Journal of Developmental Origins of Health and Disease (2015), **6**(2), 88–95.

doi:10.1017/S2040174415000124
Themed Issue: Role of Epigenetics in Mediating Programming Effects.

REVIEW

Maternal diet as a modifier of offspring epigenetics

K. A. Lillycrop[1]* and G. C. Burdge[2]

[1]*Centre for Biological Sciences, Faculty of Natural and Environmental Sciences, University of Southampton, Southampton, UK*
[2]*Human Development and Health, Faculty of Medicine, University of Southampton, Southamptom, UK*

There has been a substantial body of evidence, which has shown that genetic variation is an important determinant of disease risk. However, there is now increasing evidence that alterations in epigenetic processes also play a role in determining susceptibility to disease. Epigenetic processes, which include DNA methylation, histone modifications and non-coding RNAs play a central role in regulating gene expression, determining when and where a gene is expressed as well as the level of gene expression. The epigenome is highly sensitive to a variety of environmental factors, especially in early life. One factor that has been shown consistently to alter the epigenome is maternal diet. This review will focus on how maternal diet can modify the epigenome of the offspring, producing different phenotypes and altered disease susceptibilities.

Received 31 July 2014; Revised 5 January 2015; Accepted 26 January 2015

Key words: ageing, developmental origins of adult disease, DNA methylation, epigenetics, nutrition

Introduction

Non-communicable disease (NCD) such as diabetes, cardiovascular disease (CVD) and obesity account for 60% of all deaths globally, with the incidence of NCDs projected to rise further by 2030.[1] This increase in NCDs is not restricted to industrialized nations but is also increasingly seen in developing nations.[2] Although it is widely established that genotype plays a critical role in determining NCD risk, the failure of genome-wide association studies to find genetic variants that explain a substantial proportion of NCD risk[3] together with the rapid rise in incidence of NCDs over the past two decades have suggested that environmental factors such as diet and level of physical activity are also likely to play a major role in the development of NCDs. Early life environment in particular has been shown to play a critical role in the development of many human diseases, and the mechanism by which early life environment may influence disease risk has been suggested to involve the altered epigenetic regulation of genes. This review will focus on the evidence that early life nutrition, particularly maternal diet, can modify the epigenome of the offspring leading to persistent phenotypic changes and an altered risk for NCDs in later life.

Early life environment and future disease risk

The association between the quality of the early life environment and subsequent risk for chronic disease in later life has been well described. Such studies have shown that low birth weight even within the normal range is associated with an increased risk for CVD and the metabolic syndrome (hypertension, insulin resistance, type 2 diabetes, dyslipidaemia and obesity) in later life.[4] Although in many of these studies, a J- or U-shaped relationship between birth weight and disease risk was seen, with babies born with the highest birth weight also being at increased risk for disease.[5,6] However, birth weight in these studies is thought to be only a very crude indicator of the intrauterine environment, which may have been compromised through a variety of maternal, environmental or placental factors.[7]

One maternal factor that has consistently been shown to influence later phenotype is maternal nutrition. This has been most clearly shown in studies of the Dutch Hunger Winter, a famine, which occurred in the Netherlands during the winter of 1944. These studies have shown that individuals whose mothers were exposed to famine periconceptually and in the first trimester of pregnancy exhibit an increased risk of obesity and CVD in adulthood, whereas individuals whose mothers were exposed in the later stages of gestation showed increased incidence of insulin resistance and hypertension in later life.[8]

These findings from human epidemiological studies, which show an association between the quality of early life environment and future disease risk, have been replicated in a variety of animal models where nutrition in particular both before and after pregnancy can be precisely controlled. Early animal studies focussed on the effects of maternal undernutrition or protein restriction attempting to replicate the effects of famine on long-term offspring health, although more recently given the growing epidemic of obesity in both industrialized and developing countries, animal models have been established to investigate the effect of energy-rich diets during pregnancy on the phenotype of the offspring. Interestingly, offspring born to dams fed these different diets exhibited persistent metabolic

*Address for correspondence: Dr K. A. Lillycrop, Institute of Developmental Sciences Building (MP887), Southampton General Hospital, Tremona Road, Southampton, SO16 6YD, UK.
(Email k.a.lillycrop@soton.ac.uk)

changes often leading to features similar to human cardiometabolic disease such as obesity, insulin resistance, hypertension and raised serum cholesterol levels.[9,10] For instance, feeding rats a protein-restricted diet (PR) during pregnancy has been reported to result in impaired glucose homoeostasis,[11] vascular dysfunction,[12] impaired immunity,[13] increased susceptibility to oxidative stress,[14] increased fat deposition and altered feeding behaviour,[15,16] whereas offspring from dams fed an obesogenic diet (16% fat, 33% sugar) also produced offspring with impaired glucose homoeostasis, increased resistance artery endothelial dysfunction, hypertension, increased adiposity and reduced locomoter activity.[10] Interestingly, the metabolic alterations induced in the offspring by perturbations in the maternal diet are dependent on the timing of the nutritional challenge, which may reflect the critical phase of development of different organ systems, with respect to the timing of the nutritional constraint. Bertram *et al.*[17] have shown in the guinea pig model that female offspring born to dams fed a PR diet in the first half of pregnancy (1–35 days) have raised mean arterial blood pressure, which was associated with an increased intraventricular septum and anterior left ventricle wall (LVW) thickness. In contrast, the offspring from dams fed a PR diet in late gestation (36–70 days) were growth restricted but did not display alterations in blood pressure or LV structure. Animal studies have also shown an interaction between prenatal and postnatal environments.[18,19] For example, dyslipidaemia and impaired glucose homoeostasis induced by feeding dams a PR diet during pregnancy were exacerbated in adult male and female rats fed a diet containing 10% (w/w) fat after weaning compared with a 4% (w/w) fat post-weaning diet.[19]

Interestingly, a number of recent studies have shown that paternal diet can also influence future disease risk in the offspring. Carone *et al.*[20] showed that offspring of males fed a low-protein diet from weaning until sexual maturity exhibited an increase in the expression of many genes involved in lipid and cholesterol biosynthesis in the liver as well as differences in the levels of several lipid metabolites, whereas Ng *et al.*[21] have shown that a paternal high-fat diet (HFD) exposure induces increased body weight, adiposity, impaired glucose tolerance and insulin sensitivity in female offspring. The mechanism by which alterations in early life nutrition may induce such long-term changes in metabolism and phenotype has been suggested to involve the altered epigenetic regulation of genes.

Introduction to epigenetics

Conrad Waddington (1905–1975) first introduced the term epigenetics in the 1940s to describe the process by which the same genotype can give rise to multiple phenotypes during development.[22] However, in recent years, the definition of epigenetics has subsequently been narrowed to 'the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence'.[23] Epigenetic processes include DNA methylation, histone modifications and non-coding RNAs (ncRNAs). Together these processes determine when and where a gene is switched on and the level of gene activity. Epigenetic processes not only play a key role in regulating gene expression but are also central to the mechanism, which allow an organism to adapt to the environment.

DNA methylation

DNA methylation is a common modification in eukaryotic organisms. DNA methylation occurs primarily on the fifth position of cytosine (5mC) within a CpG (cytosine and guanine nucleotides linked by phosphate) dinucleotide, although non-CpG methylation is prevalent in embryonic stem cells.[24] Low levels of methylation at the promoter regions are associated generally with transcriptional activity, whereas high levels of methylation are associated with transcriptional silencing.[23] DNA methylation induces transcriptional silencing by preventing the binding of transcription factors to the DNA or by recruiting the methyl CpG binding protein 2 (MeCP2) to the DNA, which in turn recruits histone-modifying complexes.[25]

Methylation of CpGs is largely established during embryogenesis or during early postnatal life. Following fertilization, DNA methylation markers on the maternal and paternal genomes are largely erased with the exception of the imprinted genes. This is followed by global *de novo* methylation just before blastocyst implantation,[26] during which 70% of CpGs are methylated, mainly in the repressive heterochromatin regions and in repetitive sequences such as retrotransposable elements. Lineage-specific methylation of tissue-specific genes also occurs throughout prenatal development and early postnatal life, which is essential for cell specification. The *de novo* methylation of DNA is catalysed by DNA methyltransferases (DNMT) 3a and 3b,[27] and is maintained through mitosis by methylation of the hemi-methylated DNA by DNMT1.[28] Once DNA methylation patterns are established during development, these markers were thought to be relatively stably and generally maintained throughout life. This premise was supported by the initial failure to identify a DNA demethylase or a mechanism for DNA demethylation.[29] However, this concept has now been challenged as active demethylation and has been demonstrated in a number of studies. For example, active demethylation in mammals has been observed on the paternal genomic DNA in the zygote upon fertilization,[30] on the synaptic plasticity gene reelin in the hippocampus upon contextual fear conditioning[31] and on the interferon gamma (IFNγ) gene upon antigen exposure of memory CD8 T cells.[32] Moreover, a number of potential DNA demethylases have been identified such as ten–eleven translocation (Tet) proteins,[33,34] methyl binding domain protein (MBD)2b,[35] MBD4,[36] the DNA repair endonucleases XPG (Gadd45a)[37] and a G/T mismatch repair DNA glycosylase,[38] although these function by not removing the methyl group directly from cytosine, but through a multi-step processes linked either to DNA repair mechanisms or through further modification of 5mC.

https://doi.org/10.1017/S2040174415000124 Published online by Cambridge University Press

Histone modifications

DNA methylation works in concert with histone modifications to regulate gene expression. In eukaryotic cells, DNA is wrapped around a core of eight histone proteins, two molecules of histone H2A, H2B, H3 and H4. This forms the basic unit of chromatin, called a nucleosome. Each 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 is necessary to reduce the effective size of DNA; however, it is has now become very clear that the histones also play a critical role in regulating gene expression. The amino terminal tail domain of the histones are subjected to a large number of modifications including acetylation, methylation, ubiquitination, sumoylation and phosphorylation.[39] The establishment of these markers on the histone tails is often referred to as the histone code and leads to the binding of effector proteins that, in turn, bring about specific cellular processes. Histone acetylation, induced through histone acetyl transferases, is associated with transcriptional activity and an open chromatin state. Methylation of lysine (K) residues within the histone tail can either be an active or repressive mark depending on the specific lysine involved. For example, histone H3K4 methylation is associated with gene activation,[40] whereas histone H3K9 methylation is linked to gene silencing.[41]

Crosstalk between DNA methylation and histone modification is well established. Methylated CpGs are bound by MeCP2, which then recruits both histone deacetylases (HDACs) – which remove acetyl groups from the histones – and histone methyl transferases, this results in a closed chromatin structure and transcriptional silencing.[25] Recent studies have also shown that a number of histone modifying enzymes such as HDAC1 and HDAC2,[42–44] as well as components of the polycomb repressive histone complex 2,[45] can recruit DNMT1 to the DNA and induce DNA methylation.

ncRNA

Data from the ENCODE project have shown that, although over 74% of the eukaryotic genome is transcribed, only 1–2% of the genome encodes for proteins,[46] suggesting that a large proportion of the transcripts in the cell do not encode for proteins. These RNAs have been termed ncRNAs and are grouped into two classes. Long ncRNAs are longer than 200 nucleotides, whereas short ncRNAs are <200 nucleotides. Short ncRNAs include microRNAs (miRNAs), small interfering RNAs and piwi-interacting RNAs.[47] Small ncRNAs can induce mRNA degradation and/or translational repression,[47] and when targeted to the promoter region of a gene they can induce both DNA methylation and repressive histone modifications.[48] The mechanism by which long ncRNAss work is less well understood but may involve the recruitment of histone modifying complexes to the DNA to create repressive domains covering many kilobases. In mammals, long ncRNAs have been shown to be essential for X-chromosome inactivation and genomic imprinting.[49]

Early life nutrition and the epigenome

Although DNA methylation was originally thought to be a very stable modification and once established methylation patterns were largely maintained throughout the life-course, there is now growing evidence that a number of environmental factors such as nutrition, stress, placental insufficiency, endocrine disruptors and pollution, especially in early life, can alter the epigenome leading to long-term phenotypic changes in the offspring.[51] In this study, we will focus on the effect of early life nutrition on the epigenome and its long-term effects.

One of the best examples of how nutrition can alter phenotype through the altered epigenetic regulation of genes is seen in studies on the honeybee. Female larvae incubated in the presence of Royal Jelly predominantly develop in to Queen bees, whereas those incubated in the absence of Royal Jelly develop into sterile worker bees, although they are genetically identical.[52] However, knockdown of DNMT3, the major DNMT in bees, increased the proportion of larvae developing into queen bees as opposed to sterile workers,[53] suggesting that nutrition can profoundly affect developmental fate and it does so through the altered methylation of DNA.

Nutrition has also been shown to influence DNA methylation in rodents. In agouti viable yellow (A^{vy}) mice, coat colour is determined by the methylation status of an intracisternal-A particle (IAP) in the 5′ upstream region of the agouti gene that acts as a cryptic promoter directing expression of the agouti gene, which encodes for a paracrine signalling protein that induces follicular melanocytes to switch from producing black eumelanin to yellow phaeomelanin. Supplementation of the diet of the pregnant A^{vy} mice with folic acid, cobalamin, choline and betaine induced a graded shift in coat colour of the litter from predominately yellow (agouti) to brown (pseudoagouti).[54] This shift was accompanied by the hypermethylation of seven CpG dinucleotides, 600 bp downstream of the A^{vy} IAP insertion site.

There is also evidence in models of nutritional programming where perturbations in maternal diet are associated with persistent metabolic changes in the offspring that these metabolic changes are accompanied by epigenetic changes in key metabolic genes or genes involved in appetite control. For example, feeding pregnant rats a PR diet induced hypomethylation of the glucocorticoid receptor (GR) and peroxisome proliferator activated receptor (PPAR)α promoters in the livers of juvenile and adult offspring; this was accompanied by an increase in GR and PPARα expression and in the metabolic processes that they control.[55–57] In contrast, global dietary restriction during pregnancy increased the level of DNA methylation of PPARα and the GR in the liver of the offspring,[58] suggesting that the effects of maternal nutrition on the epigenome of the offspring depend upon the nature of the maternal nutrient challenge. Such nutrition-related responses

are consistent with the concept that induced epigenetic changes that underpin physiological change may provide a means of adapting to an adverse environment.[59]

Feeding a HFD during pregnancy and lactation has also been shown to induce epigenetic and phenotypic changes in the offspring. Vucetic *et al.*[60] showed increased expression of the μ-opioid receptor and preproenkephalin in the nucleus accumbens, prefrontal cortex and hypothalamus of mice from dams that consumed HF diet during pregnancy, and this was accompanied by the hypomethylation of the promoter regions of these genes. Maternal obesity and diabetes has also been reported to induce subtle but widespread changes in DNA methyaltion in the liver of the offpring.[61]

Although most studies to date have concentrated on identifying changes in DNA methylation associated with perturbations in maternal diet or body compoisiton, there is growing evidence that early life nutrition can also induce persistant changes in both histone modifications and miRNAs. In islet cells, a decrease in hepatic nuclear factor (HNF) 3a expression in the liver of PR offspring was accompanied by a minimal change in DNA methylation but substantial changes in histone modifications at the HNF4a promoter, such as a reduction in H3K4 methylation and an increase in H3K9me2 and H3K27me3.[62] Dietary restriction from E10 to E21, which induced an increase in IGF1A and IGF1B (insulin growth factors) expression, was also accompanied by changes in the histone markers at the IGF1 gene with a decrease in H3K4me2 and an increase in H3K4me3 being observed.[63] Marked changes in miRNA expression have also been documented in both the liver and skeletal muscles of the offspring in response to matenral undernutrition during either the peri-conceptual or the pre-implanatation period. [64,65]

Alterations in paternal diet in rodents have also been associated with epigenetic changes in the offspring. Feeding male rats a PR diet before mating induced widespread changes in DNA methylation (10–20%) in the liver of the offspring compared with controls, including an increase in methylation at an intergenic CpG island 50 kb upstream of the PPARα gene.[20] Interestingly, however, cytosine methylation patterns were highly correlative in the sperm from control, low-protein or calorie-restricted fathers, suggesting that the sperm epigenome may be more refractory to differences in diet. A decrease in H3K27 me3 levels at the monoamine oxidase (MAOA) and elongation factor Tu GTP binding domain containing 1 (EFTUD1) promoters were seen, however, in the sperm from the low-protein-fed fathers compared with controls, suggesting that differences in diet may be transmitted through modifications in the histone code.[20] Paternal high-fat feeding has been reported to induce the hypomethylation of the interleukin 13 receptor alpha 2 (IL13RA2) promoter in the islet cells of female offspring.[21] Altered expressions of 23 miRNAs were also detected in the testis of the high-fat-fed fathers, suggesting that miRNAs may play a role in the transmission of obesity and impaired metabolic health to the offspring.

Epigenome is also susceptible to nutritional factors in later life

Although many studies have shown that the epigenome is highly sensitive to environmental factors during the perinatal and prenatal period, the period of epigenetic plasticity may extend into postnatal life. Plagemann *et al.*[66] showed that neonatal overfeeding induced by raising rat pups in small litters induces the hypermethylation of two CpG dinucleotides within the pro-opiomelanocortin promoter. Folic acid supplementation in the juvenile-pubertal period has also been shown to induce the hypermethylation of the PPARα gene; this was accompanied by a decrease in PPARα expression and levels of fatty acid β-oxidation,[67] whereas Ly *et al.*[68] showed that folic acid supplementation during the peri-pubertal period led to an increased risk for mammary adenocarcinomas and a decrease in DNMT activity.

There is also evidence that even in adulthood there is some plasticity of the epigenome. For instance, feeding a diet deficient in methyl donors to post-weaning mice led to the permanent loss of IGF2 imprinting and dysregulation of its mRNA expression,[69] whereas feeding a diet deficient in choline, folate, methionine and vitamin B12 for 4 weeks to adult rats induced the hypomethylation of the proto-oncogenes c-Myc, c-Fos and c-Ras. Moreover, this effect persisted for 3 weeks after re-feeding.[70]

Macronutrient intake during adulthood can also influence the epigenome. Hoile *et al.*[71] showed that feeding adult rats fish oil-enriched diet for 9 weeks led to a transient decrease in the expression of the fatty acid desaturase 2 (FADS2) gene and increase in FADS2 promoter methylation. These effects were reversed by feeding a standard diet for a further 4 weeks. Such findings suggest that, although the epigenome may be most susceptible to environmental factors during early life when methylation patterns are being established, there is also some plasticity in later life. Although these effects may be more transient, such continued plasticity in later life does offer the opportunity for intervention to reverse or reset developmentally induced epigenetic markers.

Evidence that early nutrition can alter the epigenome in humans

Studies in animal models, where the diet pre- and post-pregnancy, as well as genetic background, can be carefully controlled, have been instrumental in demonstrating long-term effects that alterations in early life nutrition can have on the phenotype and in providing insights into the mechanism underlying this phenomena. Evidence that maternal diet in humans can induce long-term epigenetic and phenotypic changes in the offspring is more limited, although alterations in the methylation of a number of genes in DNA isolated from the whole blood of individuals who were exposed periconceptionally to famine during the Dutch Hunger Winter have been reported.[72,73] Specifically, a decrease in methylation

of the imprinted IGF2 gene and an increase in methylation of interleukin-10, leptin, ATP binding cassette A1 and guanine nucleotide binding protein were observed in those individuals whose mothers were exposed to famine during pregnancy compared with their non-exposed siblings. Interestingly, these methylation changes were predominantly seen in those individuals who had been exposed *in utero* during early gestation rather than late gestation, suggesting that the peri-conceptual period is a period of extreme sensitivity to nutritional status. Furthermore, these measurements were made 60 years after the famine, suggesting that maternal nutritional constraint induces long-term epigenetic alterations in the offspring, consistent with the observations from animal models. Altered methylation of 5 CpG sites in the IGF2 gene was also detected in the peripheral blood cells of children whose mothers took 400 μg of folic acid per day during the peri-conceptional period compared with children whose mothers did not take folic acid supplementation.[74] More recent studies have reported widespread sex-specific changes in the epigenome associated with peri-conceptual micronutrient intake[75] and an association between the DNA methylation status of human metastable epialleles with maternal nutritional intake at conception.[76] Plasticity in the human epigenome may also persist into adulthood, as studies have shown that HFD fed during adulthood induced changes in the methylation of 6508 genes in skeletal muscles, and such changes were interestingly only partially reversed after 6–8 weeks of a normocaloric diet.[77] The changes in DNA methylation in all these human studies are considerably smaller than those found in animal studies, which may reflect the fact that in animal models the perturbation in maternal diet is more defined, the postnatal environment is precisely controlled and the genetic background is identical. Interestingly, in a recent study, Teh *et al.*,[78] where they screened the genotypes and methylomes of 237 neonates, found 1423 variable methylated regions, and in 75% of the cases these were best explained by an interaction between different *in utero* environments and genotype, suggesting that the effect of early life environment is moderated by genetic background.

Epigenetic biomarkers predictive of later disease risk

If a similar mechanism does operate in humans as in animals, with early life environment inducing the altered epigenetic regulation of genes, then it should be possible to detect these altered epigenetic markers and use them as predictors of future metabolic capacity and disease risk. The potential issue with detecting such markers in humans is that there is limited tissue availability; readily accessible tissues include cord, cord blood, placenta, buccal and blood, but as DNA methylation patterns are often tissue specific, it is not clear whether the methylation status in such tissues would reflect methylation patterns in more metabolically relevant cell types. However, a number of studies have shown inter-tissue methylation correlations.[79,80] For instance, Talens *et al.*[81] have shown that, for a number of non-imprinted genes, DNA methylation levels measured in blood were also equivalent in buccal cells, despite the fact that these cell types stem from different germ layers (mesoderm and ectoderm, respectively), whereas Byun *et al.*[82] reported that, using the IlluminaGoldenGate Bead Array, which integrated 1505 CpG sites in 807 genes, the intra-individual correlations for over 11 tissues ranged from 0.738 to 0.941. It may also depend on when the environmental constraint occurred. An environmental challenge during very early development is likely to affect all germ layers and an imprint of this altered epigenetic mark maybe detectable in all tissues, whereas exposures occurring later on in gestation may induce only tissue-specific effects.

Interestingly, Godfrey *et al.*[83] have reported that the methylation of a single CpG site in the promoter region of the nuclear receptor RXRA was strongly related to childhood adiposity in both boys and girls in two independent cohorts; with RXRA promoter methylation explaining over 25% of the variance in age- and sex-adjusted fat mass in children at 6 and 9 years of age. This suggests that a far greater proportion of individual vulnerability to NCD may arise during development than has been considered previously and that developmentally induced epigenetic changes may mediate this effect. Such findings also suggest that the detection of such methylation changes even in peripheral tissues may be useful markers of later disease risk. However, contrary to many of the initial studies, which suggest that once DNA methylation patterns are established in early life they are then stably maintained, there is growing evidence that DNA methylation can be dynamically regulated in response to a number of environmental stimuli. For instance, an acute exercise bout over a 20-min period induced hypomethylation of the peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC1α) and mitochondrial transcription factor A promoters in muscle tissues,[84] whereas Tarantini[85] found an association between concentrations of PM_{10} particles over a 3-day period and levels of inducible nitric oxide synthase methylation. Such sensitivity to the environment could confound the contributions that developmentally induced epigenetic markers have on later phenotype, limiting the usefulness of any epigenetic predictive markers for disease risk. The factors that induce such dynamic changes in DNA methylation are not fully understood nor why the methylation of some CpGs appears to be stable and others very plastic, primarily as data on methylation stability from longitudinal studies, especially in children when the epigenome may well be more sensitive to the environment, are limited. One study that has examined DNA methylation stability over time in children found that methylation of the genes MAOA, dopamine receptor D4 (DRD4) and serotonin transporter (SLC6A4) is highly dynamic between the age of 5 and 10 years. Although the stability of CpG loci may be very loci-dependent, Clarke-Harris *et al.*[87] have reported year on year stability of 7 CpG sites within the PGC1α promoter in peripheral blood cells in children from 5 to 14 years of age, suggesting that at least for these CpG sites methylation is set up in early life and

stably maintained thereafter, despite changes in exercise, pollution or the onset of puberty. Moreover, four of the seven CpGs analysed at 5–7 years predicted adiposity in the children aged 9–14, consistent with the paradigm that developmentally induced epigenetic markers make a significant contribution to later phenotype. Interestingly, the CpGs analysed in this latter study were located upstream of the region shown to be hypomethylated in muscles in response to acute exercise, whether the difference in stability relates to the differential location of these CpG sites within PGC1α promoter, tissue-specific differences between blood and muscle or whether the change in methylation in response to exercise occur on top of a developmentally induced imprint is not clear. A better understanding of the factors influencing variation in DNA methylation and their role in mediating DNA methylation dynamics will be important to elucidate the role of these factors in producing DNA methylation differences that may underlie the development of complex diseases as well as for the development of epigenetic biomarkers predictive of later disease risk.

Conclusion

There is now increasing evidence to suggest that early life nutrition can induce persistent metabolic and physiological changes in the offspring through the altered epigenetic regulation of genes leading to an altered susceptibility to disease in later life. The ability of the environment to influence that epigenome may allow an organism to adapt to its environment by modulating the expression of its genes and the processes that they control. The epigenome appears to be most susceptible to environmental factors in early life when methylation markers are being established; however, it is also clear that the environment can alter DNA methylation even in adulthood, suggesting that these epigenetic processes function as part of a life-long adaptation mechanism. Preliminary studies have also suggested that it may be possible to detect these altered epigenetic markers in early life even in peripheral tissues and use these markers as potential predictive biomarkers to identify those individuals at increased risk for disease. However, there are still many factors and mechanisms that we have to learn to completely understand the exact contributions that developmentally induced epigenetic markers have on later phenotype and disease risk. For instance, how does maternal nutritional status induce epigenetic changes in the offspring, are histones or ncRNAs involved? What are the critical developmental periods? Do these differ depending on the tissue? What makes one CpG stable, another susceptible to the environment, and does this change during the lifecourse, is it tissue dependent? And can interventions be targeted to specific epigenetic markers? Understanding this relationship between epigenetics, the environment and disease susceptibility may then make it possible to make real progress in the prevention and treatment of chronic diseases and halt the rapid rise in NCDs, which are seen throughout the world at present.

Acknowledgements

None.

Financial Support

This research received no specific grant from any funding agency, commercial, or not-for-profit sectors.

Conflicts of Interest

None.

References

1. Hossain P, Kawar B, El Nahas M. Obesity and diabetes in the developing world – a growing challenge. *N Engl J Med*. 2007; 356, 213–215.
2. Ramachandran A, Snehalatha C. Rising burden of obesity in Asia. *J Obes*. 2010; 2010, vol. 2010, Article ID 868573, 8 pages, 2010. doi:10.1155/2010/868573.
3. Manolio TA, Collins FS, Cox NJ, *et al*. Finding the missing heritability of complex diseases. *Nature*. 2009; 461, 747–753.
4. Godfrey KM, Barker DJ. Fetal programming and adult health. *Public Health Nutr*. 2001; 4, 611–624.
5. Curhan GC, Willett WC, Rimm EB, *et al*. Birth weight and adult hypertension, diabetes mellitus, and obesity in US men. *Circulation*. 1996; 94, 3246–3250.
6. McCance DR, Pettitt DJ, Hanson RL, *et al*. Birth weight and non-insulin dependent diabetes: thrifty genotype, thrifty phenotype, or surviving small baby genotype? *BMJ*. 1994; 308, 942–945.
7. Hanson MA, Gluckman PD. Developmental origins of health and disease: new insights. *Basic Clin Pharmacol Toxicol*. 2008; 102, 90–93.
8. Roseboom T, de RS, Painter R. The Dutch famine and its long-term consequences for adult health. *Early Hum Dev*. 2006; 82, 485–491.
9. Bertram CE, Hanson MA. Animal models and programming of the metabolic syndrome. *Br Med Bull*. 2001; 60, 103–121.
10. Samuelsson AM, Matthews PA, Argenton M, *et al*. Diet-induced obesity in female mice leads to offspring hyperphagia, adiposity, hypertension, and insulin resistance: a novel murine model of developmental programming. *Hypertension*. 2008; 51, 383–392.
11. Burns SP, Desai M, Cohen RD, *et al*. Gluconeogenesis, glucose handling, and structural changes in livers of the adult offspring of rats partially deprived of protein during pregnancy and lactation. *J Clin Invest*. 1997; 100, 1768–1774.
12. Torrens C, Poston L, Hanson MA. Transmission of raised blood pressure and endothelial dysfunction to the F2 generation induced by maternal protein restriction in the F0, in the absence of dietary challenge in the F1 generation. *Br J Nutr*. 2008; 100, 760–766.
13. Calder PC, Yaqoob P. The level of protein and type of fat in the diet of pregnant rats both affect lymphocyte function in the offspring. *Nutr Res*. 2000; 20, 995–1005.
14. Langley SC, Seakins M, Grimble RF, Jackson AA. The acute phase response of adult rats is altered by in utero exposure to maternal low protein diets. *J Nutr*. 1994; 124, 1588–1596.

15. Bellinger L, Lilley C, Langley-Evans SC. Prenatal exposure to a maternal low-protein diet programmes a preference for high-fat foods in the young adult rat. *Br J Nutr*. 2004; 92, 513–520.
16. Bellinger L, Sculley DV, Langley-Evans SC. Exposure to undernutrition in fetal life determines fat distribution, locomotor activity and food intake in ageing rats. *Int J Obes*. 2006; 30, 729–738.
17. Bertram CE, Hanson MA. Prenatal programming of postnatal endocrine responses by glucocorticoids. *Reproduction*. 2002; 124, 459–467.
18. Ozanne SE, Hales CN. Lifespan: catch-up growth and obesity in male mice. *Nature*. 2004; 427, 411–412.
19. Zambrano E, Bautista CJ, Deas M, *et al*. A low maternal protein diet during pregnancy and lactation has sex- and window of exposure-specific effects on offspring growth and food intake, glucose metabolism and serum leptin in the rat. *J Physiol*. 2006; 571(Pt 1), 221–230.
20. Carone BR, Fauquier L, Habib N, *et al*. Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. *Cell*. 2010; 143, 1084–1096.
21. Ng SF, Lin RC, Laybutt DR, *et al*. Chronic high-fat diet in fathers programs beta-cell dysfunction in female rat offspring. *Nature*. 2010; 467, 963–966.
22. Waddington CH. Canalization of development and the inheritance of aquired characters. *Nature*. 1942; 150, 563–565.
23. Bird A. DNA methylation patterns and epigenetic memory. *Genes Dev*. 2002; 16, 6–21.
24. Ramsahoye BH, Biniszkiewicz D, Lyko F, *et al*. Non-CpG methylation is prevalent in embryonic stem cells and may be mediated by DNA methyltransferase 3a. *Proc Natl Acad Sci U S A*. 2000; 97, 5237–5242.
25. Fuks F, Hurd PJ, Wolf D, *et al*. The methyl-CpG-binding protein MeCP2 links DNA methylation to histone methylation. *J Biol Chem*. 2003; 278, 4035–4040.
26. Santos F, Hendrich B, Reik W, Dean W. Dynamic reprogramming of DNA methylation in the early mouse embryo. *Dev Biol*. 2002; 241, 172–182.
27. Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell*. 1999; 99, 247–257.
28. Bacolla A, Pradhan S, Roberts RJ, Wells RD. Recombinant human DNA (cytosine-5) methyltransferase. II. Steady-state kinetics reveal allosteric activation by methylated dna. *J Biol Chem*. 1999; 274, 33011–33019.
29. Ooi SK, Bestor TH. The colorful history of active DNA demethylation. *Cell*. 2008; 133, 1145–1148.
30. Reik W, Santos F, Mitsuya K, Morgan H, Dean W. Epigenetic asymmetry in the mammalian zygote and early embryo: relationship to lineage commitment? *Philos Trans R Soc Lond B Biol Sci*. 2003; 358, 1403–1409.
31. Miller CA, Sweatt JD. Covalent modification of DNA regulates memory formation. *Neuron*. 2007; 53, 857–869.
32. Kersh EN, Fitzpatrick DR, Murali-Krishna K, *et al*. Rapid demethylation of the IFN-gamma gene occurs in memory but not naive CD8 T cells. *J Immunol*. 2006; 176, 4083–4093.
33. Tahiliani M, Koh KP, Shen Y, *et al*. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science*. 2009; 324, 930–935.
34. Ito S, D'Alessio AC, Taranova OV, *et al*. Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. *Nature*. 2010; 466, 1129–1133.
35. Bhattacharya SK, Ramchandani S, Cervoni N, Szyf M. A mammalian protein with specific demethylase activity for mCpG DNA. *Nature*. 1999; 397, 579–583.
36. Zhu B, Zheng Y, Angliker H, *et al*. 5-methylcytosine DNA glycosylase activity is also present in the human MBD4 (G/T mismatch glycosylase) and in a related avian sequence. *Nucleic Acids Res*. 2000; 28, 4157–4165.
37. Barreto G, Schafer A, Marhold J, *et al*. Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. *Nature*. 2007; 445, 671–675.
38. Jost JP. Nuclear extracts of chicken embryos promote an active demethylation of dna by excision repair of 5-methyldeoxycytidine. *Proc Natl Acad Sci U S A*. 1993; 90, 4684–4688.
39. Turner BM. Histone acetylation and an epigenetic code. *Bioessays*. 2000; 22, 836–845.
40. Strahl BD, Ohba R, Cook RG, Allis CD. Methylation of histone H3 at lysine 4 is highly conserved and correlates with transcriptionally active nuclei in tetrahymena. *Proc Natl Acad Sci U S A*. 1999; 96, 14967–14972.
41. Nakayama J, Rice JC, Strahl BD, Allis CD, Grewal SI. Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. *Science*. 2001; 292, 110–113.
42. Fuks F, Hurd PJ, Deplus R, Kouzarides T. The DNA methyltransferases associate with HP1 and the SUV39H1 histone methyltransferase. *Nucleic Acids Res*. 2003; 31, 2305–2312.
43. Fuks F, Burgers WA, Brehm A, Hughes-Davies L, Kouzarides T. DNA methyltransferase Dnmt1 associates with histone deacetylase activity. *Nat Genet*. 2000; 24, 88–91.
44. Rountree MR, Bachman KE, Baylin SB. DNMT1 binds HDAC2 and a new co-repressor, DMAP1, to form a complex at replication foci. *Nat Genet*. 2000; 25, 269–277.
45. Vire E, Brenner C, Deplus R, *et al*. The polycomb group protein EZH2 directly controls DNA methylation. *Nature*. 2006; 439, 871–874.
46. Djebali S, Davis CA, Merkel A, *et al*. Landscape of transcription in human cells. *Nature*. 2012; 489, 101–108.
47. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell*. 2009; 136, 215–233.
48. Tan Y, Zhang B, Wu T, *et al*. Transcriptional inhibiton of Hoxd4 expression by miRNA-10a in human breast cancer cells. *BMC Mol Biol*. 2009; 10, 12.
49. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. *Nat Rev Genet*. 2009; 10, 155–159.
50. Drinkwater RD, Blake TJ, Morley AA, Turner DR. Human lymphocytes aged in vivo have reduced levels of methylation in transcriptionally active and inactive DNA. *Mutat Res*. 1989; 219, 29–37.
51. Feil R, Fraga MF. Epigenetics and the environment: emerging patterns and implications. *Nat Rev Genet*. 2011; 13, 97–109.
52. Maleszka R. Epigenetic integration of environmental and genomic signals in honey?bees. *Epigenetics*. 2008; 3, 188–192.
53. Kucharski R, Maleszka J, Foret S, Maleszka R. Nutritional control of reproductive status in honeybees via DNA methylation. *Science*. 2008; 319, 1827–1830.
54. Waterland RA, Jirtle RL. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol*. 2003; 23, 5293–5300.
55. Lillycrop KA, Phillips ES, Jackson AA, Hanson MA, Burdge GC. Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification

of hepatic gene expression in the offspring. *J Nutr*. 2005; 135, 1382–1386.
56. Lillycrop KA, Slater-Jefferies JL, Hanson MA, *et al*. Induction of altered epigenetic regulation of the hepatic glucocorticoid receptor in the offspring of rats fed a protein-restricted diet during pregnancy suggests that reduced DNA methyltransferase-1 expression is involved in impaired DNA methylation and changes in histone modifications. *Br J Nutr*. 2007; 97, 1064–1073.
57. Burdge GC, Phillips ES, Dunn RL, Jackson AA, Lillycrop KA. Effect of reduced maternal protein consumption during pregnancy in the rat on plasma lipid concentrations and expression of peroxisomal proliferator-activated receptors in the liver and adipose tissue of the offspring. *Nutr Res*. 2004; 24, 639–646.
58. Gluckman PD, Lillycrop KA, Vickers MH, *et al*. Metabolic plasticity during mammalian development is directionally dependent on early nutritional?status. *Proc Natl Acad Sci U S A*. 2007; 104, 12796–12800.
59. Gluckman PD, Hanson MA, Spencer HG. Predictive adaptive responses and human evolution. *Trends Ecol Evol*. 2005; 20, 527–533.
60. Vucetic Z, Kimmel J, Totoki K, Hollenbeck E, Reyes TM. Maternal high-fat diet?alters methylation and gene expression of dopamine and opioid-related?genes. *Endocrinology*. 2010; 151, 4756–4764.
61. Li CC, Young PE, Maloney CA, *et?al*. Maternal obesity and diabetes induces latent metabolic defects and widespread epigenetic changes in isogenic?mice. *Epigenetics*. 2013; 8, 602–611.
62. Sandovici I, Smith NH, Nitert MD, *et al*. Maternal diet and aging alter the epigenetic control of a promoter-enhancer interaction at the Hnf4a gene in rat pancreatic?islets. *Proc Natl Acad Sci USA*. 2011; 108, 5449–5454.
63. Tosh DN, Fu Q, Callaway CW, *et al*. Epigenetics of programmed obesity: alteration in IUGR rat hepatic IGF1 mRNA expression and histone structure in rapid vs. delayed postnatal catch-up? growth. *Am J Physiol Gastrointest Liver Physiol*. 2010; 299, G1023–G1029.
64. Lie S, Morrison JL, Williams-Wyss O, *et al*. Periconceptional undernutrition programs changes in insulin-signaling molecules and microRNAs in skeletal muscle in singleton and twin fetal? sheep. *Biol Reprod*. 2014; 306, E1013–E1024.
65. Lie S, Morrison JL, Williams-Wyss O, *et al*. Impact of embryo number and maternal undernutrition around the time of conception on insulin signaling and gluconeogenic factors and microRNAs in the liver of fetal?sheep. *Am J Physiol Endocrinol Metab*. 2014; 306, E1013–E1024.
66. Plagemann A, Harder T, Brunn M, *et al*. Hypothalamic proopiomelanocortin promoter methylation becomes altered by early overfeeding: an epigenetic model of obesity and the metabolic syndrome. *J Physiol*. 2009; 587(Pt 20), 4963–4976.
67. Burdge GC, Lillycrop KA, Phillips ES, *et al*. Folic acid supplementation during the juvenile-pubertal period in rats modifies the phenotype and epigenotype induced by prenatal nutrition. *J Nutr*. 2009; 139, 1054–1060.
68. Ly A, Lee H, Chen J, *et al*. Effect of maternal and postweaning folic acid supplementation on mammary tumor risk in the offspring. *Cancer Res*. 2011; 71, 988–997.
69. Waterland RA, Lin JR, Smith CA, Jirtle RL. Post-weaning diet affects genomic imprinting at the insulin-like growth factor 2 (Igf2)?locus. *Hum Mol Genet*. 2006; 15, 705–716.
70. Christman JK, Sheikhnejad G, Dizik M, Abileah S, Wainfan E. Reversibility of changes in nucleic acid methylation and gene expression induced in rat liver by severe dietary methyl deficiency. *Carcinogenesis*. 1993; 14, 551–557.
71. Hoile SP, Irvine NA, Kelsall CJ, *et al*. Maternal fat intake in rats alters 20:4n-6 and 22:6n-3 status and the epigenetic regulation of Fads2 in offspring?liver. *J Nutr Biochem*. 2013; 24, 1213–1220.
72. Heijmans BT, Tobi EW, Stein AD, *et al*. Persistent epigenetic differences associated with prenatal exposure to famine in? humans. *Proc Natl Acad Sci U S A*. 2008; 105, 17046–17049.
73. Tobi EW, Lumey LH, Talens RP, *et al*. DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. *Hum Mol Genet*. 2009; 18, 4046–4053.
74. Steegers-Theunissen RP, Obermann-Borst SA, Kremer D, *et al*. Periconceptional maternal folic acid use of 400 microg per?day is related to increased methylation of the IGF2 gene in the very young?child. *PLoS One*. 2009; 4, e7845.
75. Khulan B, Cooper WN, Skinner BM, *et al*. Periconceptional maternal micronutrient supplementation is associated with widespread gender related changes in the epigenome: a study of a unique resource in the?Gambia. *Hum Mol Genet*. 2012; 21, 2086–2101.
76. Dominguez-Salas P, Moore SE, Baker MS, *et al*. Maternal nutrition at conception modulates DNA methylation of human metastable epialleles. *Nat Commun*. 2014; 5, 3746–3753.
77. Jacobsen SC, Brons C, Bork-Jensen J, *et al*. Effects of short-term high-fat overfeeding on genome-wide DNA methylation in the skeletal muscle of healthy young?men. *Diabetologia*. 2012; 55, 3341–3349.
78. Teh AL, Pan H, Chen L, *et al*. The effect of genotype and in utero environment on interindividual variation in neonate DNA methylomes. *Genome Res*. 2014; 24, 1064–1074.
79. Murphy SK, Huang Z, Hoyo C. Differentially methylated regions of imprinted genes in prenatal, perinatal and postnatal human tissues. *PLoS One*. 2012; 7, e40924.
80. Waterland RA, Kellermayer R, Laritsky E, *et al*. Season of conception in rural gambia affects DNA methylation at putative human metastable epialleles. *PLoS Genet*. 2010; 6, e1001252.
81. Talens RP, Boomsma DI, Tobi EW, *et al*. Variation, patterns, and temporal stability of DNA methylation: considerations for epigenetic epidemiology. *FASEB J*. 2010; 24, 3135–3144.
82. Byun HM, Siegmund KD, Pan F, *et al*. Epigenetic profiling of somatic tissues from human autopsy specimens identifies tissue- and individual-specific DNA methylation patterns. *Hum Mol Genet*. 2009; 18, 4808–4817.
83. Godfrey KM, Sheppard A, Gluckman PD, *et al*. Epigenetic gene promoter methylation at birth is associated with child's later adiposity. *Diabetes*. 2011; 60, 1528–1534.
84. Barres R, Yan J, Egan B, *et al*. Acute exercise remodels promoter methylation in human skeletal?muscle. *Cell Metab*. 2012; 15, 405–411.
85. Tarantini L, Bonzini M, Apostoli P, *et al*. Effects of particulate matter on genomic DNA methylation content and iNOS promoter methylation. *Environ Health Perspect*. 2009; 117, 217–222.
86. Wong CC, Caspi A, Williams B, *et al*. A longitudinal study of epigenetic variation in?twins. *Epigenetics*. 2010; 5, 516–526.
87. Clarke-Harris R, Wilkin TJ, Hosking J, *et al*. Peroxisomal proliferator activated receptor-gamma-co-activator-1 alpha promoter methylation in blood at 5-7 years predicts adiposity from 9 to 14 years (EarlyBird?50). *Diabetes*. 2014; 63, 2528–2537.