Review Article

Nutrition and Epigenetics in Human Health

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Key Words

Epigenetics · DNA methylation · Developmental origins of health and disease · Early life environment

Abstract

Adult-onset diseases such as type 2 diabetes and cardiovascular disease are now highly prevalent in both developed and developing countries. Evidence from both human and animal studies shows that the prenatal and early postnatal environments can influence susceptibility to chronic diseases in later life. The mechanisms by which the early life environment influences future disease risk have been suggested to include the altered epigenetic regulation of gene expression. In this article, we will review how the early life environment alters the epigenome leading to an altered susceptibility to disease in later life and how our understanding of the underlying mechanisms may allow the development of new intervention strategies to reduce the burden of disease in later life.

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Introduction

Non-communicable diseases (NCD) such as diabetes, cardiovascular disease (CVD) and the metabolic syndrome, account for over a third of all deaths globally (World Health Organisation). In low- and middle-income countries, NCDs are becoming particularly important, since a rapid increase in their prevalence has been observed as these countries undergo

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Fig. 1. Weight at birth is presented has a U-shaped association with later disease risk. Nutritional exposure in the intrauterine environment has an impact upon the life course of the developing foetus. The relationship between birth weight and future risk from diseases, including CVD, diabetes and stroke, is U-shaped, with risk increasing as birth weight moves further away from a theoretical optimum. There is no specific threshold of disease, but an increasing risk at more extreme birth weights.

socio-economic improvement [1]. Fixed genomic variations such as single nucleotide polymorphisms and copy number variations explain only a fraction of the variation in NCD risk in a population [2]. However, there is growing evidence that the prenatal and early postnatal environments play important roles in influencing the risk of developing a wide range of NCDs in later life. A relationship between the quality of the early life environment and later disease risk was first shown in a Norwegian study that found a strong association of undernutrition and poverty during childhood and adolescence with later development of CVD in late middle age [3]. Subsequent work by Barker and colleagues [4–6] and Hales et al. [7] related the health of middle-aged individuals in the UK 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. Further epidemiological studies have confirmed these associations between lower birth weight and later disease risk but have also shown that babies born at the highest birth weights are also at increased risk of developing diabetes or obesity in later life (fig. 1) [8, 9].

While these epidemiological studies were the first to show a relationship between fetal growth and chronic disease risk, studies of the Dutch Hunger Winter, a famine which occurred in the Netherlands during the winter of 1944, have clearly demonstrated that maternal nutrition influences the health of the child in later life and that the timing of the environmental constraint is important. Studies from the Dutch Hunger Winter found that individuals whose mothers were exposed to famine periconceptually and in 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 [10, 11].

Comparable findings have now been replicated in a variety of animal models where, typically, rats or mice have been fed either a low-protein diet, a global dietary restriction, a high-fat or even a junk food diet during pregnancy and/or lactation [12–14]. Interestingly, the offspring exhibit similar features to human cardiometabolic disease including hypertension, dyslipidaemia, obesity and insulin resistance in later life.

The induction of different phenotypes by perturbations in early life nutrition has been suggested by Gluckman and Hanson [15] 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 to aid fitness or survival in later life. When an organism adapts to an environment and is subsequently exposed to a different envi-

Fig. 2. DNA methylation and its impact upon gene expression. **a** The most common form of mammalian DNA methylation is the addition of a methyl group (CH_3) to cytosine to form 5-methylcytosine. **b** 5-Methylcytosine occurs within the context of a cytosine immediately 5' to guanine, to form a CpG dinucleotide, with the p denoting the phosphate bond between the DNA bases. In double-stranded DNA, two reciprocally methylated CpG dinucleotides will occur opposite from each other due to base pair complementarity. **c** Transcription factors bind within the promoter region of a gene and help regulate gene expression, but DNA methylation (**d**) can block TF binding and thereby alter the transcriptional potential.

ronment after birth, a 'mismatch' occurs leaving the organism maladapted and at risk of metabolic disease in later life [16]. The mismatch between the prenatal and postnatal environments has been suggested to be central to the burgeoning rates of NCDs observed in countries undergoing socio-economic transition (e.g. populations moving from rural to urban areas) [17, 18].

Epigenetic Regulation

The mechanisms by which the early life environment may influence future disease risk have been suggested to include the altered epigenetic regulation of genes [19]. Epigenetic mechanisms have the potential to provide the required levels of both variability and rapid adaptability to allow these developmental changes to be induced and maintained throughout the life course. Epigenetic modifications, which are stably inherited through cell division without alteration of the DNA sequence, allow a large degree of control over a gene's tran-

scriptional state. Epigenetic processes include: DNA methylation, histone modifications, and non-coding RNAs. Together, they affect all aspects of gene expression and repression, controlling access to the underlying DNA sequence, and thereby defining the role of each cell within the body.

DNA Methylation

DNA methylation is the transfer of a methyl group to the 5' carbon position of cytosine, creating 5-methylcytosine [20]. In mammals, methylation of cytosine mainly occurs within the dinucleotide sequence CpG, when a cytosine is immediately 5' to a guanine. Hypermethylation is associated with gene silencing and hypomethylation with gene activation (fig. 2) [21, 22]. DNA methylation can act directly to block binding of transcription factors (TF) [23], though it is thought that its main mode of action is through promoting the recruitment of a myriad of other repressive factors that mediate local chromatin changes [24]. Methylation of DNA appears to be directly antagonistic to certain histone modifications that promote an open and accessible form of chromatin [25], and has also been shown to alter nucleosome occupancy thereby blocking TF and Pol II binding [26]. DNA methylation levels are high in both the sperm and the egg at fertilization, but global methylation levels decrease during the first few days of development, until they reach their lowest levels around blastocyst implantation, whereupon a wave of de novo methylation occurs within the inner cell mass, giving rise to lineage specific methylation patterns that are maintained in differentiated tissues [27, 28].

Histone Modifications

Two of each of the four histone proteins H2A, H2B, H3 and H4, combine together with DNA to form a nucleosome, the most basic structure of chromatin, which is then packaged into higher order chromatin structures [29, 30]. The unstructured tails of histone proteins provide a platform for modifying enzymes that catalyse the addition of different histone modifications to specific residues, which can directly affect the chromatin structure, but also provide binding sites for proteins involved in gene regulation. Together, histone modifications and DNA methylation control the chromatin structure, and therefore, the biological role played by the underlying DNA sequence [30, 31].

Non-Coding RNA

Less than half of the transcribed RNA within a cell acts as a template for production of proteins. Non-coding RNAs (ncRNAs), both long and short, are central components of the transcriptional regulation machinery, and are essential for both translational and transcriptional regulation within the cell [32–35]. Small ncRNAs 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 [36–38]. Large ncRNAs can coat regions of a chromosome, creating repressive domains encompassing many kilobases, and are essential in processes such as X-inactivation and imprinting [39–42].

Epigenetic Changes Induced by the Early Life Environment

There is an increasing body of evidence suggesting that the early life environment can alter the epigenome. One of the first examples to highlight the influence of maternal diet on DNA methylation levels in the offspring was shown by the A^{vy} mouse where coat colour is determined by the methylation status of the 5' end of the agouti gene [43–46]. Supplementation of the maternal diet with dietary methyl donors and co-factors (including folic acid, vitamin B₁₂, betaine and choline) led to an increase in the methylation of the agouti gene and a shift in coat colour of the offspring from yellow to black.

Studies in other animal models have also shown that both macro- and micronutrient intake during pregnancy can alter the methylation of key metabolic genes within the offspring. For instance, feeding a protein-restricted diet during pregnancy induced the hypomethylation of GR and PPARα genes. This decrease in DNA methylation was accompanied by an increase in GR and PPARα gene expression and persistent changes in the metabolic processes that these nuclear receptors control [47, 48]. Maternal protein restriction has also been shown to induce the hypomethylation of the angiotensin receptor promoter (AGTR1B); this was associated with a three-fold increase in gene expression. AGTR1B directly impacts the levels of adrenal aldosterone, a major factor in the development of hypertension [49].

Given the concerns in both western and modernizing societies over the increasing consumption of energy-rich diets, and their implications, a number of studies have also explored the effects of maternal high-fat feeding on DNA methylation in the offspring. Hoile et al. [12] showed that maternal high-fat feeding during pregnancy led to the reduced expression of FADS2, the rate-limiting enzyme in polyunsaturated fatty acid synthesis, and the altered methylation of key CpG nucleotides within its promoter in the offspring [50]. In contrast, high-fat feeding for a 9-week period during adulthood induced only a transient effect on FADS2 expression and methylation. This suggests that changes induced in early life persist once the cause is removed, but in adulthood, these systems are far harder to influence outside of the developmental windows of plasticity [12].

However, it has also become apparent that the period of epigenetic plasticity may extend beyond the early intrauterine period into postnatal life. Over-feeding in neonatal life was shown to induce the hypermethylation of the proopiomelanocortin (POMC) promoter, a gene that plays a key role in appetite control. Hypermethylation of the POMC promoter prevented upregulation of POMC expression despite high plasma levels of both leptin and insulin [51]. Lillycrop et al. [48] have also shown that, in rats whose mothers were fed protein-sufficient or restricted diets during pregnancy, increasing folic acid intake in the juvenile-pubertal period led to increased methylation of the promoter region of PPARα, with decreased PPARα expression and levels of β-oxidation [48]. Moreover, Ly et al. [52] have shown that folic acid supplementation during the peripubertal period led to a decrease in DNA methyl transferase activity and an increase in mammary tumorigenesis.

Effect of the Early Environment on the Human Epigenome

In humans, studies on the Dutch Hunger Winter cohort showed that periconceptual exposure to famine was associated with a small decrease in CpG methylation across the imprint control region of IGF2, while those individuals exposed to famine in late gestation showed no altered methylation at the same region [53]. This was a pattern repeated for a number of other genes examined, with exposure during the periconceptual period being associated with small DNA methylation changes within multiple loci (including leptin, IL-10, MEG3 and ABCA3) while those exposed in later pregnancy did not show these changes [54].

The association between early life exposures and epigenetic changes in key metabolic regulatory genes suggests that such changes may well underpin the long-term changes in gene expression and metabolism seen in the offspring. However, because of the technical challenges associated with changing the methylation status of a single CpG site in vivo there is yet no formal proof that these methylation changes are causal. In these studies on individuals from the Dutch Hunger Winter, methylation was measured in peripheral blood suggesting that epigenetic traits in peripheral tissues may provide useful proxy markers of future disease risk in more disease-relevant cell types [54, 55].

Recently, Godfrey et al. [56] reported in two independent cohorts that the methylation status of a single CpG site in the promoter region of the retinoid X receptor α was related positively to childhood adiposity in both boys and girls such that retinoid X receptor α promoter methylation explained over a fifth of the variance in childhood fat mass [56]. These findings not only support that hypothesis that developmentally induced epigenetic marks make a significant contribution to later phenotype but also suggest that the detection of epigenetic marks even in peripheral tissue may allow identification of individuals at increased risk of chronic disease in later life before the onset of clinical disease, and so facilitate targeted intervention strategies.

Conclusions

Epigenetic marks, laid down during the prenatal period in response to maternal nutrition, are associated with altered gene expression patterns in important metabolic tissues. The resultant alterations to both growth and metabolism are linked to later development of diabetes, hyperlipidaemia, hypertension, and CVD.

The presence of epigenetic marks, linked to later disease risk, raises the possibility of preventative medicine. Globally, health care providers are experiencing an upsurge in the diagnosis of NCDs, but by the time of diagnosis, treatment is expensive, and can only manage the condition. Epigenetic biomarkers that have the potential to be used as predictors of an individual's disease risk will allow for a far earlier detection for those most at risk, potentially allowing a more effective strategy of preventative treatment, improving the individual's quality of life and reducing the financial burden that is associated with current treatment strategies.

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