Early Factors Leading to Later Obesity: Interactions of the Microbiome, Epigenome, and Nutrition

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Obesity is a major public health problem in the United States and many other countries. Childhood obesity rates have risen extensively over the last several decades with the numbers continuing to rise. Obese and overweight children are at high risk of becoming overweight adolescents and adults. The causes are multifactorial and are affected by various genetic, behavioral, and environmental factors. This review aims to discuss a previously underrecognized antecedent of obesity and related chronic metabolic diseases such as heart disease and diabetes. Specifically, we highlight the relationship of the microbial ecology of the gastrointestinal tract during early development and the consequent effects on metabolism, epigenetics, and inflammatory responses that can subsequently result in metabolic syndrome. Although studies in this area are just beginning, this area of research is rapidly expanding and may lead to early life interventions that may have significant impacts in the prevention of obesity.

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Introduction

Obesity has become the major personal and public health problem in the United States and is burgeoning in many other countries as well.[1] Some of the urban centers in the most populous country in the world, China, are experiencing a major increase in childhood obesity.[2] Other rapidly developing countries such as Mexico, Brazil, India, and Argentina are also experiencing major increases.[3] Childhood obesity has been associated with the subsequent development of metabolic syndrome in adulthood, which includes obesity, type II diabetes mellitus, hypertension, dyslipidemia, and associated problems (Fig. 1). The long-term implications of this are concerning: the diabetic population in the Indian subcontinent alone is predicted to rise to more than 80 million by the year 2030.[4]

Causes for these increases in obesity are multifactorial and likely have little to do with genetics since these increases are occurring much faster than would be likely with genomic (DNA base pair) alterations. Some of the obvious causes include intakes of more energy than utilized with subsequent storage of the excess energy in the form of fat. Modern lifestyle factors such as lack of exercise, rich foods, and stress unquestionably play major roles. However, many other components have been found to be important in the development of obesity. Early developmental factors are now known to clearly play a role and have been studied by Developmental Origins of Health and Disease (DOHAD); an international society focused on these problems as they relate to early development.

Although storage of excess calories represents one somewhat simplistic mechanism leading to obesity, other factors are involved as well. Our intestinal microbes represent a highly genetically diverse and metabolically active biome that outnumbers our somatic cells and genes by orders of magnitude. They likely play a critical role in altering inflammatory responses, and produce highly bioactive metabolites, some of which may be involved in endocrine and metabolic alterations that may be functional in both infancy and adulthood. In addition, these microbes and their metabolic products may be involved in epigenetic mechanisms that function during critical developmental windows that profoundly affect subsequent phenotypic characteristics including obesity and other health issues during later life.

In this article, we wish to provide a summary of some of the early causes of the adult metabolic diseases, which encompass obesity, type II diabetes, hypertension, and cardiovascular disease. Here we wish to emphasize the relationship of the microbial ecology of the gastrointestinal tract during early development: in the pregnant mother and her fetus, perinatal events that may affect the microbial ecology of the gastrointestinal

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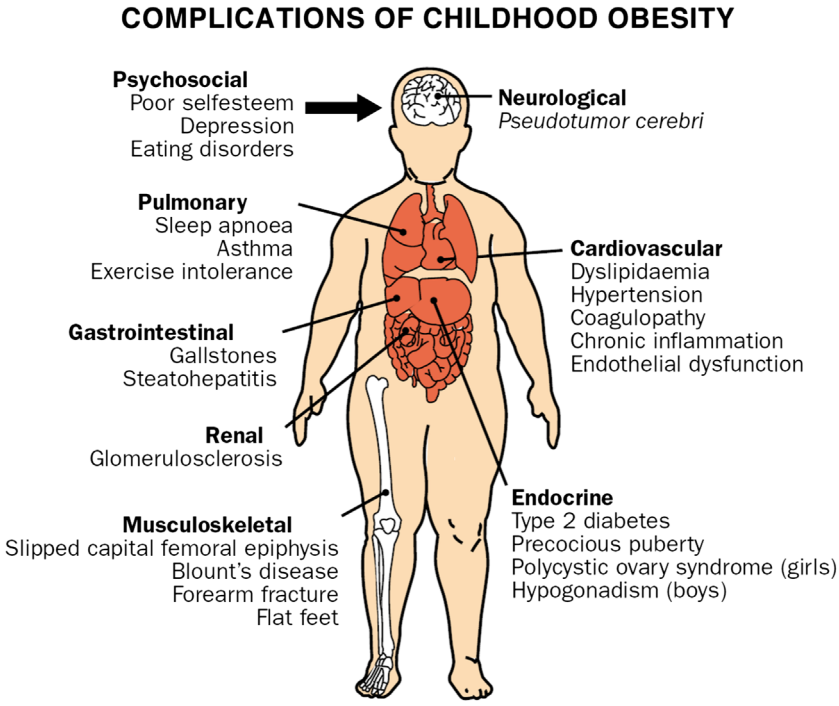

FIG 1. Complications of childhood obesity. (Adapted with permission from Ebbeling et al.[1])

tract of the developing infant, and perturbations during early infancy that alter the intestinal microbiome along with the consequent effects on metabolism, epigenetics, and inflammatory responses that can subsequently result in metabolic syndrome.

The Microbiome in Pregnancy, Peri-Partum, and Early Infancy

Over the past decade, it has become increasingly clear that the microorganisms that are closely associated with human, especially those in our gastrointestinal tracts, harbor a huge ecosystem (the "microbiome") that is highly genetically, transcriptionally, and metabolically active. The major functions of this microbiome involve symbiotic or commensal relationships with their human host. This microbiome interacts closely with a highly immunoreactive intestinal mucosal immune system and has been found to have the capability to pass traits from one generation to the next via maternal contact as well as environmental contact.[5]

The interactions between the microbiome, epigenetic, neuroendocrine, and metabolic systems are likely to play a major role in the genesis of obesity and metabolic syndrome, yet the mechanisms remain poorly delineated. Here we wish to describe some of these relationships and discuss potential pathways that require further study to better understand the actual mechanisms involved.

Here we posit that the early mechanisms leading to obesity may relate to the developing microbiome of the mother and/or fetus before birth. It appears to be dogma that the fetus commonly emerges from a sterile environment and only acquires a relationship with environmental microbes after birth. Under this paradigm, the infant's first stool (meconium) is also sterile. Recent studies contradict this dogma.[5] Microbes have been found to be present in placenta,[6] meconium,[7–9] and amniotic fluid,[10] thus suggesting that there already exists a significant interplay between the environmental microbes and the developing gastrointestinal tract of the fetus prior to delivery. The precise origin of these microbes is not fully understood but studies suggest that the vaginal tract[11] may be one potential source of these microorganisms via ascending transvaginal migration and trans-location through the chorio-decidual membranes. Another mechanism may be through the maternal gastrointestinal tract translocating directly into the maternal blood stream or via cellular transport with transporting cell probably being a form of dendritic cell.[12–14] The fact that microbes found in meconium are closely related to those found in amniotic fluid makes meconium a potentially readily

available substance to evaluate the in utero microbial environment.[9]

Whether this early fetal microbiome may play a role in the development of subsequent obesity and metabolic syndrome is poorly studied. One can consider that different microbes in the maternal gastrointestinal tract may have considerable metabolic capabilities and thus possess different capabilities for increased nutrient salvage in the gastrointestinal tract and thereby promote increased nutrient derivation from various foods such as complex carbohydrates. Furthermore, as recently reviewed by Parekkh et al.,[15] microbial metabolites may exert profound endocrine and metabolic effects that may lead to obesity and insulin resistance. Some of the most important metabolites are the short chain fatty acids (SFCAs) acetate, propionate, and butyrate. As reviewed by Parekh et al.,[15] short chain fatty acids may be a means to increase energy salvage, but concurrently may induce lipogenesis via separate mechanisms. Furthermore, these SCFAs may also regulate gut hormones such as glucagon-like-peptide and peptide YY. These intestinal hormones promote satiety. Thus, when these hormones are present at low levels, there is a predisposition to lack of satiety, hyperphagia, and obesity. Whether these mechanisms relating this complex interrelationship between nutrients, microbes, microbial production of metabolites, and subsequent response of the host play a significant role in the pregnant mother or her offspring is intriguing and further studies are needed in this area.

There are also differences in the capabilities in the production of vitamins such as folate and other highly epigenetically bioactive metabolites such as the short chain fatty acids acetate, priopionate, and butyrate. In addition to the microbiota of the pregnant mother, the microbial milieu of the fetus is also of interest in that if microbes are present in amniotic fluid, these can be swallowed by the fetus and promote priming of the gastrointestinal immune system which in turn may have a major effects on the both the neonate and the adaptive immune system postnatally.[9]

Beyond Genetics

The question arises about the interplay between the various factors that relate to the development of obesity. As already mentioned, energy intake in excess of output plays a role. However, there are numerous other factors involved (Fig. 2). Microbes are likely to play a major role in thriftiness of energy extraction from the diet. Jumpertz et al.[16] compared the fecal microbiota of 12 lean and 9 obese individuals and concurrently measured invested dietary calories and stool calories using bomb calorimetry. Changes in microbiota were seen along with changes in the microbiot and this resulted in major alterations in energy harvest.

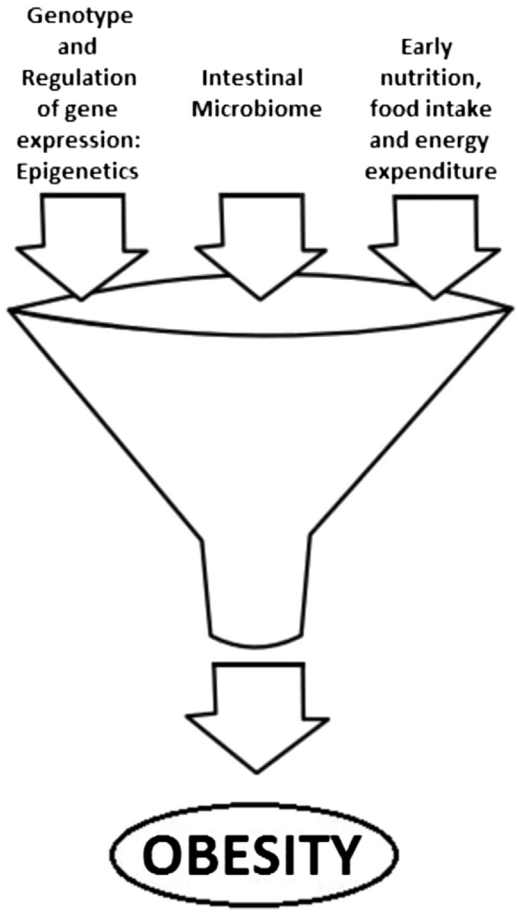

FIG 2. Epigenetic factors leading to obesity.

Various environmental factors can affect the intestinal microbiome as it relates to the subsequent development of obesity. In addition to diet, one of the most important is exposure to antibiotics. Such exposures have been increasing markedly over the past several decades, especially in children. We are also routinely exposed to low-dose antibiotics through our diet since antibiotic use has become highly prevalent in agriculture. Recent studies by Cox et al.[17] suggest that if low-dose penicillin is delivered from birth in animals, this perturbs the microbiota, affects intestinal expression of genes involved in immunity and induce sustained

effects on body composition. Furthermore, they showed that microbes associated with low-dose penicillin exposure transferred to germ free hosts induced the obese phenotype, suggesting a causal role.

The genetic predisposition to overweight and obesity is unclear because it plays a role in only a minor number of the cases, since genetic diseases that clearly relate to obesity are relatively rare. The fact that the increase in obesity worldwide is occurring over a period of a few decades underlines the role for environmental determinants rather than changes in the basic genetic code. These are likely to be highly active during early development. The role of programming during a critical window of development has been provided considerable attention. The well-known fact that intrauterine growth restriction during a critical phase of the fetal development may lead to a "thrifty phenotype" which in turn predisposes to obesity and metabolic syndrome in later life is well known.[18] The mechanisms of this effect have been investigated and studies suggest that up and down regulation of the various metabolic intermediates such as transporters of nutrients, transcription factors, and control via epigenetic changes such as DNA methylation, histone modifications that modulate transcription, and small RNAs have been implicated.

Nutrients such as methyl donors, i.e., folate, methionine, choline, betaine, and vitamin B12 as well as other factors can change DNA methylation status. This DNA methylation status alters the transcription sites of DNA to regulate the expression and timing of the specific genes during various parts of the life course and this is especially critical when considering maternal nutrition.[19] Histone modifications do not directly modify DNA as does DNA methylation. Histones are proteins closely associated with the DNA which can be modified by methylation, acetylation, and phosphorylation. This histone tail acetylation enhances accessibility of a gene to the transcription machinery. If the tails are acetylated the histone is more tightly associated to the DNA backbone and thus limits accessibility of genes to a transcription factors. Certain drugs and nutritional metabolites are known to be histone deacetylase inhibitors which act by enabling the histone transferase complex to transfer acid tail groups to histones thereby loosening their interactions with DNA and thus allowing transcription factor access and gene activation (Fig. 3).

Another epigenetic factor that is thought to be of importance relates to miRNAs which are small RNA molecules which are encoded in the genome and have profound effects on controlling gene expression. These bind to target RNA and down regulate the translation of the mRNA. These can control DNA methylation and histone modifications and thus are important epigenetic modifiers (Fig. 4). There are numerous bioactive food

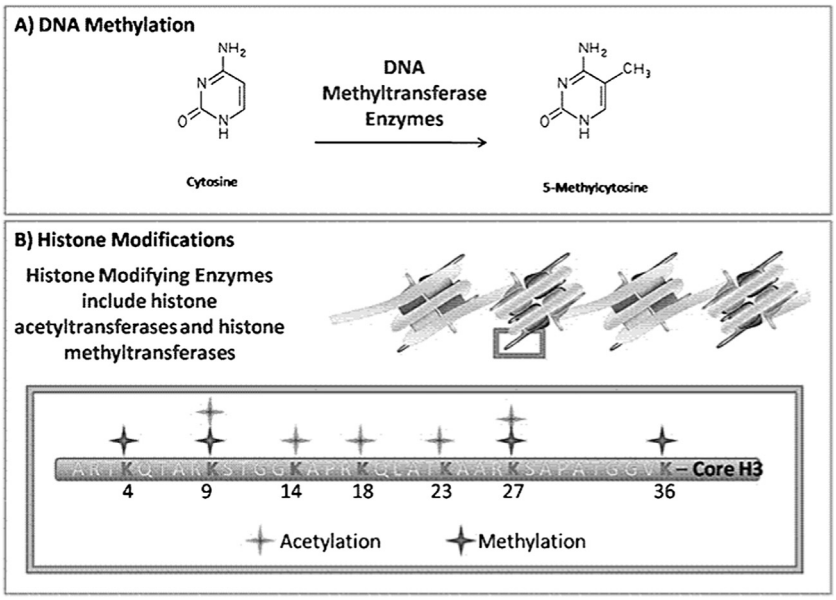

FIG 3. Epigenetic modifications to chromatin regulate gene transcription by affecting the interaction of DNA with the transcription machinery. [Adapted with permission from Wiedmeier JE, Joss-Moore LA, Lane RH, Neu J. Early postnatal nutrition and programming of the preterm neonate. Nutr Rev. 2011;69(2):76–82.]

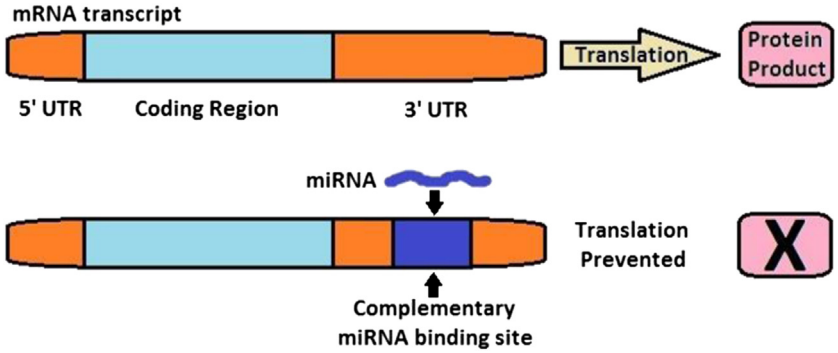

FIG 4. miRNA modifications—miRNA can base pair with a complementary region within the 3′UTR (untranslated region) of the mRNA, preventing translation into protein.

components, which are thought to act through miRNA mechanism, and these include curcumin, genistein and retinoic acid.

The Microbiota, Their Metabolites and Their Interaction via Epigenetics

Changes in the maternal microbiota during pregnancy may have an effect on colonization in the infant. Collado et al.[20] have shown that overweight and obese pregnant women have higher levels of Bacteroides, Clostridium, and Staphylococcus and lower levels of Bifidobacterium than the normal-weight women in their feces. *Akkermansia muciniphilia*, Staphylococcus, and *Clostiridum difficile* were lower in infants of normal-weight mothers and mothers with normal weight gains during pregnancy. They found that these alterations in microbiota compositions may be transferred to infants and result in an increased risk for being overweight. Although the number of bacteria in the newborn gastrointestinal tract is low and also has a relatively low diversity, this increases significantly in the first 2 years after birth.[21] The earliest colonizers may play a significant role in future development.

Early exposures even during the birth process and shortly after birth may have a major effect on the types of microbes that develop and thus the metabolic machinery of the gastrointestinal tract. The mode of delivery, C-section versus vaginal delivery, may play a significant role. Vaginally delivered babies are known to have a different microbial ecology in their gastrointestinal tract than C-section delivered babies.[22] Those infants delivered by cesarean section develop intestinal colonization pattern more closely related to the mother's skin microbes, whereas vaginally delivered babies fecal microbes more closely resemble the mothers' vaginal microbes. This colonization pattern is known to last for years after birth[23] and since the intestinal immune system develops during the first 2 years, this is known to be a very critical time in terms of immune system development. In addition, the types of microbes present may also have a major effect on the metabolic patterns present in the gastrointestinal tract. These metabolic patterns may be closely related to energy harvest, intestinal motility via potentiation of neuroendocrine responses, induction or modulation of chronic inflammation, as well as the initiation of potential for epigenetic modifications in early life that could have significant consequences of for adult disease.

Research on the microbes related to obesity have shown that obese status has been associated with the lower relative abundance of various phyla including the Bacteroides and increase in Firmicutes and that establishment in early life may potentiate the subsequent persistence of these organisms as well as their metabolic functions in adulthood.[24] Weight loss diets have been shown to lead to changes in intestinal microbiota.[25]

Of interest is that obesity-related microbiota is commonly associated with an increased energy harvest from the diet, especially from carbohydrates, with increased production of the short chain fatty acids: acetate, propionate, and butyrate. These can be readily absorbed through the intestinal mucosal surface and thus lead to it an increase in the nutrient harvest.[26] In addition to being important in nutrient harvest, these short chain fatty acids also play a major role in maintenance of the colonic epithelial epithelium, as is the case of butyrate. Butyrate is the major energy substrate for colonic epithelial cells.[27] Propionic acid

also has important effects where recently it has been found to be a major controlling factor in the use of dendritic cells as intermediates to induce the differentiation of the naïve T cells into a regulatory phenotype, which can blunt high levels of inflammation in the gastrointestinal tract as well as other organs.[28] Blunting of chronic inflammation may also play a role in the amelioration of obesity since low level inflammation has been found to be highly associated with the development of obesity, likely related to neuroregulatory mechanisms.[29]

In addition to the short chain fatty acid effects on energy harvest and the direct effects on the intestine and the immune system, these may also have major effects on the epigenetic mechanisms of the developing individual. For example, butyrate is known as a potent histone deacetylase inhibitor, thereby affecting significant metabolic changes that can the potentiate obesity and metabolic syndrome.[30]

The Special Case of the Very Low Birth Weight Infant

Infants born very prematurely should be considered at high risk for subsequent development of the obesity and metabolic syndrome. These infants, even if born appropriate for gestational age, often undergo significant periods of undernutrition during which they are not receiving similar amounts of nutrients that they would be receiving had they remained in utero. After these periods of undernutrition these infants are commonly leaving the neonatal intensive care unit at a considerably smaller size than their counterparts had they remained in utero.[31] However, these infants often undergo a period of catch-up growth when they are provided with high nutrient formulas or human milk supplements. This could be considered analogous to the catch up growth experienced by in utero growth restricted infants. There is some evidence that these infants develop a greater quantity of visceral fat than their counterparts who were delivered at term[32] and there is also some suggestion that these infants have a resultant higher incidence of cardiovascular disease and type II diabetes.[33] However, epidemiologic studies have not fully addressed the question of whether preterm infants have a greater degree of obesity then their non-preterm counterparts when they reach adulthood. Nevertheless, the fact that these infants often receive prolonged courses of antibiotics, which are known to alter their intestinal microbiota, receive intravenous nutrients that differ significantly from what these infants would be receiving in utero and thus may incur significant epigenetic consequences. These infants are also highly stressed which could lead to various neuroendocrine aberrations that result in long-term morbidity. These relationships have yet to be more fully established both from the epidemiologic as well as mechanistic perspective.

How Can We Affect the Microbiota, Metabolism and the Epigenetic Changes That Cause Obesity?

Clear strategies that will affect the development of obesity and metabolic syndrome via these mechanisms are hypothetical. Nevertheless, the hypotheses need to be stated prior to testing. Here we would like to offer some ideas that relate to these potential mechanisms. One of these mechanisms relates to the mothers' diets, epigenetics, and the microbiome. Do mothers receiving higher methyl donor diets bear infants that have less obesity? Does the microbial ecology that results in greater folate and lower butyrate production result in altered fat mass of the infant? Of interest is that there are some classic experiments which have demonstrated that providing methyl donors in mothers' diets in Agouti mice may actually prevent obesity in their offspring.[34,35] Whether this mechanism acts via the intestinal microbes has not been evaluated but in this particular model, may not play a major role. However, the intake of certain methyl donors such as folate may play a major role and there are microbes in the intestinal tract that are also producers of folate. Thus the relationship between the intestinal microbes of the mother, folate production, and the epigenetic effects on the fetus appear to be of high potential.

Delivery by cesarean section versus vaginal delivery appears to play a role in the development of the obesity and metabolic syndrome where those infants delivered by cesarean section tend have higher odds of the development of the obesity in later life.[36] Whether the microbes and their metabolic products actually play a role here is poorly understood. However, the fact that differences in microbes do exist for long periods of time after vaginal versus C-section delivery is of significance. It is clearly known that many cesarean sections are being done for nonmedical indications and of interest is the fact that in some countries and some

private clinics the rate of C-section nears 100% and almost no vaginal deliveries occur. These are clearly being done for non-medical reasons and it is a very possible that this could represent a subsequent public health hazard in the future.

The use of the antibiotics during pregnancy as well as in early childhood may have an effect on the developing microbiota. In fact, studies have shown that antibiotics in early life have a major effect on the development of obesity in animal models.[24] Furthermore epidemiologic studies have suggested that early use of antibiotics is associated with a greater risk of obesity in later life.[37]

Some evidence suggests that breast-feeding is associated with the lower risk of the development of the obesity in later life. However, there are some studies that do not fully support this contention.[38] Nevertheless recent studies have shown that microbes are present in human milk and these microbes are likely of maternal gastrointestinal origin.[39,40] Using non-culture based techniques these microbes appear to be very similar over time in each individual mother's milk,[39] however, they differ markedly from one mother to the next, hence suggesting the possibility that each mother's milk microbes may be specific for that particular mother's infant. There are multiple bioactive substances found uniquely in human milk, not found in formula, with numerous functions that contribute to overall infant health and development (Table 1). For example, human milk oligosaccharides function as prebiotic agents by serving as a food source for Bifidobacterium and Lactobacillus which are known to assist digestion of nutrients and block adhesion and colonization of pathogenic bacteria.[40]

Studies in rhesus monkeys that have been formula fed versus breast-fed have shown there is a different gut microbiota depending on type of feeding.[41] Formula-fed infant monkeys have higher levels of bacteria from the Ruminococcus genus and lower levels of bacteria from the Lactobacillus genus. The formula-fed infant monkeys are a lot bigger than the breast-fed monkeys. In addition, the formula-fed infant monkeys have higher serum insulin levels. Associated with these effects, there appears to be a higher inflammatory state in the formula fed group in the 1st month when compared to the breast-fed rhesus infants. Certain species of Lactobacillus, specifically *Lactobacillus johnssonii*, which is present in monkey milk, is known to reduce levels of the pro-inflammatory cytokines.

Interventions That may Alter the Course Toward Obesity

Because of the multivariate nature of the development of obesity, it is likely that one factor may affect other factors that are involved in the pathogenesis. For example, feeding a certain diet may affect the microbial composition of the gastrointestinal tract which in turn will result in a lean versus an obese microbial intestinal phenotype. Alteration of the microbiota with resultant alterations in metabolism may be highly resilient toward change in the adult,[42] but the early microbiota during fetal life and early infancy is much more likely to be affected than the adult microbiota when the microbial ecology of the core is largely already developed. The early composition of the microbiota especially during early infancy when differences can result from C-section versus vaginal delivery or breast-fed versus formula-fed infants may play a major role. Thus, avoiding unnecessary cesarean sections and promoting a culture of the breast-feeding could be of major benefit. Furthermore, the unnecessary use of antibiotics in pregnancy and during infancy may also play a role where the trajectory toward development of a certain core microbiota may actually be a significantly affected. In this manner, the

TABLE 1. Examples of the bioactive factors in human milk and their functions

Type of function	Selected examples
Adaptive immune factors	Secretory immunoglobulin A (sIgA), IgG, IgM, and IgE
Innate immune factors	Lactoferrin, peptides, milk fat globule, free fatty acids, pathogen-binding inhibitors—glycoproteins (e.g., bile salt-stimulating lipase), glycolipids, glycosaminoglycans, gangliosides, and mucins
Immune-modulating agents	Cytokines (e.g., TGF-β and IL-10), chemokines, macrophages, and leukocytes
Prebiotic agents	Oligosaccharides
Anti-inflammatory agents	Antioxidants (e.g., α-tocopherol, β-carotene, vitamin E, and Q10), soluble TNF-α receptors, and IL-1Rα
Growth and metabolic factors	Adipokines, insulin, epidermal growth factor, prolactin, cortisol, thyroxin, prostaglandins, and vascular-endothelial growth factor

antibiotic treated microbiota may develop into one that is conducive to an obesogenic phenotype.[24]

Nutrition for preterm infants who are hospitalized in the NICU has been evolving to a more "aggressive" approach, which involves early introduction of parenteral macronutrients as well as enteral feeding.[43] The goal of this approach is to prevent early catabolism during a highly vulnerable stage of development as well as to result in growth similar to that of normally growing fetuses of the same gestational age. Despite literature that suggests that rapid early weight gain in preterms might result in later cardiovascular[44] and metabolic problems,[45] this remains controversial. In terms of post-discharge nutrition for these infants, practice varies widely by country. These infants are often sent home on human milk alone, partially fortified human milk, preterm or term formulas. There is abundant data that these infants should be provided only human milk for the first 6 months after birth, but this is often difficult to maintain in preterm infants. As written by Lapillone et al.,[46] the preterm infants at highest risk and who have the greatest nutritional needs are, "if they: (1) are discharged well before their expected delivery date; fed predominantly human milk; (2) have fallen below the 3rd or 5th percentile in growth indices; or (3) have persistent morbidities that elevate nutritional requirements or limit the volume of feeds consumed." Breastfeeding should be promoted, but these infants also may need to have their breast milk fortified. Analysis of available studies reveals conflicting results as to whether fortification of human milk affects growth, but the European Society for Pediatric Gastroenterology Hepatology and Nutrition recommends "supplementation of human milk or provision of a special postdischarge formula with high contents of protein, mineral and trace elements as well as long chain polyunsaturated fatty acid supply, at least until a postconceptional age of 40 weeks, but possibly until about 52 weeks postconceptual age."[47] This approach appears to be prudent but whether it has any effect on long-term morbidity such as obesity or metabolic problems is not known.

Environmental influences may also play a major role. For example, the agricultural practice of the providing antibiotics to animals and plants meant for human consumption may alter the microbial environment of the intestine. There is data in animals that suggests that this results in various health problems, including behavioral problems, related to an altered intestinal microbiome.[48]

Other interventions such as the provision of probiotics, prebiotics, and synbiotics may be of use, but whether a single probiotic or prebiotic may affect the developmental microbial ecology of the intestine without perturbing the normal developmental process is a question that needs to be raised since this may affect numerous processes for the lifetime of the individual, which may not be readily reversible.

Conclusion

In summary, it is clear that although genetics may play a role in the development of obesity there are many other factors that are involved. One's genome evolves very slowly over a period of many generations and thus the effects of genetic variation over period of time occur very slowly. However, our "second genome" (the microbiome) is much more plastic and thus can be harnessed in the prevention of the obesity during the lifetime of the individual. It may actually have some beneficial effect on subsequent generations via epigenetic mechanisms. This a highly fascinating area that is in need of the additional investigation and will likely lead to important new interventions on the prevention of the this extremely important emerging health problem.

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