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Nutritional modulation of the epigenome and its implication for future health

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Non-communicable diseases (NCD) such as type-2 diabetes and CVD are now highly prevalent in both developed and developing countries. Evidence from both human and animal studies shows that early-life nutrition is an important determinant of NCD risk in later life. The mechanism by which the early-life environment influences future disease risk has been suggested to include the altered epigenetic regulation of gene expression. Epigenetic processes regulate the accessibility of genes to the cellular proteins that control gene transcription, determining where and when a gene is switched on and its level of activity. Epigenetic processes not only play a central role in regulating gene expression but also allow an organism to adapt to the environment. In this review, we will focus on how both maternal and paternal nutrition can alter the epigenome and the evidence that these changes are causally involved in determining future disease risk.

Epigenetics: DNA methylation: Biomarkers: Developmental origins of adult disease: Nutrition

Over the past two decades, there has been a rapid rise in the rates of non-communicable diseases (NCD) such as obesity, diabetes and CVD. This rise in NCD is not restricted to industrialised nations but is becoming increasingly important in both low- and middle-income countries, which demonstrate a rapid increase in NCD prevalence as they undergo socioeconomic improvement[1,2]. Such a rapid rise in the rates of these diseases cannot be accounted for purely by genetic influences but suggests that environmental factors such as the increasing consumption of energy-rich diets and lack of physical activity may also play a critical role in determining the risk of NCD. While diet and lack of physical activity in later life are certainly important risk factors of metabolic disease[3], there is growing evidence that prenatal and early postnatal environmental factors particularly nutrition also play a key role in modulating the risk of NCD in later life[4]. In this review, we will focus on how variations in maternal and paternal nutrition can affect the long-term health of the child and the evidence that epigenetic processes play a central role in transmitting the information from parental environment to the offspring.

Early-life environment and later disease risk

An association between the quality of the early-life environment and later disease risk was first shown in a Norwegian study which identified a strong association between undernutrition and poverty during childhood and adolescence, followed by later prosperity, with CVD in late middle age[5]. Subsequent work by David Barker and co-workers related the health of middle-aged individuals in Britain to their recorded birth measurements; lower birth weight was found to correlate strongly with the later risk of CVD, type 2 diabetes, hypertension and hyperlipidaemia[6–9]. Further epidemiological studies

Abbreviations: CpG, cytosine and guanine nucleotides linked by a phosphate; miRNA, microRNA; NCD, non-communicable disease; ncRNA, non-coding RNA; PR, protein restricted; tRNA, transfer RNA.

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have confirmed the associations between lower birth weight and later disease risk but importantly have also shown that babies born at the highest birth weights are also at increased risk of developing diabetes or obesity in later life[10,11]. Birth weight in these studies is thought to be an indicator of a poor intrauterine environment which may have been compromised through a variety of maternal or environmental factors such as placental insufficiency, maternal undernutrition, overnutrition, maternal obesity or gestational diabetes[12].

While these epidemiological studies were the first to show a relationship between intrauterine environment and chronic disease risk, studies of the Dutch Hunger Winter, a famine which occurred in the Netherlands during the winter of 1944, clearly demonstrated that maternal nutrition influences the health of the child in later life and that the timing of the nutritional constraint is important. Studies found that individuals whose mothers were exposed to famine periconceptually and during the first trimester of pregnancy exhibited an increased risk of obesity and CVD, whereas individuals whose mothers were exposed in the later stages of gestation showed an increased incidence of insulin resistance and hypertension in later life[13,14]. Comparable findings have now been replicated in a variety of animal models where rats or mice have been fed either a low-protein diet or a global dietary restriction during pregnancy[15–19]. Interestingly, experimental studies have also shown that the offspring from dams fed a high-fat or even junk food diet during pregnancy and/or lactation also exhibit similar features and develop hypertension, dyslipidaemia, obesity and insulin resistance in later life[20,21].

The induction of different phenotypes by perturbations in early-life nutrition has been suggested to reflect a predictive adaptive response whereby the organism, acting through the process of developmental plasticity, can adjust its developmental programme in response to environmental cues in early life to aid fitness or survival in later life[22]. When an organism adapts to one environment and is subsequently exposed to a different environment after birth, a mismatch occurs leaving the organism maladapted and at risk of metabolic disease in later life[23,24]. This mismatch between the prenatal and postnatal environments has been suggested to be central to the burgeoning rates of NCD observed in countries undergoing socioeconomic transition, for example, as populations move from rural to urban areas[25,26]. In the western world, exposure to continuous energy/sugar-rich diets may be beyond our adaptive capacity leading to pathological changes and an increased disease risk.

Epigenetic regulation

The mechanism by which early-life nutrition may influence future disease risk has been suggested to involve the altered epigenetic regulation of genes, sections of DNA that encode for proteins[27]. Epigenetic processes, which are stably inherited through cell division, regulate the expression potential of genes without altering the underlying genetic sequence. These epigenetic processes include DNA methylation, histone modifications and non-coding RNA (ncRNA). Together, they regulate all aspects of gene expression, controlling either the access of the transcriptional machinery to the underlying DNA sequence, the stability of mRNA or its translational competence, thereby defining when and where genes are expressed and levels of expression.

DNA methylation

DNA methylation is the transfer of a methyl group (CH_3) to the C_5 position of cytosine to create 5-methylcytosine[28]. In mammals, the majority of methylated cytosines are found next to a guanine and are referred to as CpG (cytosine and guanine nucleotides linked by a phosphate). In general, low levels of DNA methylation in the promoter or control regions of genes are associated with transcriptional activity, while high levels of methylation are associated with transcriptional silencing[29]. CpG promoter methylation leads to transcriptional repression by either blocking the ability of transcription factors to bind to DNA and activate gene expression or through the recruitment of histone-modifying complexes to the DNA, which leads to a closed chromatin configuration and transcriptional repression[30].

Establishment of DNA methylation patterns through development

DNA methylation is markedly reprogrammed during embryonic development in mammals. Following fertilisation, the methylation marks on the maternal and paternal genomes are largely erased[31]. Demethylation occurs rapidly on the paternal DNA, catalysed by the ten to eleven translocation proteins, while demethylation on the maternal DNA occurs more slowly over several cell divisions by a replication-dependent passive process[32–34]. However, a number of regions escape this erasure including the imprinting control regions of imprinted genes[35]. After this phase of global demethylation and around the time of blastocyst implantation, the genome then undergoes *de novo* methylation. The pluripotency genes *Oct-3/4* and *Nanog*, which are essential to maintaining the undifferentiated state of early stem cells, are methylated and silenced[36]. Methylation of lineage-specific genes also occurs, thus genes that are not required within certain cell types are methylated and silenced. In contrast, CpG islands within housekeeping genes, constitutive genes that are essential for basic cell function, are protected from this global *de novo* methylation and remain unmethylated[32].

Histone modifications

In eukaryotic organisms, DNA within the nucleus is wrapped around a histone octamer consisting of two copies of each core histone (H2A, H2B, H3 and H4) forming the basic unit of chromatin, a nucleosome. Each

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nucleosome is then folded upon itself to form a solenoid or 30 nm fibre which is then further coiled and compacted to form a 200 nm fibre. This folding and packaging of the DNA is essential to reduce its effective size[37]. However, it is now clear that histone proteins are not only important for the packaging of DNA, but also play a critical role in regulating gene expression alongside DNA methylation. Histone proteins contain two domains: a globular domain and an amino tail domain. The amino tail domains of histone proteins are rich in positively charged amino acids which interact with negatively charged DNA and are now known to be subject to a large number of post-translational modifications including acetylation and methylation[38,39]. These modifications can either directly affect chromatin structure or provide binding sites for proteins involved in gene regulation[40]. For example, acetylation of the lysine residues within histone tails, which is catalysed by histone acetyltransferases, neutralises the positive charge of the lysine, thereby reducing the interaction of the histone with DNA. This leads to an opening up of chromatin, allowing access to the transcriptional machinery. Conversely, histone deacetylases remove the acetyl groups, restoring the positive charge and leading to a closing down of the chromatin and gene repression[41]. Histone methylation is associated with both activation and repression; methylation at lysine (K) 4 on histone H3 is an activating mark, while methylation at K9 or 27 is associated with transcriptional repression[42].

Non-coding RNA

Although up to 90% of the eukaryotic genome is transcribed, only 1–2 % of the genome encodes proteins[43]. These ncRNA include microRNA (miRNA) which can induce mRNA degradation or translational repression and, when binding within the promoter region of a gene, induce both DNA methylation and repressive histone modifications resulting in reduced transcriptional activity or even complete repression[44–46]. DNA methylation, histone modification and ncRNA often work in concert with each other to regulate gene expression. Many studies, although not all, suggest that DNA methylation may consolidate changes in gene expression induced by histone modifications and ncRNA, and that DNA methylation represents the most stable of these epigenetic marks which is required for long-term persistent repression of gene expression.

Effect of maternal diet on the epigenome: evidence from experimental models

Although DNA methylation was thought to be a very stable modification and once methylation patterns were established in early life, they were then largely maintained, there is now growing evidence that a number of environmental factors such as nutrition[47], body composition[48], endocrine disruptors[49] and social environment[50] can modulate the methylome, often resulting in long-term changes in gene expression and physiology.

Alterations in maternal diet in both rats and mice have been shown to induce changes in DNA methylation in the offspring. In adult viable yellow agouti (A^{vy}) mice, supplementation of the maternal diet with dietary methyl donors and cofactors (folic acid, vitamin B_{12}, choline and betaine) shifted the coat colour of the offspring from yellow (agouti) to brown (pseudo-agouti), and this was associated with the increased methylation of the agouti gene[51]. Maternal diet can also alter the methylation of key metabolic genes within the offspring. For example, feeding rats a protein-restricted (PR) diet during pregnancy induced hypomethylation of the glucocorticoid receptor and PPAR-α promoters in the livers of juvenile and adult offspring, which was associated with an increase in glucocorticoid receptor and *Pparα* mRNA expression[47,52]. Maternal PR induced the hypomethylation of four specific CpG dinucleotides within the promoter of *Pparα*, two of which predicted the level of the mRNA transcript, in the juvenile offspring[53]. As these CpG sites lie within transcription factor binding sites, changes in the methylation status of these CpGs induced during development may affect later transcriptional induction of *Pparα* by specific stimuli and the capacity of the tissue to face the metabolic demand. In contrast to the effect of the maternal PR diet, maternal dietary restriction during pregnancy induced the hypermethylation of glucocorticoid receptor and *Pparα* promoters as well as a decrease in glucocorticoid receptor and *Pparα* expression[54]. Thus, the effects of maternal nutrition on the epigenome of the offspring may depend upon the nature of the specific maternal nutrient challenge[52,53].

Given the increasing consumption of energy-rich diets worldwide, much of the research has now focused on the effects of maternal high-fat feeding on DNA methylation in the offspring. For example, Vucetic *et al.* showed increased expression of the μ-opioid receptor and pre-proenkephalin in the nucleus accumbens, prefrontal cortex and hypothalamus of mice from dams that consumed a high-fat diet during pregnancy, which was accompanied by the hypomethylation of the promoter regions of these genes[55]. Hoile *et al.* showed that maternal high-fat feeding during pregnancy led to the reduced expression of fatty acid desaturase 2, the rate-limiting enzyme in PUFA synthesis, and the altered methylation of key CpG nucleotides within its promoter in the offspring[21,56]. Furthermore, Plagemann *et al.* showed that neonatal overfeeding induced by raising rat pups in small litters induces the hypermethylation of two CpG dinucleotides within the pro-opiomelanocortin promoter which are essential for pro-opiomelanocortin induction by leptin and insulin[57].

Although most studies have concentrated on identifying changes in DNA methylation associated with perturbations in maternal diet, maternal diet has also been shown to induce changes in both miRNA and histone modifications in the offspring. Marked changes in miRNA expression have been documented in the liver and skeletal muscle of the offspring in response to maternal undernutrition during either the periconceptual or preimplantation period[58,59], while a maternal high-fat diet during pregnancy and lactation has been shown to

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alter hepatic expression of miRNA in the adult offspring that include *lethal-7a* (*let-7a*), *let-7b* and *let-7c*[60]. Substantial changes in histone modifications at the hepatic NF 4a promoter has also been reported in offspring from dams fed a PR diet during pregnancy. Here, a decrease in hepatocyte NF 4α expression was accompanied by a reduction in histone 3 lysine 4 methylation and an increase in histone 3 lysine 9 and histone 3 lysine 27 methylation[61] with minimal changes in DNA methylation.

Changes in histone modifications induced by variations in early-life environment may precede a change in DNA methylation. Park *et al.* using a model of intrauterine ligation, which results in intrauterine growth restriction and long-term metabolic changes in the offspring, found a decrease in the expression of the transcription factor, pancreatic and duodenal homeobox 1, which plays a critical role in the differentiation and function of B cells. This was accompanied by an initial decrease in histone acetylation at the promoter of pancreatic and duodenal homeobox 1 within the intrauterine growth restriction fetus. This was followed after birth with a significant increase in the repressive histone mark histone 3 lysine 9 methylation in intrauterine growth restriction islet cells. At this stage, these epigenetic changes were reversible; however, with further accumulation of histone 3 lysine 9 methylation, and methylation of the pancreatic and duodenal homeobox 1 gene, the repression of pancreatic and duodenal homeobox 1 expression becomes irreversible[62].

The effect of maternal diet on the epigenome: evidence from human studies

There is increasing evidence that early-life nutrition can induce epigenetic alterations in human subjects. DNA methylation differences have been reported in individuals who were periconceptually exposed to famine during the Dutch Hunger Winter[63,64]. Here, a decrease in methylation of the imprinted insulin-like growth factor 2 gene and increases in the methylation of IL-10, leptin and ATP-binding cassette A1 genes in genomic DNA isolated from whole blood cells from individuals who were exposed to famine in early gestation *in utero* compared with unexposed same-sex siblings was observed[64]. Such changes in DNA methylation were only observed after exposure to famine in early gestation[63] implying that the methylome is most susceptible to alterations in maternal diet in the very early stages of development. Moreover, these measurements were made 60–70 years after famine exposure *in utero*, suggesting as in the experimental studies that variations in maternal diet can induce persistent epigenetic changes in the offspring. Waterland *et al.* have also shown altered patterns of DNA methylation in individuals conceived during the protein-limited rainy season compared with those conceived in the dry harvest season in rural Gambia[65]. DNA methylation was associated with periconceptional maternal plasma concentrations of key micronutrients involved in C_1 metabolism[66], which supplies the methyl groups for all methylation reactions, suggesting that the changes in DNA methylation found in this population may be linked not to the negative energy balance observed in mothers during the rainy season, but rather to the limited dietary levels of methyl donors and cofactors required for C_1 metabolism.

Consistent with the importance of C_1 metabolites, a number of studies have reported associations between the maternal intake and/or status of C_1 donors, cofactors and DNA methylation in the offspring. However, much of these data are highly variable in terms of the effect size, direction of effect and genes affected. For instance, periconceptional folic acid has been both positively[67] and negatively[68] associated with methylation at the insulin-like growth factor 2 locus in the offspring. However, there are also studies which have reported no effect of periconceptional folic acid exposure[69] on DNA methylation. Furthermore, there are examples of maternal macronutrient intake influencing DNA methylation in the offspring. A prenatal diet high in fat and sugar was positively associated with offspring insulin-like growth factor 2 methylation[70]; maternal carbohydrate intake during the second trimester negatively associated with retinoid X receptor α methylation at birth[71] and increased protein intake in pregnancy positively associated with *GR* methylation in the adult offspring[72]. The difficulty however with such studies is that it is not possible to know which nutrient deficits or imbalances caused the epigenetic effects.

Mechanisms by which maternal diet may alter the epigenome

The ability of nutritional factors, particularly those involved in C_1 metabolism to influence the epigenome is perhaps not unexpected, as methyl groups for all biological methylation reactions including DNA and histone methylation are primarily supplied from dietary methyl donors and cofactors via C_1 metabolism[73]. In this pathway, methionine is converted to S-adenosylmethionine, the universal methyl donor. After transferring the methyl group, *S*-adenosylmethionine is converted to S-adenosylhomocysteine, which is then converted to homocysteine. Homocysteine is either recycled to methionine by the enzyme betaine homocysteine methyltransferase which uses betaine or choline, or via a folate-dependent remethylation pathway where 5-methyltetrahydrofolate is reduced to 5,10-methylene tetrahydrofolate by 5,10-methylenetetrahydrofolate reductase. This methyl group is then used by methionine synthase to convert homocysteine to methionine using vitamin B_{12} as a cofactor.

Modulation of the epigenome may not, however, be limited to C_1 donors and cofactors, as many transcription factors which recruit the writers of the epigenetic code are regulated by nutritional factors, and indeed many of the readers and writers of the epigenetic code themselves are regulated at least in part by the concentration of specific metabolic substrates or cofactors. For instance, the PPAR family of nuclear receptors which play a key role in lipid metabolism are activated by

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PUFA[74], while the lysine-specific histone demethylase 1A, which demethylates histone 3 lysine 4 uses the reduction of the cofactor FAD to $FADH_2$. Thus, variations in dietary intake in terms of either individual components or total energy are likely to have an effect on the epigenome potentially inducing a persistent change in gene expression.

The effect of paternal diet on the offspring

To date the majority of studies have focused on the effect of maternal nutrition on the health of the child; however, it is becoming clear that paternal diet can also induce long-term effects on the health of the offspring and that these are associated with the altered epigenetic regulation of genes. Studies many years ago in Sweden showed that food availability during the pre-pubertal period of grandfathers was associated with the risk of diabetes and CVD in the grandsons, although not in the granddaughters[75,76]. Subsequent experiments in rodents have found that variations in paternal diet induced phenotypic changes in the offspring. For instance, offspring from males exposed to dietary restriction had reduced birth weight and impaired glucose tolerance[77], while feeding male rats a PR diet prior to mating led to elevated hepatic expression of genes involved in lipid and cholesterol biosynthesis and a decrease in cholesterol esters, relative to the offspring of males fed a control diet. Further studies have also reported that chronic high-fat feeding in Sprague–Dawley fathers increased body weight, adiposity and impaired glucose tolerance and insulin sensitivity in the offspring. Here, a paternal high-fat diet altered the expression of 642 pancreatic islet genes in adult female offspring; these genes were enriched for cation and ATP binding, cytoskeleton and intracellular transport. Hypomethylation of the *Il13ra2* gene, which showed the highest fold difference in expression was also demonstrated[78].

Mechanism by which paternal environment is transmitted to the offspring

To understand how alterations in paternal diet can induce epigenetic and phenotypic changes in the offspring, many groups have examined the effect of paternal diet on DNA methylation, histone modifications and/or the ncRNA content of the sperm. Carone and co-workers showed cytosine methylation patterns were highly correlative in sperm from control, low protein or energy-restricted fathers, suggesting the sperm epigenome may be more refractory to differences in diet. However, Radford *et al.* reported that sperm from mice undernourished *in utero* showed a reduced level of DNA methylation[79]. The different effects on the sperm methylome between these two studies may reflect the different diets given, exposure time to such diets and the methods used to assess DNA methylation changes. Paternal high-fat feeding has also been associated with changes in the sperm methylome. Donkin and Barres reported differential methylation of eighteen regions in the sperm of males fed a high-fat diet and their offspring, including CpGs within the solute carrier family 3 amino acid transporter heavy chain member 2, transforming growth factor β regulator 4 and major facilitator superfamily domain[80].

Variation in the paternal diet has also been linked to changes in the sperm transcriptome. The expression of twenty-three miRNA was detected in the testis of high-fat fed fathers compared with control fed males. In particular, miRNA *let-7c* has been suggested as an important mediator of paternal transmission of altered offspring phenotypes. *Let-7* miRNA, which is known to control lipid and glucose metabolism, was found to be differentially expressed in spermatozoa of high-fat diet fed rats as well as in the spermatozoa of their offspring[81]. *Let-7* miRNA were also reported to be down-regulated in spermatozoa after exposure of males to a low-protein diet[82].

Further studies have identified transfer RNA (tRNA) fragments as potential contributors of the altered offspring phenotype after paternal high-fat feeding. tRNA fragments, which range in size from ten to forty-five nucleotides, are derived from the 5′ end of either mature tRNA or pre-tRNA[82]. Chen *et al.* showed that feeding a high-fat diet to males over a 6-month period substantially altered the composition of tRNA within the sperm. Moreover, injection of tRNA fragments isolated from sperm from males fed a high-fat diet into control zygotes resulted in altered expression of metabolic genes in the early embryo and metabolic disorders in the offspring, akin to those seen after natural mating of the high-fat fed fathers to control dams[83]. These studies suggest that variations in paternal diet may alter the sperm transcriptome. Such changes may subsequently lead to the altered expression of genes and metabolism in the embryo, which may be further consolidated through modulation of histone marks and DNA methylation.

Conclusions

There is now substantial evidence that early-life nutrition is a key determinant of future disease risk, and that the underlying mechanism by which alterations in early-life nutrition can induce phenotypic changes in the offspring involves the altered epigenetic regulation of genes. Critically, although early studies demonstrated the importance of maternal diet in modulating the long-term health of the child, there is now increasing evidence which shows that paternal diet can also influence the risk of metabolic disease in the offspring and that epigenetic processes are again central to the transmission of the paternal environment to the offspring. This has important implications for public health policy and the engagement of young adults, both male and female, in discussions about healthy diet and its long-term implications for the next generation. The central role of epigenetic processes as a mechanism by which both paternal and maternal diet can influence the health of the offspring provides an opportunity for intervention aimed at reversing the adverse effects of early-life environment

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as epigenetic processes although stable have been shown to be reversible.

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Conflicts of Interest

None.

Authorship

The authors had joint responsibility for all aspects of preparation of this paper.

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