Purpose of review
The environment experienced during critical windows of development can ‘programme’ long-term health and risk of metabolic diseases such as type 2 diabetes in the offspring. The purpose of this review is to discuss potential epigenetic mechanisms involved in the developmental programming of type 2 diabetes by early nutrition.

Recent findings
Maternal and more recently paternal nutrition have been shown to play key roles in metabolic programming of the offspring. Although the exact mechanisms are still not clear, epigenetic processes have emerged as playing a plausible role. Epigenetic dysregulation is associated with several components that contribute to type 2 diabetes risk, including altered feeding behaviour, insulin secretion and insulin action. It may also contribute to transgenerational risk transmission.

Summary
Epigenetic processes may represent a central underlying mechanism of developmental programming of type 2 diabetes. During embryonic and foetal development, extensive epigenetic remodelling takes place not only in somatic but also in primordial germ cells. Therefore, concerns have been raised that epigenetic dysregulation induced by a suboptimal early environment could programme altered phenotypes not only in the first generation but also in the subsequent ones. Characterizing these altered epigenetic marks has great implications for identifying individuals at an increased disease risk as well as potentially leading to novel preventive and treatment strategies.

Keywords
developmental programming, epigenetics, metabolism, nutrition, type 2 diabetes

INTRODUCTION
On the basis of epidemiological data showing an association between low birth weight – a proxy of in-utero development – and increased risk of type 2 diabetes mellitus (T2DM) in adult life, the ‘Thrifty Phenotype Hypothesis’ was proposed more than 2 decades ago by Hales and Barker. According to this hypothesis, under suboptimal in-utero conditions, the foetus undergoes metabolic adaptations that maximize its chances of survival in a scenario of postnatal nutritional scarcity. However, in a nutritionally adequate or excessive environment, this foetally programmed thrifty phenotype would be deleterious and lead to an increased risk of metabolic diseases including T2DM [1].

Accumulating epidemiological and experimental data show that in addition to in-utero life, other developmental stages including early childhood and adolescence influence noncommunicable disease risk in adulthood and thus the field has incorporated the broader term ‘developmental origins of health and disease’ (DOHaD). According to the DOHaD concept, during early development, environmental factors can affect the individual’s disease risk trajectory through complex cellular and molecular mechanisms [2]. Maternal stress, smoking, metabolic conditions, undernutrition and obesity/overnutrition comprise important developmental programming-inducing factors [3,4]. More recently, paternal experiences, including paternal diet, have
KEY POINTS

- The environment during critical windows of development can ‘programme’ long-term health and risk of chronic metabolic diseases including T2DM in the offspring.
- Maternal and more recently paternal nutrition have been shown to play a key role in the metabolic programming of the offspring.
- Although the exact mechanisms of developmental programming are still not clear, epigenetic processes have emerged as plausible mechanisms.
- Epigenetic dysregulation has been associated with central components of T2DM nutritional programming, including feeding behaviour, key metabolic tissue dysfunction and intergenerational risk transmission.
- Characterizing altered epigenetic marks induced by early suboptimal nutrition will have great implications for identifying individuals at an increased risk and establishing novel preventive and treatment strategies.

have been shown to also impact on the offspring's health [5,6].

Adverse nutritional and hormonal perinatal environments have been proposed to programme T2DM through inducing structural and functional alterations in key metabolic systems, including the brain, muscle, liver, adipose tissue and pancreas [1,7–9]. During embryonic and foetal development, intense epigenetic remodelling is necessary to enable the establishment of transcriptional programmes associated with cellular proliferation and differentiation. During this sensitive developmental phase, the epigenome is especially plastic and prone to environment-induced disturbances [10]. Epigenetic mechanisms have thus emerged as an attractive molecular mechanism through which events that occurred early in life (i.e. suboptimal nutrition in utero) would be registered and ‘remembered’ several years later, following multiple rounds of cell division, thus permanently affecting genomic function and consequently the adult metabolic phenotype. The main epigenetic processes include DNA methylation, post-translational modifications of histone tails and noncoding RNAs (microRNAs and long noncoding RNAs). Collectively, they influence gene expression in the long-term without affecting DNA sequence [11].

T2DM is currently a major global public health problem affecting both developed and developing countries, where it poses a serious social and economic burden [12,13]. The adoption of a developmental perspective in this context could allow a better understanding of the causes of the disease, identification of individuals at an increased disease risk and establishment of innovative preventive strategies starting in early life. However, a lack of in-depth understanding of how the early life environment can have long-lasting effects on health and increased risk of T2DM in adulthood poses a major limitation to such efforts. The focus of the current review is thus to discuss recent experimental and human evidence of an epigenetic component associated with components of nutritional programming of T2DM, including altered feeding behaviour, adipose tissue and pancreatic beta cell dysfunction, and transgenerational risk transmission.

DEVELOPMENTAL PROGRAMMING OF KEY METABOLIC TISSUES

Evidence for a link between epigenetic dysregulation and programming of key metabolic tissues such as the brain, adipose tissue and endocrine pancreas is summarized below.

THE BRAIN

One main topic that has received a large amount of attention is how maternal nutrition during critical time-windows including gestation and/or lactation can influence offspring feeding behaviour [14–16]. This has been recently reviewed [8,17]. Exposure to adverse perinatal conditions such as under and over-nutrition can alter appetite and satiety regulatory systems leading to hyperphagia and increased risk of obesity in adulthood. This programming effect has been linked to disturbances in nutrient sensors, neuroendocrine levels and signalling, neurogenesis and neuropeptide levels [17].

Although the exact mechanisms by which maternal diet and metabolic state affect offspring behaviour are not clear, it has been proposed that they include indirect effects (i.e. maternal behaviour) and direct effects through alteration of the perinatal environment via increases in inflammatory cytokines, nutrients (e.g. glucose and fatty acids) and hormones (e.g. insulin and leptin) [18]. Recent evidence also indicates an important role of early ghrelin levels in the programming of hypothalamic feeding circuits [19].

Increased maternal fatty acid consumption is a topic of special interest, as their offspring have been shown to be more susceptible to developing mental health and behavioural disorders such as anxiety, depression and attention deficit hyperactivity disorder [18]. Importantly, special consideration should be directed to the specific type of fatty acid consumed, as their metabolic effects can vary depending on their dietary composition [20].
Genes and cell metabolism

The methylation status of the SP1 binding site in the promoter region of the proopiomelanocortin (POMC) gene, which encodes a neuropeptide expressed in anorexigenic neurons, was analysed in the offspring of mothers consuming conjugated linoleic acids (CLAs)-supplemented diet specifically during postnatal days 3–13 [21*]. The authors observed changes in milk composition and this was associated with increased food intake in young animals and altered metabolic parameters in adulthood including hyperglycemia and insulin resistance. Compared with control offspring, the young CLA-consuming offspring demonstrated reduced POMC expression, attenuation of Sp1–promoter interaction and hypermethylation of CpGs within the Sp1-binding site of the POMC promoter in the hypothalamus. Importantly, in cultured cells, methylation of these CpGs in the POMC promoter blocked the formation of the Sp1–promoter complex and the leptin-induced activation of POMC [21*]. As CLAs have been used as food additives, these results raise the cautionary note that special care should be taken when considering fatty acid interventions during the lactation period that represents a sensitive developmental window during which epigenetic dysregulation could occur.

Consumption of a high-carbohydrate milk formula during lactation was shown to result in chronic hyperinsulinemia and adult-onset obesity in female rats [22]. The authors examined the epigenetic status of the promoters associated with the neuropeptide Y (NPY) and POMC genes and found that only the NPY promoter presented altered methylation of specific CpG dinucleotides in the hypothalamus of young adult animals. Alterations in histone marks including acetylation but not methylation of H3K9 were also observed and suggested to be associated with NPY and POMC hypothalamic mRNA expression in the animals that consumed the milk formula compared with animals nursed by their dams [22].

ADIPOSE TISSUE

Alterations in the differentiation potential of adipocytes have been proposed to be associated with the metabolic programming effects of suboptimal nutrition in early life [23–25]. The impact of maternal obesity during gestation on adipocyte commitment and differentiation via epigenetic mechanisms in the offspring was recently investigated [26]. The authors made rats fat using a model of obesity based on the consumption of a high-calorie liquid diet via computer-controlled pumps during preconception and gestation. White adipose tissue analysis of their offspring before development of obesity revealed increased expression of key adipogenic and lipogenic transcription factors [peroxisome proliferator-activated receptor-γ (PPAR-γ) and CCAAT enhancer binding proteins (C/EBPs)], as well as specific alterations in DNA methylation of CpG sites and CGI shores proximal to developmentally important genes, including key proadipogenic factors such as Zfp423 [26]. Because of its central role in determining cellular commitment to the adipogenic lineage, particular interest has been directed towards this transcription factor in the context of metabolic programming. Yang et al. [27] showed using rats that maternal obesity promoted adipogenesis during foetal development and this was associated with increased expression of Zfp423 through reduced methylation of its promoter region.

More recently, the potential role of altered microRNA expression in the programming of adipose tissue has been described [28*]. Increased expression of mir-126 was programmed in the adipose tissue of young adult offspring of mice that were exposed to maternal obesity through consumption of high-fat, high-sucrose palatable diet. Notably, IRS-1 protein expression, a target of this microRNA, was decreased and this was a cell autonomous effect that was retained following differentiation of programmed cells in vitro [28*].

THE ENDOCRINE PANCREAS

Altered pancreatic beta cell function represents a central mechanism associated with developmental programming of T2DM [29,30]. Early life conditions including both under and overnutrition are associated with insulin resistance in key organs controlling glucose homeostasis, including liver, muscle and adipose tissue in adult life placing considerable pressure on the beta cell. Beta cells have limited proliferative and regenerative capacity and are especially prone to increased oxidative stress due to low levels of antioxidants [7]. Therefore, increased need for insulin production to compensate for the peripheral resistance will result in beta cell stress, altered function and ultimately failure. As well as effects on beta cell mass, there is accumulating evidence that the early life environment can impact on epigenetic modifications in the pancreatic beta cell, which could also influence function (reviewed in [31]).

The potential involvement of dysregulated microRNA expression in the endocrine pancreas as a mediator of early nutrition on the establishment of beta cell mass and subsequent T2DM risk has been recently examined in rodents [32**,33**]. Offspring of rats exposed to a classical maternal undernutrition
model based on the consumption of a low-protein diet during gestation presented decreased pancreatic beta cell mass and impaired function starting in foetal development [32**]. MicroRNA profiling revealed increased expression of different microRNAs including miR-375 in the pancreas of the foetuses of mothers consuming the low protein diet. Programmed altered expression of this microRNA was confirmed through sustained increased levels in neoformed islets derived from foetuses and in islets from adult offspring of undernourished mothers. Of note, the authors also demonstrated through functional analysis that normalization of miR-375 in islets could restore beta-cell proliferation and insulin secretion [32**]. These findings suggest that microRNAs may represent potential molecular targets to reverse programmed metabolic phenotypes. Exercise and dietary interventions with functional foods have been proposed as potential strategies to ‘deprogramme’ epigenetic-altered gene expression by early insults, although their efficacy remains to be evaluated [9].

THE EPIGENOME AS AN INTEGRATOR OF EARLY-LIFE ENVIRONMENTAL EXPOSURES: EVIDENCE IN HUMANS

Although most of the studies investigating the role of epigenetics in developmental programming have been conducted in animal models, an increasing number of studies have been conducted in humans [34**–36**]. One of the few studies in humans evaluating the relationship between genome-wide methylation and foetal growth was conducted in 2014 [35**]. Cord blood samples from a subset of 1046 infants from the Norwegian Mother and Child Cohort Study were analysed for CpG dinucleotide methylation across the genome using state-of-the-art technology. Particular strengths of this study include the large sample used, the homogenous nature of the population investigated and the availability of comprehensive information that allowed adjustment for different known correlates of birth weight. According to the results, associations between CpG methylation and birth weight were found for nine genes, including ARIDSB that has been associated with foetal growth and lipid metabolism [35**].

Because both in-utero nutritional deficiency and excess have been associated with increased risk of T2DM in later life [37–39], it is important to establish whether these divergent conditions leading to the same phenotypic outcome operate through similar epigenetic pathway processes. This has been examined recently in a study wherein DNA methylation was analysed in umbilical cord blood samples from infants who were born to mothers with gestational diabetes mellitus (GDM) and infants showing rapid postnatal growth after prenatal growth restraint [36**]. A genome-wide strategy was employed to study DNA methylation and it was found that eight differentially methylated loci were common to male offspring of mothers with GDM and infants with growth restriction. Among the genes with differentially methylated loci were ACYP2 (associated with coronary artery disease) and C3orf31 (a mitochondrial translocator assembly and maintenance protein associated with insulin resistance) [36**]. Although a relatively small number of samples were analysed in this study, the results are very promising and support a possible genomic commonality in metabolic programming elicited by distinct in-utero exposures. This has important implications in terms of setting preventive strategies that could be based on common pathways.

TRANSGENERATIONAL EPIGENETIC METABOLIC PROGRAMMING EFFECTS: RISK TRANSMISSION THROUGH THE FEMALE AND MALE GERMINE

The fact that epigenetic remodelling also takes place in the primordial germ cells of the developing foetus has raised concerns that suboptimal in-utero conditions not only programme the phenotype of the F1 generation but also of the subsequent generations (F2 (intergenerational epigenetic effect), F3 (nonexposed generation; transgenerational effect) and beyond [40–43]. Thus, for example, consumption of a high-fat diet by mice during pregnancy and lactation has been reported to epigenetically alter metabolism-related genes such as leptin and peroxisome proliferator-activated receptor-α (PPAR-α), but not imprinted ones, in the hepatic tissue of the female offspring as well as in their oocytes [44*].

Recent data indicate that paternal experiences including dietary habits can also influence the metabolic health of the offspring [5,6,45]. An epigenetically inherited increased metabolic risk has been proposed to be transmitted through the male germ-line, although the exact underlying epigenetic marks have not yet been characterized. The existence of specific windows of epigenetic damage inheritance susceptibility has also been suggested. This would include not only in-utero life, but also puberty, the reproductive phase and the zygotic phase [46].

Proof-of-concept that in-utero suboptimal nutritional conditions can alter the adult sperm DNA methylation profile has been recently provided [47**]. In this study, mice were exposed to an established in-utero model of intergenerational developmental programming wherein glucose
metabolic dysregulation is transmitted from F1 to F2 through the paternal germline. Importantly, the period of nutritional restriction (day 12.5–18.5 of pregnancy) coincides with the DNA methylation reprogramming in male primordial germ cells. Compared with control sperm, the authors observed that 111 regions were hypomethylated in sperm cells of adult animals that were undernourished in utero but consumed a normal diet in postnatal life [47]. Although these sperm DNA methylation alterations were not retained in the F2 somatic tissues in late gestation, these data are of great interest, as they show that the in-utero environment can have long-lasting effects on the adult male germ cells methylome and this could be a driving component in the intergenerational programming of metabolic diseases.

Additional evidence for an epigenetic intergenerational effect through the male germline was provided by a study in which pregnant mice (F0) were submitted to a 50% global caloric restriction during the last week of gestation [48]. Their offspring (F1) had a low birth-weight and developed several metabolic abnormalities, including mild hyperglycaemia and glucose intolerance. Of notice, these abnormalities were also present in the offspring (F2) of intrauterine growth-restricted males. In addition, the F2 generation presented altered hepatic lipid metabolism via decreased expression of liver X receptor (LXR) and altered methylation pattern in the loci’s regulatory region. The fact that this epigenetic signature was also observed in the sperm of the fathers reinforce the concept that in-utero induced alterations in the epigenetic programme in the male germline can be transmitted to next generation and affect its phenotype [48].

A role of microRNA dysregulation in developmental programming was further suggested by studies investigating the intergenerational transmission of obesity susceptibility and insulin resistance induced by paternal obesity in two generations of mice offspring [49]. Consumption of a high-fat diet by the F0 fathers resulted in reduced sperm microRNA content and global DNA hypomethylation of germ cells [49]. Of note, diet or exercise (swimming) interventions in obese male mice for 8 weeks (two rounds of spermatogenesis) normalized their levels of X-linked sperm microRNA,
restored insulin sensitivity and normalized adiposity in female offspring [50]. These data reinforce the potential role of paternal interventions as a strategy to improve the health of their offspring.

CONCLUSION
Maternal and more recently paternal nutrition have been shown to play a key role in the metabolic programming of the offspring. Accumulating experimental and some clinical data implicate epigenetic dysregulation as a plausible mechanism associated with central components of T2DM nutritional programming, including altered feeding behaviour, key metabolic tissue dysfunction and intergenerational risk transmission (Fig. 1). Extensive research in the field of developmental programming is still needed to better characterize these altered epigenetic marks and identify their functional consequences. Other key research needs include confirmation of the existence of true transgenerational epigenetic programming effects as well as the degree of reversibility of altered epigenetic dysregulated marks. It is anticipated that such research will have great implications for identifying individuals at an increased risk of metabolic diseases such as T2DM and establishing novel preventive and treatment strategies.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING
Papers of particular interest, published within the annual period of review, have been highlighted as:
= of special interest
= of outstanding interest

15. Ong ZY, Mullhauser BS. Consuming a low-fat diet from weaning to adulthood reverses the programming of food preferences in male, but not in female, offspring of ‘junk food’-fed dams. Acta Physiol (Oxf) 2014; 210:127–141.
This study shows that consumption of conjugated linoleic acids during lactation negatively impacts young offspring feeding behaviour and methylation status of an aromatase gene in the hypothalamus.
This is one of the first studies to show the involvement of altered adipose tissue microRNA expression in metabolic programming effects of maternal obesity.
Genes and cell metabolism


This is one of the first studies to show the involvement of altered microRNA expression in pancreatic β-cell altered function programming effects exerted by maternal undernutrition. Importantly, ex-vivo normalization of altered microRNA expression was able to partially restore β-cell functioning.


This is one of the first studies to show the involvement of altered microRNA expression in pancreatic β-cell altered function programming effects exerted by maternal undernutrition.


This is one of the few studies investigating the association between maternal nutrition and epigenetic status in the offspring in humans. The data from this study show that maternal nutrition, particularly methyl donor nutrients, is associated with the epigenetic status of metastable epialleles.


This is one of the few studies in humans evaluating the association between genome-wide methylation and foetal growth. Associations were described for some genes involved with foetal growth and lipid metabolism.


This is one of the few studies investigating the association between maternal nutrition and epigenetic status in the offspring in humans. The data from this study show that infants who were submitted to conditions of under or overnutrition present some common differentially methylated genes.


43. Sarkar DK. Male germ line transmits fetal alcohol epigenetic marks for multiple generations: a review. Addict Biol 2015. [Epub ahead of print]


This is one of the first studies to show that in-utero exposure to suboptimal nutritional conditions can alter epigenetic marks in germ and somatic cells of the female offspring.


This study provides proof-of-concept that in-utero suboptimal nutritional conditions can alter adult sperm DNA methylation. Results provided by this study show that epigenetic alterations in the male germ line could be a driving force in transgenerational metabolic programming.


This is one of the first studies showing that in-utero suboptimal nutritional conditions can alter adult sperm DNA methylation. Results provided by this study show that epigenetic alterations in the male germ line can be transmitted to offspring somatic tissue.


This study shows the relevance of dietary and exercise interventions in obese fathers during preconception as a potential strategy to prevent metabolic programming effects in the offspring.