International Journal of Obesity (2011) 35, 72–83

0307-0565/11
www.nature.com/ijo

PEDIATRIC REVIEW

Epigenetic changes in early life and future risk of obesity

KA Lillycrop[1] and GC Burdge[2]

[1]*School of Biological Sciences, University of Southampton, Southampton, UK and* [2]*Institute of Human Nutrition, School of Medicine, University of Southampton, Southampton, UK*

The rapid increase in incidence of obesity 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 environments also strongly influence the risk of developing obesity in later life. Initially, human studies showed that low birth weight was associated with an increased risk of obesity but increasingly there is evidence that overnutrition in the early life can also increase susceptibility to future obesity. These findings have now been replicated in animal models, which have shown that both maternal under- and overnutrition can induce persistent changes in gene expression and metabolism. 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. In this review, we discuss the recent evidence that shows that early-life environment can induce altered epigenetic regulation leading to the induction of an altered phenotype. The demonstration of a role for altered epigenetic regulation of genes in the developmental induction of obesity opens the possibility that interventions, either through nutrition or specific drugs, may modify long-term obesity risk and combat this rapid rise in obesity.
International Journal of Obesity (2011) **35,** 72–83; doi:10.1038/ijo.2010.122; published online 15 June 2010

Keywords: DNA methylation; fetal development; cardio-metabolic disease; folate

Introduction

The incidence of obesity has risen sharply over the past 20 years and has now reached epidemic proportions, with more than 1 billion adults overweight and 300 million adults clinically obese worldwide.[1] This increase in obesity is not limited to industrialized nations but is also increasingly seen in developing nations. Obesity is known to be a major risk factor for a number of chronic non-communicable diseases such as type 2 diabetes, hypertension, cardiovascular disease (CVD), osteoarthritis and certain forms of cancer, including breast, colon and prostate cancers, and thus poses a major public health problem. Although it is well established that the risk of an individual developing obesity is dependent on the interaction between their genotype and lifestyle factors such as an energy-rich diet and sedentary behaviour, the increase in obesity over the past two decades has occurred too rapidly to be explained solely by such factors. There is now substantial evidence that the fetal and early postnatal environments strongly influence the risk of developing obesity and that altered epigenetic regulation of specific genes is central to this process. This review will focus on the role of altered epigenetic processes in developmental induction of obesity and how these processes may make a significance contribution to the obesity epidemic.

Correspondence: Dr KA Lillycrop, School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton SO16 7PX, UK.
E-mail: kal@soton.ac.uk
Received 9 December 2009; revised 16 April 2010; accepted 18 April 2010; published online 15 June 2010

The developmental origins of human metabolic disease

It has been known for almost 100 years that the environment in which the embryo and fetus develops can induce variations in the phenotype of the offspring without changing the genome. This is illustrated by experiments such as those described by Stockard,[2] which show that modest environmental constraint during specific periods in the development of the embryo can induce dramatic changes in the phenotype of the offspring. For example, lowering the oxygen tension of water surrounding gastrulating trout embryos induces conjoined fry. There are also a number of well-documented examples from natural history that show variation in the phenotype of the offspring in response to changes in the maternal environment during pregnancy.[3–5] It is, therefore, unsurprising that the quality of

the developmental environment to which humans embryos are exposed is able to induce lifelong changes in the phenotype of the children both in terms of morphology[6,7] and metabolism. Gross morphological variation in response to the early-life environment is relatively rare. However, variation in the quality of the intrauterine environment, for example, the availability of nutrients and oxygen, and exposure of hormones, has been demonstrated repeatedly as a causal factor in differential risk of metabolic disease.

The association between the quality of the early-life environment and subsequent risk of cardio-metabolic disease has been described in a series of epidemiological studies by David Barker and Osmond[8] in the United Kingdom. They found a strong geographical relationship between infant mortality and risk of CVD disease 50–60 years later. Subsequent retrospective studies in cohorts across the globe in developed and developing nations, including the United Kingdom, North America, India and China,[9] have shown consistently that lower birth weight within the normal range for a particular population is associated with an increased risk in later life of CVD and the metabolic syndrome (hypertension, insulin resistance, type 2 diabetes, dyslipidaemia and obesity).[8–10] 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.[11,12] It should be noted that birth weight is a proxy marker of the degree on constraint of the intrauterine environment rather than the mechanism by which the phenotype of the child is induced.[13] However, a female who is born small and remains small throughout life may constrain the growth and development of her child during pregnancy.

The timing of the nutritional constraint during pregnancy is important in determining the future risk of disease. Studies of individuals exposed to famine *in utero*, which occurred in the Netherlands during the winter of 1944, show that the risk of obesity and its associated conditions is related to the timing of nutrient constraint. Individuals whose mothers were exposed to famine periconceptually and in the first trimester of pregnancy did not have reduced birth weights compared with unexposed individuals, but as adults, exhibited increased risk of obesity and CVD. 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.[14] These findings are in agreement with those of Stockard[2] who showed that induction of cycolpia or two-headed trout by variations in the temperature or oxygen concentration in the water only occurred during the first 24 h after fertilization. Prenatal or postnatal undernutrition in sheep has also been shown to induce distinct changes in growth and vascular function.[15] One implication of these studies is that the mechanism that underlies the induction of an altered phenotype must exhibit differential activity as development proceeds.

In humans, weight gain up to 25 weeks gestation is primarily due to linear growth. Accumulation of body fat is initiated at about 25 weeks of gestation[16] and $\sim$40% of the variation in birth weight reflects differences in the magnitude of fat deposition.[17] Thus, infants born with a lower birth weight are likely to have a reduced fat mass. Small babies who undergo early catch-up growth that is characterized by a greater accumulation of fat mass relative to lean body mass have an increased risk of becoming obese in later life compared with those born at higher birth weights.[18–20] Early catch-up growth in infants born pre-term, who also have a reduced fat mass at birth, and who were fed formula milk also show increased risk of cardio-metabolic disease in later life,[21,22] including obesity.[23] A number of studies have also shown a greater incidence of obesity in adults who were formula fed as opposed to breast fed during infancy,[23,24] although not all studies found this.[25] Fat mass is important for the onset of reproductive function, particularly in females.[26] In an evolutionary context, it is logical that catch-up growth in children born with a lower birth weight is characterized 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.

Overnutrition 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[27] have reported that children of obese women are more likely to become obese and develop insulin resistance in later life. Gestational weight gain irrespective of pre-pregnancy weight is positively associated with obesity at 3 years old[28] and even moderate weight gain between successive pregnancies has been shown to result in a significant increase in large for gestational age infants.[29] However, maternal weight loss through bariatic surgery prevents transmission of obesity to children compared with the offspring of mothers who did not undergo the surgery and remained obese.[30] Maternal gestational diabetes can also promote the development of obesity and insulin resistance in offspring. Children born to mothers with gestational diabetes are frequently macrosomic, the greater the level of hyperglycaemia in pregnancy producing a greater risk of childhood obesity.[31] Such children also show a greater gain of body mass by 4 years of age relative to children of non-diabetic mothers.[32] Children who were born large for gestational age to mothers with gestational diabetes or obesity also show increased risk of cardio-metabolic disease.[33]

Experimental models of the developmental induction of obesity

Animal models have been used extensively to investigate the mechanism by which the early-life environment induces

persistent alterations in the metabolism and physiology of the offspring. 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. These offspring to varying extents exhibit characteristics of humans with cardio-metabolic disease, including obesity, insulin resistance, hypertension and raised serum cholesterol levels. Together, these studies support the hypothesis that nutritional imbalance during prenatal and early postnatal life can induce long-term metabolic changes and increased susceptibility to metabolic disease in later life.

The best-studied and most characterized animal model of nutritional induction of an altered metabolic phenotype is feeding rats with a protein-restricted (PR) diet from conception throughout pregnancy. In some studies, this nutritional constraint is continued during lactation. Offspring from PR dams show a number of features of human cardio-metabolic disease, including hypertension,[34] increased fat deposition and altered feeding behaviour,[35–37] impaired glucose homeostasis, dyslipidaemia,[38] vascular dysfunction,[35,39] impaired immunity[40] and increased susceptibility to oxidative stress.[41] The phenotype of the offspring, however, does vary according to the exact composition of the diet.[42] This indicates that even small variations in maternal diet can affect the risk of disease in later life.

Some of the alterations in the metabolism and physiology of the offspring induced by maternal protein restriction have been shown to be accompanied by stable alterations in the transcription of genes involved in metabolic regulation and homeostatic processes.[43] These include increased glucocorticoid receptor (GR) expression and reduced expression of the enzyme, which inactivates corticosteroids, 11β-hydroxysteroid dehydrogenase type II, in the liver, lung, kidney and brain in the offspring.[44] In the liver, increased GR activity upregulates phosphoenolpyruvate carboxykinase expression and activity and so increases the capacity for gluconeogenesis. This may contribute to the induction of insulin resistance in this model.[45]

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 with a PR diet during pregnancy and lactation.[46] The offspring of rats fed with a PR diet during pregnancy show increased blood triacylglycerol (TAG) and non-esterified fatty acid (NEFA) concentrations.[42,47] Peroxisomal proliferator-activated receptor (PPAR)-α expression was increased in the liver of the offspring of rats fed with a PR diet during pregnancy and was accompanied by upregulation of its target gene acyl-CoA oxidase (*AOX*), while PPARγ1 expression was unchanged.[48,49] In contrast, in adipose tissue, the expression of the PPARγ adipose-specific isoform PPARγ$_2$ was reduced.[48,49] The different effects of maternal PR diet on PPARγ expression in the liver and adipose tissue may reflect the fact that the PPARγ isoforms, PPARγ$_1$ and PPARγ$_2$, expressed in the liver and adipose tissue, respectively, are generated from different promoters. If so, this suggests a mechanism by which the process that induces altered gene expression, and hence phenotype, may result in different effects on the expression of the same gene in different tissues. Increased PPARα expression would be expected to increase TAG clearance. However, increased hepatic TAG synthesis may result from increased flux of NEFA from adipose tissue as a result of reduced expression of PPARγ expression[48] and insulin resistance[45] and may have exceeded the capacity of fatty acid clearance pathways regulated by PPARα. Overall, the offspring of dams fed with a PR diet during pregnancy show impaired lipid homeostasis.[50]

Global nutrient restriction to 30% of *ad libitum* fed throughout gestation induces a more severe change in phenotype compared with the maternal PR diet, which is comparable to intrauterine growth retardation.[51] Offspring born to dams fed with 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. Although the nutritional constraint in this model is more severe than that which leads to variation in risk of cardio-metabolic disease within the normal range of birth weights, the overall reduction in energy intake is comparable to that to which pregnant women were exposed during the Dutch Hunger Winter.[52] However, even modest global nutrient restriction during pregnancy have been shown to induce alterations in metabolism and the hypothalamic–pituitary–adrenal axis. In guinea pigs fed with an 85% of *ad libitum* diet throughout gestation, alterations in postnatal cholesterol homeostasis were observed in the male offspring.[53] Long-term changes in gene expression have also been reported in adult offspring of dams fed with a global nutrient restriction during pregnancy. Gluckman *et al*.[54] have showed that expression of PPARα and GR are both downregulated in adult offspring born to dams fed with a global nutrient-restricted diet of 30% *ad libitum* during pregnancy.

Animal studies have also shown a clear interaction between prenatal and postnatal environments.[55,56] Even modest variations in the diet fed after weaning can exacerbate the effects of maternal undernutrition on the phenotype of the offspring. For example, dyslipidaemia and impaired glucose homeostasis induced by feeding dams with a PR diet during pregnancy were exacerbated in adult male and female rats fed with a diet containing 10% (w/w) fat after weaning compared with a 4% (w/w) fat post-weaning diet.[42]

Animal models of overnutrition

A number of animal models of overnutrition during pregnancy have also been reported. Feeding rats with 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 with a PR or globally restricted diet during gestation. Feeding an obesogenic diet to female rats before mating and through lactation has also been shown to lead to maternal obesity as well as hyperphagia, increased adiposity, decreased muscle mass, reduced locomoter activity and accelerated puberty in the offspring.[57] Samuelsson *et al*.[58] have also shown that offspring from pregnant rats fed with a 'junk food diet' of 16% fat and 33% sugar throughout pregnancy and lactation exhibited higher blood pressure, greater adiposity and insulin resistance in comparison with control offspring. There were also persistent alterations in the expression of PPARγ2, 11β-hydroxysteroid dehydrogenase type I and the β2 and β3 adrenoreceptors in adipose tissue of the offspring, which may lead to increased adipogenesis and decrease lipolysis in these rats.

Feeding pregnant mice with a high-fat diet also induces fatty liver and hepatic inflammation if the offspring were also fed with a high-fat diet in postnatal life.[59] The mechanism underlying non-alcoholic fatty liver disease involves dysregulation of hepatic lipid metabolism and reduced activity of mitochondrial oxidative phosphorylation complexes. Interestingly, studies by Khan *et al*.[60] have shown that continued high-fat feeding in postnatal life conferred protection against endothelial dysfunction, suggesting that an adaptive mechanism might operate, enabling the fetus to adjust at least some of its metabolic processes to be better suited to survive in a high-fat postnatal environment. The type of fat however may also be important as dams fed with diets high in essential fatty acids throughout late gestation and lactation produce offspring that eat less, are leaner and have improved insulin sensitivity as adults.[61] High carbohydrate diets are also protective and produce offspring that remain lighter in weight.[62]

There is also evidence that the suckling period is critical in the developmental induction of obesity. Studies of rats in cross-fostering experiments show that high-fat feeding in the suckling period leads to an increase in adiposity, hyperleptinaemia and hypertension in the adult offspring fed with a normal diet after weaning.[63–65] Schmidt *et al*.[66] have also shown that overfeeding rats during the suckling period by rearing them in small litters produces hyperphagia and obesity in adults. In addition, maternal gestational diabetes has also been shown to produce obesity in the adult offspring[67] and even transient neonatal hyperinsulinaemia can induce obesity in early adulthood.[23] Waterland *et al*.[68] have also shown transgenerational amplification of the obesity phenotype in agouti viable yellow (A^{vy}) mice through three generations. There is also growing evidence that overnutrition during prenatal and/or early postnatal life alters the maturation of the appetite- and energy-regulating neural network in the hypothalamus. The effect of overnutrition on hypothalamus function has been observed both in sheep in which the neural network is relatively mature at birth as in humans[69] and in rodents in which the circuits are not fully mature until postnatal day 16.[70] For example, overfeeding rat pups by rearing them in small litters leads to an increased food intake in the perinatal period and this was also associated with a persistent increase in appetite drive in later life.[64–66,70] In rodents, exposure to a diabetic environment before birth or exposure in early postnatal life results in significant changes in the architecture of the hypothalamus including a decrease in neuronal density within the ventromedial hypothalamic nucleus, which is associated with the inhibition of food intake and pancreatic insulin secretion, and an increase in neuronal density within the dorsomedial hypothalamic nucleus, which is involved in promoting body weight gain and food intake.[71] In adult offspring of gestational diabetic rats, there was also an increase in neuropeptide Y and galanin neurons in the arcuate hypothalamic nucleus, suggesting that gestational maternal diabetes promotes the malformation of the hypothalamic neurons and the dysregulation of the appetite-controlling regulatory network, resulting in obesity and metabolic disturbances in later life.[72]

Epigenetic mechanisms in induced risk of obesity

Together, these findings demonstrate that the prenatal and early postnatal periods have a critical role in the developmental induction of obesity. There is now however increasing evidence that epigenetic processes are central to the mechanism by which the early nutritional environment can increase susceptibility to obesity in later life (Figure 1). The term epigenetics literally means on top of genetics and refers to processes that induce heritable changes in gene

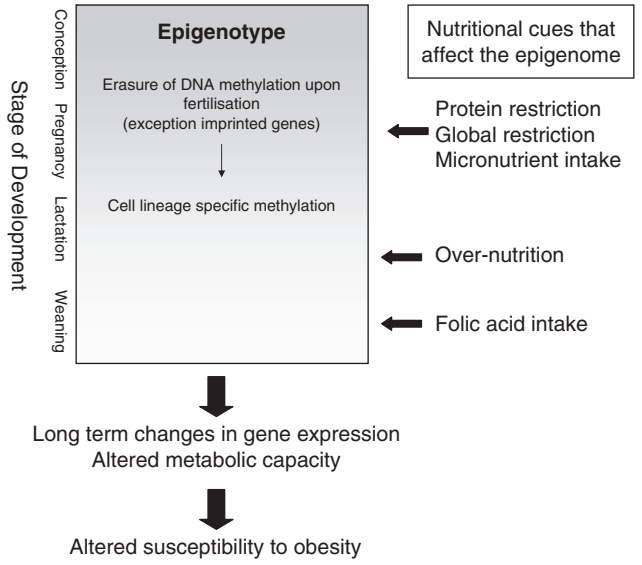

Figure 1 Alterations in the epigenome induced by nutritional challenges results in an altered metabolic capacity and altered susceptibility to developing obesity in later life. The sensitivity of the epigenome to the environment (represented by the shading) decreases during postnatal life.

expression without altering the gene sequence. Epigenetic processes are integral in determining when and where specific genes are expressed.[73] Alterations in the epigenetic regulation of genes may lead to profound changes in phenotype. The major epigenetic processes are DNA methylation, histone modification and microRNAs. To date, most studies on the effect of early-life nutrition on the epigenetic regulation of genes have focussed on DNA methylation.

Epigenetic mechanisms and gene regulation. Methylation at the 5′ position of cytosine in DNA within a CpG (cytosine and guanine nucleotides linked by phosphate) dinucleotide (the 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.[74] Methylation of CpG dinucleotides *de novo* is catalysed by DNA methyltransferases (Dnmts) 3a and 3b, and is maintained through mitosis by gene-specific methylation of hemimethylated DNA by Dnmt1.[75] 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, whereas hypomethylation of CpG islands is associated with transcriptional activation.[76] DNA methylation can induce transcriptional silencing by blocking the binding of transcription factors and/or through promoting the binding of the methyl CpG-binding protein (MeCP2).[75] The latter binds to methylated cytosines and, in turn, recruits histone-modifying complexes to the DNA. MeCP2 recruits both histone deacetylases (HDACs), which remove acetyl groups from the histones, a signal of trasncriptionally active chromatin,[77–79] and histone methyl transferases such as Suv39H1,[80] which methylates lysine 9 on H3, resulting in a closed chromatin structure and transcriptional silencing. Recent studies have however shown that Dnmt1 is recruited by a number of histone-modifying enzymes such as HDAC1 and HDAC2, and the histone methyl transferases SUV39 and EZH2,[81–83] suggesting that chromatin structure may also determine DNA methylation status and that there is a reciprocal relationship between these two processes.

DNA methylation is important for asymmetrical silencing of imprinted genes,[84] X-chromosome inactivation and silencing of retrotransposons.[85,86] DNA methylation is also critical for cell differentiation by silencing the expression of specific genes during the development and differentiation of individual tissues.[74] Methylation of CpGs is largely established during embryogenesis or in early postnatal life. Following fertilization, maternal and paternal genomes undergo extensive demethylation followed by global methylation *de novo* just before blastocyst implantation[87] during which 70% of CpGs are methylated, mainly in repressive heterochromatin regions and in repetitive sequences such as retrotransposable elements.[87] Lineage-specific methylation of tissue-specific genes occurs throughout prenatal development and early postnatal life and determines developmental fates of differentiating cells. For example, Oct-4 is permanently silenced by hypermethylation around E6.5 in the mouse,[88] whereas HoxA5 and HoxB5 are not methylated and silenced until early postnatal life.[89] In contrast, phosphoenolpyruvate carboxykinase and δ-crystallin-2 are methylated in early embryos, but undergo progressive demethylation during development.[90,91] 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.

Early-life environment and altered epigenetic regulation. Two non-nutritional models have shown that the prenatal and neonatal environments modify the epigenetic regulation of specific genes. In an elegant study of the effect of maternal behaviour during suckling on the development of stress response in the offspring, Weaver *et al*.[92] showed that pups raised by rat dams that showed poorer nurturing had an increased stress response. The effect was due to hypermethylation of specific CpG dinucleotides within the promoter of the GR gene in the hippocampus of the offspring. These changes were reversed in adult brain by intracranial administration of the HDAC inhibitor, trichostatin A, and L-methionine.[93] Others have shown that ligation of a uterine artery in the rat decreases p53 expression in the kidney of the offspring, associated with increased apoptosis and reduced nephron number.[94]

Studies on isolated embryos first supported the hypothesis that variations in nutrient availability can alter the epigenome. Mouse embryos cultured in Whitten's medium without amino acids showed biallelic expression of the imprinted *H19* gene, whereas those cultured in medium containing amino acids showed monoallelic expression.[95] Differential methylation of the insulin-like growth factor-2 (*IGF-2*) and *H19* genes also occurred when embryos were cultured with or without fetal calf serum.[96] In humans, assisted reproductive technologies using *in vitro* fertilization and intracytoplasmic sperm injection are associated with increased risk of Angelman's syndrome[97] and Beckwith–Weidemann syndrome,[98] which are caused by decreased methylation of the regulatory regions of the UBE3A, and *H19* and *IGF-2* genes.[97,98] Alterations in the epigenetic regulation of imprinted genes produce dramatic alterations in 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 induced by maternal nutritional imbalance during pregnancy associated with increased susceptibility to obesity, which are not associated with gross structural abnormalities.

Maternal diet and altered DNA methylation. Differences in the maternal intake of nutrients have been shown to alter

Table 1 Examples of genes involved in metabolic, vascular or endocrine function whose methylation status is altered by changes in diet either during prenatal or early postnatal life

Gene	*Methylation*	*Species*	*Nutritional challenge*	*Tissue analysed*	*Age of offspring at the time of measurement*	*Reference*
Agouti gene	Increased	Mouse	Maternal methyl donor	Tail, liver, kidney, brain	Adult	Waterland and Jirtle[85]
Glucocorticoid receptor	Decreased	Rat	Maternal PR	Liver	Juvenile adult	Lillycrop *et al.*[49]
PPAR-α	Decreased	Rat	Maternal PR	Liver	Juvenile adult	Lillycrop *et al.*[49]
Angiotensin receptor 1b	Decreased	Rat	Maternal PR	Adrenal	Adult	Bogdarina *et al.*[103]
Glucocorticoid receptor	Increased	Rat	Maternal UN	Liver	Adult	Gluckman *et al.*[54]
PPAR-α	Increased	Rat	Maternal UN	Liver	Adult	Gluckman *et al.*[54]
Proopiomelanocortin	Increased	Rat	Overnutrition/suckling	Hypothalamus		Plagemann *et al.*[106]
Glucocortociod receptor	Increased	Rat	Pubertal folic acid supplementation	Liver	Adult	Burdge *et al.*[117]
PPAR-α	Increased	Rat	Pubertal folic acid supplementation	Liver	Adult	Burdge *et al.*[117]
Insulin receptor	Decreased	Rat	Pubertal folic acid supplementation	Adipose	Adult	Burdge *et al.*[117]
Insulin-like growth factor II	Decreased	Human	Maternal UN	Whole blood	Adult	Heijmans *et al.*[104]
Interleukin 10	Increased	Human	Maternal UN	Whole blood	Adult	Tobi *et al.*[105]
Leptin	Increased	Human	Maternal UN	Whole blood	Adult	Tobi *et al.*[105]
ATP-binding cassette A1	Increased	Human	Maternal UN	Whole blood	Adult	Tobi *et al.*[105]
Guanine nucleotide binding protein	Increased	Human	Maternal UN	Whole blood	Adult	Tobi *et al.*[105]
Maternally expressed 3	Increased	Human	Maternal UN	Whole blood	Adult	Tobi *et al.*[105]

Abbreviations: PPAR-α, peroxisomal proliferator-activated receptor-α; PR, protein restricted; UN, undernutrition. For rats: juvenile, offspring <40 days postnatal; adult, offspring >40 days postnatal.

the methylation of non-imprinted genes resulting in subtler effects on fetal and offspring development. A summary of genes involved in metabolic, vascular or endocrine function whose methylation status is altered by changes in diet either during prenatal or early postnatal life is shown in Table 1. Variations in the maternal diet of nutrients involved in one-carbon metabolism during pregnancy in the agouti mouse have been shown to induce differences in the coat colour of the offspring. In agouti mice A^{vy}, there is an insertion of an intracisternal A particle (IAP) retrotransposon in the 5′ end of the *Agouti* gene, which acts as a cryptic promoter directing expression of the agouti gene that encodes a paracrine signalling molecule that induces melanocytes to produce pheomelanin, a yellow pigment instead of the black or brown pigment eumelanin. The methylation status of the IAP element produces as range of coat colours between yellow (unmethylated) and pseudo-agouti (methylated).[85,99] Supplementation of the maternal diet with methyl donors such as betaine, choline, folic acid and vitamin B_{12} shifted the distribution of coat colour of the offspring from yellow (agouti) to brown (pseudo-agouti).[99] This shift is due to increased methylation of the IAP element.[85] Thus, maternal intake of nutrients involved in one-carbon metabolism can induce graded changes to DNA methylation and gene expression in the offspring, which persist into adulthood.

Feeding pregnant rats with a PR diet induced hypomethylation of the GR and PPARα promoters in the livers of juvenile and adult offspring, which were associated with increased GR and PPARα mRNA expression and an increased expression of their target genes, namely, phosphoenolpyruvate carboxykinase and *AOX*.[49,100] 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, which facilitate transcription, acetylation of histones H3 and H4, and methylation of histone H3 at lysine K4, whereas those that suppress gene expression were reduced or unchanged.[101] 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 and this persisted in adults.[102] As the altered CpGs 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 the transcriptional 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.[103]

Maternal global undernutrition also induces a phenotype that resembles human metabolic syndrome in the offspring. 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.[54] Thus, the effects of maternal nutrition on the epigenome of the offspring depends on the nature of the maternal nutrient challenge.

In humans, Heijmans *et al.*[104] has reported hypomethylation of the imprinted *IGF-2* gene in genomic DNA isolated

from whole blood from individuals who were exposed to famine *in utero* during the Dutch Hunger Winter compared with unexposed same-sex siblings. The same group also found that[105] IGF-2 was hypomethylated in individuals whose mothers were periconceptually exposed to famine, whereas interleukin-10, leptin, ATP-binding cassette A1, guanine nucleotide-binding protein and maternally expressed 3 (meg3) were hypermethylated.[105]

There is also recent evidence that an overrich early nutritional environment can alter the epigenetic regulation of genes and that this change in gene methylation may have a role in the altered metabolic phenotype of the offspring. Plagemann *et al.*[106] showed that neonatal overfeeding induced by raising rat pups in small litters induces the hypermethylation of two CpG dinucleotides within the pro-opiomelanocortin (POMC) promoter, which are essential for POMC induction by leptin and insulin. Consequently, POMC expression is not upregulated in these rats despite hyperinsulinaemia and hyperleptinaemia. This clearly shows that overfeeding during early postnatal life when the neural network within the hypothalamus is still developing can alter the methylation of POMC, a gene critical for body weight regulation resulting in alterations in appetite and energy homeostasis and an increased prevalence of obesity in later life.

Mechanisms for induced changes in the epigenome. Methylation of CpG dinucleotides *de novo* is catalysed by Dnmts 3a and 3b, and is maintained through mitosis by gene-specific methylation of hemimethylated DNA by Dnmt1.[74] Although DNA methylation is considered as a stable epigenetic mark, a number of DNA demethylases have been proposed, including MBD2b,[107] MBD4,[108] the DNA repair endonucleases XPG (Gadd45a)[109] and a G/T mismatch repair DNA glycosylase.[110] Although the evidence that they fulfill this role is at present very limited, there is clear evidence that demethylase activity exists in cells during development. Active demethylation of paternal genomic DNA in the zygote on fertilization,[87] of the synaptic plasticity gene reelin in the hippocampus on contextual fear conditioning[111] and of interferon-γ on antigen exposure of memory CD8 T cells has been observed.[112] Syzf has also proposed that the methylation status of CpGs in post-mitotic cells may represent an equilibrium state dependent on the relative activities of Dnmt1 and demethylases.[113] Thus, environmental exposures that alter the activity of the Dnmts and/or demethylases provides a possible mechanism by which a change in methylation could occur and, in turn, the level of transcriptional activation of a gene (Figure 2).

Pregnant rats fed with a PR diet show increased blood homocysteine (Hcyst) concentration in early gestation,[114] and as Dnmt1 expression is negatively regulated by Hcyst and increased by folic acid, modulation of Dnmt1 expression by differences in one-carbon metabolism may provide a link between maternal diet and epigenetic regulation of gene expression in the fetus. This is supported by the finding that feeding a PR diet to rats during pregnancy induced a reduction in Dnmt1 expression and in binding of Dnmt1 at the GR promoter.[101] However, the expression of Dnmt3a, Dnmt3b and MBD2, and the binding of Dnmt3a at the GR promoter were unaltered.[101] This suggests that hypomethylation of the GR promoter in the liver of the offspring, and probably other genes including PPARα, is induced by the maternal diet as a result of lower capacity to maintain patterns of cytosine methylation during mitosis. Although a reduction in Dnmt1 might be expected to result in global demethylation, loss of Dnmt1 has been shown to result in only a subset of genes being demethylated.[115] 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.[81–83] Interestingly, hyperglycaemia and

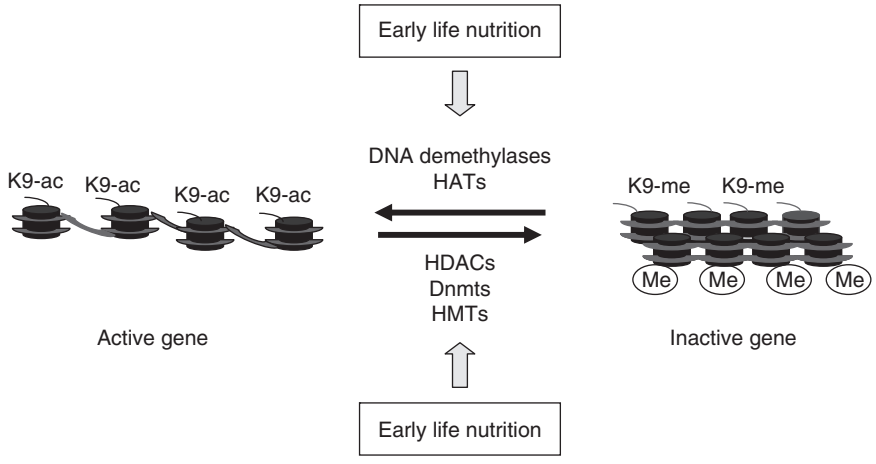

Figure 2 Epigenetic programming by early-life nutrition. Nutrition in early life determines the balance between gene methylation and demethylation. HDACs, HMTs and Dnmts promote histone deactylation, histone K9 and DNA methylation, resulting in a closed chromatin structure and gene silencing. Histone acetyl transferases (HATs) and DNA demethylases induce histone acetylation and DNA demethylation resulting in an open chromatin structure and gene transcription.

hyperinsulinaemia have been reported to enhance Hcyst remethylation, leading to increased intracellular concentrations of *S*-adenosylmethionine and enhanced DNA methyl transferase activity,[116] supporting the findings from previous undernutrition studies that the methylation balance and regulation of DNA methyl transferases are sensitive to nutritional environmental cues.

Prevention and reversal of an altered phenotype and epigenotype. A number of studies have shown that despite the apparent stability of methylation marks, alterations in DNA methylation induced by maternal diet can be prevented and even reversed by interventions even in postnatal life. Supplementation of the maternal PR diet with folic acid prevents hypertension, vascular dysfunction and dyslipidaemia in the adult offspring.[42] Increasing the folic acid content of the PR diet also prevented the hypomethylation of the PPARα and GR promoters and restored the levels of GR and PPAR expression to levels seen in control offspring. Folic acid supplementation of PR diet during pregnancy also upregulated Dnmt1 expression.[49] This suggests that impaired one-carbon metabolism has 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. Interestingly, 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 CpGs were hypermethylated.[102] Thus, increasing maternal folic acid intake does not simply prevent the effects of the PR diet, but may induce subtle changes in gene regulation. Burdge *et al.*[117] have also shown that folate supplementation during the juvenile–pubertal period altered both the phenotype and epigenotype induced by a maternal PR diet. These results showed that in contrast to supplementation of the maternal PR diet with folic acid, supplementation during the juvenile–pubertal period induced impaired lipid homeostasis, including downregulation of hepatic fatty acid β-oxidation, hepatosteatosis and increased weight gain irrespective of the maternal diet. This was associated with altered methylation of specific genes, including hypermethylation of PPARα in the liver and hypomethylation of the insulin receptor in adipose tissue. These findings suggest that the period between weaning and adulthood in rats represents a period of increased epigenetic plasticity, and it may be possible to reverse the adverse effect of prenatal nutrition by nutritional interventions before adulthood, the design of any supplementation regimen for use in humans would need to include careful consideration of the timing and magnitude of the intervention.

Supplementation of the maternal diet with methyl donors also prevented the transgenerational amplification of obesity observed in A^{vy} mice.[68] The effect of methyl supplementation on body weight was independent of epigenetic changes at the A^{vy} locus, suggesting that maternal obesity may alter the epigenetic regulation at other sites within the genome and that methyl donor supplementation during pregnancy can block such epigenetic dysregulation and the propensity to become obese in later life. Altered epigenetic gene regulation may therefore be a key mechanism not only by which the fetus makes adaptations to its metabolism and physiology in response to variations in maternal diet, but may also be part of the mechanism by which maternal obesity can influence the metabolic capacity of the offspring.

Treatment with leptin between postnatal days 3 and 13 of neonatal rats born to dams that experienced 70% global reduction in food intake during pregnancy normalized caloric intake, locomotor activity, body weight, fat mass and fasting plasma glucose, insulin and leptin concentrations in adult offspring, in contrast to saline-treated offspring of undernourished dams that developed all these features on a high-fat diet.[118] This again shows that developmental metabolic programming is potentially reversible by an intervention late in the phase of developmental 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 parallel by effects on methylation and expression of PPARα and GR.[54] This suggests that neonatal leptin intervention may exert its corrective adaptive effects through epigenetic mechanisms.

Conclusions

There is now substantial evidence that early nutritional environmental of the fetus and/or infant can influence susceptibility to obesity in later life and that epigenetic processes have a key role in this pathway. Altered epigenetic regulation has been shown to be induced by both maternal under- and overnutrition within genes that control lipid and carbohydrate metabolism and within genes involved in the central appetite–energy balance neural network. In many cases, the epigenetic status of the same genes is altered both by maternal under- and overnutrition, although the direction of the epigenetic change is different. Although our understanding of how these nutritional cues induce the altered epigenetic regulation of genes is only just beginning, these findings do suggest that the current rapid increase in incidence of obesity may be, in part, explained by fetal and early-life events, which, through epigenetic mechanisms, result in lifelong changes in the metabolism of the offspring. However, despite the apparent stability of these epigenetic marks induced by early nutritional challenges, there is clear evidence that these marks are reversible, and this therefore opens the possibilities that nutritional or pharmacological intervention may combat this rapid rise in obesity.

Conflict of interest

The authors declare no conflict of interest.

References

1 WHO. *Obesity. Preventing and Managing the Global Epidemic*. WHO: Geneva, 1998.
2 Stockard CR. Developmental rate and structural expression: an experimental study of twins, double monsters and single deformities, and the interaction among embryonic organs during their origin and development. *Am J Anat* 1921; **28**: 115–277.
3 Lee TM, Zucker I. Vole infant development is influenced perinatally by maternal photoperiodic history. *Am J Physiol* 1988; **255**: R831–R838.
4 Pener MP, Yerushalmi Y. The physiology of locust phase polymorphism: an update. *J Insect Physiol* 1998; **44**: 365–377.
5 Laforsch C, Tollrian R. Embryological aspects of inducible morphological defenses in *Daphnia*. *J Morphol* 2004; **262**: 701–707.
6 Brent RL. Environmental causes of human congenital malformations: the pediatrician's role in dealing with these complex clinical problems caused by a multiplicity of environmental and genetic factors. *Pediatrics* 2004; **113**: 957–968.
7 Delaval K, Wagschal A, Feil R. Epigenetic deregulation of imprinting in congenital diseases of aberrant growth. *Bioessays* 2006; **28**: 453–459.
8 Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet* 1986; **1**: 1077–1081.
9 Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of *in utero* and early-life conditions on adult health and disease. *N Engl J Med* 2008; **359**: 61–73.
10 Godfrey KM, Barker DJ. Fetal programming and adult health. *Public Health Nutr* 2001; **4**: 611–624.
11 McCance DR, Pettitt DJ, Hanson RL, Jacobsson LT, Knowler WC, Bennett PH. Birth weight and non-insulin dependent diabetes: thrifty genotype, thrifty phenotype, or surviving small baby genotype? *BMJ* 1994; **308**: 942–945.
12 Curhan GC, Willett WC, Rimm EB, Spiegelman D, Ascherio AL, Stampfer MJ. Birth weight and adult hypertension, diabetes mellitus, and obesity in US men. *Circulation* 1996; **94**: 3246–3250.
13 Hanson MA, Gluckman PD. Developmental processes and the induction of cardiovascular function: conceptual aspects. *J Physiol* 2005; **565**: 27–34.
14 Painter RC, Roseboom TJ, Bleker OP. Prenatal exposure to the Dutch famine and disease in later life: an overview. *Reprod Toxicol* 2005; **20**: 345–352.
15 Cleal JK, Poore KR, Newman JP, Noakes DE, Hanson MA, Green LR. The effect of maternal undernutrition in early gestation on gestation length and fetal and postnatal growth in sheep. *Pediatr Res* 2007; **62**: 422–427.
16 Poissonnet CM, LaVelle M, Burdi AR. Growth and development of adipose tissue. *J Pediatr* 1988; **113**: 1–9.
17 Catalano PM, Kirwan JP. Maternal factors that determine neonatal size and body fat. *Curr Diab Rep* 2001; **1**: 71–77.
18 Rolland-Cachera MF, Deheeger M, Bellisle F, Sempe M, Guilloud-Bataille M, Patois E. Adiposity rebound in children: a simple indicator for predicting obesity. *Am J Clin Nutr* 1984; **39**: 129–135.
19 Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. *BMJ* 2000; **320**: 967–971.
20 Ong KK. Size at birth, postnatal growth and risk of obesity. *Horm Res* 2006; **65**: 65–69.
21 Singhal A, Cole TJ, Fewtrell M, Lucas A. Breastmilk feeding and lipoprotein profile in adolescents born preterm: follow-up of a prospective randomised study. *Lancet* 2004; **363**: 1571–1578.
22 Singhal A. Early nutrition and long-term cardiovascular health. *Nutr Rev* 2006; **64**: S44–S49.
23 Harder T, Bergmann R, Kallischnigg G, Plagemann A. Duration of breastfeeding and risk of overweight: a meta-analysis. *Am J Epidemiol* 2005; **162**: 397–403.
24 Owen CG, Martin RM, Whincup PH, Smith GD, Cook DG. Effect of infant feeding on the risk of obesity across the life course: a quantitative review of published evidence. *Pediatrics* 2005; **115**: 1367–1377.
25 Owen CG, Martin RM, Whincup PH, vey-Smith G, Gillman MW, Cook DG. The effect of breastfeeding on mean body mass index throughout life: a quantitative review of published and unpublished observational evidence. *Am J Clin Nutr* 2005; **82**: 1298–1307.
26 Gluckman PD, Hanson MA. Evolution, development and timing of puberty. *Trends Endocrinol Metab* 2006; **17**: 7–12.
27 Dorner G, Plagemann A. Perinatal hyperinsulinism as possible predisposing factor for diabetes mellitus, obesity and enhanced cardiovascular risk in later life. *Horm Metab Res* 1994; **26**: 213–221.
28 Oken E, Taveras EM, Kleinman KP, Rich-Edwards JW, Gillman MW. Gestational weight gain and child adiposity at age 3 years. *Am J Obstet Gynecol* 2007; **196**: 322–328.
29 Villamor E, Cnattingius S. Interpregnancy weight change and risk of adverse pregnancy outcomes: a population-based study. *Lancet* 2006; **368**: 1164–1170.
30 Kral JG, Biron S, Simard S, Hould FS, Lebel S, Marceau S *et al*. Large maternal weight loss from obesity surgery prevents transmission of obesity to children who were followed for 2–18 years. *Pediatrics* 2006; **118**: 1644–1649.
31 Hillier TA, Pedula KL, Schmidt MM, Mullen JA, Charles MA, Pettitt DJ. Childhood obesity and metabolic imprinting: the ongoing effects of maternal hyperglycemia. *Diabetes Care* 2007; **30**: 2287–2292.
32 Silverman BL, Rizzo TA, Cho NH, Metzger BE. Long-term effects of the intrauterine environment. The Northwestern University Diabetes in Pregnancy Center. *Diabetes Care* 1998; **21**: B142–B149.
33 Boney CM, Verma A, Tucker R, Vohr BR. Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. *Pediatrics* 2005; **115**: 290–296.
34 Langley-Evans SC, Phillips GJ, Jackson AA. *In utero* exposure to maternal low protein diets induces hypertension in weanling rats, independently of maternal blood pressure changes. *Clin Nutr* 1994; **13**: 319–324.
35 Lucas A, Baker BA, Desai M, Hales CN. Nutrition in pregnant or lactating rats programs lipid metabolism in the offspring. *Br J Nutr* 1996; **76**: 605–612.
36 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.
37 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 (Lond)* 2006; **30**: 729–738.
38 Burdge GC, Slater-Jefferies JL, Grant RA, Chung WS, West AL, Lillycrop KA *et al*. Sex, but not maternal protein or folic acid intake, determines the fatty acid composition of hepatic phospholipids, but not of triacylglycerol, in adult rats. *Prostaglandins Leukot Essent Fatty Acids* 2008; **78**: 73–79.
39 Torrens C, Brawley L, Anthony FW, Dance CS, Dunn R, Jackson AA *et al*. Folate supplementation during pregnancy improves offspring cardiovascular dysfunction induced by protein restriction. *Hypertension* 2006; **47**: 982–987.
40 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.

41 Langley-Evans SC, Sculley DV. Programming of hepatic antioxidant capacity and oxidative injury in the ageing rat. *Mech Ageing Dev* 2005; **126**: 804–812.
42 Burdge GC, Lillycrop KA, Jackson AA, Gluckman PD, Hanson MA. The nature of the growth pattern and of the metabolic response to fasting in the rat are dependent upon the dietary protein and folic acid intakes of their pregnant dams and post-weaning fat consumption. *Br J Nutr* 2008; **99**: 540–549.
43 Burdge GC, Hanson MA, Slater-Jefferies JL, Lillycrop KA. Epigenetic regulation of transcription: a mechanism for inducing variations in phenotype (fetal programming) by differences in nutrition during early life? *Br J Nutr* 2007; **97**: 1036–1046.
44 Bertram C, Trowern AR, Copin N, Jackson AA, Whorwood CB. The maternal diet during pregnancy programs altered expression of the glucocorticoid receptor and type 2 11beta-hydroxysteroid dehydrogenase: potential molecular mechanisms underlying the programming of hypertension *in utero*. *Endocrinology* 2001; **142**: 2841–2853.
45 Burns SP, Desai M, Cohen RD, Hales CN, Iles RA, Germain JP *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.
46 Maloney CA, Gosby AK, Phuyal JL, Denyer GS, Bryson JM, Caterson ID. Site-specific changes in the expression of fat-partitioning genes in weanling rats exposed to a low-protein diet *in utero*. *Obes Res* 2003; **11**: 461–468.
47 Burdge GC, Delange E, Dubois L, Dunn RL, Hanson MA, Jackson AA *et al*. Effect of reduced maternal protein intake in pregnancy in the rat on the fatty acid composition of brain, liver, plasma, heart and lung phospholipids of the offspring after weaning. *Br J Nutr* 2003; **90**: 345–352.
48 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.
49 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.
50 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.
51 Woodall SM, Johnston BM, Breier BH, Gluckman PD. Chronic maternal undernutrition in the rat leads to delayed postnatal growth and elevated blood pressure of offspring. *Pediatr Res* 1996; **40**: 438–443.
52 Roseboom T, de RS, Painter R. The Dutch famine and its long-term consequences for adult health. *Early Hum Dev* 2006; **82**: 485–491.
53 Kind KL, Clifton PM, Katsman AI, Tsiounis M, Robinson JS, Owens JA. Restricted fetal growth and the response to dietary cholesterol in the guinea pig. *Am J Physiol* 1999; **277**: R1675–R1682.
54 Gluckman PD, Lillycrop KA, Vickers MH, Pleasants AB, Phillips ES, Beedle AS *et al*. Metabolic plasticity during mammalian development is directionally dependent on early nutritional status. *Proc Natl Acad Sci USA* 2007; **104**: 12796–12800.
55 Ozanne SE, Hales CN. Lifespan: catch-up growth and obesity in male mice. *Nature* 2004; **427**: 411–412.
56 Zambrano E, Bautista CJ, Deas M, Martinez-Samayoa PM, Gonzalez-Zamorano M, Ledesma H *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**: 221–230.
57 Sloboda DM, Howie GJ, Pleasants A, Gluckman PD, Vickers MH. Pre- and postnatal nutritional histories influence reproductive maturation and ovarian function in the rat. *PLoS ONE* 2009; **4**: e6744.
58 Samuelsson AM, Matthews PA, Argenton M, Christie MR, McConnell JM, Jansen EH *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.
59 Bruce KD, Cagampang FR, Argenton M, Zhang J, Ethirajan PL, Burdge GC *et al*. Maternal high-fat feeding primes steatohepatitis in adult mice offspring, involving mitochondrial dysfunction and altered lipogenesis gene expression. *Hepatology* 2009; **50**: 1796–1808.
60 Khan I, Dekou V, Hanson M, Poston L, Taylor P. Predictive adaptive responses to maternal high-fat diet prevent endothelial dysfunction but not hypertension in adult rat offspring. *Circulation* 2004; **110**: 1097–1102.
61 Korotkova M, Gabrielsson BG, Holmang A, Larsson BM, Hanson LA, Strandvik B. Gender-related long-term effects in adult rats by perinatal dietary ratio of n-6/n-3 fatty acids. *Am J Physiol Regul Integr Comp Physiol* 2005; **288**: R575–R579.
62 Kozak R, Burlet A, Burlet C, Beck B. Dietary composition during fetal and neonatal life affects neuropeptide Y functioning in adult offspring. *Brain Res Dev Brain Res* 2000; **125**: 75–82.
63 Khan IY, Dekou V, Douglas G, Jensen R, Hanson MA, Poston L *et al*. A high-fat diet during rat pregnancy or suckling induces cardiovascular dysfunction in adult offspring. *Am J Physiol Regul Integr Comp Physiol* 2005; **288**: R127–R133.
64 Plagemann A. Perinatal programming and functional teratogenesis: impact on body weight regulation and obesity. *Physiol Behav* 2005; **86**: 661–668.
65 Plagemann A, Harder T, Rake A, Melchior K, Rohde W, Dorner G. Increased number of galanin-neurons in the paraventricular hypothalamic nucleus of neonatally overfed weanling rats. *Brain Res* 1999; **818**: 160–163.
66 Schmidt I, Fritz A, Scholch C, Schneider D, Simon E, Plagemann A. The effect of leptin treatment on the development of obesity in overfed suckling Wistar rats. *Int J Obes Relat Metab Disord* 2001; **25**: 1168–1174.
67 Franke K, Harder T, Aerts L, Melchior K, Fahrenkrog S, Rodekamp E *et al*. 'Programming' of orexigenic and anorexigenic hypothalamic neurons in offspring of treated and untreated diabetic mother rats. *Brain Res* 2005; **1031**: 276–283.
68 Waterland RA, Travisano M, Tahiliani KG, Rached MT, Mirza S. Methyl donor supplementation prevents transgenerational amplification of obesity. *Int J Obes (Lond)* 2008; **32**: 1373–1379.
69 Muhlhausler BS. Programming of the appetite-regulating neural network: a link between maternal overnutrition and the programming of obesity? *J Neuroendocrinol* 2007; **19**: 67–72.
70 Davidowa H, Plagemann A. Decreased inhibition by leptin of hypothalamic arcuate neurons in neonatally overfed young rats. *Neuroreport* 2000; **11**: 2795–2798.
71 Plagemann A, Harder T, Rake A, Janert U, Melchior K, Rohde W *et al*. Morphological alterations of hypothalamic nuclei due to intrahypothalamic hyperinsulinism in newborn rats. *Int J Dev Neurosci* 1999; **17**: 37–44.
72 Plagemann A, Harder T, Melchior K, Rake A, Rohde W, Dorner G. Elevation of hypothalamic neuropeptide Y-neurons in adult offspring of diabetic mother rats. *Neuroreport* 1999; **10**: 3211–3216.
73 Goldberg AD, Allis CD, Bernstein E. Epigenetics: a landscape takes shape. *Cell* 2007; **128**: 635–638.
74 Bird A. DNA methylation patterns and epigenetic memory. *Genes Dev* 2002; **16**: 6–21.
75 Razin A, Szyf M. DNA methylation patterns. Formation and function. *Biochim Biophys Acta* 1984; **782**: 331–342.

76 Bird A, Macleod D. Reading the DNA methylation signal. *Cold Spring Harb Symp Quant Biol* 2004; **69**: 113–118.
77 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 USA* 1999; **96**: 14967–14972.
78 Lachner M, O'Carroll D, Rea S, Mechtler K, Jenuwein T. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* 2001; **410**: 116–120.
79 Zegerman P, Canas B, Pappin D, Kouzarides T. Histone H3 lysine 4 methylation disrupts binding of nucleosome remodeling and deacetylase (NuRD) repressor complex. *J Biol Chem* 2002; **277**: 11621–11624.
80 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.
81 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.
82 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.
83 Vire E, Brenner C, Deplus R, Blanchon L, Fraga M, Didelot C *et al*. The Polycomb group protein EZH2 directly controls DNA methylation. *Nature* 2006; **439**: 871–874.
84 Li E, Beard C, Jaenisch R. Role for DNA methylation in genomic imprinting. *Nature* 1993; **366**: 362–365.
85 Waterland RA, Jirtle RL. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol* 2003; **23**: 5293–5300.
86 Walsh CP, Chaillet JR, Bestor TH. Transcription of IAP endogenous retroviruses is constrained by cytosine methylation. *Nat Genet* 1998; **20**: 116–117.
87 Reik W, Dean W, Walter J. Epigenetic reprogramming in mammalian development. *Science* 2001; **293**: 1089–1093.
88 Gidekel S, Bergman Y. A unique developmental pattern of Oct-3/4 DNA methylation is controlled by a cis-demodification element. *J Biol Chem* 2002; **277**: 34521–34530.
89 Hershko AY, Kafri T, Fainsod A, Razin A. Methylation of HoxA5 and HoxB5 and its relevance to expression during mouse development. *Gene* 2003; **302**: 65–72.
90 Benvenisty N, Szyf M, Mencher D, Razin A, Reshef L. Tissue-specific hypomethylation and expression of rat phosphoenolpyruvate carboxykinase gene induced by *in vivo* treatment of fetuses and neonates with 5-azacytidine. *Biochemistry* 1985; **24**: 5015–5019.
91 Grainger RM, Hazard-Leonards RM, Samaha F, Hougan LM, Lesk MR, Thomsen GH. Is hypomethylation linked to activation of delta-crystallin genes during lens development? *Nature* 1983; **306**: 88–91.
92 Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR *et al*. Epigenetic programming by maternal behavior. *Nat Neurosci* 2004; **7**: 847–854.
93 Weaver IC, Champagne FA, Brown SE, Dymov S, Sharma S, Meaney MJ *et al*. Reversal of maternal programming of stress responses in adult offspring through methyl supplementation: altering epigenetic marking later in life. *J Neurosci* 2005; **25**: 11045–11054.
94 Pham TD, MacLennan NK, Chiu CT, Laksana GS, Hsu JL, Lane RH. Uteroplacental insufficiency increases apoptosis and alters p53 gene methylation in the full-term IUGR rat kidney. *Am J Physiol Regul Integr Comp Physiol* 2003; **285**: R962–R970.
95 Doherty AS, Mann MR, Tremblay KD, Bartolomei MS, Schultz RM. Differential effects of culture on imprinted H19 expression in the preimplantation mouse embryo. *Biol Reprod* 2000; **62**: 1526–1535.
96 Khosla S, Dean W, Reik W, Feil R. Culture of preimplantation embryos and its long-term effects on gene expression and phenotype. *Hum Reprod Update* 2001; **7**: 419–427.
97 Cox GF, Burger J, Lip V, Mau UA, Sperling K, Wu BL *et al*. Intracytoplasmic sperm injection may increase the risk of imprinting defects. *Am J Hum Genet* 2002; **71**: 162–164.
98 DeBaun MR, Niemitz EL, Feinberg AP. Association of *in vitro* fertilization with Beckwith-Wiedemann syndrome and epigenetic alterations of LIT1 and H19. *Am J Hum Genet* 2003; **72**: 156–160.
99 Wolff GL, Kodell RL, Moore SR, Cooney CA. Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. *FASEB J* 1998; **12**: 949–957.
100 Burdge GC, Slater-Jefferies J, Torrens C, Phillips ES, Hanson MA, Lillycrop KA. Dietary protein restriction of pregnant rats in the F0 generation induces altered methylation of hepatic gene promoters in the adult male offspring in the F1 and F2 generations. *Br J Nutr* 2007; **97**: 435–439.
101 Lillycrop KA, Slater-Jefferies JL, Hanson MA, Godfrey KM, Jackson AA, Burdge GC. 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.
102 Lillycrop KA, Phillips ES, Torrens C, Hanson MA, Jackson AA, Burdge GC. Feeding pregnant rats a protein-restricted diet persistently alters the methylation of specific cytosines in the hepatic PPARalpha promoter of the offspring. *Br J Nutr* 2008; **100**: 278–282.
103 Bogdarina I, Welham S, King PJ, Burns SP, Clark AJ. Epigenetic modification of the renin-angiotensin system in the fetal programming of hypertension. *Circ Res* 2007; **100**: 520–526.
104 Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES *et al*. Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc Natl Acad Sci USA* 2008; **105**: 17046–17049.
105 Tobi EW, Lumey LH, Talens RP, Kremer D, Putter H, Stein AD *et al*. DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. *Hum Mol Genet* 2009; **18**: 4046–4053.
106 Plagemann A, Harder T, Brunn M, Harder A, Roepke K, Wittrock-Staar 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**: 4963–4976.
107 Bhattacharya SK, Ramchandani S, Cervoni N, Szyf M. A mammalian protein with specific demethylase activity for mCpG DNA. *Nature* 1999; **397**: 579–583.
108 Zhu B, Zheng Y, Angliker H, Schwarz S, Thiry S, Siegmann M *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.
109 Barreto G, Schafer A, Marhold J, Stach D, Swaminathan SK, Handa V *et al*. Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. *Nature* 2007; **445**: 671–675.
110 Jost JP. Nuclear extracts of chicken embryos promote an active demethylation of DNA by excision repair of 5-methyldeoxycytidine. *Proc Natl Acad Sci USA* 1993; **90**: 4684–4688.
111 Miller CA, Sweatt JD. Covalent modification of DNA regulates memory formation. *Neuron* 2007; **53**: 857–869.
112 Kersh EN, Fitzpatrick DR, Murali-Krishna K, Shires J, Speck SH, Boss JM *et al*. Rapid demethylation of the IFN-gamma gene occurs in memory but not naive CD8 T cells. *J Immunol* 2006; **176**: 4083–4093.
113 Szyf M. The dynamic epigenome and its implications in toxicology. *Toxicol Sci* 2007; **100**: 7–23.
114 Petrie L, Duthie SJ, Rees WD, McConnell JM. Serum concentrations of homocysteine are elevated during early pregnancy in rodent models of fetal programming. *Br J Nutr* 2002; **88**: 471–477.

115 Jackson-Grusby L, Beard C, Possemato R, Tudor M, Fambrough D, Csankovszki G *et al*. Loss of genomic methylation causes p53-dependent apoptosis and epigenetic deregulation. *Nat Genet* 2001; **27**: 31–39.
116 Chiang PK, Gordon RK, Tal J, Zeng GC, Doctor BP, Pardhasaradhi K *et al*. S-Adenosylmethionine and methylation. *FASEB J* 1996; **10**: 471–480.
117 Burdge GC, Lillycrop KA, Phillips ES, Slater-Jefferies JL, Jackson AA, Hanson MA. 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.
118 Vickers MH, Gluckman PD, Coveny AH, Hofman PL, Cutfield WS, Gertler A *et al*. Neonatal leptin treatment reverses developmental programming. *Endocrinology* 2005; **146**: 4211–4216.