Conference on 'Nutrition and health: cell to community'

Symposium 1: Nutrition and epigenetics
Effect of maternal diet on the epigenome: implications for human metabolic disease

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The rapid increase in the incidence of chronic non-communicable diseases over the past two decades cannot be explained solely by genetic and adult lifestyle factors. There is now considerable evidence that the fetal and early postnatal environment also strongly influences the risk of developing such diseases in later life. Human studies have shown that low birth weight is associated with an increased risk of CVD, type II diabetes, obesity and hypertension, although recent studies have shown that over-nutrition in early life can also increase susceptibility to future metabolic disease. These findings have been replicated in a variety of animal models, which have shown that both maternal under- and over-nutrition can induce persistent changes in gene expression and metabolism within the offspring. The mechanism by which the maternal nutritional environment induces such changes is beginning to be understood and involves the altered epigenetic regulation of specific genes. The demonstration of a role for altered epigenetic regulation of genes in the developmental induction of chronic diseases raises the possibility that nutritional or pharmaceutical interventions may be used to modify long-term cardio-metabolic disease risk and combat this rapid rise in chronic non-communicable diseases.

Epigenetics: DNA methylation: Fetal development: CVD: Obesity

The incidence of chronic non-communicable disease has risen sharply over the last 20 years with an estimated 18 million people dying from CVD per year[1]. This rise in CVD has been amplified by a rapid rise in the rate of obesity and type II diabetes across the world, which are major risk factors for CVD. This increase in the incidence of chronic non-communicable diseases over the past two decades cannot be explained solely by genetic and adult lifestyle factors. There is now substantial evidence that the fetal and early postnatal environment strongly influences the risk of developing cardio-metabolic disease. Epidemiological studies show that a poor intra-uterine environment induced by maternal diet, placental insufficiency or endocrine factors, such as stress, induces changes in the embryo and fetus, which increase its future risk of a range of non-communicable diseases[2]. These findings have been replicated in animal models where restricted nutrition during pregnancy induces dyslipidaemia, obesity, hypertension, hyperinsulinaemia and hyperleptinaemia in the offspring[3,4]. This association between poor intra-uterine growth and increased risk of disease in later life may reflect a mismatch between the future environment 'predicted' by the embryo/fetus, based on signals from the mother during gestation, and the actual environment experienced in later life[5]. The mechanism by which cues about nutrient availability in the postnatal environment are transmitted to the fetus and the process by which different, stable phenotypes are induced are beginning to be understood. A number of recent studies suggest that changes in the epigenetic regulation of genes in the embryo are central to the induction of a phenotype that persists into adulthood[5]. Because the phenotypic attributes induced include

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responses to environmental challenges such as diet, these epigenetic processes affect the risk of later disease.

The developmental origins of human cardio-metabolic disease

Human epidemiological studies in the UK provided the first evidence that early-life environment was associated with later disease risk. David Barker and colleagues found a strong geographical relationship between infant mortality and the incidence of CVD 50–60 years later, suggesting that a poor early-life environment increased susceptibility to CVD in later life[6]. Subsequent retrospective studies in both developed and developing countries have confirmed this association between birth weight and CVD, and have also shown that low birth weight is associated with an increased risk of hypertension, insulin resistance, type II diabetes, dyslipidaemia and obesity in later life[2,6]. This association between birth weight and metabolic disease in later life was observed even within the normal birth weight range and has been shown to have a continuous relationship with disease risk. At the highest birth weight, the risk of disease again increased, resulting in a U- or J-shaped relationship between birth weight and later disease risk[7,8]. However, birth weight in all these studies is thought to be simply a crude indicator of the intra-uterine environment that might have been compromised through a variety of maternal, environmental or placental factors[9]. There is growing awareness that the factors that compromise fetal growth or nutrition need not be severe; for example, fetal growth is known to be constrained in those who are born to smaller mothers, in primigravida or in very young mothers[10].

The role of maternal diet, however, on subsequent disease susceptibility has been most clearly shown in studies of the Dutch Hunger Winter, a famine that 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 did not have reduced birth weights compared to unexposed individuals, but did as adults exhibit an increased risk of obesity and CVD, whereas individuals whose mothers were exposed in the later stages of gestation had reduced birth weights and showed an increased incidence of insulin resistance and hypertension[11], suggesting that the timing of the nutritional constraint during pregnancy is also important in determining the future risk of disease.

Early catch-up growth in infants born pre-term, and who were fed formula milk also show increased risk of cardio-metabolic disease in later life[12,13], including obesity[14]. A number of studies have also shown greater incidence of obesity in adults who were formula fed as opposed to being breast fed during infancy[14,15], although not all studies have found this[16]. Fat mass is important for the onset of reproductive function, particularly in females[17]. In an evolutionary context, it is logical that catch-up growth in children born with a lower birth weight is characterised by greater adiposity relative to lean body mass, possibly as a mechanism to reach puberty at a similar age to peers born at greater weights. Although obesity is a risk factor for CVD, it has little negative effect in terms of potential reproductive success and so the trade-off associated with this strategy in terms of fitness is small.

Over-nutrition in early life also increases susceptibility to future obesity which may account for the U- or J-shaped relationships observed between birth weight and risk of obesity or insulin resistance in later life. Dorner and Plagemann[18] have reported that children of obese women are themselves more likely to become overweight and develop insulin resistance in later life[18]. Gestational weight gain irrespective of pre-pregnancy weight is positively associated with obesity at the age of 3 years[19] and even moderate weight gain between successive pregnancies has been shown to result in a significant increase in large for gestational age births[20]. However, maternal weight loss through bariatric surgery prevents the transmission of obesity to children compared to the offspring of mothers who did not undergo the surgery and remained obese[21].

Experimental models of induced cardio-metabolic disease

Animal models have been used extensively to investigate the mechanism by which nutrition in early life induces persistent alterations in the metabolism and physiology of the offspring[4]. These studies have generally been performed using sheep or rodents and have involved feeding either a low-protein diet, a global dietary restriction or even a high-fat or junk food diet through pregnancy and/or lactation. Interestingly, offspring born to such dams fed these different diets exhibit to varying extents characteristics of human subjects with cardio-metabolic disease including obesity, insulin resistance, hypertension and raised serum cholesterol levels.

Maternal protein restriction

The best-studied and most characterised animal model of nutritional induction of an altered metabolic phenotype is feeding rats a protein restricted (PR) diet from conception throughout pregnancy[22]. In some studies, this nutritional constraint continued during lactation. Offspring from PR dams show a number of features of human cardio-metabolic disease including hypertension[23], increased fat deposition and altered feeding behaviour[24–26], impaired glucose homeostasis, dyslipidaemia[27], vascular dysfunction[28], impaired immunity[29] and increased susceptibility to oxidative stress[30]. The phenotype of the offspring, however, does vary according to the exact composition of the diet[31]. This indicates that even small variations in maternal diet can affect the risk of disease in later life.

Animal studies have also shown a clear interaction between prenatal and postnatal environments[32,33]. Even modest variations in the diet fed after weaning can exacerbate the effects of maternal under-nutrition on the phenotype of the offspring. For example, dyslipidaemia and impaired glucose homeostasis 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 to a 4 % (w/w) fat post-weaning diet[31].

Global dietary restriction

A number of groups have also used global dietary restriction during pregnancy to investigate how maternal diet can influence disease susceptibility in later life. Woodall and co-workers used a global nutrient restriction of 30 % *ad libitum* fed throughout gestation, which results in a rat model of intra-uterine growth retardation[34]. Offspring born to dams fed this diet during pregnancy are significantly smaller at birth than control offspring. They also exhibit higher systolic blood pressure, hyperinsulinaemia, hyperleptinaemia, hyperphagia, reduced locomotion and obesity. These metabolic alterations are all augmented by feeding a high-fat postnatal diet. However, even modest global nutrient restriction during pregnancy has been shown to induce alterations in metabolism and the hypothalamic–pituitary–adrenal axis. In guinea pigs fed an 85 % *ad libitum* diet throughout gestation, alterations in cholesterol homeostasis was observed in the male offspring[35]. In sheep, a 15 % global nutrient restriction during the first half of pregnancy led to reduced adrenocorticotrophic hormone and cortisol responses to exogenous corticotropin-releasing hormone and arginine vasopressin administration, and also a blunted cortisol response to adrenocorticotrophic hormone[36].

High-fat diet during pregnancy

With the current rapid rise in incidence of obesity and type II diabetes across the globe, a number of new animal models of over-nutrition during pregnancy have also been developed. Feeding rats a diet high in saturated fats during pregnancy produces offspring with insulin resistance, abnormal cholesterol metabolism and raised adult blood pressure, interestingly very similar outcomes to those observed in offspring born to dams fed either a PR or globally restricted diet during gestation[37,38]. For instance, offspring from rats fed a 'junk food diet' of 16 % fat, 33 % sugar throughout pregnancy and lactation exhibited higher blood pressure, greater adiposity and insulin resistance in comparison with control offspring[39].

Phenotype induction and altered transcription

The induction during early life of persistent changes to the phenotype of the offspring by perturbations in maternal diet implies stable alterations to gene transcription which, in turn, results in altered activities of metabolic pathways and homeostatic control processes. Feeding a PR diet to pregnant rats increased glucocorticoid receptor (GR) expression and reduced expression of 11β-hydroxysteroid dehydrogenase type II, the enzyme that inactivates corticosteroids, in the liver, lung, kidney and brain in the offspring[3]. In the liver, increased GR activity up-regulates phosphoenolpyruvate carboxykinase expression and activity and so increases capacity for gluconeogenesis. This may contribute to the induction of insulin resistance in this

model[40]. Altered expression of GR has also been reported in the lung, liver, adrenal and kidney of the offspring of sheep fed a restricted diet during pregnancy[40–43]. Feeding a PR diet to pregnant rats up-regulates glucokinase expression in the liver of the offspring, which implies increased capacity for glucose uptake[44].

Restricting maternal protein intake during pregnancy and/or lactation in rats also alters the expression of specific genes involved in lipid homeostasis. Expression of acetyl-CoA carboxylase and fatty acid synthase were increased in the liver of the offspring of rats fed a PR diet during pregnancy and lactation[45]. PPAR-α expression has also been reported to be increased in the liver of the offspring of rats fed a PR diet during pregnancy and was accompanied by up-regulation of its target gene acyl-CoA oxidase[46,47].

Long-term changes in gene expression have also been reported in adult offspring of dams fed a global undernutrition or a high-fat diet during pregnancy. Gluckman *et al.* have showed that expression of PPARα and GR are both down-regulated in adult offspring born to dams fed a global nutrient restricted diet of 30 % *ad libitum* during pregnancy[48], while in offspring from dams fed a 'junk food diet' during pregnancy there were persistent alterations in the expression of PPARγ2, 11β-hydroxysteroid dehydrogenase type I and the β2 and β3 adrenoreceptors in adipose tissue[39]. Long-term changes in the expression of PPARγ2 and the adrenoreceptors may lead to increased adipogenesis and decrease lipolysis in these rats.

To gain further understanding of the mechanisms by which maternal diet may induce such changes to the expression of genes, recent studies have investigated the specificity of induced changes in the transcriptome of the offspring using microarray analysis in animal models. The analysis of the changes in the hepatic transcriptome of adult offspring from control and PR-fed dams showed that only 1·3 % of genes were changed in response to maternal protein restriction[49], suggesting that maternal protein restriction alters the expression of a relatively small subset of genes. Significant alterations in pathways involved in ion transport, developmental process and response to steroid hormone and reactive oxygen species pathways were observed in PR *v.* control offspring. Alterations in these pathways are consistent with previous phenotypic studies, which show that ion transport[50], cell commitment in blastocysts[51], response to reactive oxygen species[30] and steroid hormones[52,53] are altered by maternal diet.

Developmental plasticity

These findings together demonstrate that the prenatal and early postnatal period play a critical role in the induction of metabolic disease in later life. Gluckman and Hanson have suggested that the changes induced by maternal under- or over-nutrition may reflect an adaptive response of the fetus to environmental cues acting through the process of developmental plasticity which allows an organism to adjust its developmental programme resulting in long-term changes in its metabolism and physiology in order to be better adapted to the future environment[54]. For instance, poor maternal nutrition may signal to the fetus

that nutrients are scarce and an uncertain life course lies ahead. The fetus may then adapt its metabolism to conserve energy demands, increase its propensity to store fat, accelerate puberty and invest less in bone and muscle mass. If in the postnatal environment nutrition is indeed poor, then the organism's metabolism will be matched to the environment and that individual would be of low disease risk. In support of this, there is evidence that in both rat and pig models of maternal over-nutrition during pregnancy that continued high-fat feeding in postnatal life does not lead to deleterious effects[55,56]. However, if the offspring does not predict correctly the environment experienced after birth, then it is at increased risk of developing CVD and metabolic disease because its metabolism and homoeostatic capacity is mismatched to that environment. This mismatch pathway may explain why a nutritional constraint in early life followed by an adequate or nutritionally rich postnatal diet will result in an increased risk of metabolic disease in later life. This would also explain why human populations undergoing socio-economic change or migration from rural to urban areas show increased risk of chronic disease[57]. One important feature of such adaptive changes during development is that different phenotypes can be generated from a single genome depending on the environment that the organism experiences and there is now increasing evidence that the mechanism by which different phenotypes are generated from a single genome is through the altered epigenetic regulation of genes.

Epigenetic mechanisms and regulation of transcription

The term epigenetics literally means on top of genetics and refers to processes that induce heritable changes in gene expression without altering the gene sequence. The major epigenetic processes are DNA methylation, histone modification and microRNA. Epigenetic processes are integral in determining when and where specific genes are expressed. Alterations therefore in the epigenetic regulation of genes may lead to profound changes in phenotype[58–60]. To date, most studies on the effect of early-life nutrition on the epigenetic regulation of genes have focused on DNA methylation.

Methylation at the 5′ position of cytosine in DNA within a CpG dinucleotide (p denotes the intervening phosphate group) is a common modification in mammalian genomes and constitutes a stable epigenetic mark that is transmitted through DNA replication and cell division[61]. CpG dinucleotides are not randomly distributed throughout the genome but are clustered at the 5′ ends of genes/promoters in regions known as CpG islands. Hypermethylation of these CpG islands is associated with transcriptional repression, while hypomethylation of CpG islands is associated with transcriptional activation[61,62]. DNA methylation can induce transcriptional silencing by either blocking the binding of transcription factors or through promoting the binding of the methyl CpG-binding protein 2. The latter binds to methylated cytosines and, in turn, recruits histone modifying complexes to the DNA[63]. Methyl CpG-binding protein 2 recruits both histone deacetylases, which remove acetyl groups from the histones, and histone

methyl transferases that methylate Lys9 on His3, resulting in a closed chromatin structure and transcriptional silencing[63–65]. MicroRNA, which are small non-coding RNA, can also regulate gene expression, they have been shown to modulate gene expression at the post-transcriptional level through the induction of mRNA degradation or translational repression of a target mRNA[66]. However, more recent studies have shown that the human microRNA can also induce chromatin remodelling[67] and direct DNA methylation[68], suggesting that DNA methylation, histone modifications and microRNA may work in concert to regulate gene expression.

DNA methylation is important for asymmetrical silencing of imprinted genes[58], X chromosome inactivation and silencing of retrotransposons[69,70]. DNA methylation is also critical for cell differentiation by silencing the expression of specific genes during the development and differentiation of individual tissues[61,62]. Methylation of CpG is largely established during embryogenesis or in early postnatal life. Following fertilisation, maternal and paternal genomes undergo extensive demethylation. Following this, global *de novo* methylation occurs[71,72] during which 70% CpG are methylated, mainly in repressive heterochromatin regions and in repetitive sequences such as retrotransposable elements. At these early stages of development, the polycomb proteins, which are a group of histone modifying proteins play a critical role in maintaining the pluripotent nature of the embryonic stem cells by silencing cell determination genes such as *Pax*, *Hox* and *Dlx*[73], which are required for development, through polycomb-induced methylation of Lys27 on histone His3. As development proceeds, loss of polycomb proteins from their target genes[74] together with lineage-specific DNA methylation lead to the establishment of structurally and functionally distinct cell types. These epigenetic marks are essentially maintained throughout life. However, environmental perturbations during periods when methylation patterns are induced may impair the programme of gene silencing or activation with potential long-term adverse consequences.

Environmental challenges in early life alter the epigenome

A number of studies on isolated embryos have shown that variations in nutrient availability can alter the epigenome. Mouse embryos cultured in Whitten's medium without amino acids showed bi-allelic expression of the imprinted H19 gene, while those cultured in medium containing amino acids showed mono-allelic expression[75]. Differential methylation of the insulin-like growth factor-2 and H19 genes also occurred when embryos were cultured with or without fetal calf serum[76]. In human subjects, assisted reproductive technologies using *in vitro* fertilisation and intracytoplasmic sperm injection are associated with increased risk of Angelman's syndrome[59,77] and Beckwith–Weidemann syndrome[60] which are caused by decreased methylation of the regulatory regions of the UBE3A, and H19 and insulin-like growth factor-2 genes[59,60]. However, whether these effects are due to the nutrient composition of the medium or some other aspect of the *in vitro* environment is not known. Alterations to the

epigenetic regulation of imprinted genes produce dramatic alterations to the phenotype of the offspring including structural abnormalities in the skeleton and other tissues, and metabolic defects that are evident at birth. Such changes are in marked contrast to the effects of environmental constraint associated with cardio-metabolic disease which are not associated with gross structural abnormalities.

Maternal diet and altered epigenetic regulation

Differences in the micronutrient intake during pregnancy in the agouti mouse have been shown to induce differences in the coat colour of the offspring. The murine Agouti viable yellow mutation results from the insertion of an intracisternal-A particle retrotransposon upstream of the agouti gene, which regulates the production of yellow-pigmented fur. Supplementation of pregnant mice with methyl donors and cofactors, betaine, choline, folic acid and vitamin B_{12} shifted the distribution of coat colour of the offspring from yellow (agouti) to brown (pseudo-agouti)[78]. This shift is due to increased methylation of seven CpG dinucleotides 600 bp downstream of the Agouti viable yellow intracisternal A particle insertion site that acts as a cryptic promoter directing the expression of the agouti gene[70].

We have also shown that feeding pregnant rats a PR diet-induced hypomethylation of the GR and PPARα promoters in the livers of juvenile and adult offspring, which was accompanied by increased mRNA expression of these genes[47,79]. This was the first evidence that moderate changes in macronutrient intake during pregnancy can alter the epigenome. This was associated with an increase in histone modifications at the GR promoter that facilitate transcription; acetylation of histones H3 and H4 and methylation of histone H3 at Lys4, while those that suppress gene expression were reduced or unchanged[80]. Altered methylation status of the liver PPARα promoter was due to hypomethylation of four specific CpG dinucleotides, two of which predicted the level of the mRNA transcript, in juvenile offspring, which persisted in adults[81]. Because the altered CpG corresponded to transcription factor-binding sites, this suggests a mechanism by which changes in the epigenetic regulation of genes established during development determines changes in transcription in response to specific stimuli, and thus the capacity of the tissue to respond to metabolic challenge. The angiotensin receptor 1b promoter is also hypomethylated in adrenal glands from PR offspring[82]. Maternal global under-nutrition also induces in the offspring a phenotype that resembles human metabolic syndrome[83]. In contrast to the effect of the maternal PR diet, adult female offspring of dams which experienced 70% reduction in total nutrient intake during pregnancy showed hypermethylation and decreased expression of the GR and PPARα promoters in their liver[48]. Thus, the effects of maternal nutrition on the epigenome of the offspring depend on the nature of the maternal nutrient challenge.

In human subjects, Heijmans and co-workers have reported hypomethylation of the imprinted insulin-like growth factor-2 gene in genomic DNA isolated from whole blood from individuals who were exposed to famine *in utero* during the Dutch Hunger Winter compared to unexposed same-sex siblings[83]. The same group also found that the insulin-like growth factor promoter was hypomethylated in individuals whose mothers were peri-conceptually exposed to famine, while IL-10, leptin, ATP-binding cassette A1 and the guanine nucleotide-binding protein were hypermethylated[84]. These studies show that a nutritional challenge in early life can result in a change in DNA methylation which is detectable 60 years later, suggesting, as in the animal studies, early-life environment can induce long-term alterations to the epigenetic regulation of genes.

There is also evidence that an over-rich early nutritional environment can alter the epigenetic regulation of genes. Plagemann *et al.*[85] 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[85]. Consequently, Pro-opiomelanocortin expression is not up-regulated in these rats despite hyperinsulinaemia and hyperleptinaemia[85]. Thus, overfeeding during early post-natal life when the circuitry within the hypothalamus is still developing can alter the methylation of a gene critical for appetite control, resulting in the long-term altered regulation of this system and an increased disposition towards obesity in later life.

Reversal or prevention of altered phenotype and epigenotype

Induction of an altered phenotype in the offspring of rats fed PR diet during pregnancy can be prevented by supplementation of the PR diet with glycine or folic acid[31,86,87]. Increasing the folic acid content of the PR diet (PR supplemented with folic acid) also prevented the hypomethylation of the PPARα and GR promoters and restored levels of GR and PPARα expression to levels seen in control offspring. Folic acid supplementation of PR diet during pregnancy also up-regulated DNA methyltransferase (Dnmt) 1 expression[80]. This suggests that impaired 1-carbon metabolism plays a central role in the induction of the altered epigenetic regulation of GR and PPARα and in the induction of an altered phenotype by maternal protein restriction. A comparison of the hepatic transcriptome in offspring from control, PR and PR supplemented with folic acid dams revealed, while 1·3% of genes were altered in response to maternal PR, only 0·7% of genes in the liver were changed between control and PR-supplemented-with-folic-acid offspring[49], suggesting that while folic acid supplementation can prevent many of the changes induced by maternal protein restriction, it cannot prevent all changes and induces folate-specific changes in gene expression. Moreover detailed analysis of the PPARα promoter showed that although increased maternal folic acid intake prevented hypomethylation of the majority of CpG dinucleotides induced by the PR diet alone, two CpG were hypermethylated[81]. Thus, increasing maternal folic

acid intake does not simply prevent the effects of the PR diet, but may induce subtle changes in gene regulation.

However, it has also become apparent that the period of epigenetic plasticity may extend beyond the early intra-uterine period. Burdge et al.[88] have shown that increasing folic acid intake in the juvenile-pubertal period in rats whose mothers were fed protein sufficient or restricted diets during pregnancy, altered their phenotype and epi-genotype[88]. However, supplementation of the diet of juvenile-pubertal rats of dams fed a PR diet did not simply reverse the altered epigenotype induced by maternal PR, but induced a different pattern of epigenetic changes including hypermethylation of the PPARα promoter[88]. These results showed that in contrast to supplementation of the maternal PR diet with folic acid, supplementation during the juvenile-pubertal period induced, irrespective of the maternal diet, impaired lipid homeostasis including down-regulation of hepatic fatty acid β-oxidation, hepa-tosteatosis and increased weight gain. These findings sug-gest that the period between weaning and adulthood in rats represents a period of increased plasticity, where it may be possible to reverse the adverse effects of prenatal nutrition by nutritional interventions before adulthood. However, these data also indicate that any intervention must be undertaken with care with respect to the intervention and background diet to avoid inducing further deleterious epigenetic changes.

Supplementation of the maternal diet with methyl donors has also been shown to prevent the transgenera-tional amplification of obesity observed in Agouti viable yellow mice[89], supporting the hypothesis that altered epigenetic regulation also underlies the mechanism by which maternal obesity can increase the risk of offspring obesity. The effect of methyl supplementation on body weight was independent of epigenetic changes at the Agouti viable yellow locus, suggesting that maternal obesity alters the epigenetic regulation at other genetic loci which may influence appetite and energy homeostasis and that methyl supplementation blocks such epigenetic dysregulation.

Treatment with leptin between postnatal days 3 and 13 of neonatal rats born to dams which experienced 70 % global reduction in food intake during pregnancy normal-ised energetic intake, locomotor activity, body weight, fat mass and fasting plasma glucose, insulin and leptin con-centrations in adult offspring in contrast to saline-treated offspring of undernourished dams which developed all these features on a high-fat diet[90]. This again shows that developmental metabolic programming is potentially reversible by an intervention late in the phase of develop-mental plasticity. The ability of leptin to reverse these metabolic effects has been suggested to occur as a result of leptin administration giving a false developmental cue signalling adiposity to the pups that were actually thin and thus therefore setting their metabolic phenotype to be more appropriate to a high-nutrition environment. Strikingly, the corrective effects of leptin were paralleled by effects on methylation and expression of PPARα and GR[48]. This suggests that neonatal leptin intervention may exert its corrective adaptive effects through epigenetic mechanisms.

Mechanisms for induced changes in the epigenome

Methylation of CpG dinucleotides is catalysed by Dnmt3a and Dnmt3b, and is maintained through mitosis by gene-specific methylation of hemimethylated DNA by Dnmt1[91]. Although traditionally DNA methylation has been regarded as a stable epigenetic mark, active deme-thylation has been observed for paternal genomic DNA in the zygote upon fertilisation[72]. Rapid demethylation has also been reported of the synaptic plasticity gene reelin in the hippocampus upon contextual fear conditioning[92] and on interferon-γ upon antigen exposure of memory CD8 T cells[93]. A number of DNA demethylases have now been proposed; these include MBD2b[94], MBD4[95], the DNA repair endonucleases XPG (Gadd45a)[96] and a G/T mis-match repair DNA glycosylase[97].

The mechanism by which nutrition in early life alters the epigenome is not known but feeding a PR diet to rats during pregnancy induced a reduction in Dnmt1 expres-sion, but not in the expression of Dnmt3a and Dnmt3b[80]. This suggests that hypomethylation of the GR and PPARα promoters in the liver of the offspring may be induced by a failure to maintain methylation patterns during mito-sis[80,98]. This is supported by the finding that a decrease in Dnmt1 expression induced by the maternal PR diet was prevented by increasing the folic acid content of the PR diet during pregnancy. Folic acid supplementation of the PR diet during pregnancy also prevented the hypomethyl-ation of GR and PPARα and many of the phenotypic changes induced by PR[80]. Although a reduction in Dnmt1 activity might be expected to result in global demethyla-tion, abolition of Dnmt1 expression appears only to affect a subset of genes[99]. This indicates that Dnmt1 is targeted to specific genes, and there are now a number of reports that have shown that Dnmt1 interacts with a number of histone-modifying enzymes and is targeted to specific DNA sites[100,101]. As recent findings have suggested that DNA methylation may involve continual demethylation–remethylation cycles[102,103], nutritional challenges in early life which alter the activity of the Dnmt1 may shift this equilibrium towards demethylation.

Conclusion

Traditionally DNA sequence was believed to be the sole determinant of phenotype and phenotypic variation was a result of genetic mutation or recombination. There is now evidence that epigenetic mechanisms allow the developing fetus to adapt to nutritional cues from the mother and adjust its developmental trajectory to produce a phenotype matched to the predicted postnatal environment. Studies from both animal and human subjects suggest that these altered epigenetic marks induced by early environmental challenges are stably maintained throughout the life course raising the possibility that these altered marks may be used as predictive markers of later phenotype and disease risk. Animal studies also suggest that these altered epigenetic marks can be prevented and/or reversed at specific time periods implying that it may be possible either through nutritional or pharmaceutical interventions to reverse such epigenetic marks and reduce the incidence of

non-communicable diseases. However, there is still much we have to learn in terms of which early-life exposures can alter the epigenome, which pathways are affected, what are the critical developmental periods and when the epigenome is most susceptible to environmental cues and can interventions be targeted to specific epigenetic marks. With this increased level of understanding of the relationship between epigenetics, the environment and disease susceptibility, it may be possible to make real progress in the prevention and treatment of chronic diseases and halt the rapid rise in non-communicable diseases currently seen throughout the world.

Acknowledgements

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