenic Pollution

Environmental pollution due to human activities such as domestic sewage, effluents originating from agricultural, concentrated animal feed (CAF) operations for dairy and meat production, illicit discharge of construction and industrial wastewaters and other pollutants originating from storm water, heavy floods, and recreational activities may reduce availability of acceptable quality raw water for human consumption (Dokulil et al. 2000).

Water Scarcity Due to Physical and Political Barriers

In some cases, water resources may be available in adequate quantities but at a distance that cannot be economically reachable (Molden 2007). This is particularly true for rural communities in underdeveloped countries. This can be named as a physical barrier. This also relates to geographical barriers across the nations that will not permit transport of water. The political barrier relates to those conflicts arising from the political interests between the water-rich and water-scarce regions.

Issues Caused by Water Scarcity and Drought

Lack of access to drinking water and basic sanitation: Water scarcity causes lack of access to clean drinking water required for basic sanitation (water needs for bathing, cooking, and cleaning) and hygiene. This results in use of available impaired waters which causes development of new diseases and other issues public health issues. Many children and infants loose lives due to consumption of unclean water.
Hunger and poverty: Regions facing drought and water scarcity often have limited production of essential crops and meat. This causes hunger and impacts the morality

of the people in these regions. Prosperity and well-being is affected severely leaving populations in poverty. Poverty and the drive for ensuring reliable supplies of food will cause internal and external conflicts.

Education: Poverty and lack of basic sanitation will lead to lack of education. This causes the communities lacking education are not necessarily knowledgeable in managing the local, limited water supplies. They need education to understand the value of water and to manage this precious source in an equitable and beneficial manner.

Water Quality in Drought Areas

Groundwater Quality

Increasing withdrawals of freshwater due to population explosion and industrialization from current dwindling surface water sources have led the populations around the world to depend on the ground water sources for domestic and agricultural uses. These resources are finite and deplete with continued activity called as "mining" for our increasing demands. Fig. 1 shows the regions in the USA where groundwater depletion is highly pronounced. In some regions, though they were originally water-rich, the depletion rates are very high (Konikow 2013). This is usually associated

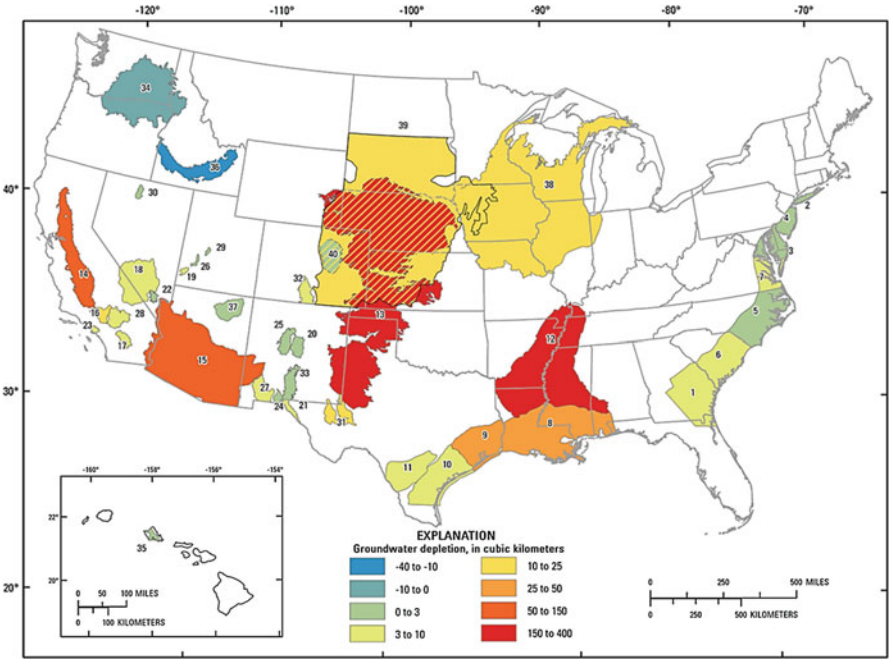

Fig. 1 Groundwater depletion in the United States between 1900 and 2008 (Konikow 2013, USGS)

with increased withdrawals for irrigation as in the case of Mississippi. In some cases, it is compounded by the water needs due to excessive population growth, for example, the water crisis in Central valley of California.

As the quantity declines in many groundwater reserves, quality of the source becomes unsuitable for many purposes when other undesired groundwater chemistry that could take place when soil and water contact. In many parts of the world, ground water is not suitable for direct consumption. They are impaired either due to naturally occurring hazardous substances such as uranium, fluoride, and arsenic (Yarlagadda et al. 2011). In some cases, the impairment is caused by illicit discharges by industrial sectors. Often, these ground waters also contain high dissolved solids, leading to their categorization as brackish waters. Traditional treatment techniques are not adequate to remove the dissolved solids. For instance, two-thirds of the continental United States including New Mexico has large volumes of saline water sources. The total volume of ground water in aquifers in New Mexico is estimated to be 20 billion acre-feet; however, 75% of the groundwater is too saline (10,000–35,000 ppm) for most uses and the remaining 25% of the ground water contains dissolved concentrations of lower than 2000 mg/L.

Brackish Groundwater

Although there are many groundwater sources available in some parts of the world, they are often not suitable for direct use. These sources are highly saline which requires for additional treatment to remove the salts to produce potable water or water suitable for other nonpotable uses. Similar to many parts of the world, some regions of the United States, especially southwestern part, are in critical need of potable water supplies. As mentioned earlier, extreme population growth and industrial development coupled with unsustainable practices have resulted in unmanageable water demands. As shown in Fig. 2a, some of the highly populated cities are located in water-scarce regions. In addition, about 50% of the population is concentrated in coastal regions where access to freshwater is an issue (Fig. 2b). Other regions that are in desperate need for freshwater are the inland communities which are committed to agriculture and irrigation as shown in Fig. 2c. It can be noted that groundwater sources in many of these regions contain high dissolved solids concentrations which render them not suitable for most potable uses including irrigation. Nontraditional technologies such as membrane and thermal desalination processes should be considered to treat these water.

Water Scarcity Around the World

At present, one-third of the world population is lacking access to clean water and by 2025, 1.8 billion people will experience absolute water scarcity, and two-thirds of the global population will be living under water-stressed conditions (Macedonio et al. 2012). Many parts of the world are reaching a critical situation of water scarcity (see Fig. 3). Water scarcity is classified into physical water scarcity and economic water scarcity (Rijsberman 2006). Physical water scarcity means that there are simply no adequate water resources to meet the current needs. The economic water scarcity refers to a situation where the water sources are available in abundance (water resource

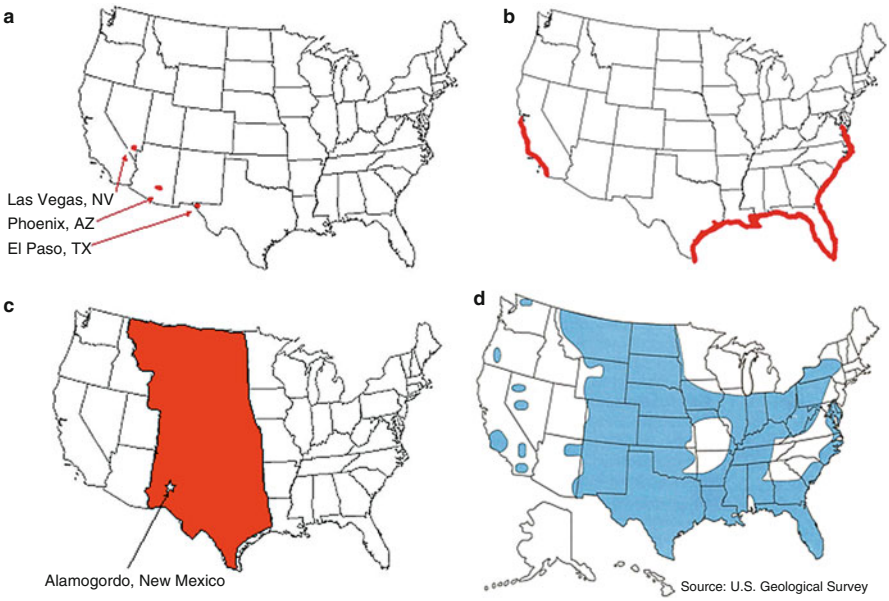

Fig. 2 Water-scarce regions and ground water quality in the USA: (**a**) major water-scarce, populated inland cities in USA, (**b**) highly populated coastal communities, (**c**) water-scarce inland rural communities due to excess irrigation needs, and (**d**) brackish water reserves in the USA

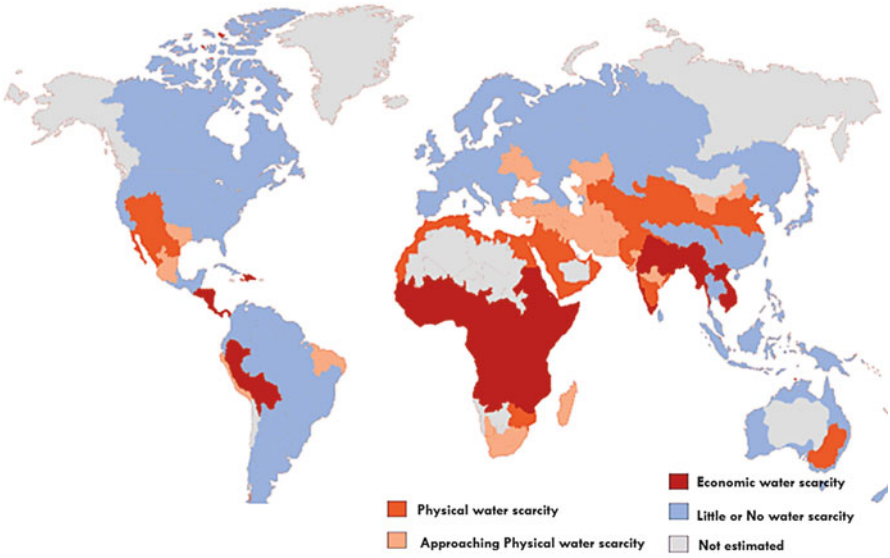

Fig. 3 Different types of water scarcity around the world (Fewresources.org)

availability is higher than the withdrawal, or the resource capacity is underutilized), but the mechanisms to acquire access to these sources have not been established or are influenced by human, institutional, and financial capital limitations. This could be because of the economic issues, polluted water resources, and political and social and climatic issues. In most cases, the economic water scarcity refers to a situation where the affordability to obtain clean water has been jeopardized due to economic issues. Physical water scarcity is currently experienced in southwestern region of the United States which includes California, Arizona, New Mexico and Texas and Mexico, Middle Eastern and North African countries (MENA region) and southeastern European countries, Southern India, Pakistan, Mongolia, Afghanistan, Kazakhstan, Turkmenistan, Uzbekistan, Tajikistan, and western Australia. Economic water scarcity is experienced in central and Latin America including Caribbean region, South and central Africa, North India, some parts of China and some other Asian countries. In some of these countries, desalination supported by renewable energy sources could be implemented as a sustainable option, but many nontechnological barriers need to be overcome.

Water Stress Regions in the World

Over the past century, the worldwide population has tripled, while the water use or water withdrawals increased more than six-fold which suggests increasing water consumption mostly driven by improved living standards and industrialization all over the world. Many regions realize the inadequacy of existing freshwater sources to meet ever growing water demands. In some cases, sufficient surface and ground water sources to even meet current demands for water supplies are not available. This situation is called stress. Fig. 4 shows the water stress regions around the world. It can be noted that the high populated countries with economic development are facing severe stress, while some developed countries such as Australia are facing water stress due to climate change and desertification.

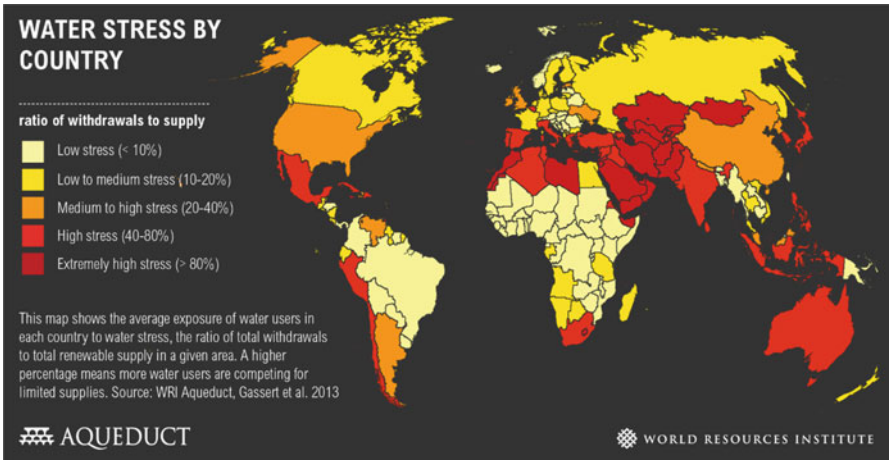

Fig. 4 Water stress levels around the world (wri.org)

Solutions to Water Supply Issues in Drought Areas

Water supply issues in drought areas can be addressed using different approaches. This is a multifaceted problem which requires involvement from users, governing bodies, industrial partners, and research and development and regulatory agencies. Figure 5 shows the approaches that should be considered for a holistic management of the problem. The solution includes mitigation of current demands where possible with proper governance and supply enhancement to meet the demands by management and technological approaches.

Demand Mitigation

Conservation activities such as retrofitting water-saving fixtures in domestic applications, industrial production, and other public amenities where water use is a must. Agricultural sector contributes to most of the water loss followed by power production industry. Water conservation practices in irrigation practices can make significant improvement in this aspect. About 30–75% of the water withdrawals are lost in power production, especially in cooling applications. Water losses due to evaporation can be reduced by considering other novel approaches such dry cooling and among others (Gude 2015a).

Water is often considered abundant natural source available freely in abundance. This is a misperception that many of the communities carry across generations. Water should be realized as a precious and dwindling commodity to ensure its availability for future generations to come. This can only be achieved by enforcing appropriate water pricing dependent on the socioeconomics of a community. This will enable improve user behavior in terms of responsible use and concern for its protection and management. This approach requires active involvement of public,

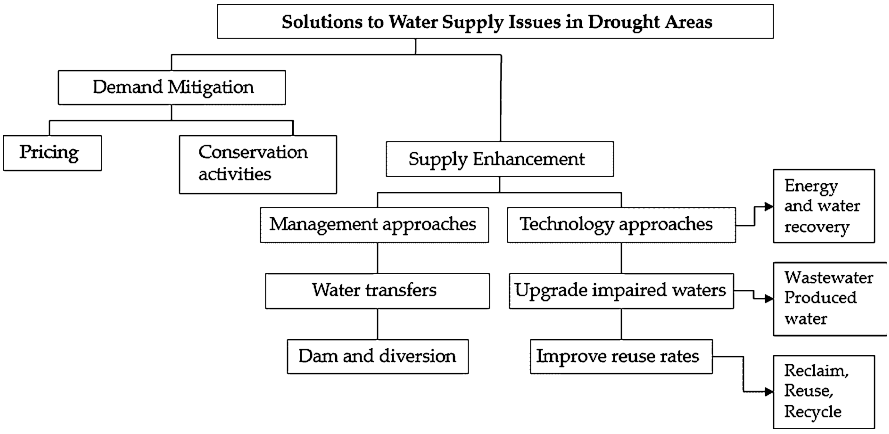

Fig. 5 Solutions to holistic management of water supply issues in drought areas

administrators, and industrial partners. Public education and outreach is a key to achieve success. The main drawback is that the progress can be slow as it depends on the user psychology, preparation levels, and willingness to protect the valuable resource.

Demand mitigation approaches do not solve the supply problem directly, but they can establish a system in which water supply options can become more feasible and affordable.

Supply Enhancement

Water supplies can be enhanced following management and technological approaches. Water management approaches include immediate or near-future construction activities or those intended for long-term sustainability. Short-term and immediate approaches involve water transfer acts across the regions. The main mechanisms in which water supply enhancement can be done is by: intercepting (rain harvesting), diverting (water diversions), storing (dams and reservoirs), and transferring water (transport water among basins or regions). The following paragraphs discuss the

Water transport – Water management can be done by considering transport of water from water-rich region to a water-scarce region if affordable water costs can be achieved through this mean. For example, many countries such as Singapore, USA, and other European countries already practice this approach as a water supply alternative (Gude 2016). International transfer and interstate transfer is possible depending on the situation. The main drawback with this option is that this arrangement may create some political stress and economic dependence on water-rich countries. Construction of dams and water flow diversion can be considered as other feasible approaches when dealing with the surface water sources with seasonal and geographical changes.

Technology development – Technological approaches include innovation in the present technology based on scientific discovery and practical feasibility. Many technologies are available that produce high quality and safe drinking water or water suitable for other beneficial uses. Technological approaches depend on the type of source water as well as the purpose of the end product. Surface and ground water sources with less than 3000 mg/L total dissolved solids (TDS) can be treated with conventional technologies such as coagulation, flocculation, lime-softening, and sedimentation and filtration processes. Membrane technologies can also be used to remove the TDS effectively. Ground waters and seawaters having TDS higher than 3000 mg/L should be treated using membrane or thermal desalination technologies.

Energy efficiency and water recovery – As shown in Fig. 5, energy efficiency and water recovery are the two important factors in determining the feasibility of a desalination technology. Membrane technologies offer superior performance at lower specific energy consumption, while thermal technologies require both thermal and electrical energy. In general, both technologies are energy-intensive and cost-prohibitive. However, the absolute necessity for water makes these technologies viable. In some regions where low-cost thermal energy is available as a result of power generation, thermal

technologies are more feasible. The reason for utilization of thermal technologies is also often associated with high TDS seawater and seasonal fluctuations in temperatures. Energy recovery schemes have offered a less energy-intensive desalination option in both membrane and thermal technologies. Reuse and recycling is the fundamental principle followed in thermal technologies, whereas the advent of energy recovery devices has made the membrane desalination a feasible option despite high capital and maintenance costs. More details on the energy recovery aspect are not within the scope of this chapter and hence not discussed here. However, readers are referred to other publications in this area (Gude 2011, 2015a, b).

Rain harvesting – Some drought regions face water scarcity not because of low precipitation levels, rather due to lack of methods for capturing the excess water during rainy events which is usually drained or lost to the environment. Rain harvesting has been practiced for centuries as a simple, proactive alternative to deal with the dry seasons. Rain harvesting can be done at very small to large-scale applications depending on the water needs. Capturing the rain water itself can be done many ways (Sharma and Smakhtin 2006) which are in situ harvesting, cisterns/tamks/kunds, Khadin system, Nadis/tobas, check dams, and percolation tanks.

In situ rainwater harvesting is practiced all over the world by developing field bunding, contour bunding, ridging, conservation furrows, key line, and contour cultivation as measures. Vegetative barriers to replace or supplement earthen bunds that emerged in 1980s have been tried in a number of countries with mixed results. Constructed microcatchments is another method to capture the rain water in in situ conditions. Cisterns/tankas/kunds that are essentially underground storage tanks used most commonly for centuries and more predominant in the Indian arid zone, Pakistan, Sri Lanka, China, and several other countries, generally constructed for storage of surface runoff.

Run-off water harvesting based farming (khadin farming) for rain water capture and moisture conservation is well suited in deep soil plots surrounded by some sort of natural catchment zone. Nadis/tobas are small to medium sized excavated or embanked village ponds, for harvesting meager precipitation to mitigate the scarcity of drinking water and domestic needs in water scarcity regions. Pond water is available for periods from 2 months to a year after rain, depending upon the catchment characteristics and amount and intensity of rainfall. The nadis range from 1.5 to 12 m in depth, 400 to 700,000 m^3 in capacity and have drainage basins of various shapes and sizes (8 to 2000 ha) (Sharma and Smakhtin 2006).

Series of check dams on natural streams provide for artificial recharge by restricting the surface run off and by making additional water available for percolation. The surface water is impounded during monsoon behind the structure and spread over the entire streambed and thereby increasing the wetted area. The impounded water helps in replenishment of groundwater. Percolation tanks are generally constructed on the small streams or rivulets with adequate catchment for impounding surface runoff. These tanks are used entirely for recharging the aquifer through percolation. In comparison to ponds, percolation tanks conserve water to a greater extent because the filling and recharge occur mostly during the monsoon when the evaporation rate is about the half of potential rate in summer through which ponds contain water.

Some of the above methods can be implemented at small-scale, while the others are more suitable for large-scale water management. Large-scale rain harvesting in drought-prone areas can be realized as a strategic tool for drought mitigation, if it can be implemented through the adoption of relevant policies and investments at different levels such as user, watershed, urban locality, district, state, and a country.

Water reclamation and reuse – Supply enhancement can be achieved by utilizing impaired waters such as treated secondary effluents (wastewater) for water reuse related to nonpotable uses, and other impaired waters used in process cooling and heating applications and produced waters from shale gas production. As the conventional water supplies are diminishing and the need for water reuse is inevitable, water reclamation and reuse allows for securing the longevity of available water resources for more critical needs, while the recycled water can be used for nonpotable uses. The most common nonpotable uses include irrigation, for example, agricultural, horticultural, and green spaces; environmental purposes; and domestic nonpotable uses such as third pipe systems. These uses are often employed to reduce the pressures on other existing water sources or to provide a water source where a suitable one does not already exist.

Water reuse has been practiced for over 5000 years; however, during the last 100 years, efforts have been made in many regions of the world, for the production of high-quality reused water, following strict quality guidelines (Angelakis and Gikas 2014). Wastewater sources for water reuse can be of four types: municipal wastewater, industrial wastewater, agricultural wastewater, and grey water (wastewater from clothes washers, bath tubs, showers, and sinks). Reclaimed water reuse for other potable uses has been very well accepted in many parts of Australia (Dillon 2009). Grey water reuse is widely practiced in United States, Europe, and many other countries in Middle East. Around 75% of wastewater is reused in the Middle Eastern countries. United States has a long history of water reuse beginning in 1912 for watering the lawns in California. USEPA has set the guidelines for the water reuse practice based on the type of use. The quality of the product water for reuse differs for various applications such as urban reuse, agricultural reuse, recreational impoundments, construction uses, cooling uses, groundwater recharge of potable aquifers, and augmentation of surface suppliers (WEF 2006). Kuwait and Qatar take lead in water reuse program with more than 10% of reclaimed water out of total water supply (Madwar and Tarazi 2003).

Water reuse implementation – Water reclamation and reuse programs have been widely adopted across the world in view of the increasing demands for water supplies. However, the contribution from this approach still remains to be unsubstantial (Miller 2006). Including water reuse option in the water supply and management portfolio helps to realize the true value of the contribution from this approach. It can be instrumental in water policy development and its implementation, which may lead to long-term sustainability (especially, availability) of these resources. However, including water reuse in the overall water supply and management portfolio can create many challenges rising from water governance, health risks, regulatory aspects, and public perception. Other requirements would be the need for developing innovative technologies, technology transfer, and novel applications and public education. Novel technologies are essential to remove the emerging contaminants such as endocrine disrupting chemicals and pharmaceutical and

personal care products. In some regions, groundwater recharge or aquifer storage is considered a long-term sustainability approach.

Options for water reuse implementation – Several options can be considered to make the water reuse and recycle programs to be effective (see Fig. 6). These options are education and outreach, incentives, removing barriers, and mandates and regulations (GE 2015).

Education and Outreach may include programs that promote certification programs and recognition awards. Programs to disseminate the scientific information and general benefits and essential facts about the water reuse by education and outreach efforts. Educating the users on water withdrawal trends, consumption, discharge, and reuse data can help create interest in water reuse implementation.

An incentive program including direct subsidies, reductions in payments to the government, payments for water reuse, adjusting pricing mechanisms, regulatory relief for recycled water users, government procurement of water recycling/reuse equipment, and structuring of water rights to reduce the use of potable water can be beneficial in supporting the water reuse implementation.

Regulatory reliefs may include modification of local regulations depending on the application especially in terms of water quality standards, revising plumbing codes to allow dual piping, and monitoring permitting and inspection requirements for recycled water.

Creating mandatory requirements should consider utility planning and management to develop plans for recycled water, restricting potable water to human or food-related uses, and requiring the use of recycled water for agriculture and other nonpotable uses and requiring water recovery systems.

Role of desalination technologies – Finally, combining water reuse and desalination mechanisms may help achieve supply enhancement (Gude et al. 2010).

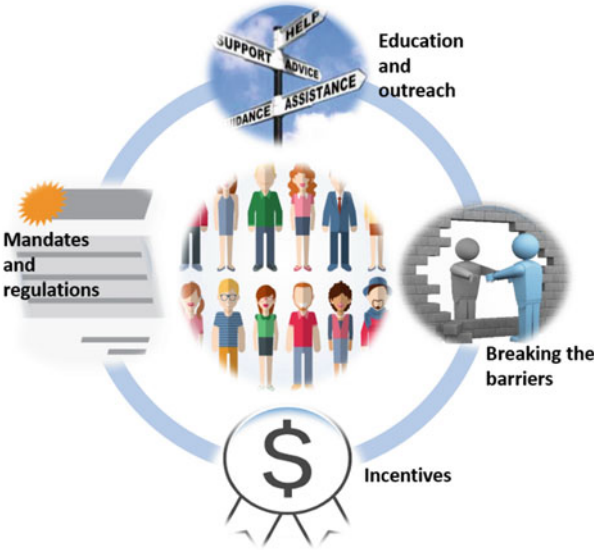

Fig. 6 Options for water reuse implementation

Technologies available for these two approaches make it possible to convert wastewater into high-quality water that suits various industrial applications because these technologies share similar characteristics. Feed source for both is an impaired water source whether it is seawater or produced water. The technology that is used most widely for water reuse or desalination is based on membranes for several reasons. Desalination by reverse-osmosis membranes combined with the use of conventional pretreatment units or modern pretreatment technologies are now well-established methods of wastewater desalination techniques (Madwar and Tarazi 2003). Microbial retention is a major concern in water reclamation which can be achieved by microfiltration (MF) and ultrafiltration (UF) (Côté et al. 2004). MF and UF are employed as preferred pretreatment processes for nanofiltration (NF) or reverse osmosis (RO), i.e., the quaternary treatment step which can produce drinking or ultrapure process water quality. This dual membrane treatment concept plays now a major role in water reclamation schemes that are aimed at advanced levels of purification. Applications include several aquifer recharge projects (one even for indirect potable reuse) and dual water systems in households and industrial process water, or for mixed urban and agricultural uses. Water production costs to treat the effluent from the conventional activated sludge followed by membrane bioreactors through RO process have been compared with the seawater process. The total life cycle costs for the produced water from secondary effluent (RO) are two times cheaper than the traditional desalination process from seawater (SWRO). The advantages of the reclaimed process over the traditional seawater process are the following: no pretreatment required, higher recovery with higher flux rates at lower operating pressures (energy savings) and longer membrane life.

Affordability of Desalination as Water Supply Alternative

Energy requirements for desalination have fallen significantly in recent years. Although the energy budget for domestic desalination units appears to be reasonable, many communities around the world cannot afford these energy demands and associated costs. Figure 7 depicts the desalinated water affordability around the world. The vertical axis presents the cost per capita of desalinated water to all at all times. The horizontal axis depicts the ability to pay for water and it increases from left to right.

Four basic quadrants can be identified. The bottom right quadrant depicts situations where the costs of supplying water in a sustainable manner are low and countries' ability to pay is high. These situations are limited largely to rich humid regions, particularly in Western Europe and North America. In these countries, the main cost may pertain to assuring adequate water quality and pollution prevention. The top right quadrant pertains to regions where the per capita cost of clean water supply is high, but as these regions are rich these costs are still only a small share of the total gross national product (GNP) per capita. Thus, countries in this quadrant, such as the arid but rich Gulf oil states, can afford the high cost of clean water supply even when this includes large-scale seawater desalination. These countries may enjoy the desalinated water, despite their extreme first-order scarcity due to availability of ample nonrenewable energy sources and inexhaustible seawater sources. The bottom left area

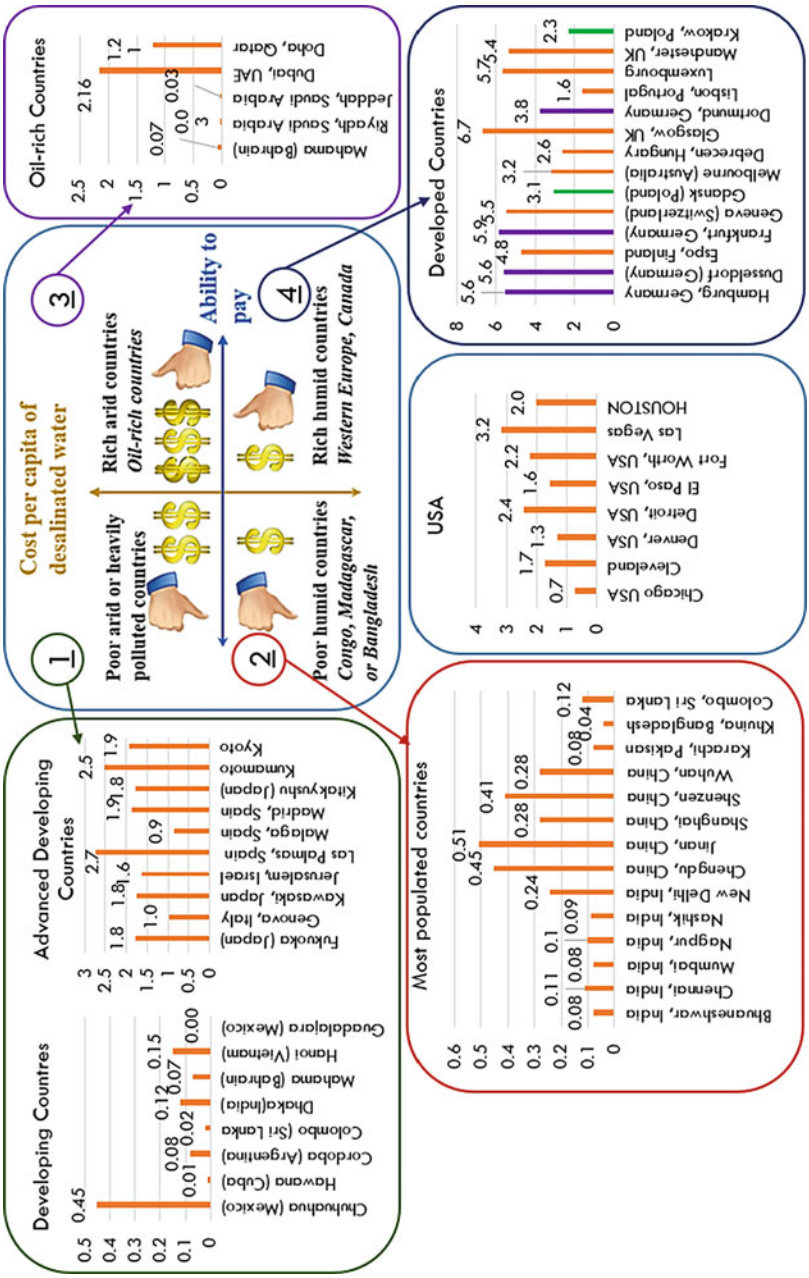

Fig. 7 Desalination affordability around the world (adapted from Gude 2016)

depicts poor regions with ample water resources. In these regions, the cost of desalinated water supply may be low, but as the GNP is also low even these comparatively low costs may become a significant burden. These countries do not suffer from first-order scarcity. Because of second-order scarcity and particularly low financial capacity, desalination may not be an attractive alternative. Examples of such countries would include Congo, Madagascar, or Bangladesh. The worst situation, however, is depicted by the upper left quadrant. This is the situation where the per capita cost of sustainable clean water to all people at all times would require high cost per capita and in which such costs would be a large share of the per capita GNP. Thus, regions in this situation may find that the costs of sustainable clean water are unaffordable for their citizens and especially through desalination technologies. The countries most prone to this situation are, naturally, poor countries in arid or semi-arid regions. Such countries may have had relatively high adaptive capacity, as demonstrated by traditional societal institutions and infrastructures, and yet may not have the financial resources to cope with rapid increases in population or pollution due to a weak economic base. Desalination alternative may not be meaningful for many low income countries due to very low GNP where other meaningful indicators could make more sense (Feitelson and Chenoweth 2002).

Protocol

Determining the Water Supply Options

This chapter described various alternatives and pathways for expanding freshwater supplies for drought conditions. It is important to consider demand reduction alternatives prior to considering the supply enhancement schemes. Water reuse and recycle programs reduce the stress on water sources, while the residual demands can be met with desalination alternative which should be considered a final resort.

Policies

Local Governmental Policy for Water Reuse Implementation

This chapter included four major approaches that local governments should consider to encourage water reuse and recycling alternatives. These include:

- Education and outreach (making public aware of water supply issues and need for reuse)
- Breaking the barriers (providing funding and technological support to implement)
- Incentive program (such as tax reliefs and carbon credits for water reuse)
- Regulatory reliefs (related to water quality for reuse options)
- Mandates and regulations (strictly enforcing water reuse as mandatory for new developments).

Dictionary of Terms

- **Desalination** – Is a process that removes dissolved salts and minerals from saline waters. Saline waters include brackish groundwater and seawater.
- **Brackish water** – Is water that has higher concentrations of salt than in freshwater, but not as much as seawater.
- **Microfiltration** – Is a type of physical filtration process where a contaminated fluid is passed through a special pore-sized membrane to separate microorganisms and suspended particles from process liquid.
- **Nanofiltration** – Is a relatively recent membrane filtration process used most often with low total dissolved solids water such as surface water and fresh groundwater, with the purpose of softening (polyvalent cation removal) and removal of disinfection by-product precursors such as natural organic matter and synthetic.
- **Reverse osmosis** – A process by which a solvent passes through a porous membrane in the direction opposite to that for natural osmosis when subjected to a hydrostatic pressure greater than the osmotic pressure.

Summary Points

- By 2050, the world's population will have grown from 7 to 9 billion. This enormous upsurge means the need for water will increase by over 50%, if we continue our consumption at the current rate. Many places in the world just do not have enough water. This is partly due to climate change, but especially due to increasing urbanization.
- Increasing demands for freshwater supplies can be met by management approaches based on the elements of demand mitigation and supply enhancement.
- Public education about the value of water and adverse effects of water impairment and water scarcity will help implement some of the management strategies for demand mitigation.
- Water reclamation, recycle, and reuse should be considered as a primary alternative to demand mitigation. Suitable technologies should be developed for this approach.
- Supply enhancement can be achieved by developing novel technologies. Technology advancement for treating high saline ground and surface waters should be considered for efficient utilization of these sources
- Improving water conservation practices can be the first step in demand mitigation. Sustainable water management practices should be explained to the farmers and other industrial users to reduce the water withdrawals and consumption.
- Improving sewage system as well as supporting clean water initiatives will help both ensure healthy surface waters which in turn can serve as safe reserves and sources for freshwater supplies.
- Governance and financing the clean water ad environment initiatives can help implement a long-term sustainable freshwater supply system in drought influenced regions.

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Technical Approaches for Desalination and Water Supplies for Drought

120

Veera Gnaneswar Gude

Contents

Abstract

Providing clean water for human consumption has become a major challenge at local, regional, national, and global levels due to excess population growth. The direct domestic water demand and the indirect industrial, agricultural, and environmental water needs to sustain this growth is expected to place serious strains on the currently available water resources. Water reuse and desalination technologies can provide a solution to this issue if implemented in a sustainable manner. Provision of clean water inevitably requires energy, which is currently being

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provided essentially by nonrenewable fossil fuels which is not a sustainable approach. This chapter discusses various options available for enhancing water supply in drought regions. Water reuse and desalination technologies have been discussed in detail. Energy needs and integration of renewable energy sources, energy recovery and process integration concepts have been discussed. Future research directions to develop energy-efficient water supply technologies are provided.

Keywords
Desalination · Drought · Water scarcity · Water reuse · Soil treatment · Wetlands · Cogeneration · Reverse osmosis · Membranes · Thermal desalination · Renewable energy · Microfiltration · Ultrafiltration · Disinfection · Ozonation

Introduction

Freshwater is an essential commodity for continued existence of human kind. Recent statistics show that currently 2.3 billion (~35 % of current world population) people live in water stressed areas and among them 1.7 billion (~25% of current world population) live in water scarce areas (Misra and Kupitz 2004; Greenlee et al. 2009). The situation is going to worsen further in the coming years. It has been estimated based on the current demographic trajectory; the human population will surpass 10 billion by the year 2030 (Ehrlich and Ehrlich 1993; McFalls 1991). Since 1990, the global rate of water withdrawal from surface and groundwater sources has increased six-fold, while the world population increased only three-fold. Water withdrawal rates are projected to at least double in the next two decades. Renewable water resources are on the decline in many countries due to increased population and rapid industrialization. Traditional fresh water resources such as lakes, rivers, and groundwater are overused or misused; as a result, these resources are either diminishing or becoming saline. As countries continue to develop and cities expand, few new water resources are available to support daily fresh water needs. Some areas of the world have a very low renewable energy sources (Greenlee et al. 2009), for instance, total annual renewable water resources 5500 m^3 per capita in Africa, about 3500 m^3 per capita in Asia, and as low as 636 m^3 per capita in the Arab world. The increased use of the fixed water resource in response to rising demands is not only reducing water availability, but also jeopardizing water quality (Hamoda 2004). While water conservation and reclamation/recycling practices provide partial solution to the water scarcity, appropriate technological solutions are considered to augment water resources through nonconventional sources (Hamad and Abdul-Karim 2005). Many of these technologies are based on desalination principle, separation of salts and/or other ions from seawater or brackish ground waters. As discussed in the previous chapter, water supply issues can be addressed by demand mitigation and supply enhancement. Demand mitigation can be implemented by water reclamation and reuse, and supply enhancement can be achieved through desalination processes which will be discussed in this chapter.

Water Reuse Technologies

Various chemical and microbial contaminants should be removed to make the wastewater sources suitable for potable or nonpotable uses. Advanced treatment using chemical and physical separation mechanisms is often considered as a treatment scheme. Numerous treatment options, including engineered and natural treatment processes, are available to achieve this goal. Selection of a suitable treatment scheme depends on the specifics of water quality objectives intended for specific uses (Miller 2006). Advanced treatment processes are also capable of removing emerging pathogens or contaminants such as endocrine disrupting chemicals and pharmaceutical and personal care products (Fast et al. 2017).

Natural systems are considered in treatment schemes where the water quality objective is a potable water reuse. These involve "natural barriers" such as soil aquifer treatment, wetlands, and other land application for soil infiltration to provide natural treatment of organic and nutrient compounds and microbial contaminants (Gude et al. 2013; Martinez-Guerra et al. 2015). This is achieved by providing a retention time suitable for natural attenuation of contaminants followed by blending with the water sources. Application of natural systems is subject to climate and hydrogeological conditions. These systems are not the preferred method currently due to lack of standardized guidelines for design and operation.

Engineered solutions are designed by taking the advantages of chemical, physical, and characteristics of the contaminants in the water (Gude 2015a). Conventional sand filtration is commonly used to remove the suspended solids, but membrane filtration processes such as microfiltration and ultrafiltration have become a more common practice in recent years. Chemical coagulation, precipitation, neutralization, oxidation, hydrolysis, and other reactions are considered for organic contaminant removal. Trace organics including volatile contaminants can be removed using granulated organic carbon (GAC) filters and ion exchange columns. Nutrients and other microbial contaminants can be removed using natural systems such as wetlands, soil application, and air-stripping and reverse osmosis processes. Disinfection is performed using chlorination, and/or ozonation, and/or ultraviolet exposure (UV), depending on the specific objective of the water reuse application. Table 1 shows the different membrane technologies and their specific characteristics in water treatment and desalination applications (Baker 2000; Tsuru 2001; WEF 2006). Microfiltration and ultrafiltration are used in lieu of conventional sand filtration process due to higher efficiency and smaller footprint. Nanofiltration can be used to remove some specific contaminants at a molecular weight cutoff with much lower specific energy consumption compared to reverse osmosis. Salts and other dissolved and micro- or trace contaminants can be removed using reverse osmosis membrane filtration.

Some examples of water reuse applications in the United States are shown in Table 2. It can be noted that lime coagulation, clarification, and media filtration are used in plants constructed in 1960s through 1990s. Membrane filters have become more dominant in the plants constructed after 1990s, mainly due to development of highly efficient membrane materials with lower energy consumption. Soil aquifer

Table 1 General characteristics of membrane technologies for water treatment and desalination (Adham et al. 1996)

Membrane type	Particle capture size	Typical contaminants removed	Typical operation pressure ranges	Key applications
Microfiltration	0.1–10 μm	Suspended solids, bacteria, protozoa	0.1–2 bar (1–30 psi)	Water treatment plants, pretreatment in desalination plants, the preparation of sterile water for industries, such as pharmaceuticals
Ultrafiltration	ca. 0.003–0.1 μm	Colloids, proteins, polysaccharides, most bacteria, viruses (partially)	1–5 bar (cross-flow) 0.2–0.3 bar (dead-end and submerged) (3–80 psi)	Drinking water treatment, the pretreatment process in desalination, and membrane bioreactors
Nanofiltration	ca. 0.001 μm	Viruses, natural organic matter, multivalent ions (including hardness in water)	5–20 bar (70–220 psi)	Treatment of fresh, process, and wastewaters
Reverse osmosis	ca. 0.001 μm	Almost all impurities, including monovalent ions	10–100 bar (800–1200 psi)	Treatment of fresh, process and wastewaters, desalination of sea water

treatment is also used often in plants where the intended end use is the recharge of local water supply aquifers.

Desalination Technologies

Desalination is a process that separates dissolved solids (mostly salts) from a saline water source to produce fresh water driven by an evaporative process (thermal desalination) or a mechanical filtration (membrane separation) process. Thermal desalination is based on the principle of evaporation of freshwater in the form pure water vapors from the saline water and condensation of the same on a cold surface to produce nearly pure water free of dissolved solids. The membrane processes employ a physical barrier (membrane) which allows the water molecules to permeate through to produce permeate with considerably low concentration of dissolved solids. Thermal processes require large quantities of heat energy to evaporate the pure water and the membrane processes require high-quality electrical energy to apply the mechanical pressure on the membrane to cause separation.

Table 2 Examples of water reuse plants and their operating schemes in the United States (National Research Council 2012)

Location	Type of indirect reuse	Project size MGD (10^3 m^3/day)	First installation Year	Current status	Treatment technologies				
					Suspended solids	Organic compounds	Residual nutrients	Residual salts	Pathogens
Montebello Forebay, County Sanitation Districts of Los Angeles County, CA	Groundwater recharge via soil aquifer treatment	44 (165)	1962	Ongoing	Media filtration	Soil-aquifer treatment	Soil-aquifer treatment	None	Chlorination, soil-aquifer treatment
Water Factory 21, Orange County, CA	Groundwater recharge via seawater barrier	16 (60)	1976	Terminated 2004	Lime clarification	GAC filtration; reverse osmosis; UV/AOP	Air stripping; reverse osmosis	Reverse osmosis	Lime clarification, chlorination, UV
Upper Occoquan Service Authority, VA	Surface water augmentation	54 (204)	1978	Ongoing	Lime clarification, media filtration	GAC filtration	Ion exchange (optional)	None	Chlorination

(*continued*)

Table 2 (continued)

Location	Type of indirect reuse	Project size MGD (10^3 m^3/day)	First installation Year	Current status	Treatment technologies				
Hueco Bolson Recharge Project, El Paso Water Utilities, TX	Groundwater recharge via direct injection	10 (38)	1985	Ongoing	Lime clarification, media filtration	Ozonation, GAC filtration	PAC augmented activated sludge system	None	Ozonation, chlorination
Clayton County Water Authority, GA	Surface water augmentation	18 (66)	1985	Ongoing	Land application system and wetlands	Land application system; wetlands	Land application system; wetlands	None	Chlorination, UV
West Basin Water Recycling Plant, CA	Groundwater recharge via direct injection	12.5 (47)	1993	Ongoing	Microfiltration	Reverse osmosis; UV/AOP	Reverse osmosis	Reverse osmosis	Microfiltration chloramination, UV
Gwinnett County, GA	Surface water augmentation	60 (227)	1999	Ongoing	Ultrafiltration	Pozonation; GAC filtration	Chem. Premoval	None	Ultrafiltration, Ozone
Scottsdale Water Campus, AZ	Groundwater recharge via direct injection	14 (53)	1999	Ongoing	Media filtration, microfiltration	Reverse osmosis	Reverse osmosis		Microfiltration Chlorination

Los Alimitos Barrier Water Replenishment District of So. CA	Groundwater recharge via direct injection	2.7 (10)	2005	Ongoing	Microfiltration	Reverse osmosis, UV	Reverse osmosis	Reverse osmosis	Microfiltration UV
Arapahoe County/ Cottonwood, CO	Groundwater recharge via spreading operation	9 (34)	2009	Ongoing	Media filtration	Reverse osmosis, UV/AOP	Reverse osmosis	Reverse osmosis	Chlorination
Permian Basin, Colorado River Municipal Water District, TX	Surface water augmentation	2.5 (9.4)	2012	Ongoing	Ultrafiltration	Reverse osmosis, UV-AOP	Reverse osmosis	Reverse osmosis	Chlorination

Thermal desalination technologies include solar distillation (SD) such as solar stills and active and passive solar desalination systems, multieffect evaporation/distillation (MED), multistage flash distillation (MSF), thermal vapor compression (TVC), and mechanical vapor compression (MVC). Membrane processes include electrodialysis (ED) and reverse osmosis (RO). Other processes that involve a combination of the two principles in a single unit or in sequential steps to produce pure or potable water include membrane distillation (MD) and reverse osmosis combined with MSF or MED processes (Gude 2015b).

Desalination has become an important source of freshwater supply and has taken place for a long time, predominantly by means of thermal processes (over the past 60 years) such as multistage flash distillation (MSF) or multieffect distillation (MED), followed by membrane processes developing over the past 40 years (Hamad and Abdul-Karim 2005; Gleick 2006). Desalination of all types is both cost- and energy-intensive (Pettersen et al. 1996). The process typically requires the conveyance of the water to the desalination plant, pretreatment of the intake water, separation of salts, disposal of the concentrate (brine), and process maintenance (Shannon et al. 2008). The energy requirements for desalination vary in the range of 3.5–25 kWh/m^3 depending on the source of water, desalination method, and capacity. Membrane technologies represent the lower end of the scale with specific energy consumption in the range 3–9 kWh/m^3, while thermal technologies are on the higher end of the scale with specific energy consumption in the range 10–25 kWh/m^3. For membrane technologies, the energy requirements vary with feed water concentration as it is the direct relation between the osmotic pressure and the dissolved salt concentration. This is the reason that reverse osmosis or nanofiltration units are able to remove the salts from low salt concentration waters with specific energy consumption as low as 0.5–3 kWh/m^3 of freshwater (NRC 2008).

Membrane Desalination Technologies

With increasing energy costs and availability of improved membranes properties, it has been recognized long ago that membrane-based desalination plants will provide cost-effective and energy saving solution for freshwater supply. Membrane processes are widely used for desalination due to their simplicity in operation, relatively lower capital and operating costs, and lower energy requirements. The dominance of membrane technologies in seawater, brackish water and surface water desalination has already shown its presence in many areas of the world. For example; in the United States, the share of membrane technology-based desalination among existing desalination plants is 75%. The share of MSF, a predominant thermal process, has decreased by 10%, whereas about a similar percentage of increase has been noted for RO, a leading membrane technology for desalination in Middle Eastern countries (Mehdizadeh 2006).

Membrane processes offer several advantages for desalination applications. The advantages of the membrane processes can be listed as (1) low energy consumption, (2) moderate costs (lower capital and operating costs), (3) easier operation and

maintenance, (4) compact and modular units, flexibility in capacity expansion, short construction periods, (5) lower start up and delivery times, (6) advances in RO membranes and technology, (7) decoupling of power and desalination plants (due to water demand growth factor of 11% over 4% for power), (8) possible hybridization of three or more processes, (9) ambient temperature process, and (10) lower environmental impacts. Apart from the above, the membrane technologies have wide range of applicability, such as desalting, disinfection by-product (DBP) control, disinfection (pathogen removal), clarification, and removal of inorganic and synthetic organic chemicals which make the use of membrane processes universal (Jacangelo et al. 1997; Mohsen and Al-Jayyousi 1999; Duin et al. 2000; Glucina et al. 2000; Darwish et al. 2003; Judd and Jefferson 2003; Cath et al. 2005; Mehdizadeh 2006; NRC 2008; Bayod-Rujula and Martinez-Gracia 2009).

The disadvantages of membrane technologies are, in general, RO is not generally favored for seawater desalination due to high salinity (45,000 ppm of TDS in the Persian Gulf), high temperatures (40 °C in the Persian Gulf), high silt density, high bacteria activity, and pollution. However, reverse osmosis is being considered recently for seawater desalination as a first option. The most important disadvantage of membrane systems is the problem of fouling. Pretreatment is very important for RO systems. However, there has been a recent inclination towards using RO in seawater desalination, both for new plants and in connection with present MSF plants, due to a reduction in energy requirements and lower operation costs (Mehdizadeh 2006).

Predominant membrane technologies for desalination are namely reverse osmosis (RO) (seawater and brackish water), electrodialysis (ED) (brackish water), and membrane distillation (MD) (seawater and brackish water). Reverse osmosis and electrodialysis processes are well established worldwide. Membrane distillation is an emerging technology with great potential for large-scale applications. General characteristics of these membrane technologies are presented in Table 1 (Tsuru 2001; WEF 2006; Baker 2000).

Reverse osmosis (RO): Reverse osmosis process is a nonphase change operation where a semi-permeable membrane (allowing water to pass through but not the salts) is used to separate the freshwater from the saline feed water. An external pressure is applied to exceed the osmotic pressure of the feed water to allow the freshwater to pass through the membrane (Gude 2015b). The amount of energy required for mechanical pumping to create the external pressure depends on the feed water salt concentration. This process does not require heating or phase-change of the feed water. A typical RO plant consists of four major components: feed water pretreatment, high-pressure pumping, membrane separation, and permeate posttreatment (Ayyash et al. 1994). The pretreatment step involves removal of large suspended solids, bacteria, and colloidal matter that may cause damage to the membrane operations. A typical pretreatment includes chlorination, coagulation, acid addition, multimedia filtration, micron cartridge filtration, and dechlorination (Al-Sheikh 1997; Durham and Walton 1999). Additionally, the fouling problems should be avoided by using cleaning solutions (Amjad 1997). The type of pretreatment mainly depends on the feed water characteristics, membrane type and

configuration, recovery ratio, and product water quality (Gude 2011). Post-treatment of the permeate (fresh water) is done by re-carbonation and blending with feed water (Gude 2011).

Reverse osmosis and nanofiltration processes require severe pretreatment of the feed water to avoid membrane fouling associated maintenance and replacement issues including the costs. Chemical coagulation followed by conventional sand filtration or membrane filtration can be used for the pretreatment. Table 3 shows a comparison of the conventional vs. membrane filtration technologies. In several aspects, conventional filtration works similar to the membrane filtration. Conventional filtration requires lower capital costs but with larger footprint and less reliable microbial separation. Membrane filtration is expensive when compared with conventional filtration but provides higher quality effluent.

Electrodialysis (ED): The operating principle of this method is based on the migration of ionic salts towards their respective counter charge electrodes. Selective membranes that allow passage of either anions or cations in an alternating fashion result in concentrate and product streams. The anions can pass through the anion-selective membrane, but are not able to pass by the cation-selective membrane, which blocks their path and traps the anions in the brine stream (Strathmann 1993). Similarly, cations move in the opposite direction through the cation-selective membrane under a negative charge and are trapped by the anion-selective membrane.

A typical ED system includes a membrane stack with a number of cell pairs, each consisting of a cation transfer membrane, a demineralized flow spacer, an anion transfer membrane, and a concentrate flow spacer. Compartments for the electrodes are at opposite ends of the stack. The electrodes need regular flushing to reduce fouling or scaling (Gude 2015b). Recycling the concentrate stream and discharging concentrate to waste or blowdown is common and called feed-and-bleed mode. This is necessary because of the fact that there are sharp differences in flow rates between the product and brine streams. Diluate flow is about 10 times the flow of the brine stream; this difference in flows creates pressure imbalances, requiring concentrate recycle (Gude 2015b). An ED unit can remove about 50% to 94% of dissolved solids from a feed water, up to 12,000 mg/L TDS. Voltage input, and process configuration (number of stacks or stages), raw water quality, and membrane selection determine the salt removal efficiency of the process.

TDS removal is generally limited by economics. The cost of ED increases as the feed water TDS increases. Electrodialysis reversal (EDR) is similar to ED, but the polarity of the electrodes is regularly reversed, thereby freeing accumulated ions on the membrane surface. This process minimizes the effect of inorganic scaling and fouling by converting product streams into waste streams. This process requires increases membrane life and does not require chemical addition and improves membrane and electrode cleaning.

Membrane distillation (MD): Membrane distillation process can be described as a hybrid process since it combines thermal evaporation and membrane separation principles in a single process unit. The feed water (saline water) is heated by an

Table 3 Comparison between conventional pretreatment and membrane pretreatment (Rosberg 1997; Wolf et al. 2005; WEF 2006; Vedavyasan 2007)

Parameter	Conventional treatment	MF	UF
Membrane pore size	NA (media effective size 350–2000 μm and smallest particle removed 8–12 μm)	0.03 to 10 μm	0.002 to 0.1 μm
Feed pressure (psi)	64		82
Chemicals required		Coagulant or powder activated carbon	
10 log removal	1 to 2	1 to 2	> 5.8
Membrane fouling index	1.5–3.0	0.4–0.7	0.12–0.2
Salt density index	5 to 6	2	< 1
System recovery (%)	40	55	55
Energy requirements	Less than MF/UF due to gravity flow	Higher than conventional (requires pumping water through the membranes)	
Capital costs	Competitive with MF/UF	Slightly higher than conventional pretreatment	
Chemical costs	High due to coagulant and process chemicals	Chemical use is low dependent on raw water quality	
Foot print	Larger foot print	Significantly smaller foot print (30–50% of conventional filters)	
Membranes used		*Memcor, Zeeweed*	*Norit/X flow, Aquasource, Zeeweed-UF Pall and Koch*
Applications	Traditional surface water treatment and wastewater treatment	Reduce turbidity, remove suspended particles, algae, and bacteria	Removal of contaminants that effect color, high-weight dissolved organic compounds, bacteria, and some viruses
Other applications			Recycling of backwash from sand filters, recycling of industrial and municipal waste water
RO capital cost	Higher than MF/UF since RO operates at lower flux	Higher flux with lower capital costs (due to lower SDI values, RO can be operated at 20% higher flux reducing capital costs)	
RO operating costs	High (due to higher potential of membrane fouling resulting in higher operating pressures and frequent cleaning of membranes)	Lower operating costs due to less fouling potential and longer membrane life (membrane cleaning is reduced by 10-100% reducing system downtime and prolonged membrane life)	

external heat source often derived from solar energy or process waste heat and is passed through the hot side of the unit to allow for the water vapors to raise and diffuse through the membrane barrier and condense in the permeate flow on the cold side of the unit (Camacho et al. 2013). Because the hot (saline water feed) and cold (permeate or freshwater) streams are separated by a membrane barrier, a very low temperature differential of 10 °C is sufficient to produce freshwater through this process. The membrane applied in this process should be porous enough allow the water vapor to pass through but not allowing the liquid and preferably should not be wetted by the process liquids. There are four main configurations of the MD process: (1) direct contact membrane distillation (DCMD), air gap membrane distillation (AGMD), vacuum membrane distillation (VMD), and sweep gas membrane distillation (SGMD) (Yarlagadda et al. 2009). In DCMD, the membrane is in direct contact with the liquid phases, while in AGMD, an air gap is introduced between the membrane and condensation surface to improve the energy efficiency. In VMD, the permeate side is maintained at a lower pressure by mechanical pumping to increase the permeate flux, while in the SGMD, a stripping or carrier gas is used to collect the vapor produced. Among these methods, DCMD operates at a reasonable efficiency and a higher permeate rate. MD systems can be operated standalone using solar energy (Qtaishat and Banat 2013; Saffarini et al. 2012). The permeate flux is very low for the AGMD. VMD can produce higher permeate but at the expense of higher energy requirements and is suitable for feed waters with volatile contaminants (Yarlagadda et al. 2011).

As of June 2011, there were 15,988 desalination plants worldwide which combined produce a total of 65.2 million m^3 of freshwater equivalent to 17.5 billion US gallons in over 150 countries supporting 300 million people (Gude 2016a). Out of these desalination plants, reverse osmosis with about 60% share currently dominates the other desalination technologies (Gude 2016b) and this trend is expected to continue into the future followed by well-established MSF technology (26.8%) and MED technology (8%) with the remaining 5% contributed by electrodialysis and other hybrid technologies. Sixty percent of the desalination plants process seawater to produce freshwater followed by brackish water (21.5%), river water (8.3%), wastewater recovery/reuse (5.7%), and other water sources (4.5%). Energy requirements for desalination technologies vary significantly in quantity and quality (Gude 2016a).

Thermal Desalination Technologies

Multistage flash distillation (MSF): MSF distillation involves flash evaporation where seawater is evaporated by reducing the pressure in successive effects (stages). The energy economy is achieved by successive regenerative heating where the seawater flashing in each flash chamber (effect or stage) rejects heat to the feed water, thereby heating the incoming seawater (Gude 2015b; El-Nashar 2001). Seawater prior to the introduction in the first stage is heated by external heat sources, such as low pressure steam, form power plants or an extraction steam from a steam

turbine plant. This heated seawater then enters the flash evaporation chambers with falling operating pressures in the successive stages; 15 and 30 stages in modern large-scale MSF plants are common to achieve optimum energy recovery (Khawaji et al. 2008). The operating temperatures vary between 90 °C and 120 °C (also known as top brine temperatures), which depends on the quality of heat source available in the first stage.

Multieffect evaporation/distillation (MED): Similar to MSF process, MED process operates in a number of effects to increase the energy efficiency and has higher thermodynamic efficiency when compared with an MSF process (Al-Shammiri and Safar 1999). However, in contrast to MSF process, the preheated seawater is sprayed onto the tubes in the first evaporation chamber which are heated by external heat source derived from a power plant steam (Ophir and Lokiec 2005). These are operated in a dual purpose power plant scheme. The freshwater vapor evaporated in the first evaporation chamber is allowed to pass through a condenser which serves as evaporating surface in the next effect. The remaining brine from the first evaporation chamber is passed through the next effect where it is sprayed on the evaporating tubes at lower temperatures and lower pressures. This process continues in the successive effects as long as suitable temperature gradient is available for freshwater evaporation. The energy or steam economy is proportional to the number of effects. The total number of effects is limited by the total temperature range available and the minimum allowable temperature difference between successive effects (Michels 1993). Typical number of effects in MED process varies between 4 and 21, which again depends on the heat source temperature and the top brine temperature in the first effect. The top brine temperatures are usually around 90 °C for MED processes, but a lower top brine temperature of 70 °C is also possible which are called low-temperature MED (LTMED) (Ophir et al. 1994). Although thermodynamically efficient, this process requires large heat transfer areas. The heat transfer areas for LTMED are considerably higher than MED process often varying between 25% and 40%.

Solar distillation (SD): Solar distillation refers to solar stills and active or passive solar desalination systems that are supported either by direct solar energy or indirect solar energy (Gude et al. 2010). Indirect solar energy means that the solar energy harvested in the solar collectors (flat panel collectors, parabolic trough collectors, etc.) is supplied to the desalination unit. These applications depend on the type of solar energy harvesting technology and a suitable desalination mechanism. The solar stills are the simplest and cheapest direct solar harvesting desalination units. These units utilize the direct solar energy to evaporate the freshwater from salt water leaving behind the concentrated brines. Solar stills incorporate the evaporating and condensing units into a single chamber. Often, the glass roof of the unit serves as the condensing surface which rejects the latent heat to the ambient.

The energy efficiency of typical solar stills varies between 30% and 40% (Tiwari et al. 2003). The stills with an external condensing surface maintained in shade or at lower temperatures showed higher energy efficiencies up to 70%. The energy efficiency and the distillate product can be improved by adding multiple effects in the same unit. The energy efficiency is calculated as the ratio of the amount of the

distillate produced to the amount of solar energy received by the solar still unit. Other indirect solar desalination systems include multieffect distillation or evaporation system, low temperature multieffect desalination, humidification-dehumidification, and membrane distillation systems (Sampathkumar et al. 2010; Kalogirou 1997).

Energy Requirements for Desalination Processes

Energy requirements for desalination vary from process to process (Gude et al. 2010; Gude 2011). Thermal desalination processes require both thermal and electrical energy for evaporation, process hydraulic flow, and transport of the feed and product water. Membrane desalination processes require electrical energy to supply the mechanical energy for membrane separation and pumping in and distribution out of the plant. Figure 2 shows the specific energy consumption for thermal and membrane desalination processes in terms of kJ of energy required for producing one unit of freshwater in kilogram (Mehdizadeh 2006). It can be noticed that MSF process has the highest specific energy consumption, while the seawater reverse osmosis (SWRO) process has the lowest specific energy consumption. For this reason along with its inherent simplicity, the SWRO process is the preferred desalination method for both brackish and seawater desalination around the world. Table 4 shows the equivalent electrical energy requirements for the desalination processes expressed in kWh/m^3 of freshwater production (Mehdizadeh 2006). From this table, it can be inferred that membrane processes require higher electrical energy for freshwater production for both with and without energy recovery options. It should also be noted that electrical energy is a prime (highest form) quality energy that could probably be used for other high value beneficial uses. At the same time, for thermal processes, the availability of steam is important whether it is produced for desalination solely or in conjunction with power production.

Renewable Energy Powered Desalination

The potential renewable energy-desalination technology combinations are shown in Fig. 1. The renewable energy sources (RES) should be integrated with the relevant desalination technology that has the best capability to utilize the energy in the most effective manner (Gude 2015b). Some renewable energy source-dependent desalination technologies must be placed on the same site (co-location) and some do not have this requirement. Accordingly, the following thermal desalination-renewable energy combinations require colocation (located on same site):

(a) Wind–shaft–MVC
(b) Solar thermal–TVC
(c) Solar thermal–MSF
(d) Solar thermal–MED

Table 4 Equivalent electrical energy consumption for desalination processes (Data taken from Gude 2016b)

Process	Steam energy kWh/m^3	Electrical energy kWh/m^3	Equivalent electrical energy kWh/m^3	Equivalent CO_2 emissions kg CO_2/m^3
MSF	7.5–11	2.5–3.5	10–14.5	0.09
MED	4–7	2	6–9	0.04
VC	–	7–15	7–15	0.051
SWRO	–	4–6 (with ER) 7–13 (without ER)	4–6 (with ER) 7–13 (without ER)	0.032
BWRO	–	0.5–2.5	0.5–2.5	
ED	–	0.7–2.5	0.7–2.5	0.038

(e) Solar thermal–SD
(f) Geothermal–TVC)
(g) Geothermal–MSF or MED

The other electricity-driven combinations that do not require co-location are (a) wind–MVC, (b) wind–RO, (c) solar PV–RO, (d) solar PV–MVC, (e) geothermal–MVC, and (f) geothermal–RO. More details on this topic can be found in Gude (2015b).

Energy Recovery and Integrated Concepts

Thermal desalination technologies are generally considered energy-intensive and therefore cost-prohibitive. However, these technologies are more acceptable in regions where thermal energy sources can be accessed at low cost especially in oil-rich regions of Middle Eastern countries. Low-temperature thermal desalination processes are considered energy-efficient (Gude and Nirmalakhandan 2008), which can also utilize waste heat sources such as reject heat from the domestic air-conditioning units, and heat harvested by solar collectors and photovoltaic thermal collectors (Gude et al. 2011a, b, 2012). Often thermal desalination technologies are co-located with power plants, the waste steam from the condenser is utilized as a heat source for MSF or MED processes. In thermal desalination technologies, energy recovery and recycling is done through successive stages. However, energy source utilization must be maximized. As shown in Fig. 2, it can be noted that state-of-the-art power plants have a high energy efficiency of 35–40%, whereas a cogeneration plant can achieve an energy efficiency of 75–80%. If the beneficial uses can be extended through heating and cooling applications in a configuration called "Poly generation," energy efficiencies as high as 90% can be reached.

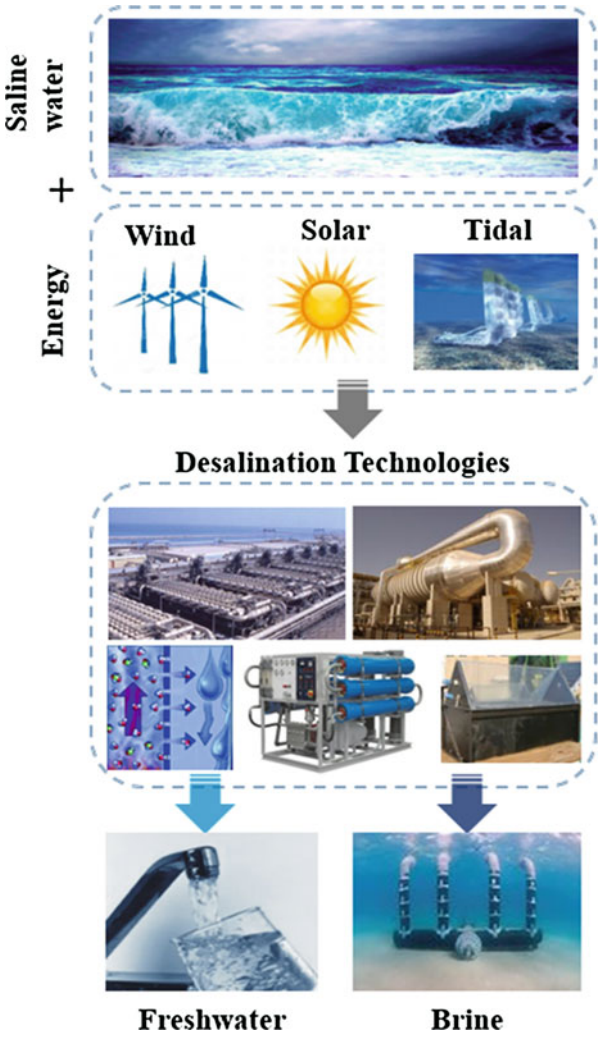

Fig. 1 Renewable energy applications for desalination (Adapted from Gude 2015b)

Membrane desalination technologies are also considered energy-intensive because they utilize electrical energy which is a high-quality energy. Energy recovery in membrane processes is a widely studied concept. Various types of energy recovery devices have been developed over the past few decades to minimize the specific energy consumption in membrane processes. Because minimum theoretical energy consumption cannot be achieved due to other constraints such as membrane scaling and biofouling, water recovery in multiple pass configurations can help reduce the specific energy consumption as well. For more information on this topic, the readers are suggested to refer to the contribution by Gude (2011).

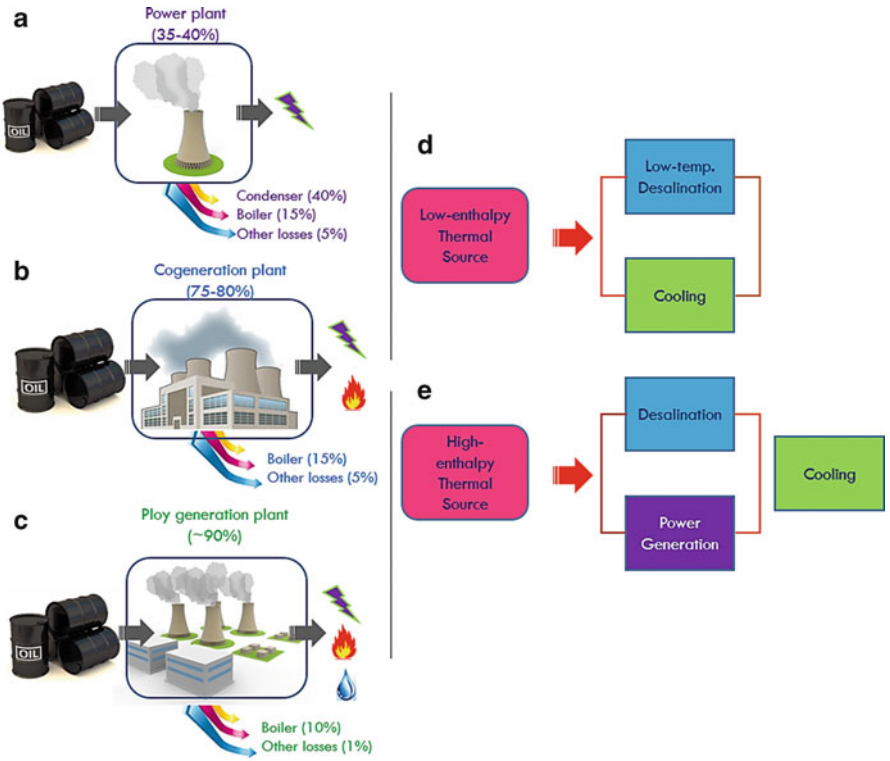

Fig. 2 Energy efficiency in power plants and desalination industry. (**a**) conventional power plant, (**b**) cogeneration plant, (**c**) polygeneration plant with multiple benefits, (**d**) low enthalpy heat source for LT-MED process and cooling application, and (**e**) high enthalpy heat source for desalination, power generation, and cooling application

Other Considerations for Desalination

The major drawback with desalination technologies is one of the end products which is highly concentrated with salts called "brine" or "concentrate." Disposal and management of brine streams is an ongoing issue for these facilities. Coastal desalination plants have the privilege of considering ocean discharge for brine disposal. However, inland desalination plants are faced with the dilemma of options that are usually not so environmentally friendly. Evaporation, deep well injection, discharge to sewers, land application, and valuable chemical recovery through further processing are the available options, all of which are again energy- and cost-intensive. Environmental permits are another major hurdles that these facilities need to consider. Concentrate disposal into surface waters or sewers increases salt concentrations of existing water supplies, whereas an impermeable barrier is required for evaporation and land application options.

Protocol

Desalination technology selection

This chapter described various alternatives for desalination and water reuse. Membrane technologies are more suitable for water reuse applications to recover potable water from wastewater effluents, while desalination technologies should be used for high saline ground water or seawater as water supply sources. Water reuse and recycle programs should be given priority before considering desalination alternative for water supply enhancement. Energy recovery and integrated configurations along with enhanced water recovery should be implemented in desalination.

Policies

Governmental policy for water reuse and desalination

This chapter described various membrane and thermal desalination technologies. As desalination processes are energy-intensive, the following should be considered.

- Priority should be given to renewable energy driven desalination configurations
- Mandatory regulations and incentive programs should be implemented.
- Environmental impacts of desalination plants should be thoroughly evaluated.

Dictionary of Terms

- **Feed water** – Supply water that is fed into the RO system to be treated
- **Permeate** – A portion of the feed water that passes through a series of membranes and is returned as purified water
- **Concentrate** – A portion of the feed water that is rejected by the membrane and contains the solution of impurities that have been filtered out of the permeate
- **Water flux** – The rate of permeate production typically expressed as the rate of water flow per unit area of membrane (e.g., gallons per square foot per day)
- **Recovery rate** – The ratio of permeate flow to feed water flow, which indicates the overall water efficiency of the system
- **Reclaimed water** – Municipal wastewater that has been treated to meet specific water quality criteria with the intent of being used for beneficial purposes. The term *recycled water* is synonymous with reclaimed water.
- **Water reclamation** – The act of treating municipal wastewater to make it acceptable for beneficial reuse.
- **Water reuse** – The use of treated wastewater (reclaimed water) for a beneficial purpose. Synonymous with the term *wastewater reuse*.

- **Potable reuse** – Augmentation of a drinking water supply with reclaimed water.
- **Nonpotable reuse** – All water reuse applications that do not involve potable reuse (e.g., industrial applications, and irrigation).

Summary Points

- Supply enhancement can be done by considering water reuse technologies such as advanced chemical and membrane treatment processes or desalination technologies.
- Because desalination technologies are energy-intensive, it is important to reduce their nonrenewable energy input by integrating solar and wind energy sources.
- Future research efforts in desalination technology development should focus on development of energy-efficient and high recovery process configurations including membrane materials with high rejection capacities and water recovery.
- Membrane technologies such as forward osmosis and nanocomposite membranes show the potential for wide application. Forward osmosis is a desired process to recover water from impaired sources such as wastewaters and other oil-/gas-produced waters. Capacitive deionization process still shows promise for future applications especially for energy savings.
- Hybrid and trigeneration configurations including membrane/thermal and physico-chemical processes should be considered for enhancing the water recovery and supply process benefits.

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Severe Protein-Calorie Malnutrition After Bariatric Surgery

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Reginaldo Ceneviva, Vivian Marques Miguel Suen, and Camila Scalassara Campos

Contents

Abstract

Obesity is an important medical and social problem due to its severity and increasingly high prevalence, which has led to a significant increase in the number of bariatric surgeries.

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Bariatric surgeries are the most efficacious treatment for obesity, leading to a recognized improvement in health and quality of life and heightened patient survival, but they may result in several complications. Protein-calorie malnutrition is a serious major complication, particularly in its severe form. Few evidence-based studies have been found in the literature, and the most relevant ones helped guide the choice of strategies and procedures.

Keywords

Obesity · Bariatric surgery · Roux-en-Y gastric bypass · Malabsorptive bariatric procedures · Postoperative support · Bariatric surgery complications · Malnutrition · Protein-calorie malnutrition · Protein-energy malnutrition · Conservative treatment · Medical treatment

List of Abbreviations

A.S.P.E.N.	American Society of Parenteral and Enteral Nutrition
AIDS	Acquired immunodeficiency syndrome
AL	Alimentary limb
BMI	Body mass index
BPD	Biliopancreatic diversion
BPD/DS	Biliopancreatic diversion/duodenal switch
BPL	Biliopancreatic limb
CC	Common channel
DRYGB	Distal Roux-en-Y gastric bypass
HDL-c	High-density lipoprotein-cholesterol
IFSO	International Federation for the Surgery of Obesity and Metabolic Disorders
LAGB	Laparoscopic adjustable gastric banding
PCM	Protein-calorie malnutrition
RMR	Resting metabolic rate
RYGB	Roux-en-Y gastric bypass
SG	Sleeve gastrectomy
SPCM	Severe protein-calorie malnutrition

Introduction

Obesity is a significant medical and social problem due to its severity and high and increasing prevalence.

The severity of obesity, especially in its visceral form, is related to increasing risk of several diseases and metabolic and physical disorders, such as those associated with metabolic syndrome: systemic arterial hypertension, dyslipidemia, type 2 diabetes mellitus, increase in total cholesterol, decrease in HDL-c levels, and abdominal circumference. Other disorders may also develop, such as cardiovascular diseases (acute myocardial infarction, cerebrovascular accidents, and venous stasis),

pulmonary dysfunction, degenerative arthropathies, gastroesophageal reflux disease, nonalcoholic fatty liver disease, cholelithiasis, abdominal hernias, higher risk of some malignancies, female sexual dysfunction, worsening of psychological disorders, and social discrimination.

Obesity is an epidemic condition with a worldwide exponentially increasing incidence over the last few years. At least 2.8 million people in the world die each year from overweight or obesity complications (Masters et al. 2013). The proportion of obese male adults in England increased from 13.2% in 1993 to 26% in 2013 and from 16.4% to 23.8% among females in the same period (Lifestyles Statistics Team, Health, and Social Care Information Centre 2015). http://www.hscic.gov.uk/pubs/sopad15.

Bariatric surgery is considered to be the most efficacious treatment for severe obesity because of its good results regarding the resolution of comorbidities and the consequent improvement in health (Buchwald et al. 2004) and quality of life for most patients (Karlsson et al. 2007), in addition to increasing survival (Sjöström 2013). However, it is not free from complications. Nutritional deficiencies are the most important long-term complications of bariatric surgery (Tack and Deloose 2014). Severe protein-calorie malnutrition (SPCM) is not common after bariatric surgery but is a complication of an often difficult solution, besides being potentially lethal (Wade et al. 2010).

Although the definitions of protein-calorie malnutrition (PCM) are not uniform, this condition may be described as "protein, energy and micronutrient deficiency, depending on the inability of the body to satisfy the protein and energy fuel requirements through diet" (Torún 2009).

Severe PCM is characterized by a marked protein loss resulting in a generalized muscle and fat loss and signs and symptoms such as diarrhea; sparse, fine, and dry hair; fine and dry skin; weakness; apathy; and a substantial reduction in physical capacity.

This chapter focuses on SPCM after bariatric surgery, its causes, diagnosis, and medical treatment. Published studies on PCM, especially in its severe form, are relatively scarce, most of which are retrospective and almost always do not define the type and severity of malnutrition.

Surgical treatment is indicated for patients with a body mass index (BMI) equal or greater than 40 kg/m^2 or above 35 kg/m^2 when associated with comorbidities that do not respond to conservative treatment. There has been an increasing demand for bariatric surgery given the positive results regarding both weight loss and improvement in comorbidities. Those results have surpassed those of conservative treatment in various prospective and randomized trials (Karlsson et al. 2007; Ikramuddin et al. 2013; Sjöström 2013). Since the late 1990s, there has been a marked increase in the number of bariatric surgeries performed, from approximately 10,000 in 1998–100,000 in 2003 (Buchwald et al. 2004) and from 145,301in 2003-340,768 in 2011 (Buchwald and Oien 2013).

To better understand PCM after bariatric surgery, it is important to bear in mind the anatomical basis and the main physiological consequences of the surgical techniques currently in use.

Bariatric Surgeries

The surgical techniques can be performed by means of laparoscopy or laparotomy and are classified as follows, according to their objective:

Restrictive: based on the reduction of gastric capacity, resulting in early satiety and a marked decrease in food intake. The number of vertical banded gastroplasty and laparoscopic adjustable gastric banding (LAGB) has fallen, whereas that of vertical gastrectomy, also called sleeve gastrectomy (SG) (Fig. 1a), has increased.

Malabsorptive: based on the exclusion of an ample small bowel segment from the food transit, resulting in a marked reduction of food absorption capacity. Jejunoileal and jejunocolic bypasses are currently proscribed due to their high rates of long-term nutritional and metabolic complications.

Mixed: they involve both gastric restriction and intestinal malabsorption and may have a predominant component – restrictive (Roux-en-Y gastric bypass (RYGB) or malabsorptive (biliopancreatic diversion (BPD) and its variant duodenal switch (BPD/DS)). The small bowel segment between the anastomosis of the alimentary limb (AL) with the biliopancreatic limb (BPL) and the ileocecal valve is called common limb or common channel (CC). It corresponds to the portion of the small bowel where the food starts to come in contact with the bile and the digestive enzymes from the pancreatic juice.

RYGB (Fig. 1b) is the universally more accepted procedure for the surgical treatment for obesity and involves vertical gastroplasty (restrictive component) and Roux-en-Y gastrojejunal bypass (malabsorptive component). The weight loss is usually within the 30–40% range, and the loss of excess weight is within the 60–75% range, for 1 and 2 years (Pajecki et al. 2007). RYGB and SG induce alterations in hormonal secretion

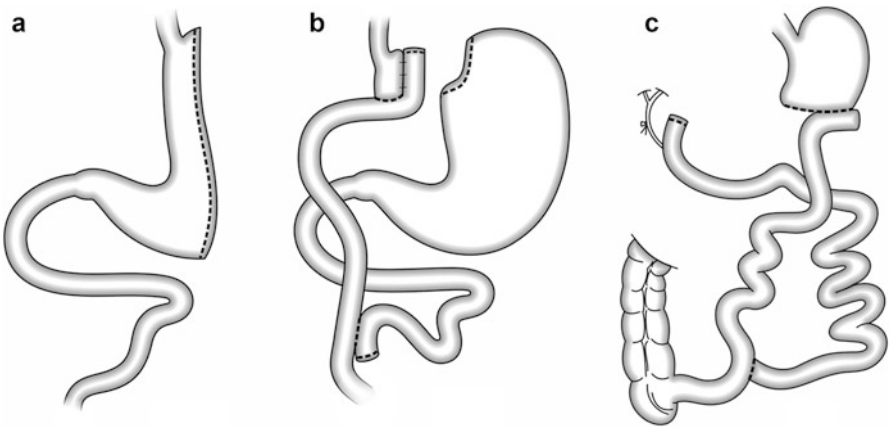


Fig. 1 Bariatric surgeries. (**a**) Sleeve gastrectomy. (**b**) Roux-en-Y gastric bypass. (**c**) Biliopancreatic diversion

such as increase in glucagon-like peptide and peptide YY and a decrease in ghrelin levels, leading to satiety and a favorable effect on glycemic levels.

With the distal Roux-en-Y gastric bypass (DRYGB), a modification of standard RYGB is made by increasing the malabsorptive component through the elongation of the AL or the BPL, alone or combined, with a consequent shortening of the CC, thus effecting a reduction of the absorptive area of the bowel.

The biliopancreatic diversion (BPD) based on the Scopinaro technique (Fig. 1c) consists of a distal gastrectomy with a Roux-en-Y gastroileal anastomosis. The biliopancreatic diversion based on the Marceau-Hess technique, also called duodenal switch (BPD/DS), is a modification of Scopinaro's technique. It consists of SG and duodenoileal anastomosis, while preserving the pylorus, with the ileum anastomosed to the duodenum at 200 cm from the ileocecal valve and enteroenterostomy located at 100 cm from the ileocecal valve. The DRYGB scheme is shown in Fig. 2 and the BPD/DS in Fig. 3.

With the biliopancreatic diversions, the partial resection of the stomach effects the restriction, which is greater with the BPD/DS than the BPD, and the intestinal bypass causes malabsorption, especially of fats and carbohydrates. The weight loss is usually within 35–40% range and the loss of excess weight within 75–80% range, about 1–2 years after surgery. A surgery inducing loss of more than 50% excess weight is considered valid (Pajecki et al. 2007), a result not always achieved by using purely restrictive operations. The BPD with or without DS, due to the restriction of food intake and mainly to the malabsorption of both macro- and micronutrients, requires close monitoring. Biliopancreatic diversions cause a marked and lasting weight loss, but malabsorption, especially of fats, can also cause side effects that limit its use, such as increased frequency of daily evacuations of fetid feces and flatus. Besides that, various nutritional deficiencies and metabolic changes can occur leading to higher mortality as compared to alternative procedures. RYGB involves a

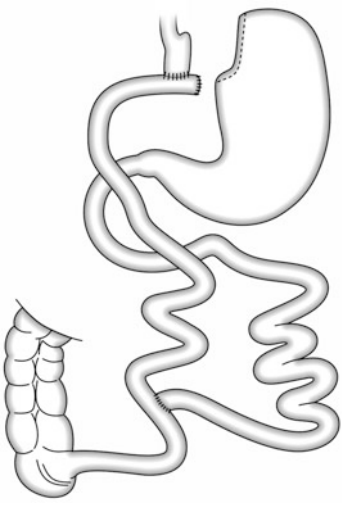


Fig. 2 Distal Roux-en-Y gastric bypass

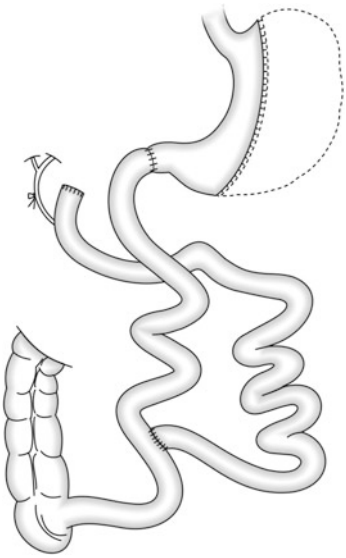


Fig. 3 Biliopancreatic diversion with duodenal switch

greater risk of nutritional deficiencies than purely restrictive surgeries but a lower risk than biliopancreatic diversions (Søvik et al. 2013; Suarez Llanos et al. 2015).

Buchwald and Oien (2013) studied the relative distribution of 340,768 metabolic/bariatric procedures conducted in 42 nations or national groupings in 2011. They observed that the more frequently used surgeries were RYGB (46.6%), SG (27.8%), adjustable gastric banding (17.8%), and BPD/DS (2.2%), with a marked increase in SG. Welbourn et al. (2017) described demographic data from the report of IFSO Global Registry including 54,490 patients from 31 countries operated in the 3 years 2013–2015. Gastric bypass was the most prevalent operation (49.4%), followed by sleeve gastrectomy (40.7%) and gastric banding (5.5%) (Welbourn et al. 2017, 1). Scott et al. (2017, 2935) used the Premier database that collects data from community and teaching hospitals and large health systems in the USA and found that the proportion of SG went up from 39% to 63%, while that of RYGB decreased from 44% to 30% and gastric banding from 13% to 2% over the period 2012–2015.

The postoperative mortality rate of bariatric procedures is of 0.1–2%, and a non-negligible number of patients suffer from acute or late surgical or clinical complications (Karlsson et al. 2007).

Protein-Calorie Malnutrition

Many patients seeking bariatric surgery already have nutritional deficiencies, including hypoproteinemia and lean mass depletion, resulting from bad eating habits and previous restrictive diets (Ernst et al. 2009; Kulovitz et al. 2014). Thus, attention should be paid to the patient's nutritional status before and after performing a bariatric surgery (Lopez et al. 2007; Santarpia et al. 2014; Dietch et al. 2015).

Malnutrition may occur as a consequence of the restrictive and/or malabsorptive effects of the bariatric surgery itself and also results from other causes: reduction in nutrient intake, due to decreased tolerance to protein-rich foods, besides mechanical problems resulting from benign processes (peptic or caustic stenosis) or malignant ones (tumor-induced stenosis), in the upper digestive tract, or yet surgical sequels; reduction in intestinal absorption, due to Crohn's disease, AIDS, and celiac disease, among others; and increase in metabolic demands, resulting from severe infections, burns, cancer, trauma, or neurological injuries (Enomoto et al. 2013).

The decrease in protein levels after various bariatric procedures may occur in the early postoperative period as a result of several conditions occurring at this phase, such as increased metabolic stress and accelerated protein catabolism, besides anorexic state and decreased tolerance to protein-rich foods.

Restrictive surgeries may lead to a reduction in protein intake, although usually without any clinical or laboratory repercussion. Vomiting contributes to the development of malnutrition. If postoperative vomiting is intense and persistent, nutritional deficiency may occur, especially regarding vitamins and minerals. The nonacceptance of, or non-compliance with the diet directions, and the intolerance to protein-rich foods, also not rare after RYGB, may lead to insufficient protein intake and so aggravate malnutrition. This occurs between 3 and 6 months after surgery but almost always resolves after 1 year (Bock 2003; Moize et al. 2003). Postoperative nutritional support should be provided by encouraging patients to ingest at least 60 g protein per day, even if this should require supplementation with formulas with high-protein content (Schollenberger et al. 2016). Protein malnutrition after SG or LAGB is rare.

Protein-calorie malnutrition after RYGB may result from several causes, such as stenosis of the gastrojejunal anastomosis, psychiatric disorders, intestinal bacterial overgrowth, diarrhea due to bile salts, chronic dumping, and some early complications, such as dehiscence of the anastomosis and/or a digestive fistula (Akusoba et al. 2016). With the mixed procedures, the measurement of the total length of the small bowel is essential to prevent the inadvertent constructing of too short a CC.

The investigation of diarrhea caused by RYGB entails several exams, among which a radiologic study of the intestinal transit, qualitative and quantitative examination of fecal fat, and tests of bacterial overgrowth. While the cause is being investigated and the clinical and laboratory data available are being analyzed, nutritional support may be provided, as well as experimental treatment with antibiotics, probiotics, or pancreatic enzymes (Machado et al. 2008; Chen et al. 2016). Diarrhea secondary to bacterial overgrowth in the small bowel ($>10^5$–10^6 microorganisms/mL) may be the result of the reduction in gastric acidity and/or stasis in the afferent loop. Weight loss induced by diet or bariatric surgery may modify the gut microbiota composition (Damms-Machado et al. 2015).

The standard test for diagnosing intestinal bacteria overgrowth is the microbial investigation of jejune aspirates. Quantitative culture of small bowel bacteria would be indicated for diagnosis confirmation, but this is a laborious test. The diagnostic hypothesis can be tested out by the expired hydrogen assay after glucose or lactulose ingestion (Machado et al. 2008), however keeping in mind some recent queries

about this exam, reported in the literature (Ishida et al. 2014). The prophylactic use of probiotics has shown promising results (Chen et al. 2016).

Patients submitted to RYGB are at low risk of protein malnutrition. PCM is detected in less than 5% of patients with a Roux loop of 150 cm or less (Faintuch et al. 2004; Isom et al. 2014) and in 13% of patients with a Roux limb >150 cm (Isom et al. 2014). With techniques using a significant malabsorptive component such as BPD, BPD/DS, and DRYGB, there is a greater chance of diarrhea and a drop in albumin content, depending on the length of the absorptive bowel to be maintained (Scopinaro 2006, Ciovica et al. 2008; Currò et al. 2015). Often diarrhea demanding careful control may lead to severe protein-energy malnutrition (Buchwald and Oien 2013). With the technical standardization of the length of common loops being about 50–100 cm, the incidence of various degrees of PCM is of about 40%, especially in the first 2 years after operation (Sethi et al. 2016). Hypoalbuminemia in itself tends to cause a worsening of diarrhea and aggravation of PCM, due to edema of the intestinal loops and the consequent reduction in the absorptive area.

The severity of PCM can be measured by the assessment of the patient's nutritional status based on their clinical history, anthropometric measurements, and on laboratory tests.

Significant short-term weight loss indicates nutritional risk. In non-bariatric hospitalized patients, the interpretation of values related to weight loss and time of evolution can be made according to the classification proposed by Blackburn et al. (1977) (Table 1). It is common knowledge that patients who underwent RYGB have usual postoperative weight loss rate of about 13% at 3 months, 23% at 6 months, and 30–40% within 1 and 2 years (Pajecki et al. 2007). Much greater losses than these indicate risk of malnutrition.

Severe malnutrition relates not only to low BMI but also to the percentage of short-term weight loss. The speed of malnutrition installation and low BMI are associated with the risk of patient's death.

Nutritional history and physical presentation are usually sufficient for the diagnosis of SPCM, which should be confirmed, evaluated, and monitored by means of laboratory tests. Besides confirming the clinical diagnosis of PCM and making possible the monitoring of nutritional interventions, laboratory tests can detect subclinical nutritional deficiencies.

Care about hydration is important, and protein intake should be 60–80 g per day after RYGB or 1.1–1.5 g/kg of ideal body weight (BMI = 25 and 90–120 g per day after BPD/DS (Slater et al. 2004; Dagan et al. 2017). Supplementation with vitamins and minerals is essential for a daily routine nutrition after mixed bariatric surgeries, especially those with the predominance of the malabsorptive component, by

Table 1 Malnutrition risk according to the percentage of unintentional weight loss in relation to the elapsed time for non-bariatric hospitalized patients

Elapsed time	Significant weight loss	Severe weight loss
1 week	1–2%	>2%
1 month	5%	>5%
3 months	7.5%	>7.5%
6 months	10%	>10%

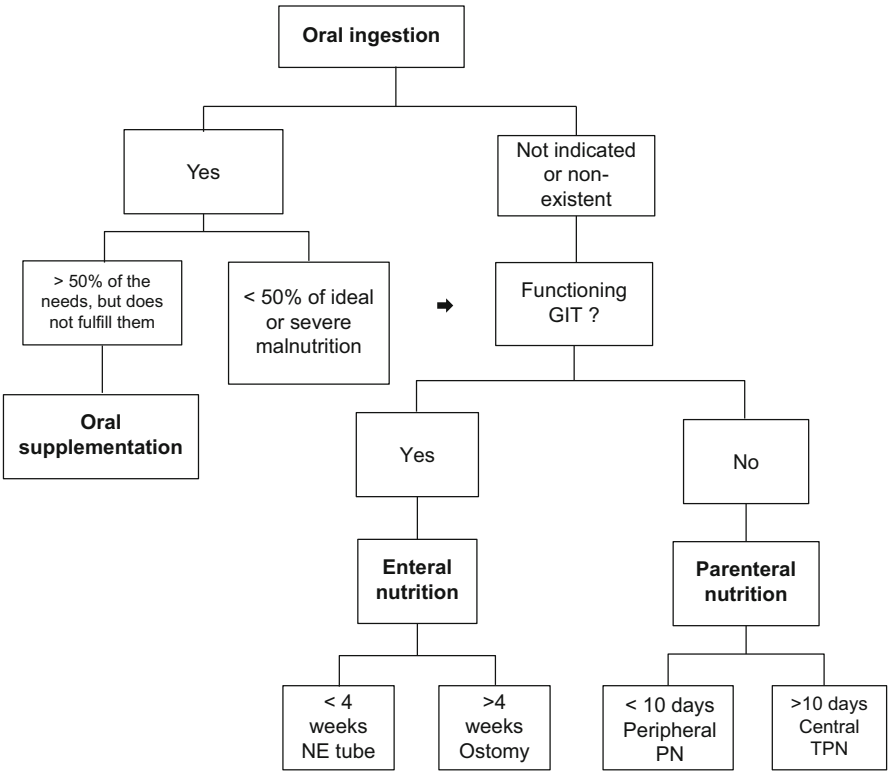
administration of commercial products containing those micronutrients in adequate quantity. The prescription may be calcium (500 mg two to three times/day or more), vitamin D3 (cholecalciferol) (2000–3000 U/day or more; titration should be done to reach normal concentrations of 30 ng/ml), and vitamin B12 (250–350 mcg/day or 1000 mcg/week sublingual or 3000 mcg intramuscular every 6 months). Vitamin B1 (thiamin), folic acid (400 mcg/day), fat-soluble vitamins, zinc, copper, and vitamin C should also be routinely recommended (Dagan et al. 2017). There may be changes in this prescription according to clinical laboratory evaluation of each patient. The diet of those patients, who frequently have inadequate intake of macronutrients and micronutrients, should receive special attention (Campos et al. 2008). Patient follow-up and clinical laboratory assessment at short intervals are necessary for an early diagnosis and correction of nutritional deficiencies (Bavaresco et al. 2010; Thibault et al. 2016). In addition to hypoproteinemia and possibly associated anemia, deficiency of other nutrients should be searched, among them vitamin B12; folate; vitamins A, B1, and D; calcium; copper; iron; zinc; and selenium, especially in critically ill patients (Donadelli et al. 2011; Rosa et al. 2011; Choban et al. 2013; Salgado et al. 2014; Kwon et al. 2014; Santarpia et al. 2014; Smelt et al. 2016).

Medical Treatment

Mild and moderate uncomplicated malnutrition can be treated at an outpatient service since hospitalization increases the risk of infections and also anorexia. Critically ill patients should be hospitalized, and their treatment for existent diarrhea may involve administration of pancreatic enzymes and antibiotics, as well as protein-calorie nutritional support by oral, enteral, or parenteral route, each alone or in combination. Nutritional therapy for malnourished patients after bariatric surgery may be guided by flowcharts (Flowchart 1), which should be modified with adequate adaptations to the conditions of each patient. The choice of route to be followed for the supplying of nutrients is based on several parameters, including the feasibility of the route, the sufficiency of supply for energy needs, and the evolution of clinical and laboratory conditions of the patient.

Nutritional therapy by oral route progresses from a fractionated clear liquid diet for the first days up to foods of reasonable consistency. Vitamin and mineral supplements should be administered routinely, and attention should be paid to protein intake (Lopez et al. 2007).

When the digestive tract can be used but the patient with PCM is unable to ingest an appropriate quantity of nutrients for 7–14 days, enteral nutrition therapy is indicated (Isom et al. 2014). When the patient's physical condition after bariatric surgery is precarious or when the recovery period has been predicted to be long enough to permit a revision surgery without posing a high risk, gastrostomy at the remnant stomach should be considered for enteral nutrition. It should persist until satisfactory results are achieved. The administration of enteral diet through gastrostomy or a nasoenteric tube should start with small volumes and gradually increase to prevent refeeding syndrome (Akusoba et al. 2016). The enteral diet, also



Flowchart 1 Flowchart of nutritional therapy for the malnourished patient. *GIT* gastrointestinal tract, *EN* enteral nutrition, *PN* parenteral nutrition, *TPN* total parenteral nutrition

containing appropriate quantities of vitamins and minerals, should be slowly and continuously administered, in order to optimize the absorption process, preferably by using an infusion pump, which would also facilitate appropriate monitoring. Whole nutrients may aggravate malabsorption with an unfavorable course of nutritional status, with oligomer diets being then indicated.

Parenteral nutrition is indicated after bariatric surgery when severe complications follow, leading to intestinal insufficiency or failure which may occasionally become chronic (Abdal Raheem et al. 2014). Few studies are found in the literature regarding the indication of prolonged parenteral nutrition after bariatric surgery. Abdal Raheem et al. (2014) reported on six patients who became dependent on parenteral nutrition after bariatric surgery, with four of them suffering from short bowel syndrome. The response of patients to nutritional therapy should be monitored mainly if hypoalbuminemia occurs. A shorter half-life protein such as prealbumin followed over time may be a useful parameter for monitoring the response of the patient to nutritional therapy (Koch and Finelli 2010).

With nutrition therapy, it is interesting to consider the BMI. Protein supply recommendations are $\geq$1.9 g/Kg for class I and II obesity (BMI 30–40(Kg/m^2)

and ≥2.5 g/Kg of ideal body weight for class III obesity (BMI ≥40 Kg/m^2) (Choban and Dickerson 2005). Moizé et al. (2013) recommended a supply of calorie of 25–30 Kcal/kg weight/day and of protein about 1.2 g/Kg weight/day. No consensus was observed to exist as to which correction factors should be used to establish the total energy requirements for enteral or parenteral feeding, since malnourished patients are at risk for suffering complications related to refeeding syndrome. On the other hand, A.S.P.E.N. guidelines recommend starting with a hypocaloric, high-protein feeding for the critically ill, obese patients, with normal renal and hepatic function, aiming at preventing overfeeding and hyperglycemia and achieving a net protein anabolism (Choban et al. 2013; Isom et al. 2014).

The best method for measuring energy needs is the indirect calorimetry. When this is not possible, the measurement of energy requirements for the critically ill, adult, obese patients may be done by estimation of the resting metabolic rate (RMR) by using predictive equations involving some variables according to the patient's characteristics (Mifflin et al. 1990; Ireton-Jones and Jones 2002; Choban et al. 2013; Isom et al. 2014; Beebe and Crowley 2015) (Table 2). Based on the physiological and anatomical effects of bariatric surgery, Isom et al. (2014) suggest that recent guidelines for critically ill, obese patients can be applied to all patients undergoing bariatric surgery.

The most frequently used equations to estimate energy expenditure by obese patients are shown in Table 2. The choice of predictive equations should be based on patient's clinical status (Mifflin et al. 1990; Breen and Ireton-Jones 2004; Isom et al. 2014).

The A.S.P.E.N. Clinical Guidelines for Nutrition Support of Hospitalized Adult Patients with Obesity recommend the use of the Penn State University 2010 equation (PSU) for critically ill, ventilated, obese patients ≤60 years of age and the PSU modified equation for patients >60 years of age (Isom et al. 2014). If the PSU equation cannot be used or patients are not ventilator-dependent, the use of the Mifflin-St. Jeor (MSJ) equation should be recommended (Mifflin et al. 1990; Choban et al. 2013; Isom et al. 2014). For those equations, the actual body weight should be used. The calculated resting metabolic rate (RMR) should be multiplied by the appropriate activity and stress factors, such as sex (male = 1, female = 0), trauma (present = 1, absent = 0), and burn (present = 1, absent = 0) (Ireton-Jones and Jones 2002; Choban et al. 2013; Isom et al. 2014; Beebe and Crowley 2015).

For critically ill patients, the A.S.P.E.N. guidelines recommend starting with hypocaloric, high-protein feedings providing ≤14 Kcal/Kg of the actual body weight. The protein requirements for this type of feeding are 1.2 g/Kg of actual weight and 2–2.5 g/Kg of ideal body weight (Isom et al. 2014). In the trauma intensive care unit, class I or class II obese patients receiving hypocaloric, high-protein enteral feedings need a minimum of 1.9 g/Kg ideal body weight/d to achieve nitrogen equilibrium. For the class III obesity patients, a minimum of 2.5 g/Kg ideal body weight/day or more are necessary (Choban and Dickerson 2005).

Laboratory monitoring of malnourished patients after bariatric surgeries should be done to search for good results. Nutrition supervision is of great value, as malnutrition and vitamin and micro- and macronutrient deficiencies may lead to

Table 2 Predictive equations of energy requirements for adult obese patients

PSU 2010 (≤60 years)	RMR (Kg/day) = MSJ (0.96) + Tmax (167) +VE (31) − 6212
PSU (modified) (>60 years)	RMR (Kg/day) = MSJ (0.71) + Tmax (85) + VE (64) + 3085
MSJ (men)	RMR = (10 × Weight [Kg]) + [6.25 × Height ([cm)] − (5 × Age) + 5
MSJ (women)	RMR = [10 × Weight (Kg)] + [6.25 × Height (cm)] − (5 × Age) – 161
Ireton-Jones (mechanical ventilation)	EE(v) = 1925 – 10(Age) + 5 [(eight [Kg]) + 281(S) + 292(T) + 851(B)
Ireton-Jones (spontaneous breathing)	EE (s) = 629 – 11(Age) + 25 [Weight (Kg)] – 609(O)

PSU (Penn State University), *MSJ* (Mifflin-St Jeor), *RMR* (resting metabolic rate), *Tmax* (maximum temperature in the past 24 h), *VE* [minute ventilation (L/min)], *EE* (energy expenditure), *v* (mechanically ventilated), *s* (spontaneous breathing), *S* [sex (male = 1, female = 0)], *T* (trauma), *B* [burn (present = 1, absent = 0)], *O* [obesity (present = 1, absent = 00)]

deleterious consequences (Handzlik-Orlik et al. 2015). Isom et al. (2014) and Handzlik-Orlik et al. (2015) suggest that vitamins B1, B12, and D, calcium, folic acid, and iron should be monitored before bariatric surgery and at 3, 6, and 12 months and yearly after; vitamin A before and yearly after bariatric surgery, and vitamin K, zinc, and copper yearly after the surgery. The measurement of the micronutrients may be done at shorter intervals or at any moment if necessary, mainly for malnourished patients.

Revision surgery is frequently indicated in cases of SPCM. If anatomic causes occasionally continue after bariatric surgery, malnutrition may persist or be reestablished, even after a favorable response to conservative treatment has occurred. According to Akusoba et al. 2016, it is of interest to perform revision surgery after a significant improvement of a SPCM. It is also crucial to perform this procedure at an opportune time without over insisting on protein replacement therapy when the latter proves to be inefficient, and a delay might be dangerous. Potentially fatal protein-calorie malnutrition can occur after gastric bypass or after biliopancreatic diversions (Wade et al. 2010), and revision procedures can be an obligation (Buchwald 2015).

Policies and Protocols

Policy

Obesity is one of the current problems of public health, especially in the industrialized and developing countries. Due to its frequent comorbidities, obesity exerts a substantial negative impact on the quality of life and causes reduction in the patient's life expectancy, besides being a significant economic burden to society because of the lower service income of the obese and higher public health

expenditure. In this chapter, we reported that bariatric surgery is the most effective method of treating severe obesity, but it only solves the problem of the relatively few patients selected. The governmental acts of obesity care have been directed almost exclusively to the treatment of the already obese. However, in order to fully tackle the problem, several issues should be addressed, such as acknowledging that the medical treatment of patients with severe obesity results in limited and transient weight loss, with almost constant failures, surgical treatment is not free from complications, and public hospitals are unable to meet the full demand for bariatric surgery. Based on the importance of those facts, it is essential that governments should optimize their actions aiming at the prophylaxis of obesity through building awareness of the problem and providing guidance to the population at various levels, besides implementing resources of care conductive to a healthy lifestyle.

Protocol

Obesity is characterized by BMI values higher than 30 Kg/m^2. In this chapter, we reported that the BMI is the best indicator for measuring obesity and its prevalence. However, it is important to have in mind that visceral and subcutaneous adipose tissues are morphologically and functionally different, including metabolic differences. The preponderant accumulation of visceral fat over peripheral fat is expressed in the increase in waist circumference, defining the so-called visceral or central obesity. Central obesity, more common in men than women, is more often associated with metabolic syndrome and, consequently, poses higher cardiovascular risk. People with BMI higher than 30 kg/m^2, however, may have normal body fat and increased muscle mass, such as athletes, and people with normal BMI may have excess visceral fat and reduced muscle mass, thus facing higher risk of cardiovascular diseases. These data illustrate the risk of taking the BMI alone as a reference for the definition of obesity and its severity. Waist circumference and body composition measurements are important auxiliary indicators to identify the severity of obesity. The evaluation of body composition depends on the use of a special device for measuring the bioelectric impedance, while that of abdominal circumference is simpler and faster to be done, using only a tape measure.

For adult men, a short waist circumference is considered to be less than 94 cm long, a long circumference from 94 to 102 cm, and a very long over 102 cm. For adult women, a short waist circumference is considered to be less than 80 cm, a long one from 80 to 88 cm, and a very long more than 88 cm. Several anatomical references have been reported for the measurement of abdominal circumference. One method that seems most reliable is the tape measure, positioned at the midpoint between the lower edge of the last ribs and the upper pole of the iliac crest, after normal inspiration. For a more reliable assessment of obesity and its prevalence, a simple measurement of the abdominal circumference, associated with BMI, should be considered.

Dictionary of Terms

- ***Obesity*** – a chronic metabolic disease of multifactorial causes, with genetic and environmental determinants, characterized by excess body fat associated with weight gain in relation to height. Obesity is defined as BMI values equal or higher than 30 kg/m^2.
- ***Body Mass Index*** (BMI) – is considered the best indicator for measuring obesity and overweight and their prevalence and is calculated by dividing the body weight (in kilograms) by the square of height (in meters). The normal BMI values are between 18.5 and 24.9 kg/m^2. It is important to keep in mind the additional value of the measurements of abdominal circumference and body composition.
- ***Bariatric*** – the word was coined in the 1960s, from the Greek BAROS, "weight," and a derivative of IATRÓS, used with the meaning of "medical practice." The word bariatric refers to the branch of medicine that encompasses the analysis and treatment of obesity. This would include studying the causes of obesity, its symptoms, physical conditions, and commonly associated treatments, as well as other aspects of the disease.
- ***Intestinal bacterial overgrowth*** – is the excessive growth in the number of bacteria that inhabit the stomach, duodenum, jejunum, and proximal ileum and/or alteration in the type of bacteria. Diagnosis of intestinal bacterial overgrowth can be accepted as the concentration of $\geq 10^5$ bacteria/mL in the proximal jejune aspiration.
- ***Refeeding syndrome*** – a potentially fatal condition that occurs within the first few days after initiation of enteral or parenteral nutrition therapy, in which there is acidosis, increased intravascular volume, hyperinsulinemia, hypoglycemia, and severe disorder of electrolytes, body fluids, and micronutrients, besides metabolic abnormalities. Patients commonly have low levels of serum phosphate, potassium, and magnesium.
- ***Metabolic syndrome*** – is characterized by a genetic and/or acquired decrease in insulin sensitivity, notably in the muscles, liver, and adipose tissue, which leads to elevated glycemic levels.

The III Adult Treatment Panel of the National Cholesterol Education Program proposed a simple scheme for the routine diagnosing of metabolic syndrome, according to which diagnosis can be established if there are at least three of the following five risk factors (American Association 2002):

- Increased abdominal circumference ($\geq$102 cm in men and $\geq$88 cm in women)
- Increased triglycerides ($\geq$150 mg/dl)
- Reduction of HDL cholesterol ($<$40 mg/dl in men and $<$50 mg/dl in women)
- Increased blood pressure ($>$130/85 mmHg or under treatment for hypertension)
- Increased glucose ($\geq$100 mg/dl)

Summary Points

- This chapter focuses on severe protein-calorie malnutrition (SPCM) after bariatric surgery, its causes, diagnosis, and medical treatment.
- A literature review was conducted to identify studies reporting the causes, severity, and medical management of protein-calorie malnutition (PCM), as well as the results of treatment. Little information is available about evidence-based studies, especially on the severe form of malnutrition.
- Protein-calorie malnutrition may be described as protein, energy, and micronutrient deficiency, depending on the inability of the body to satisfy the protein and energy fuel requirements through diet (Torún 2009).
- According to the World Health Organization, the diagnosis of SPCM can be made for BMI <16 kg/m^2, but there are error factors associated with body weight. Although the disproportion between the body mass distribution and the hydric balance is cited as an error factor, the changes in the body weight may reflect an insufficient energetic or protein absorption.
- Severe protein-calorie malnutrition is a marked protein and energy deficiency resulting in a generalized loss of muscle mass, decline in nutritional status, weakness, apathy, and a significant reduction in physical capacity with the risk of death.
- The severity of PCM can be established by the assessment of nutritional status based on the clinical history of the patients, on their anthropometric measurements, and on laboratory tests.
- The relation of the percent weight loss to the time of malnutrition development is of great value for assessing PCM risk.
- Speed of installation of malnutrition and low BMI are associated with the risk of patient death.
- Medical therapy for PCM after bariatric surgery does not differ much from that used for PCM caused by other factors than bariatric surgery and emphasizes nutritional support.
- Surgical revision for SPCM after bariatric surgery is indicated when there is a failure of medical treatment or a prevailing anatomical cause, such as anastomosis stenosis refractory to endoscopic dilatation.

Case Report

A 51-year-old obese woman, with a BMI of 50 Kg/m^2, underwent RYGB surgery and 1.5 months later presented vasomotor symptoms suggestive of early dumping syndrome such as tachycardia and intensive sweating and also nausea. To avoid those symptoms, she started restricting food intake, skipping meals, and eating small quantities. Six months after the surgery, the conditions evolved with diarrhea mounting up to ten daily liquid evacuations. However, she did not look for medical assistance for over 2 years after surgery, when she eventually was referred to hospital with complaints of fatigue, difficulty in walking, hair loss, edema, and diarrhea.

Table 3 Nutrition oral and parenteral therapy

Days after hospital admission	Oral	Parenteral			Parenteral + Oral Kcal/day
	Low CH, low fat Kcal/day	Protein (20%) g (Kcal/day)	Carbohydrates (50%) g (Kcal/day)	Lipids (20%) g (Kcal/day)	
10–13	600	45 (180)	135 (459)	30 (57)	1116
14–16	600	70 (280)	150 (510)	38 (72)	1182
17–20	600	85 (340)	180 (612)	42 (80)	1292
21–30	600	85 (340)	210 (714)	48 (91)	1405

CH Carbohydrates

On physical examination, she showed signs of severe protein-calorie malnutrition, including generalized depressive edema, pleural effusion, bruises, and thin and brittle hair. BMI was 24 Kg/m^2. Laboratory data showed hypoalbuminemia (1.7 g/dL), macrocytic anemia, lowered red blood cells count (2,35 10^6 U/L), hemoglobin (8 g/dL), hematocrit (24%), a medium corpuscular volume slightly increased (102), and lowered copper content (44.2 ug%). Sudan test was positive. Reduced oral food intake and mainly diarrhea after RYGB were hypothesized as the main causes of SPCM. Due to the severity of the clinical picture, intestinal transit time and breath test to exclude bacterial overgrowth syndrome were not done at that moment.

Nutrition support was started immediately through enteral nutrition associated with oral diet. A standard enteral nutrition of 200 mL in 24 h was initiated, as well as a sucrose-free and low-fat oral diet, split into six meals per day. The enteral nutrition was continuously administered through a nasoenteric tube with an infusion pump. On the second hospitalization day, the patient had diarrhea with liquid and loose stools, abdominal distension, and flatulence. Then, the enteral diet was discontinued, and the oral diet was maintained and slowly increased. The maximum volume tolerated by the patient was 1200 mL/day, corresponding to about 1200 kcal/day. The stools were still watery and loose, and the daily episodes were around 7 to 8.

Since the patient was not showing any improvement in nutritional status, parenteral nutrition was initiated on the tenth hospitalization day through central venous access. Calorie and protein contents of parenteral and oral nutrition are summarized in Table 3.

Parenteral nutrition was provided for 27 days, and oral diet was maintained in small quantities. After this period, enteral nutrition was restarted, 600 mL/day being administered with continuous infusion. During the parenteral and oral nutrition period, the patient's edema gradually decreased, the bruises disappeared, and the hair started growing again. The patient also had an improvement on the diarrhea. The evacuations decreased to two to three episodes a day, and the stool consistency became thicker. With the decreasing of the edema and the improvement in the nutritional status, the enteral nutrition was gradually increased to 1350 kcal/day. The oral diet was maintained and provided around 600 kcal/day. The hair was thicker, and the stools were no longer loose. The laboratory tests showed normal results for serum albumin (4.1 g/dL), hemoglobin (13 g/dL), hematocrit (38%), red blood cells (4 10^6 U/L), and medium corpuscular volume (94) and a significant increase in copper (60.4 ug%). As the patient showed clinical and nutritional

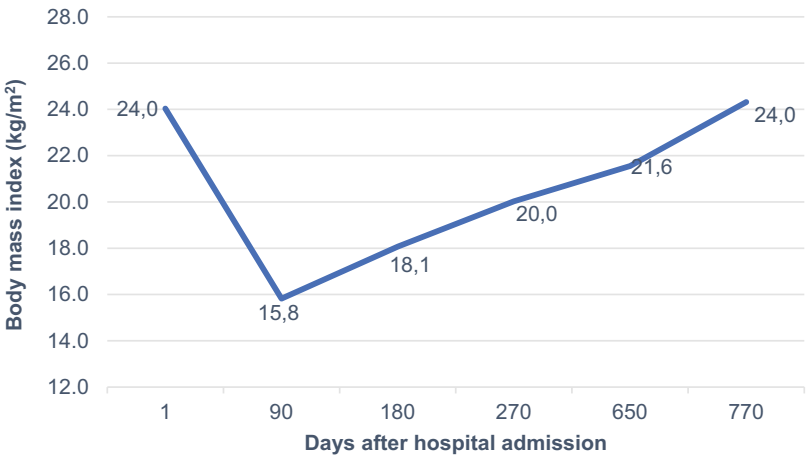


Fig. 4 Body mass index evolution

improvement and increased oral intake, the enteral nutrition was gradually reduced and discontinued at the time of the patient's discharge, after 90 days of hospitalization. The patient was instructed to maintain the oral diet at home, low in sucrose and fat, split in six meals a day.

She continues taking multivitamin and minerals supplementation and being attended at the outpatient bariatric service. Along 2 years after discharge, the patient has had discrete increases of the BMI. After 770 days following discharge, she eats seven meals a day and takes oral protein supplement mixed with fruit twice a day. She does not exhibit clinical signs of protein-calorie malnutrition, her BMI is about 24 Kg/m^2, and her corporal weight is 60 Kg. The most recent laboratory tests revealed normal serum albumin (4.0 g/dL), red blood cells (3.52 10^6 U/L), hemoglobin (11.5 g/dL), and hematocrit (36%). Figure 4 represents the changes in the BMI, 24 Kg/m^2 on admission reduced to 15.8 Kg/m^2 on discharge, probably due partially to the decrease in edema, and increased to 24 Kg/m^2 after 25 months, probably due the increase in corporal muscular mass and fat.

Although the BMI presented normal value on hospital admission (24 Kg/m^2), there are mistake factors associated with body weight. The patient presented edema, but although the disproportion between the body mass distribution and the hydric balance is cited as an error factor, the body weight changes reflect an insufficient energetic or protein intake. There was an important reduction in the preoperative BMI (52%), greater than that expected for patients undergoing RYGB, for whom the weight loss has been referred between 30% and 40% within 1–2 years after the surgical procedure.

The purpose of presenting this case was to emphasize the occurrence of serious malnutrition and anemia due to reduction in food ingestion and mainly to diarrhea in a patient submitted to RYGB and the successful result of treatment just through nutritional support and correction of the dietary mistakes. The patient recovered the normality of her nutritional status, shown by clinical and laboratory results. The question remains whether the long hospital stay could

have been reduced with the administration of pancreatic enzymes and/or antibiotics. The diarrhea episodes were reduced, but the patient still presents a frequency of around three to four daily evacuations of softened stools, and malnutrition and anemia may return if there is non-compliance with the scheduled nutrition recommendations. The occasional recurrence of diarrhea and malnutrition would require a better investigation of the various hypothetical causes of diarrhea, among them intestinal bacterial overgrowth, low digestive action of pancreatic enzymes, and short bowel syndrome. Based on the diagnosis, the proper treatment should be done, including revision surgery.

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National Programs and Policies to Address Child Malnutrition in India: Challenges and Opportunities

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Abstract

Despite India's progress in launching and scaling up programs aimed at achieving optimal child nutrition over the last decade, India fell short of the 2015 Millennium Development Goals. This chapter reviews existing national level programs that address child nutrition, details their performance, and recommends changes that can enhance their impact on child nutrition. Specifically, we identify challenges and opportunities in the implementation and operation of national level programs, which currently function in silos and lack a cohesive coordinating strategy. This chapter also documents the palpable interest in advancing maternal and child health through national policy, advocacy, and social engagement that pervades India.

Keywords

Child nutrition · India · Health policy · National programs · Nutrition framework · Governance · Implementation science · Advocacy · Rural-urban disparities · Social determinants of health

List of Abbreviations

ASHA	Accredited Social Health Activists
ICDS	Integrated Child Development Services
MAA	Mothers Absolute Affection
NHM	National Health Mission
NRC	Nutritional Rehabilitation Centers
NREGA	National Rural Employment Guarantee Act
NRHM	National Rural Health Mission
ORS	Oral Rehydration Salts
PDS	Public Distribution System
PRISM	Practical, Robust Implementation and Sustainability Model
UNICEF	United Nationals International Children's Emergency Fund
WHO	World Health Organization

Introduction

In 2017, India continues to be at the epicenter of the child undernutrition epidemic. The intransigent nature of child undernutrition in India poses a vexing issue for public health experts, politicians, economists, and philanthropists, alike. Over the past decade, the proportion of stunted children only decreased by 10% (48–38%). Meanwhile, the proportion of children suffering from wasting increased by 1% (19.8–21.0%). Evidently, gaps persist in the knowledge-base and implementation know-how, which are preventing national-level programs from being more impactful in addressing the crisis. This chapter details existing national-level programs and policies aimed at addressing child undernutrition in India.

We organize this discussion of policies around the 2013 evidence-based framework on child malnutrition, which draws attention to the multifactorial causes of child malnutrition and the importance of identifying potential entry points for interventions (Fig. 1) (Black et al. 2013). According to this model, optimal child nutritional status can be achieved by three interdependent pathways improving (1) adequate dietary intake, (2) feeding and caregiving practices, and (3) overall health status (Black et al. 2013). A robust enabling environment at a national level that can support and sustain public health interventions and programs underlies the promise to affect change (Gillespie et al. 2013). This enabling environment can lead to nutrition-sensitive and nutrition-specific public health programs that can improve nutrition through the three aforementioned pathways. Nutrition-sensitive interventions address underlying determinants of malnutrition (Ruel and Alderman 2013), whereas nutrition-specific address immediate-determinants of malnutrition (Bhutta et al. 2013). Therefore, this framework facilitates a comprehensive discussion of child nutrition in India ranging from public policy to personal practice. Figure 1 lists major existing nutrition-sensitive and specific programs in India (bold).

National Policies and Programs

Creating an Enabling Environment

India is uniquely positioned to invest in its maternal and child health due to the capital generated by its booming economy. Led by commendable advocacy efforts of stakeholders, which include human-rights activists, economists, and academicians, nutrition has gained prominence in India's national agenda over the last decade (Swaminathan 2009). A more notable example of this advocacy is the Supreme Court case filed by activists to recognize the right to food as a legal right which was litigated for almost 10 years (2001–2011) (Mander 2012). Eventually, the Indian Supreme Court justices not only ruled in favor of the petition but also mandated enforcement of programs that provide food to underprivileged populations as their legal rights.

Despite these achievements, Indian government's commitment to address nutrition ranks 30th out of 45 nations with severe or alarming undernutrition status (Institute of Development Studies, UK Aid and Irish Aid 2015). This nutrition index considers 12 indicators based on legal decisions, public policies, and public expenditure. A closer look at this index reveals that significant areas of improvement for India at a governance level include developing multisector policy coordination and establishment of time-bound nutrition targets. To accentuate this point, consider Fig. 2 that provides an overview of national programs for addressing child undernutrition. The eight programs described in this figure and detailed below are run by a total of seven different departments from seven different ministries. This lack of horizontal integration leads to redundancy and competing agendas, which may lead to confusion and suboptimal implementation of policies.

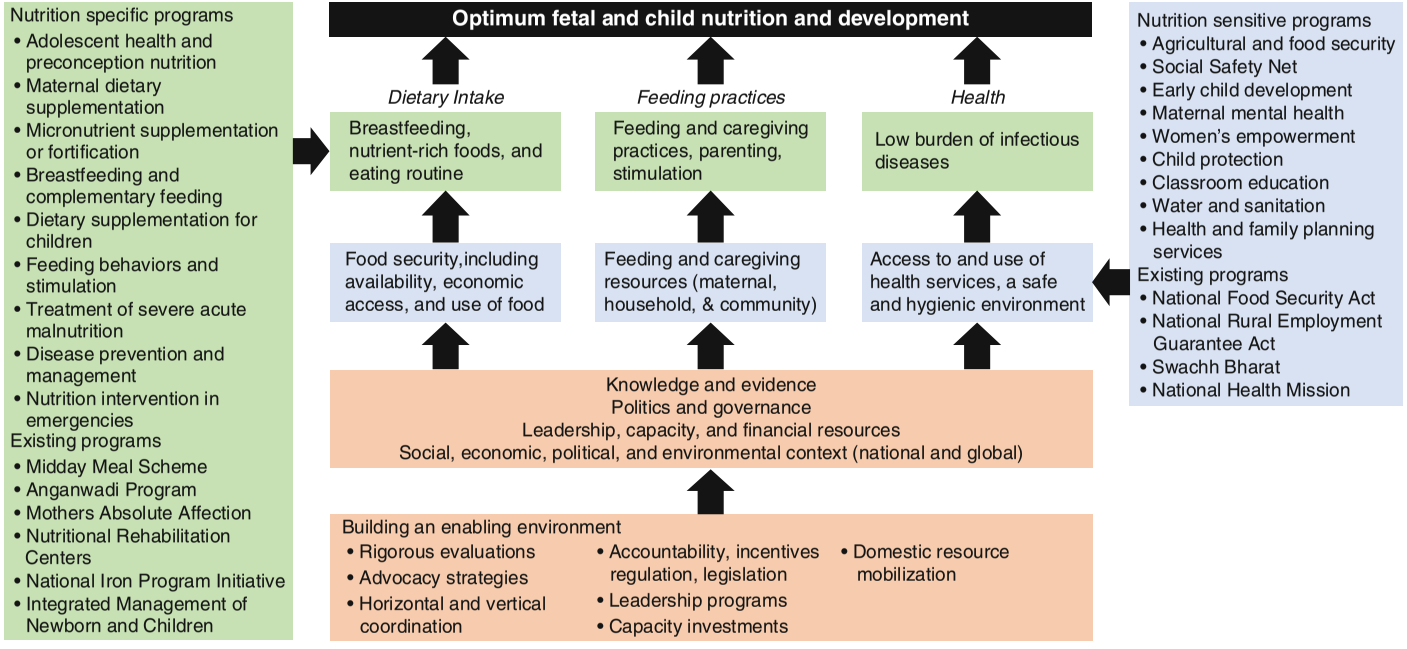

Fig. 1 Framework for optimum child nutrition and development. Modified version of the framework for achieving optimal child nutrition proposed by Black et al. (2013) in a series of articles for child nutrition in the Lancet. Key adaptions in this figure include categorization of existing programs in India at their respective level of action (Reprinted from Black et al. (2013), with permission from Elsevier)

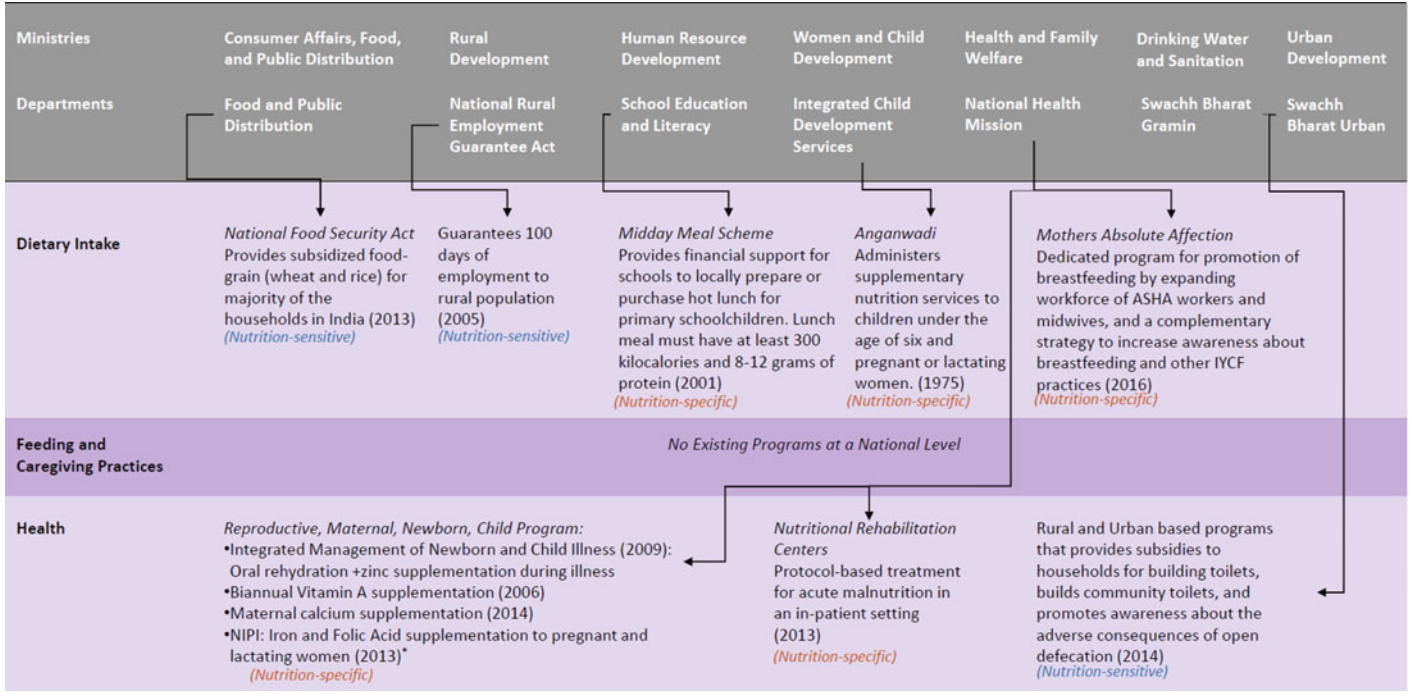

Fig. 2 An overview of national programs in India addressing child nutrition. Existing Indian government programs to support at-scale coverage of evidence-based interventions. The programs are organized based on the pathways (Fig. 1) through which they affect child undernutrition (Credit: Unpublished work by authors, Key: NIPI = National Iron Plus Initiative. ASHA = Accredited Social Health Activists)

Improving Infant and Young Child Feeding Practices: Breastfeeding and Complementary Feeding

Background: National level data from the 2005 National Family Health Survey spurred a nationwide effort to promote Infant and Young Child Feeding practices. According to these results, although the majority (87%) of mothers reported breastfeeding their child in the first year of life, less than a quarter reported early initiation of breastfeeding and less than half reported exclusive breastfeeding for children under the age of 6 months (Menon et al. 2015). Based on the most recent data available from 13 states for which the 2014–2015 National Family Health Survey data have been released, the proportion of children who were breastfed within 1 h of birth and those who were exclusively breastfed has increased by 13.4% (range: −5.1 to 30.9) and 18.4% (range: −6.3 to 43.2), respectively. Conversely, the proportion of children with timely initiation of complementary foods has decreased. In 2005, roughly two out of every three children received solid, semi-solid, or soft food between 6 to 8 months and, while 2014–2015 data are available for only 11 states, it suggests an average decrease of 9.7% (range: −23.8 to 4.9) (Menon et al. 2015). This decline is concerning particularly when considering evidence from randomized controlled trials suggesting that timely initiation of complementary feeding is more effective in preventing stunting than breastfeeding (Jones et al. 2003).

Anganwadi centers: Named after the Hindi word for court shelter, Anganwadi centers are the primary platform for delivery of the Integrated Child Development Services (ICDS). There are more than 1 million Anganwadi centers across India, and their workers are employed by the Ministry of Women and Child Development to administer supplementary nutrition services to children under the age of six and pregnant or lactating women (Bredenkamp et al. 2006; Saxena 2016). Additionally, Anganwadi workers provide nonformal preschool education, health education, immunization, health check-up, and referral services. Initially, these centers started as a program for households that are below the poverty line; however, legislative amendments in 2014 have expanded the eligibility to all children under the age of six.

The full impact of ICDS on child undernutrition is blunted by poor quality and coverage of nutrition-specific programs. In 2011–2012, only 39% of all eligible children had ever received a take-home food ration or prepared hot meals and only 14% of children had received it in the previous month (Kandpal 2011; Desai and Vanneman 2015). Interviews of Anganwadi workers suggests that they are overburdened with the task of deploying multiple programs from different departments and onerous documentation takes away from time that could be used providing services (Programme Evaluation Organisation: Planning Comission 2011). Because allocation of resources and funding to Anganwadi centers is contingent upon documentation, gaps in the effective functioning of these centers have emerged and persisted over time, particularly in high-demand regions (Programme Evaluation Organisation: Planning Comission 2011).

Considering the prominent role played by Anganwadi centers in combating child undernutrition in India, it is important to focus on overcoming process-related challenges by strengthening the workforce at a local level and leveraging technology to mitigate the burden of administrative responsibilities. The newly established

Mothers Absolute Affection program aims to increase local workforce for maternal and child nutrition, specifically for promoting infant and young child feeding practices. More details on this program are described below. The impact of this program's initiative towards meeting the nutrition needs of its community and its coverage of Anganwadi-based programs remains to be seen.

Mothers absolute affection (MAA): MAA is an intensive nationwide effort that was implemented in August 2016 to enhance breastfeeding and other infant and young child feeding practices (Ministry of Health and Family Welfare and Mission 2016). MAA proposes to strengthen the workforce of Accredited Social Health Activists (ASHA: *Hindi word for hope*) and Auxiliary Nurse Midwives at a community level and train them to provide lactation support services to young mothers. The program has dedicated funding to generate awareness about the importance of breastfeeding through mass-media activities that include TV commercials, radio spots, and print media. Additionally, ASHA workers are tasked to conduct regular meetings with mothers from their community to promote breastfeeding practices and reinforce this message during their home visits as part of the checkup for newborns. The MAA program proposes to recruit and train additional ASHA and auxiliary nurse midwives to saturate the villages by August 2017.

A review of the operational guidelines released by National Health Mission raises many concerns. First, the program is centered on breastfeeding practices, and no specific plan is proposed for promoting other Infant and Young Child Feeding practices. Considering that complementary feeding practices and dietary diversity have decreased in the past 10 years, a lack of focus on these indicators is troublesome. Second, the program is not grounded on a cohesive implementation framework. Although there is a concerted effort to consider program activities at a macro-, meso-, and microlevel, a lack of framework impairs the ability to define the implementation processes and evaluate performance in a time-sensitive manner. The dynamic ability to generate feedback during the implementation stage and initiate corrective actions at the regional level is very important in India's socio-culturally diverse context. Lastly, the MAA program is primarily operated by the National Health Mission and misses the mark on horizontal integration with ICDS services and the Anganwadi program. The MAA program largely depends on developing new interactions and contact-points with reproductive-aged women and does not leverage existing partnerships developed by Anganwadi workers.

These limitations notwithstanding, the MAA program has tremendous potential to improve maternal and child nutrition based on its scope and the resources allocated for the program. The program's reach and impact could be enhanced by employing a more dynamic implementation framework, increasing collaboration with ICDS services, and integrating evidence-based interventions in its platform.

Promoting Dietary Diversity

Background: Diversity in mother and child's diet is an important determinant for child undernutrition (Bhutta et al. 2008, 2013). A review of national level data from the past 30 years suggests that dietary diversity has steadily declined in India, as has

overall dietary intake. This decrease is observed in both rural (2150 calories/person/day in 1993–1994 vs. 2020 in 2009–2010) and urban regions (2071 vs. 1946) in India (National Sample Survey Organisation 2012). Dietary diversity, as measured by the proportion of the population consuming at least four food groups (milk, vegetables, fruit, and pulses), has decreased over the same time period as well (Gaiha et al. 2013; Desai and Vanneman 2015). Remarkably, the proportion of women of reproductive age and children with low hemoglobin levels (< 10 mg/dl) has also increased over time (Desai and Vanneman 2015).

National Food Security Act: This new legislation, passed in 2013, strengthens the existing Public Distribution System (PDS) and expands the proportion of the country's households that can benefit from it. As a result, the PDS is now the largest safety net program in the world. It provides five kilograms of cereals per person per month at the subsidized rate of 1 to 3 rupees per kilogram to two-thirds of the country's population (Mishra 2013). However, this reform might be a double-edged sword. Recent scholarship based on data from 2011 to 2012 suggests that families receiving food through PDS predominantly used the subsidized cereals and had reduced dietary diversity (Desai and Vanneman 2015). After accounting for potential confounders, investigators found that on a national average, in comparison to nonbeneficiaries, beneficiaries consumed less milk (780 ml per capita) and less pulses (30 g per capita) on a monthly basis. The assumption that by providing substantial subsidies for cereal, families would use saved capital to consume diverse foods may be inaccurate. Considering the cereal-centric composition of India's staple diet, reduced consumption of milk and vegetables, secondary to a program aimed at enhancing food security, is concerning because dietary diversity is a crucial determinant of children's nutritional status. A higher proportion of children under the age of five from households that utilized Public Distribution System (40.2%) were underweight in comparison to children from households that did not take advantage of the program (33.4%). Adjusting for potential confounders reduced the difference between the two groups (39.4 vs. 37.4), but did not eliminate or reverse it (Desai and Vanneman 2015). Taken together with the evidence for the importance of a micronutrient-rich and diverse diet, there is an urgent need to amend this well-intentioned program to promote consumption of milk, fruits, and pulses.

National Rural Employment Guarantee Act (NREGA): This national employment program is another form of social safety net and guarantees rural households 100 days of unskilled manual work per year (Ministry of Law and Justice 2005). This program is implemented by local governments at the village level, and the costs are covered by the state and national governments. By 2014, 9 years after its inception, NREGA had been implemented across all 673 districts in India; 50 million households participated in the program that year, 48% of whom were women; and the program has generated a total amount of 46 billion US dollars (Ministry of Rural Development 2015). A cross-sectional study of 540 households from Rajasthan, one of the first states to implement NREGA, suggests that female participation is inversely associated with acute forms of child malnutrition but not with chronic forms (Nair et al. 2013). However, other states have not implemented this program to its fullest potential. According to a recent analyses of the program,

20 out of the 31 states in India have suboptimal implementation and can maximize their rural employment gains by increasing engagement of low caste populations and women (Singh 2016). In comparison to efficient states, inefficient states had 133% less involvement of women and 75% less involvement of low caste populations (Singh 2016). Thus, the promise of this program for socioeconomic empowerment remains unfulfilled and concerted efforts to engage women and low caste people in this program, especially in historically poor states, should be prioritized. One possible solution for this challenge is to expand the scope of job opportunities to include apprenticeships for women with female health workers supporting Anganwadi, MAA, and National Health Mission program activities.

Midday meal scheme: Based on a 2001 Supreme Court decision in 2001, the national government funds provision of cooked meals consisting of at least 300 kcal and 8–12 g of protein to children at school. These meals are prepared locally either by the schools themselves or in collaboration with (NGOs) (Khera 2006; Chutani 2012). As of 2015, more than 136 million primary-school going children in India receive meals because of this program (Kumar 2016). A review of survey-based studies have found that there is a rise in attendance and enrollment rates, especially among girls and low caste people after the implementation of this program (Khera 2006). Receipt of Midday meals is associated with a reduced gap between recommended dietary allowance and actual daily intake of children of 30% for total calories, 10% for iron, and almost entirely for protein (Afridi 2010). Another study of children living in drought-prone regions of Andhra Pradesh found that Midday Meals blunted the adversarial effect of drought on children's nutritional status (Singh et al. 2014a). Midday Meal Scheme has also faced substantial controversy due to reports of unsanitary cooking conditions resulting in illness among children across the country. In one such episode in Bihar, 22 children died after consuming meals cooked with adulterated oil (Singh 2013; Pain 2014). The governing body responsible for Midday Meal Scheme has endeavored to set cooking standards and has established an expert committee for ensuring quality and proper sanitation (Kumar 2016). Unfortunately, calls for fortification of food grains were dismissed during the most recent committee meeting for this program (Kumar 2016).

National iron program initiative: The Indian government launched this program as part of their comprehensive strategy to address iron deficiency anemia in the subcontinent. The most recent national-level estimates of anemia and iron deficiency are based on 2005–2006 National Family Health Survey data which revealed that roughly 70% of children under the age of five were anemic and that the prevalence of anemia among mother and child had increased since 1998–1999 (Deb 2015). Under this program, different recommendations are proposed for providing iron and folic acid supplementation for five different groups: children aged 6–59 months, children aged 5–10 years, adolescents aged 10–19 years, pregnant or lactating women, and nonpregnant, nonlactating women in reproductive age (20–49 years). However, there are no explicit guidelines for coordination with other nutrition-specific programs (i.e., Anganwadi, MAA), and there is a paucity of evidence for the effectiveness or coverage of such micronutrient supplementation efforts. As of 2015, almost all states had reported providing supplements to lactating and pregnant mothers, but

only a few states provide supplements to children aged 6 months to 5 years (Assam, West Bengal, Uttarakhand, and Odisha) and even less provide supplements to children aged 5–10 years old (Assam and Odisha) (Deb 2015).

Biannual vitamin A supplementation program: Initially started as a prophylaxis program against nutritional blindness in 1970, the present-day national program for Vitamin A supplementation has undergone significant changes. In 2006, based on a workshop on micronutrients, the Indian Ministry of Health and Family Welfare instituted a policy for bi-annual vitamin A supplementation (Ministry of Health and Family Welfare, Department of Family Welfare and Child Health Division 2006). The program is implemented using the Government's ASHA workers, and in 2011, 62 million children (~66%) received two doses in 1 year. There are no publicly available evaluations of this program's coverage and effectiveness at a local or national level.

Promoting Water and Sanitation Hygiene

Background: The tremendous burden of poor sanitation and hygiene in India is highlighted by two remarkable statistics: (1) a third of worldwide population lacks access to proper sanitation and lives in India and (2) roughly two-thirds of rural Indian population defecate in the open, which highlights the tremendous burden of poor sanitation and hygiene in India (UNICEF and WHO 2012).

Swachh Bharat Program: The current government program to improve sanitation facilities across the rural regions of the country, officially launched on 2 October, 2014 (Mahatma Gandhi's 145th birth anniversary), revamps existing programs dating back to the 1986 (Hueso et al. 2013). In 1986, to reduce open defecation, the Indian government launched a top-down program to provide families with subsidies towards building a toilet. The program invested 6 billion rupees and constructed over 9 million latrines in rural areas but did not produce much change in the sanitary practices as only 22% of rural households reported access to a toilet in 2001 (Department of Drinking Water and Sanitation and Ministry of Rural Development 2012). In response, India launched a rigorous campaign in the 2000s that aimed at enhancing knowledge and awareness of the importance of proper sanitation among rural households (Hueso et al. 2013). However, this effort also fell short of its expectation and the rural sanitation coverage in 2011 improved only by 9% points (Chandramouli 2012).

The current iteration, the Swachh Bharat Program, is informed by lessons learned from these previous experiences and employs a subsidy-based and public-private partnership approach that is supplemented by a massive campaign for generating demand through Information, Education, and Communication tools (Ministry of Urban Development 2014; Ministry of Drinking Water and Sanitation 2016; National Sample Survey Organisation and Ministry of Statistics and Programme Implementation 2016). The goal of Swachh Bharat Program is to eradicate open defecation within 5 years, by October 2, 2019, which marks the 150th birth anniversary of Mahatma Gandhi.

It is unclear whether previous attempts to reduce open defecation by building more toilets resulted in a decreased incidence of diarrhea in children and a subsequent improvement in their nutritional status. Two separate cluster randomized controlled trials to evaluate the effect of Total Sanitation Campaign revealed that although the Government program was associated with an increase in individual household latrines, there were no discernible improvements observed for child health outcomes (Clasen et al. 2014; Patil et al. 2014). A possible reason for this disconnect between improved toilet facilities and health outcomes within the Indian context is the role of hygiene behavior and environmental presence of pathogens (Dutta et al. 2016). A trial to improve nutritional status of school-aged children by providing iron-fortified lunches reported an increase in total body iron but did not improve hemoglobin levels, possibly due to presence of soil-transmitted helminths (Osei et al. 2010). Therefore, the momentum towards improving sanitation facilities at a household level following Swachh Bharat Mission guidelines must be complemented with a systematic effort to help improve individual hygiene for caregivers and children. The scope and reach of MAA program focused on improving infant and young child feeding practices must be explored as a possibility to deliver messages for safe hygiene practices.

Nutrition During Illness and Management of Acute Malnutrition

National Health Mission (NHM): Similar to other nutrition-related programs discussed earlier in this chapter, NHM is an expansion and intensification of an existing program called National Rural Health Mission (NRHM) (Ministry of Health and Family Welfare 2012). NRHM was originally formed in 2005 to address the rural health needs of 18 states, where public health indicators were weak (National Rural Health Mission 2005). In 2013, NRHM was expanded to all 29 states and covered urban regions as well (Ministry of Health and Family Welfare 2012). Several successful maternal and child health programs that were started under NRHM or as experiments in other states were coalesced to form a comprehensive Reproductive, Maternal, Newborn, Child, and Adolescent health strategy grounded in the lifecycle framework for health (Black et al. 2008; Ministry of Health and Family Welfare 2012). Overall, there are more than 20 different child-health-related programs and interventions. We briefly describe selected activities that affect nutritional status of the child before presenting a detailed analysis of Nutritional Rehabilitation Centers, NHM's primary program to combat acute malnutrition (Fig. 2).

Surveillance and supplementation: Leveraging the workforce of nearly 1 million ASHA workers and more than 200,000 auxiliary nurse midwives, NHM aims to track all pregnancies in rural and underserved urban areas using its web-based Maternal and Child Tracking system and promote antenatal checkups. NHM has provisions for free institutional delivery and newborn care for 1 year at public institutions, along with an implementation of an evidence-based newborn care package such as vitamin K injections, support for Kangaroo Mother Care, and administration of antibiotics to prevent neonatal sepsis. Additionally, ASHA workers

conduct scheduled home-based newborn care visits within the first 6 months to identify early danger signs. Auxiliary nurse midwives throughout the country provide oral swallowable calcium tablets to pregnant women and ask them to take it daily (1 g calcium/day) from 14 weeks of pregnancy to 6 months postpartum (National Health Mission 2014). At the time of initiation in second trimester, 12 strips with 15 tablets each are provided by the auxiliary nurse midwives and supplemented during third trimester, at the time of zero dose of polio for the infant, and at the time of diphtheria, pertussis, and tetanus vaccination for the infant. ASHA and Anganwadi workers are tasked with the responsibility of performing house visits to check adherence to twice-daily regimen.

Treatment of acute illness: NHM developed Integrated Management of Newborn and Childhood Illness guidelines to provide field training for ASHA workers in indentifying acute cases of childhood diarrhea and treat them with WHO formulated Oral Rehydration Solution packets and zinc tablets. ASHA workers also collaborate with Anganwadi workers to conduct household visits during peak infectious disease season (July–August) to identify cases of childhood diarrhea and provide oral rehydration and zinc therapy. According to government's estimates, 200 million families were reached and 3.6 million children received ORS-zinc therapy in 2014 (Department of Health and Family Welfare 2015). No independent, peer-reviewed national-level assessment of this intervention has been reported. However, an assessment of adherence to national guidelines for zinc supplementation (10 mg/day for ages 2–6 months and 20 mg/day for ages 7–59 months for 14 days) among districts in the state of Uttar Pradesh revealed that less than half (47.8%) of the children prescribed the treatment were able to complete it within 14 days (Lamberti et al. 2015).

To provide protocol-based treatment for severe acute malnutrition, NHM has established a total of 891 Nutritional Rehabilitation Centers (NRC). Anganwadi workers are primarily responsible for monitoring growth of the children in rural settings and referring them to the NRC program if they are severely underweight as per weight-for-height standardized score or mid-upper arm circumference measurements (National Health Mission 2011). After referral, NRC staff evaluate the child for severe acute malnutrition based on a list of criteria that includes the presence of bilateral pitting edema with any of the following complications: anorexia, fever, persistent vomiting, severe dehydration, hypoglycemia, anemia, or any other indication based on the clinician expertise. Once admitted, guidelines from WHO and Indian academy of Pediatrics are followed until the child is more than −1 standardized deviation for weight/height. WHO recommended F-75 and F-100 diets are prepared by cooks using locally available products; however, recipes are not standardized or published in the operating guidelines for NRCs (National Rural Health Mission 2011). If the child does not meet the threshold within 2 weeks, they are discharged with instructions for preparing nutrition-dense foods. Anganwadi workers are tasked with the responsibility to provide home-based care and monitor nutritional intake of these children postdischarge. ASHA workers are responsible for facilitating four follow-up visits to NRC at 15-day intervals (National Rural Health Mission 2011).

Evaluation and limitations: A handful of regional assessments of NRCs have reported mixed results (Aguayo et al. 2013; Singh et al. 2014b, 2016). Overall, there are reports of modest weight-gain during hospitalization: an average of 9.3 and 12.1 g/kg/day in two separate studies (Singh et al. 2014b, 2016). Less than half of the children who were hospitalized met the threshold for improvement prior to discharge and less than 20% completed all four visits postdischarge (Aguayo et al. 2013; Singh et al. 2014b, 2016). However, there is consensus that NRCs help reduce mortality among children experiencing severe acute malnutrition (Aguayo et al. 2013; Singh et al. 2014b, 2016). The number of NRCs needs to dramatically increase, especially in tribal and historically underserved regions, to effectively care for approximately 6 million children in India who suffer from severe acute malnutrition. In the meanwhile, improvements in the current NRC model can fill some coverage gaps.

Programmatically, there are three major inadequacies in the current NRC program. First, the reliance on Anganwadi workers, who are supported by a different administrative branch and are already overburdened with an outstanding number of responsibilities, to identify and refer children possibly experiencing acute malnutrition creates the possibility of missed or improper identification. Indeed, majority (up to 75%) of the children referred to NRC do not qualify for hospitalization (Prasad et al. 2012). These children are sent back with instructions for preparing nutrition-rich food at home and sometimes, though not always, with treatment for any underlying illness. This experience, especially if it recurs, could breach the relationship between the family and Anganwadi workers, which could lead to decreased attendance in the Anganwadi. Second, the criteria for discharging children after 14 days of stay at the NRC result in almost half of the children returning home with suboptimal nutritional status (Dasgupta et al. 2014; Singh et al. 2014b, 2016). Currently, Anganwadi workers are responsible for conducting home visits to monitor nutritional intake, and ASHA workers facilitate follow-up visits with NRCs (National Health Mission 2011). This generates coverage gaps and may be the cause for the substantial attrition observed in follow-up. Third, there is a need for community-based program that treats moderate acute malnutrition or uncomplicated severe acute malnutrition. Currently, families of children who do not qualify for hospitalization at NRCs or are discharged from NRCs without recovery receive training to prepare nutrition-rich foods, and Anganwadi workers are responsible for monitoring their progress. However, ASHA workers are better-suited to perform home visits, while Anganwadi workers are more effective at their own centers. Management of community-based program can be achieved by pairing Anganwadi workers with ASHA workers such that WHO recommended nutrition-rich foods and fluids can be provided to children either at-home by ASHA workers or at the Anganwadi centers by Anganwadi workers their staff. Development of a community-based program can also alleviate concerns about improper referral by Anganwadi workers to NRCs and can shift the task of preparing nutrition-dense food from families to NHS or ICDS programs. A handful of states have implemented experimental programs for community-based management of acute malnutrition with the support of Médecins Sans Frontières (Bihar), Department for International Development – United Kingdom (Madhya Pradesh and Odisha), and UNICEF (Andhra Pradesh) (Dasgupta et al. 2014;

Dasgupta et al. 2015). These programs show promise for the management of mild-to-moderate acute malnutrition by implementing ready-to-use therapeutic food. There is strategic involvement from India's public policymakers and the existing workforce of Anganwadi and ASHA workers, which is being leveraged to support these state-led experiments (Feeney and Lee 2012). However, the heterogeneity in the implementation of each of these experiments limits the potential for scaling-up similar programs across the country (Dasgupta et al. 2015).

Summary

See Table 1.

The Way Forward

The lack of horizontal integration across different programs (Fig. 2) limits accountability and impairs the full promise of each individual program. An example of a harmonious coordination between these programs to affect change would include co-opting increased workforce from NREGA to develop local centers for preparing and delivering nutrition-rich meals to different programs: Midday Meals, Anganwadi, Hospital, and Community-Based NRCs, etc. Such an approach, employing rural Indian women for nonlabor work, has the potential to empower women by increasing their self-esteem and equipping them with a skillset to seek additional economic opportunities.

The field of implementation science offers an opportunity to enhance horizontal integration across different programs and can facilitate equitable coverage of services. For example, grounding the MAA program on an implementation framework such as Practical, Robust Implementation, and Sustainability Model (PRISM) can promote adaptability of the program at a local level, identify agents of change that can support the initial implementation, and define processes that can help sustain it over time (Feldstein and Glasgow 2008). Under this framework, local stakeholders can be engaged prior to implementation and help identify members from within the community that can help implementation of the program. This grassroots approach can generate buy-in from the community and enhance its uptake. Additionally, this process can also help identify parallel activities within the community, which can be paired with the implementation of MAA program. Moreover, this approach also provides a predefined framework for program evaluators to assess the impact of the program consistently across different settings.

In addition to improved governance of existing programs, equitable coverage of services for tribal and low-caste people must be prioritized. While discrimination based on caste is outlawed in India, low caste members of Indian society still experience it on a daily basis (Haq 2013; Thomas et al. 2013). In a study of 565 villages studied across 11 states, 33% of villages have public health workers who

Table 1 Impact of selected national-level programs on the coverage of evidence-based interventions and recommendations for improving child nutrition in India

Topic	Program	Coverage	Recommendation
Management of Severe Acute Malnutrition	Nutritional rehabilitation centers (NRCs)	1. Close to a thousand nutritional rehabilitation centers across the country 2. Overall reduction in mortality among children with severe acute malnutrition, but low cure rate (<50%), and poor follow-up (<20%).	1. Expansion of NRCs to increase coverage, especially in tribal and historically underserved regions 2. Establishing standards for preparing nutrition-dense foods using local products
	Swachh Bharat program (Gramin and urban) (***Nutrition-sensitive***)	1. Approximately 27 and 25 million more toilets built in rural and urban regions, respectively 2. Impact of the Swachh Bharat program on child growth is unclear, but studies based on previous versions of similar programs showed an improvement in toilet use but were unable to find improvements in child growth or health status	1. More evaluation of the impact of Swachh Bharat program is needed to assess its impact on incidence of infectious diseases and growth failure 2. Programs to promote healthy water and sanitation hygiene can enhance the potential health benefits of increasing toilet use throughout the country
Preventive zinc supplementation	Integrated Management of Newborn and Child Illness	1. Approximately, 3.6 million children received ORS-zinc therapy in 2014 2. A single-state study assessed adherence of national guidelines to zinc supplementation and found 47.8% of children completed treatment	1. Additional evaluation of preventive zinc supplementation is needed 2. Coordination with the ramp-up of community-based management of acute malnutrition
Promotion of breastfeeding	Mothers absolute affection	1. Too early to report program-based findings because it was launched in august 2016 2. Based on data from 2014 to 2015 NFHS, less than 50% of infants from high-priority regions received breastmilk within the first hour 3. Overall, breastfeeding practices increased from 2004 to 2005 while complementary feeding decreased	1. Enhancing emphasis on timely initiation of complementary feeding and dietary diversity in light of a declining trend in the last 10 years 2. Adopt a defined implementation strategy to identify pitfalls in a timely manner allowing for modification at a local level 3. Integrate ICDS and Anganwadi programs in a more defined and specific manner

(*continued*)

Table 1 (continued)

Topic	Program	Coverage	Recommendation
Appropriate complementary feeding	Anganwadi program	1. Recipients of services have improved nutritional status but coverage is poor, especially in high-demand regions. 2. In 2011–2012, less than half of the eligible children had ever received take-home food ration	1. Capacity building to increase coverage of services and lessen administrative burden of Anganwadi workers 2. Partnering with ASHA workers to deliver nutritional services at home
	Midday meal scheme (***Nutrition-sensitive***)	1. Evidence suggesting the effectiveness of the program in closing the nutritional gap, particularly for protein 2. Multiple reports of poor sanitary conditions leading to serious illness and deaths among children	1. Establish safety and sanitation standards for preparing meals (already in place) 2. Incorporate micronutrient-fortified food products in school lunches 3. Promote healthy handwashing and safety hygiene to children
Management of Mild acute malnutrition	No National-Level Programs	1. Five states have implemented experimental programs in collaboration with international health organization and support from Indian government	1. Standardization of protocol to allow for a scale-up of similar programs across other states
Periconceptual folic acid supplementation or fortification	National Iron Program Initiative (includes folic acid)	1. Since the program's launch in 2013, no national-level estimates of anemia has been reported 2. As of 2015, most states provide supplements to pregnant and lactating mothers but only a handful are providing supplements to children	1. Coordination with MAA program, midday meal scheme, and Anganwadi programs can help improve the coverage of this intervention
Maternal balanced energy protein supplementation	No National-Level Programs		
	National Food Security act (***Nutrition-sensitive)***	1. 30 states and union territories are providing subsidized food, 2 states (Chandigarh and Puducherry) are providing direct cash transfers 2. Likely to reduce dietary diversity because beneficiaries over-depend on subsidized cereal	1. Subsidize other food groups (milk, fruits, pulses) 2. Explore partnership opportunities with local dairy and farms

(*continued*)

Table 1 (continued)

Topic	Program	Coverage	Recommendation
	National Rural Employment Guarantee act (***Nutrition-sensitive)***	1. Some evidence for link between female participation and reduces odds of acute child malnutrition 2. Inefficient implementation in several states, not equitable participation from women and low caste people	1. Expand the types of job available to include support staff for Anganwadi and ASHA workers 2. Prioritize employment for women and low caste people in inefficient states
Maternal multiple micronutrient supplementation	No National-Level Programs	1. Several different programs provide micronutrient supplementation (iron, folic acid, Iodine, calcium) during peripartum period	1. A unifying strategy for providing multiple micronutrients can enhance efficiency and coverage
Vitamin A supplementation	Bi-annual vitamin A supplementation program	1. Number of children receiving two doses per year increased from 25% in 2005 to 66% in 2011 2. 62 million children received two doses in 2011	1. Evaluation of the program's impact on night blindness and child mortality/morbidity is needed
Maternal calcium supplementation	Calcium supplementation during pregnancy and lactation	1. 2014 guidelines dictate complete coverage of twice daily oral chewable tablets that are provided by auxillary nurse midwives 2. Surveillance by ASHA and Anganwadi workers to assess compliance with supplementation regimen	1. Pair the program with other existing programs for providing maternal micronutrient supplementation to facilitate a comprehensive strategy 2. Evaluation of the program's impact and implementation process is needed

This table provides a summary of the national-level programs, their impact on the coverage of evidence-based interventions, and finally, recommendations for enhancing their impact (Credit: Unpublished work by authors)

refuse to enter homes of Scheduled Caste people, 25% forbid Scheduled Caste from buying milk, 48.4% do not allow Scheduled Caste access to water supplies, 73% do not allow Scheduled Caste to enter homes of other castes, and in 37.8% of the villages, children of Scheduled Caste families are made to sit separately in government schools (Thomas et al. 2013). A recent study of pregnant women from rural western India found that in comparison to General Caste, women of Other Backward Castes were twice as likely to report experiencing discrimination and women of

Scheduled Castes or Tribes were four times as likely (Khubchandani et al. 2017). Thus, there is a need for a systematic approach to address maternal and child health in low-caste and historically underserved populations.

Conclusions

India has made tremendous progress over the last decade in launching and scaling-up programs aimed at achieving optimal child nutrition by improving dietary intake and health status of children. Although India fell short of the Millennium Development Goals, there is a renewed interest in advancing maternal and child health through advocacy and social engagement. It is important for India to act now and institute support for ongoing successful programs. In addition, India should endeavor to improve the implementation and governance of programs, which lack horizontal integration with other similar projects. An example of a step towards this goal is the MAA program, which aims to improve coordination between the existing workforce and programs focused on maternal and child health. As India sets its agenda towards meeting the Sustainable Development Goals, it is important to have a clear strategy for addressing the social determinants of health and implementing policies focused on health equity. Both of which will be crucial in helping to close the gap in child malnutrition.

In this decade of nutrition, India could be a beacon of light for the rest of the world with concerted efforts to reduce the huge burden of undernutrition and micronutrient deficiencies. To do this, there must be a massive investment in addressing social determinants, especially issues of women's empowerment and huge inequities in access and opportunity. The initiatives undertaken in health must be expanded to address food insecurity, gender, religious, ethnic, and caste disparities as well as targeting interventions and safety nets to reach those who need it most. There is the political will at hand and even resources to address this issue within our life time.

Policies

The eight national-level programs covered in this chapter are run by a total of seven different departments from seven different ministries. This lack of horizontal integration leads to redundancy and competing agendas, which leads to confusion, loss of accountability, and suboptimal implementation of policies. Therefore, in our review of these programs and their impact on child nutrition, we identify potential areas of opportunities for enhancing the performance of each program. Broadly, our recommendations fall within these categories:

- **Expanding effective programs to reach rural, tribal, and historically underserved populations**: Nutritional rehabilitation centers have shown promising results, but their coverage is limited and prohibitive for families leaving in geographically remote regions.

- **Increasing evaluation and oversight of programs to allow community-specific adaptation**: National Rural Employment Guarantee Act is well received but has failed to engage women and low-caste workers in states, especially those with higher burden of child undernutrition.
- **Coordinating activities across different programs to leverage existing resources and infrastructure**: Anganwadi program holds promise for delivering nutrition-specific intervention at scale if its activities are coordinated with MAA program.

In addition to the recommendations for individual programs, our chapter also highlights the importance for governmental leadership to define a comprehensive strategy that is grounded in implementation science and thus can prespecify opportunities for adaptation, intervention, and evaluation of each of these programs.

Dictionary of Terms

- **Child nutrition**: Science of nutrients and nutrition of children necessary for proper health and growth
- **India**: A country located in South Asia
- **Health policy**: A set of decisions and plans that dictate action of and related to health and healthcare
- **National programs**: A set of activities decided by central government of a country (India) that is applied throughout the country
- **Nutrition framework**: A conceptual model to understand the factors that affect nutrition
- **Governance**: The process through which health policy is translated into national programs
- Implementation science: A study of methods and results of implementing conceptual ideas and interventions into real-world settings
- **Advocacy**: The act of coalescing support and providing recommendations on behalf of a particular cause or health policy
- **Rural-urban disparities**: Differential circumstances experienced by people living in rural areas in comparison to urban areas
- **Social determinants of Health**: Nonmedical factors, such as where people are born, live, and work, as well as who they interact with, that affect their health

Summary Points

- Buoyed by support from general population, public health experts, politicians, and economists, India has enacted or augmented several national level programs to address child undernutrition in the last decade.

- However, most of these programs are run by different departments and lack coordination with each other limiting accountability and impairing the full promise of each individual programs.
- In 2011–2012, only 39% of all eligible children had ever received a take-home food ration or prepared hot meals and only 14% of children had received it in the previous month from Anganwadi workers.
- Interviews of Anganwadi workers suggest that they are over-burdened with the task to deploy multiple programs from different departments and have to spend disproportionate time towards documentation at the expense of providing services.
- The central assumption of National Food Security Act that by providing substantial subsidies for cereal, families would use saved capital to consume diverse foods may be inaccurate based on food-consumption data.
- Considering the cereal-centric composition of India's staple diet, reduced consumption of milk and vegetables, secondary to a program aimed at enhancing food security is concerning because dietary diversity is a crucial determinant of children's nutritional status
- According to a recent analyses of the NREGA program, 20 out of the 31 states in India have suboptimal implementation and can maximize their rural employment gains by increasing engagement of low caste population and women. In comparison to efficient states, inefficient states had 133% and 75% less involvement of women and low caste people, respectively.
- The momentum towards improving sanitation facilities at a household level following Swachh Bharat Mission guidelines must be complemented with a systematic effort to help improve individual hygiene for caregivers and children.
- The NRC program relies on already overburdened Anganwadi workers for case identification which can lead to missed or improper identification.
- The NRC program's limit of at most 14 days of hospitalization leads to almost of half of its children returning home with suboptimal nutritional status.
- There is an urgent need to complement NRC with home or community-based nutritional rehabilitation program.
- The field of implementation science offers an opportunity to enhance horizontal integration across different programs and can facilitate equitable coverage of services.

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Improving Infant and Young Child Nutrition in a Highly Stunted Rural Community: A Practical Case Study from Guatemala

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Contents

Abstract

Stunting is a major public health concern because of its association with child mortality and morbidity and later adult economic and health. Despite Guatemala's important contributions on child nutrition research, the indigenous Maya population in Guatemala has one of the highest stunting rates in the world. This chapter contextualizes the global problem of child stunting with a close

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look to Guatemala's historical nutrition research and policy, followed by a description of a comprehensive nutrition programming model for highly stunted communities developed by the nongovernmental healthcare organization Wuqu' Kawoq | Maya Health Alliance and a single-community case study of the model implementation. The model includes both preventative and curative evidence-based interventions to target food security, environmental factors, access to health services, and caregiver education and empowerment. Frontline workers with cultural and linguistic competency deliver individualized education and nutrition assessments. Over a 2-year implementation of the approach in a Maya community, the overall prevalence of stunting under 5 declined 20% and the prevalence of severe stunting 35%. In this and other similar settings, holistic, primary care approaches to malnutrition can have significant impact on child health.

Keywords
Stunting · Diet quality · Feeding practices · Case study · Guatemala · Indigenous · Food security · Community-based nutrition · Nongovernmental organization · Primary care

List of Abbreviations

FANTA	Food and Nutrition Technical Assistance
INE	Instituto Nacional de Estadística
LMIC	Low- and Middle-Income Country
MHA	Wuqu' Kawoq | Maya Health Alliance
MSPAS	Ministerio de Salud Pública y Asistencia Social
NGO	Nongovernmental Organization
PEC	Expansion of Coverage Program
PPHO	Plan de Pacto Hambre Cero
USAID	United States Agency for International Development
WHO	World Health Organization
ZHP	Zero Hunger Plan

Introduction

More than 150 million children younger than 5 years of age worldwide suffer from chronic malnutrition, or stunting (UNICEF 2016). Stunting results from a complex interplay of social, economic, environmental, and biological factors that lead to restricted linear growth (Reinhardt and Fanzo 2014). Stunting not only is a risk factor for early childhood morbidity and mortality (Black et al. 2013) but also confers deleterious long-term effects on cognitive development, adult health, and economic productivity (Hoddinott et al. 2008; Adair et al. 2013; Sawaya et al. 2003). Although the global prevalence of stunting has been declining since 1990, rates remain high in many low-and middle-income countries (LMIC) (Stevens et al. 2016;

UNICEF 2016). While less than 50% of all children under 5 years of age live in LMICs, 98% of the world's stunted children live in these countries (Black et al. 2013; WHO 2016).

This chapter focuses on child nutrition in Guatemala, a Central American LMIC with a population of 16 million people. With a prevalence of stunting of 46% in children under 5 years of age, Guatemala has the highest stunting rate in the Western Hemisphere (MSPAS 2017; USAID 2016). Additionally, national-level data tend to obscure the disproportional burden of stunting throughout the country. The poorest sectors of Guatemala's population, who are largely rural indigenous Maya, have much higher stunting rates, making them the most stunted group in the world according to some estimates (Black et al. 2013). Such health indicators are deeply intertwined with the longstanding economic, social, and political marginalization of Maya communities in Guatemala, which includes state-sponsored genocide during a civil war from 1960 to 1996 (Oglesby and Nelson 2016).

We first briefly review the history of nutrition research in Guatemala before leading to a synthesis of the current state of the country's nutrition politics and policy. The historical overview showcases how Guatemala has served as a global nutrition research laboratory from the 1940s up to the present day. Among other significant work, we review how the Institute of Nutrition of Central America and Panama (INCAP) Cohort Study and its follow-up iterations, which are still ongoing, have had a remarkable influence on the scientific understanding of child malnutrition and on global nutrition policy. We then describe how findings from the INCAP study and related research have been put into practice within the contemporary Guatemalan health system under the "First Thousand Days" framework. Underlying this analysis is the uncomfortable reality that although Guatemala has served as an extremely productive nutrition research setting, stunting rates in the country persist at extraordinarily high levels.

After our discussion of nutrition research and policy in Guatemala, we provide a practical overview of a model of nutrition programming for highly stunted Maya communities developed by our nongovernmental organization, Wuqu' Kawoq | Maya Health Alliance (MHA). We then turn to a single-community case study of the implementation of this model to demonstrate its impact on child health. We conclude by reflecting on our experiences and discussing future research and programmatic priorities with regards to nutrition in Guatemala.

Stunting in Guatemala

Guatemala has an estimated population of 16.5 million people. The country has one of the largest indigenous populations in Latin America, with nearly 50% of the population self-identifying as indigenous Maya (Chary and Rohloff 2015; MSPAS 2017). Around the world, there are profound inequalities in health indicators and access to healthcare services for indigenous populations (Anderson et al. 2016; Gracey and King 2009). In Guatemala, stunting is a significant example of these

disparities, as 58% of indigenous Guatemalan children are stunted as opposed to 34% of nonindigenous children (MSPAS 2017). As mentioned above, according to some analyses, the poorest quintile of Guatemalans (mostly rural and indigenous) are the most stunted population in the world (Black et al. 2013).

Stunting is a proxy for many significant short-term and long-term health risks. In the short term, stunted children are more likely to face illness, die in childhood, and have early cognitive and neurodevelopmental delays (Black 2017). In the long term, these children tend to not reach their full academic, economic, and health potential. They have decreased school performance, worse economic prospects, and face increased reproductive risks as adults (Adair et al. 2013; Dewey and Begum 2011; Hoddinott et al. 2008). Additionally, an emerging literature suggests that children who are chronically malnourished have higher risks of chronic noncommunicable diseases as adults, such as obesity, cardiovascular disease, and diabetes (DeBoer et al. 2012).

Guatemala's Historical Nutrition Research Overview

Since the late 1940s, Guatemala has made important contributions to the global study of childhood malnutrition. At that time, research conducted by INCAP in Maya communities demonstrated how the provision of malnutrition preventative services and improvements in basic community living conditions could have greater impacts on children's nutritional status than curative services alone (Mata 1978; Scrimshaw et al. 1969). Findings from these earlier studies formed the basis for the conceptualization of the INCAP Oriente Study, a nutrition trial that began as a supplementary feeding program in eastern Guatemala villages from 1969 to 1977 (Martorell et al. 1995; Stein et al. 2008). The Oriente Study is one of the most famous and oft-cited longitudinal nutrition studies in the world. Longitudinal follow-up from the original cohort and subsequent generations have generated strong evidence on the longer-term positive effects of early nutrition supplementation (Martorell 2010). In addition, the study clearly documented for the first time that child malnutrition in Guatemala is primarily a problem of endemic stunting (low height-for-age) rather than acute malnutrition (wasting, low weight-for-height) or underweight (low weight-for-age) (Ruel et al. 1995) and that exposure to chronic infectious disease and low-quality diets were the principal causes of children's impaired growth (Martorell 2010). The latest wave of data collection in the Oriente Study is currently being carried out, and using detailed biologic samples, researchers hypothesize that better child nutrition will be protective against adult obesity and related noncommunicable chronic diseases (Stein 2014). An important final note is that although prior INCAP research had included the study of child nutrition in indigenous Maya communities, the Oriente Study included only Spanish-speaking (i.e., nonindigenous) communities due to logistical barriers and the unavailability of unbiased cognitive tools for this population (Flood 2015).

Guatemala's Historical Nutrition Policy Overview

The INCAP Oriente Study demonstrated that growth faltering of stunted children in Guatemala occurs early, usually in the first year of life (Ruel et al. 1995). Nevertheless, historically much of Guatemala's official nutrition policy remained focused on school feeding programs (Nutrinet 2009). However, since 2006, nutrition policy in Guatemala has finally seen important policy shifts toward more preventive and comprehensive interventions targeting the early childhood period. These national changes derive from advocacy by major international donors and policy makers, including the United States Agency for International Development (USAID) and the World Food Program, in favor of early childhood programs in "the first 1,000 days of life" window (FANTA 2010). In 2012, the government of President Otto Pérez Molina made the prevention of early childhood malnutrition a major priority, disseminating a new multilevel strategic plan called "Plan Hambre Cero" (Zero Hunger Plan, ZHP), derived from the global "Thousand Days Initiative" framework (Thousand Days 2014). ZHP was launched with the goal to reduce stunting rates by 10% by the end of 2015 from a baseline rate of nearly 50% (PPHO 2012). The ZHP strategy emphasized global evidence-based interventions such as the distribution of micronutrient powders, deworming, provision of supplementary fortified flour blends, growth monitoring, and maternal education.

The ZHP was designed to function within the government's existing rural health system known as the "Programa de Extension de Cobertura" (Expansion of Coverage Program, PEC). PEC was established in the late 1990s, after the conclusion of the Guatemalan civil war, as a system to provide basic preventative health services for rural underserved indigenous populations. However, after allegations of institutional corruption within PEC, the government dismantled the program in early 2015 (Avila et al. 2015; Espina 2015). Uncertainty and political instability followed, and basic healthcare services in rural areas were suspended for months. A government corruption scandal led to the impeachment and resignation of the Guatemalan president, vice president, and several other major government officials; among the allegations were that public health funds had been diverted to private coffers (Galicia 2016). The rural system has subsequently been reconstituted, but this back and forth political drama simply underscores the fact that rural health programs remain incredibly underfunded and extremely limited in the provision of quality services.

An important, probably unintended side effect of implementing the Thousand Days framework in Guatemala has been an inflexible prioritization by nutrition and government stakeholders on the prevention of stunting in children under 2, limiting consideration of other pediatric health issues including strategies to improve outcomes in the very large number of Guatemalan children who are already stunted. While prevention of stunting is highly cost-effective, this does not indicate that there is no social or economic benefit to policies that also include catch-up growth in already stunted children, or in children older than 2 years. Indeed, historical data

from the INCAP Oriente Study in Guatemala, as well as high-quality evidence from other countries, show that catch-up growth is possible and significant (Prentice et al. 2013; Roberts and Stein 2017; Stein et al. 2010).

Finally, the emphasis within Guatemala's rural health system of utilizing front-line health workers to deliver narrow, technical nutrition interventions to Guatemalan infants and children reflects a final point of departure from more holistic community health models pioneered in Guatemala many years ago. For example, in 1962, Dr. Carroll Behrhorst founded the Behrhorst Development Foundation, which attempted to address the underlying factors causing poverty and poor health among the rural Guatemalan population through community participation, empowerment, and integrated development programs. Central to the Behrhorst model were rural Maya community health workers trained to deliver curative and preventative medicine in their communities (Horton 1987). A case study of the Foundation was included in Kenneth Newell's 1975 book *Health by the People* (Newell and WHO 1975), which became highly influential during the primary health care movement in the lead-up to the Declaration of Alma Ata in 1978. In 1980s, the Foundation was severely impacted by the Guatemalan civil war, and after Behrhorst's death in 1990, decreases in international funding lead the Foundation to shift away from its original mission of community-based development (Maupin 2015).

Community-Based Nutrition Programming

Based on the foregoing analysis, we have been working for the last decade with rural Maya communities to reformulate community-based nutrition programming in a way that reclaims a focus on primary, holistic care; includes both preventative strategies and catch-up growth interventions for stunted children; and has a strong focus on caregiver education and empowerment. In this section, we describe the formative nutrition research and nutrition delivery model that we have developed, finally, moving to a detailed case study of its implementation in one rural community. The goal is to provide practical insights into comprehensive nutrition program design, as well as the potential impact of the approach, which will be of use to other practitioners around the world.

All of this work has been conducted within the institutional umbrella of Wuqu' Kawoq | Maya Health Alliance (MHA). MHA is a primary care organization, founded by ourselves and others in 2007, to provide culturally and linguistically nuanced services to rural Mayan-speaking populations. Over the years, MHA has grown into a full-service organization in central Guatemala delivering services in 20 rural highland communities to more than 20,000 patients per year. MHA also performs ethnographic and health services research relevant to Maya populations. With regards to child nutrition, MHA operates a large nutrition delivery program and has conducted numerous research studies, including clinical trials, on nutrition in this setting.

Formative Nutrition Research

Formative nutrition research in the years leading up to our nutrition program design has focused on ground-level factors that we have observed in our programmatic work to contribute to improved nutrition outcomes. Themes have included gender roles, food insecurity, breastfeeding, feeding practices, and community perceptions of stunting.

Importantly, a better understanding of the timing and onset of growth faltering has been a major concern. As also previously demonstrated in the INCAP Oriente Study (Ruel et al. 1995), we have noted that growth faltering in Maya communities starts usually at 6 months of age, corresponding to the time of transition to complementary foods, and negatively correlates with the household financial stability and availability of high-quality infant foods (Chary et al. 2011). For many indigenous families with young infants who are experiencing growth faltering, meeting food-based dietary recommendations is difficult due to the lack of economic resources for purchasing nutritious foods and the wide availability of alternative low-cost processed foods (Brown et al. 2014; Chary et al. 2013).

We have also investigated how poor child feeding indicators exist even in rural communities devoted to agricultural production, largely due to the pressures to enter the export agricultural market. For example, a recent study we conducted in a rural Maya community where most families dedicated themselves to agricultural production revealed that less than 3% of the children under the age of five met World Health Organization (WHO) standards for dietary diversity and meal frequency. In this community, most harvested produce was directed to exportation with minimal local consumption available for children (Webb et al. 2016). This is not really a matter of negligence so much as the fact that when the prevalence of child stunting is extremely high, its deleterious impacts on child health become normalized and are not easily perceived by caregivers (Brown et al. 2014; Chary et al. 2013). Finally, discrimination against Maya mothers by the formal health system exacerbates the impact of stunting by discouraging Maya caregivers from seeking out primary healthcare services (Chary et al. 2011).

Finally, we have explored strategies to overcome barriers to suboptimal breastfeeding and complementary feeding practices among Maya caregivers. For example, we have shown how mothers' relationships with their male partners and mothers-in-law contribute to feeding practices. Male partners often control all household funds, and mothers-in-law often make most food purchasing and preparation decisions. These family-level factors have a significant impact on child health and must be taken into consideration when designing programs (Brown et al. 2014; Chary et al. 2011).

Nutrition Program Model

Based on the results of this formative research, MHA has worked to design and adapt nutrition programs to meet the needs and sensibilities of clients and families in rural Maya communities. This work began in 2009 at a very small scale, serving

2 communities and approximately 70 children and their families. The program has continued to grow steadily; in 2016, it covered 20 rural communities and 1500 children under 2 years in three provinces (Chimaltenango, Sololá, Suchitepéquez).

The program model is designed to decrease the community prevalence of stunting through both universal preventative and targeted recuperative (catch-up growth) services. A basic package of preventative services is offered to all children under 5 years of age. This package is evidence-based (Bhutta et al. 2008) and in line with the Guatemalan government's own recommendations under the ZHP initiative (PPHO 2012), including growth monitoring, basic nutrition education for caregivers, micronutrient supplementation, and deworming. Notably, the provision of services for children older than 2 years of age is a departure from the thousand days framework (which recommends services up to 2 years as a policy priority) and is based on our experiences over the last 10 years that communities and families strongly prefer this programatic decision and that the more inclusive approach improves community buy-in. It is also in part a response to the evidence that catch-up growth is possible in stunted children older than 2 years (Prentice et al. 2013; Roberts and Stein 2017; Stein et al. 2010). In addition to this preventative community-based package, a more intensive 6-month catch-up growth intervention is targeted to already-stunted children younger than 2 years of age; this package is described in more detail below. All services are free-of-charge.

The program operates with indigenous auxiliary nurses and community health workers (CHW) as front-line workers, to whom we further henceforth as "nutrition technicians." Nutrition technicians are bilingual in Mayan and Spanish languages and use standardized clinical algorithms for the screening, management, and treatment of stunting, as well as for delivering nutrition education to the children's caregivers. In Fig. 1, nutrition technicians from MHA perform children's growth monitoring activities in rural Maya communities.

Nutrition technicians are supervised and supported by physicians and nutritionists, who perform monthly evaluation meetings. The nutritionists and physicians are on call for emergencies, and they evaluate children not responding as expected to the nutrition interventions. Staff physicians perform further medical evaluations for complex consultations or for primary care issues beyond the nutrition technicians' scope of practice. Integrating nutrition programming into this more comprehensive primary care safety net is, we feel, critical. Despite the ubiquitous nature of stunting in Guatemala, other treatable pediatric diseases such as inborn errors of metabolism or endocrine disorders can go undiagnosed without the input of expert practitioners (King et al. 2016). Care by nutrition technicians is primarily delivered by home visits, an approach which enables health care provision to patients who live far away from health care facilities.

The program's clinical protocols were derived from national, regional, and international recommendations for the prevention and treatment of childhood malnutrition (Bhutta et al. 2008; Dewey 2003; MSPAS 2010). In addition to preventative services mentioned above, which are provided to all children, stunted children under 2 years receive additional services. These include monthly home visits for more intensive, individualized nutrition assessment and counselling and

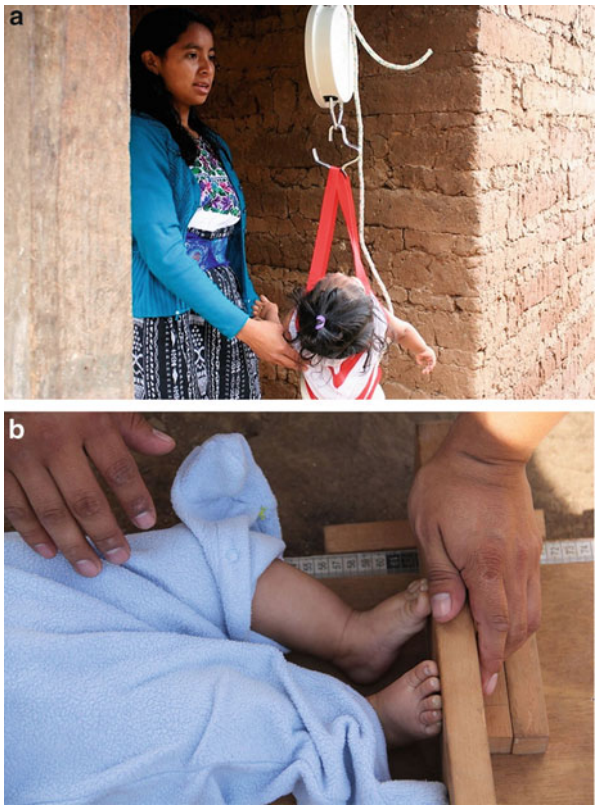

Fig. 1 Growth monitoring. Nutrition technicians perform children's growth monitoring activities of weight (**a**) and length (**b**) in rural Maya communities during home visits

food supplementation with a family food ration to offset food insecurity and to supplement the child's diet with specific quality calories and nutrients (particularly, protein) often lacking in the diet (FANTA 2010). Children who are wasted in addition to stunted also receive therapeutic milk formula (F-100) (Ashworth et al. 2003) or ready-to-use therapeutic foods (RUTF) (Ciliberto et al. 2005) as indicated by a staff nutritionist. As shown in Fig. 2, these services all together form a comprehensive primary care approach to child malnutrition, with key interventions designed to address four key drivers of malnutrition: food security, environmental health, health services, and caregiver's empowerment and education. To promote interinstitutional collaboration, individual nutrition technicians establish community-level partnerships with community leadership councils and local government health-care staff.

During home visits with stunted children, nutrition technicians perform individualized dietary counseling focused on two primary indicators – dietary diversity (number of food groups per day) and meal frequency (number of meals per day). To guide this interaction, they conduct a 24 hour dietary recall at each visit, based on the WHO's published template (WHO 2008). Through systematic collection of dietary recall data, nutrition technicians can identify deficiencies in each child's diet and

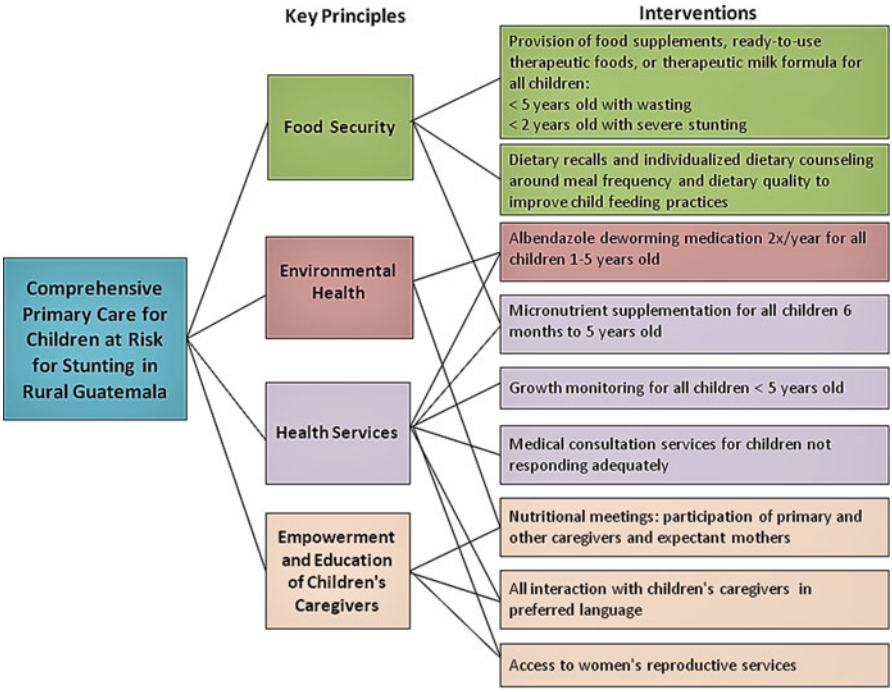

Fig. 2 Key drivers of child nutrition. Theoretical overview of a comprehensive primary care-focused model for treating child malnutrition in rural Guatemala developed and implemented in several rural-Maya communities by Maya Health Alliance

Fig. 3 Nutrition counseling Nutrition technician guides children's caregivers through child nutrition improvement strategies during a home visit

guide the primary caregiver through improvement strategies. Conducting this work in the home facilitates confidentiality. Over time, caregivers gradually feel more comfortable asking questions and sharing their experiences, concerns, and expectations. Finally, the home setting allows nutrition technicians to learn about each child's living situation, food accessibility, economic resources and family dynamics, and tailor interventions accordingly (Fig. 3).

Table 1 Themes covered during group educational meetings. Themes covered in the nutrition curriculum used for group meetings are shown. Caregivers and other participants attend five weekly sessions. Each session last 2–3 hours and ends with a food preparation activity

Session Theme	Description
1. The importance of good nutrition	Nutrition during pregnancy. Food classification in terms of micro- and macronutrients (vitamin A and C, iron, folic acid, protein, carbohydrates, fats). Hygiene and water purification
2. Breastfeeding and its values	Frequency and duration of breastfeeding episodes. Helpful posture and problem-solving techniques for quality breastfeeding. Common breastfeeding myths
3. Complementary feeding	Adequate frequency, quantity, quality, and consistency of complementary feeding. Strategies for improving diet diversity with inexpensive locally available fresh foods
4. Childhood malnutrition	Acute and chronic malnutrition, anemia. Causes, consequences, and solutions. Review of the value of micro and macronutrients for an adequate growth
5. Complete review	General review of each previous class. Time for additional questions and sharing of feedback. Closing ceremony

In addition to preventative and recuperative child-focused activities, the model concentrates heavy investments in caregiver education; all primary caregivers in the community with children younger than 5 years, as well as expectant mothers and other important family caregivers (such as grandmothers), are invited to participate in a weekly nutritional education program facilitated by the nutrition technician for five consecutive weeks. These sessions aim to empower the caregivers by increasing their ability to select a variety of high-quality foods for home consumption and diversifying their food preparation methods. Nutrition education meetings are conducted in a highly participatory manner, with usually 8–10 participants. As outlined in Table 1, we use our understanding of the local context of poverty and food insecurity to provide relevant and practical tips for improving child diets, placing emphasis on utilizing potential "hidden family resources" such as shifting household expenses away from junk food purchases (Brown et al. 2014). These meetings also always include meal preparation activities (Fig. 4), using only locally available food and resources.

Finally, because the nutrition model exists within a larger institutional primary care framework, nutrition technicians can connect other members of the family with other health care needs to potential medical resources. This is most important for female caregivers, who can access a full range of reproductive health services – including comprehensive reproductive education, cancer and sexual transmitted diseases' screening and treatment, and family planning – which are important elements for caregiver empowerment (Fig. 2).

Case Study

To provide additional programmatic details on the implementation of comprehensive, community-based nutrition programming, we present here a brief case study of the implementation of a nutrition program in one rural Maya community,

Fig. 4 Meal preparation activities. Nutrition technician performs a food preparation demonstration for children's caregivers, during these activities special focus is placed on the use of locally available foods and the use of possible hidden-resources

Table 2 Characteristics of Xik'injuyu' case study community prior to beginning nutrition programming. Basic demographic and nutrition-related characteristics are shown for the case study community. These characteristics are typical of the region and help to illustrate the stressors and risk factors of populations at high risk of stunting

Characteristics (n = 167)	Value
Household size (mean ± SD)	3.99 ± 1.93
Maternal illiteracy (%)	28
Adequate child diet (%)	49
Child nutrition status (%)	
Stunted	52
Severely stunted	22

Key: *n* sample size, *SD* standard deviation

Xik'injuyu', located in the western Guatemala piedmont (Table 2). MHA began building a primary care program – primarily focused on child nutrition and reproductive health services – in collaboration with the community in 2014. The community consists of approximately 360 families, most of K'iche' Maya descent. Potable water services are scarce. Most male heads-of-households work as seasonal agricultural day laborers on rubber, banana, or coffee plantations, while most female

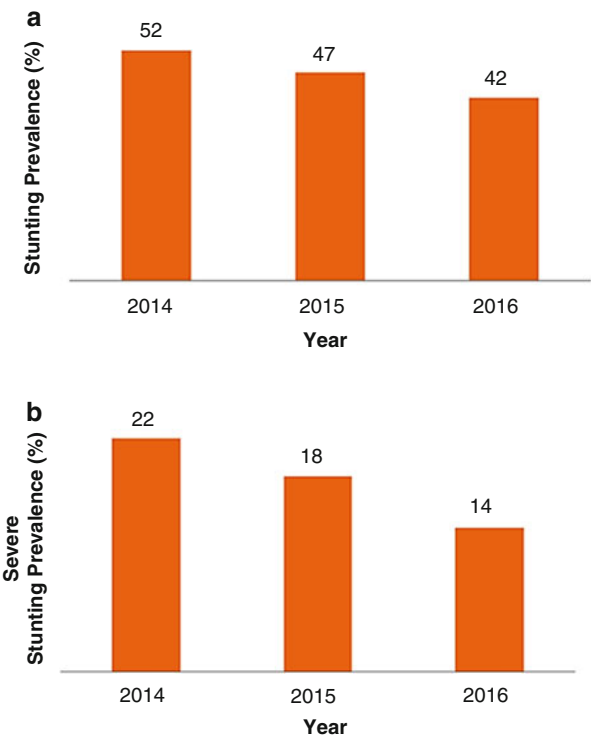

Fig. 5 (a) Change in stunting prevalence and (b) severe stunting prevalence over 2 years in Xik'injuyu'. Longitudinal change in the prevalence of stunting (**a**) and severe stunting (**b**) for children younger than 5 years in the community of Xik'injuyu' are shown. Note the decrease of both since the implementation of the Wuqu' Kawoq | Maya Health Alliance nutrition program in 2014

residents are homemakers. Underemployment is common and around 70% of families live in poverty (less than $2 USD/day).

When beginning our collaboration with the community, families reported to us how they struggled with access to nutritious food, especially due to droughts, increases in food prices, and periods of unemployment, and more than 60% reported family-level food insecurity. Very few families (less than one-third) own land, and so producing their own food is an unavailable strategy. Many families told us that they resorted to purchasing low cost processed foods at local stores, as these were less costly and more readily available than high-quality fresh foods. Because of these stressors, prior to initiating joint efforts in the community, the overall prevalence of stunting (height-for-age Z score [HAZ] < -2) based on WHO Child Growth Standards (De Onis et al. 2006) was 52%, and a full 22% of children were severely stunted (HAZ < -3).

Over the last 2 years implementing the primary care approach described in this chapter, we have seen important improvements in rates of malnutrition. As shown in Fig. 5a, the rate of overall stunting in children under 5 has declined from 52% to 42%, nearly a 20% improvement. The rate of severe stunting has declined even more rapidly, from 22% to 14%, a roughly 35% improvement (Fig. 5b).

Final Reflexions

In this chapter, we contextualize the global problem of child stunting with a close look at its history and dynamics in rural Guatemala, which suffers from some of the highest rates of stunting in the world. In the first section, we offer a review of nutrition research in Guatemala and a synthesis of the government's nutrition policy, which only recently has officially recognized the country's staggering stunting problem. However, national nutrition programs and policies have suffered from intermittent and ineffective deployment, a narrow focus on stunting prevention to the exclusion of treatment, and failure to address the root causes of stunting in a holistic fashion.

In the second part of this chapter, we offer a detailed, practical description of work in rural Guatemala with Wuqu' Kawoq | Maya Health Alliance, a nonprofit healthcare organization, to deploy evidence-based interventions for stunting within a comprehensive primary care model. Various premises of the model will be generalizable and useful to practitioners in other settings. First, well-supported nutrition technicians, who speak indigenous Maya languages, can provide high value nutritional care and are highly motivated to carry out time-consuming, high-contact activities such as home visits and education sessions. This is in line with the overall consensus in the literature that community-based interventions led by nurses and CHWs are critical for improving health outcomes (Lassi et al. 2016). Second, nutrition technicians can adopt and successfully utilize advanced interview techniques, such as 24-h dietary recalls, to better customize and individualize the interventions they provide to children and caregivers. Finally, by emphasizing home visits and respectful caregiver education, nutrition programming can promote an inclusive, collaborative environment, where caregivers feel comfortable sharing their experiences without fear of punishment or stigma, thereby learning about nutrition and building a network of trust and support.

In the final part of the chapter, we share additional details from a single rural community where we recently initiated a primary care nutrition program. In this program, over a space of 2 years, the overall prevalence of stunting declined 20% and the prevalence of severe stunting 35%. These findings are typical of our experiences in multiple Maya communities over the last 10 years. First, they reinforce that holistic, primary care approaches to malnutrition can have a significant impact on child health in these settings. At the same time, they also underscore that no existing stunting intervention is able to completely eradicate the problem. This fact is ultimately motivating, as it serves as a call for ongoing research, increased advocacy, and sustained commitments from healthcare organizations, civil society, governments, and policymakers together until every child's and every family's nutritional future is made secure.

Policies and Protocols

In this chapter, we have described practical details of how to implement culturally and linguistically sensitive, comprehensive nutrition programs for rural areas of Guatemala. Many of these details are also readily applicable to other settings and contexts. Here we highlight three of these:

1. Frontline nutrition workers should be cognizant of the culture and language of their clients. Administrators from both government and civil society groups should prioritize hiring practices that ensure these linguistic and cultural competencies. Monitoring for program quality should include provision of services in the client's preferred language.
2. Individualized education can improve caregiver engagement and child feeding practices. Implementers should consider focusing effort on training frontline workers to provide personalized interventions. A key area is dietary recalls to better focus assessments of dietary quality. Existing materials developed by the World Health Organization (WHO 2008) can easily be adapted.
3. In areas of high stunting prevalence, programming should include both preventative and (if resources allow) recuperative interventions. Home-based visits by nutrition technicians are an excellent strategy for implementing recuperative interventions, which can include more intensive feeding or education strategies.

Dictionary of Terms

- **Dietary diversity** – A term for dietary quality from the perspective of the regular consumption of a diversity of food items. Can be easily assessed on a dietary recall by counting the number of distinct food groups consumed in a given time period. The World Health Organization has published useful guidelines detailing methods for assessing food group consumption in young children (WHO 2008).
- **Meal frequency** – An important indicator of overall caloric adequacy in a child's diet, which can be assessed on a 24-h dietary recall. Each meal should consist of solid food items, not liquids. The World Health Organization has published detailed guidelines for the required number of meals based on the child's age (WHO 2008).
- **Stunting** – Also known as chronic malnutrition. Defined as a height-for-age Z-score of less than −2, compared to World Health Organization Child Growth Standards (De Onis et al. 2006). Severe stunting is defined as a Z-score of less than −3.
- **Underweight** – Defined as a weight-for-age Z-score of less than −2, compared to World Health Organization Child Growth Standards (De Onis et al. 2006). Severe underweight defined as a Z-score of less than −3.
- **Wasting** – Also known as acute malnutrition. Defined as a weight-for-length or weight-for-height Z-score of less than −2, compared to World Health Organization Child Growth Standards (De Onis et al. 2006). Severe acute malnutrition is defined as a Z-score of less than −3

Summary Points

- This chapter focuses on stunting, one of most important pediatric growth disorders in the world.

- Stunting causes early morbidity and mortality in childhood, as well as developmental delay, reduced school achievement, and loss of adult economic potential.
- The indigenous Maya population in Guatemala has one of the highest rates of stunting in the world.
- Although Guatemala has been an important source of child nutrition research, this has not translated into improved stunting outcomes for rural indigenous communities.
- Interventions to target stunting should take a comprehensive approach, targeting food security, environmental factors, access to health services, and caregiver education and empowerment.
- Frontline workers, such as nurses and community health workers, can effectively deliver nutrition interventions, especially if they have cultural and linguistic competency.
- Individualized education and nutrition assessments are an important part of nutrition programming, because they improve caregiver empowerment and the effectiveness of interventions.
- In high stunting prevalence communities, programs should ideally have both preventative and curative interventions.

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Resources in Famine, Starvation, and Nutrient Deprivation

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Abstract

There is no greater major threat to public health worldwide than nutrient deprivation. Presently nutrient deficiency affects around 800 million people worldwide. Since time immemorial nutrient deprivation or overt starvation has caused organ dysfunction or death. Nutrient deficiency is not only the consequence of drought, famines, or civil upheaval. Malnutrition may arise for a number of

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reasons. For example, some studies have reported that in hospitals as many as half of patients may be malnourished or at risk of malnutrition. The term malnutrition however is very broad and covers undernutrition, imbalance, and overnutrition. This book focuses on the former two umbrellas and coverage ranges from cellular events to public health policies. The detailed molecular events arising from nutrient deprivation are relatively recent discoveries in the overall time scale of research into malnutrition. At the other end of the spectrum, public health policies are constantly being updated to take into account prevailing social, geographical, political, and economic profiles of communities and countries. Within this are policies which encompass future trajectories which aim to prevent famines or undernutrition. Thus there is a complex continuum of knowledge that stretches from our understanding of how molecules in cells behave to the imposition of policies. To further aid colleagues in the acquisition of this knowledge, this chapter lists the resources on the regulatory and professional bodies, journals, books, and websites that are relevant to an evidence-based approach to famine, starvation, and nutrient deficiency, from cells to policy.

Keywords
Books · Evidence · Famine · Journals · Nutrient deprivation · Professional societies · Regulatory bodies · Resources · Starvation

Introduction

Nutrient deprivation is a major threat to global public health. Starvation is the most extreme nutrient deprivation. It results from severe restriction of dietary energy and macro- and micronutrient intake below that needed to sustain life. Accurate data on the extent of starvation is relatively difficult to obtain. However, less severe nutrient deficiency affects around 815 million people on a global basis (Food and Agriculture Organization of the United Nations et al. 2017).

Famine has caused starvation and nutrient deprivation in humans since time immemorial. However, the understanding of the molecular changes relevant to the effects of worsening nutrient deprivation is relatively recent discoveries in the overall time scale of research in this field. It is important to remember that nutrient deprivation arises in a number of scenarios other than due to famine per se. These include undernutrition due to illness, poverty, societal imbalance, conflict, self-starvation, and so on. Nutrient deprivation is under-recognized in modern medicine and to some extent does not receive the attention it deserves. For example, over 20 years ago, Powell Tuck (1997) highlighted that the prevalence of malnutrition in hospital patients is between 19 and 55% using various criteria. Despite this,

Table 1 This table lists the regulatory bodies, professional societies, and organizations involved with famine, starvation, and nutrient deprivation

Regulatory bodies, professional societies, and organizations
Action against hunger www.actionagainsthunger.org.uk
American Society for Nutrition (ASN) www.nutrition.org
Austrian nutrition society www.oege.at
Concern worldwide www.concern.org.uk
Dutch academy of nutritional sciences www.voedingsacademie.nl/english
Dutch malnutrition steering group www.fightmalnutrition.eu
Famine early warning system network www.fews.net
Federation of European Nutrition Societies www.fensnutrition.eu
Finnish society for nutrition research www.protsv.fi/sry
Food and agriculture organization www.fao.org
Food fortification initiative (FFI) www.ffinetwork.org
German nutrition society www.dge.de
Global alliance for improved nutrition (GAIN) www.gainhealth.org
International zinc nutrition consultative group (IZiNCG) www.izincg.org
International Union of Nutritional Sciences (IUNS) www.iuns.org
Iodine global network (IGN) www.ign.org
Malnutrition task force www.malnutritiontaskforce.org.uk
Micronutrient forum micronutrientforum.org
National Institutes of Health www.nih.gov
Nutrition international www.nutritionintl.org
Secure nutrition www.securenutrition.org

(*continued*)

Table 1 (continued)

Regulatory bodies, professional societies, and organizations
Sight and life www.sightandlife.org
Societe Francaise de nutrition www.sf-nutrition.org
Swiss society for nutrition www.sge-ssn.ch
The Danish nutrition society www.sfe.dk/en
The hunger project www.thp.org
The nutrition society www.nutritionsociety.org
United Nations Children's fund (UNICEF) www.unicef.org
United Nations Office for the coordination of humanitarian affairs (OCHA) www.unocha.org
United Nations University www.unu.edu
United States Agency for International Development (USAID) www.usaid.gov
WHO www.who.int
World food Programme
www1.wfp.org

undernutrition in the hospital setting still remains problematic (Kruizenga et al. 2016; Morris et al. 2018).

The treatment of starvation and nutrient deprivation may seem obvious and straightforward. However, the relatively recent discovery of the phenomenon known as the refeeding syndrome underlines the importance of gradual replacement of the calorie deficit (Friedli et al. 2017). Yet although refeeding syndrome was described more than 70 years ago, there is still no standard definition or recommendations for its prevention or treatment (Friedli et al. 2017).

Regardless there has recently been an explosion in the knowledge and understanding of nutrient deprivation and starvation. It is now difficult even for experienced scientists to remain up-to-date. To assist colleagues who are interested in understanding more about this field, we have therefore produced tables containing up-to-date resources in this chapter. The expert who assisted with the compilation of these tables of resources is acknowledged below.

Table 2 Journals publishing original research and review articles related to nutrient deprivation, famine, or starvation. We list the top 20 journals which have published the most number of articles over the past 5 years. Although we used Scopus to generate this list, other databases or the use of refined search terms will produce different results

Journals relevant to nutrient deprivation, famine, or starvation
Plos one
Nutricion Hospitalaria
Clinical nutrition
Scientific reports
The lancet
Nutrition
Autophagy
Journal of nutrition health and aging
Journal of parenteral and enteral nutrition
Nutrition in clinical practice
Nutrients
Proceedings of the National Academy of Sciences of the United States of America
Food and nutrition bulletin
European journal of clinical nutrition
Journal of biological chemistry
Maternal and child nutrition
Bioresource technology
Public health nutrition
American journal of clinical nutrition
BMC public health

Resources

Tables 1, 2, 3, and 4 list the most up-to-date information on the regulatory bodies and professional societies (Table 1), journals on nutrient deprivation (Table 2), books (Table 3), and online resources (Table 4) that are relevant to an evidence-based approach to nutrient deprivation.

Summary Points

- Nutrient deprivation is a major threat to global public health.
- Starvation is the most extreme nutrient deprivation.
- Nutrient deprivation is under-recognized in modern medicine.

Table 3 This table lists books on nutrient deprivation due to famine or starvation

Relevant books
Biology of Starvation in Humans and Other Organisms. Merkin TC. Nova science publishers, 2011.
Comparative Physiology of Fasting, Starvation, and Food Limitation. McCue MD. Springer, 2012.
Contemporary Famine Analysis. Rubin O. Springer 2016
Diet, Nutrition, and Fetal Programming. Rajendram R, Preedy VR, Patel VB (editors). Springer, 2017.
Essentials of Pediatric Nutrition. Samour PQ, king K (editors). Jones & Bartlett Learning, 2013.
Famine: As a Geographical Phenomenon. Currey C (editor). Springer, 2012
Feast, Famine or Fighting?: Multiple Pathways to Social Complexity. Chacon RJ and Mendoza RG (editors). Springer, 2017
Global Child Health. Subrahmanian K, Swamy P. Springer, 2018.
Malnutrition in Chronic Diet-Associated Infantile Diarrhea: Diagnosis and Management. Lifschitz CH (editor). Academic press, 2013.
Molecular, Genetic, and Nutritional Aspects of Major and Trace Minerals. Collins JF. Academic press, 2016.
Nutrition in the Prevention and Treatment of Disease, 4th ed. Coulston AM, Boushey CJ, Ferruzzi M, Delahanty L (editors). Elsevier, 2017.
Pediatric Nutrition, 4th ed. Samour PQ, king K (editors). Jones & Bartlett Learning, 2012.
Practice-Based Nutrition Care, An Issue of Medical Clinics of North America, Kahan S, Kushner RF. Elsevier, 2016.
Present Knowledge in Nutrition, 10th ed. Erdman JW, Macdonald IA, Zeisel SH. ILSI press, 2012.
Protein-Calorie Malnutrition. Olson R (editor). Academic press, 2012.
Searching for Medical Truths. Metabolism in health and disease states: Obesity, alcoholism, diabetes and starvation. Owen OE. Infinity publishing.Com, 2006.
Starvation: New Insights for the Healthcare Professional. QA Acton. Scholarly editions, 2012.
The State of Food Security and Nutrition in the World 2017. Building resilience for peace and food security. Food and agriculture Organization of the United Nations (FAO), the International Fund for Agricultural Development (IFAD), the United Nations Children's fund (UNICEF), the world food Programme (WFP), and the World Health Organization (WHO). FAO, Rome, 2017.
The Vitamins: Fundamental Aspects in Nutrition and Health. Combs Jr. GF, McClung JP. Academic press, 2017.

- Nutrient deprivation arises in a number of scenarios other than due to famine, in illness, poverty, societal imbalance, self-starvation, etc.
- This chapter lists resources relating to the regulatory and professional bodies, societies, journals, books, and websites that are relevant to an evidence-based approach to nutrient deprivation, starvation, and famine.

We would like to thank the following author for contributing to the development of this resource. Dr Valeria Galetti.

Table 4 This table lists some internet resources relevant to nutrient deprivation, famine, or starvation. Sites listed in Table 1 may also have tools or resources within them. For example, the NIH listed in Table 1 will have information on dietary reference intakes (DRIs), nutrition in cancer care, screening tools, and so on within the domain of its websites

Relevant online resources and information
Detecting and managing malnutrition: Malnutrition universal screening tool (MUST) www.southampton.ac.uk/medicine/research/impact/must_a_new_tool_for_combatting_malnutrition_in_uk_and_overseas.page
Demographic and health surveys program www.dhsprogram.com
Dutch malnutrition steering group – Screening tools www.fightmalnutrition.eu
e-library of evidence for nutrition actions (eLENA) www.who.int/elena/en
Evidence-informed policy network (EVIPNet) www.who.int/evidence/en
Food and agricultural organization – Fighting famine www.fao.org/emergencies/crisis/fightingfamine/en
Food and nutrition technical assistance – Measuring household food insecurity www.fantaproject.org/research/measuring-household-food-insecurity
Food Standards Agency www.food.gov.uk
Global database on the implementation of nutrition action (GINA) http://www.who.int/nutrition/gina/en
Global fortification data exchange www.fortificationdata.org
Global targets 2025 www.who.int/nutrition/global-target-2025/en
International clinical trials registry platform (ICTRP) www.who.int/ictrp/en
Landscape analysis on countries' readiness to accelerate action in nutrition www.who.int/nutrition/landscape_analysis/en
Malnutrition task force – Resources www.malnutritiontaskforce.org.uk
Nutrition landscape information system (NLiS) apps.who.int/nutrition/landscape/search.aspx
ReliefWeb www.reliefweb.int
The British nutrition foundation-resources www.nutrition.org.uk/healthyliving/resources.html
The World Bank www.worldbank.org
UNICEF data: DefInfo devinfo.org/unicefdata/libraries/aspx/home.aspx
UNICEF data: Monitoring the situation of children and women www.data.unicef.org

(*continued*)

Table 4 (continued)

Relevant online resources and information
UNICEF-WHO-World Bank joint child malnutrition estimates 2017 apps.who.int/gho/data/node.wrapper.nutrition-2016?lang=en
WHO child growth standards www.who.int/childgrowth/en
WHO country profiles apps.who.int/nutrition/landscape/report.aspx
WHO global data Bank on infant and young child feeding www.who.int/nutrition/databases/infantfeeding/en
WHO global database on child growth and malnutrition www.who.int/nutgrowthdb/en
WHO mortality database apps.who.int/healthinfo/statistics/mortality/whodpms
WHO tracking tool www.who.int/nutrition/trackingtool/en
WHO vitamin and mineral nutrition information system (VMNIS) www.who.int/vmnis/en

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Friedli N, Stanga Z, Sobotka L, Culkin A, Kondrup J, Laviano A, Mueller B, Schuetz P (2017) Revisiting the refeeding syndrome: results of a systematic review. Nutrition 35:151–160

Kruizenga H, van Keeken S, Weijs P, Bastiaanse L, Beijer S, Huisman-de Waal G, Jager-Wittenaar H, Jonkers-Schuitema C, Klos M, Remijnse-Meester W, Witteman B, Thijs A (2016) Undernutrition screening survey in 564,063 patients: patients with a positive undernutrition screening score stay in hospital 1.4 d longer. Am J Clin Nutr 103:1026–1032

Morris NF, Stewart S, Riley MD, Maguire GP (2018) The burden and nature of malnutrition among patients in regional hospital settings: A cross-sectional survey. Clin Nutr ESPEN 23:1–9

Powell-Tuck J (1997) Penalties of hospital undernutrition. J R Soc Med 90:8–11

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