

REVIEW

Diet induced epigenetic changes and their implications for health

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Abstract

Dietary exposures can have consequences for health years or decades later and this raises questions about the mechanisms through which such exposures are ‘remembered’ and how they result in altered disease risk. There is growing evidence that epigenetic mechanisms may mediate the effects of nutrition and may be causal for the development of common complex (or chronic) diseases. Epigenetics encompasses changes to marks on the genome (and associated cellular machinery) that are copied from one cell generation to the next, which may alter gene expression, but which do not involve changes in the primary DNA sequence. These include three distinct, but closely inter-acting, mechanisms including DNA methylation, histone modifications and non-coding microRNAs (miRNA) which, together, are responsible for regulating gene expression not only during cellular differentiation in embryonic and foetal development but also throughout the life-course. This review summarizes the growing evidence that numerous dietary factors, including micronutrients and non-nutrient dietary components such as genistein and polyphenols, can modify epigenetic marks. In some cases, for example, effects of altered dietary supply of methyl donors on DNA methylation, there are plausible explanations for the observed epigenetic changes, but to a large extent, the mechanisms responsible for diet–epigenome–health relationships remain to be discovered. In addition, relatively little is known about which epigenomic marks are most labile in response to dietary exposures. Given the plasticity of epigenetic marks and their responsiveness to dietary factors, there is potential for the development of epigenetic marks as biomarkers of health for use in intervention studies.

Keywords DNA methylation, epigenetics, histone modifications, nutrition.

Chronic diseases including cardiovascular disease, diabetes and cancers account for more than half of the deaths worldwide and are responsible for a growing proportion of the global burden of disease (World Health Organisation, 2003). Dietary and other lifestyle factors are the major determinants of obesity and of the risk of chronic diseases, many of which are more prevalent in the obese (Report 2007, Whitlock *et al.* 2009), and so offer attractive targets for effective interventions to reduce the morbidity and mortality due to chronic diseases (Danaei *et al.* 2009). In the

developing world, poorer communities suffer from the double burden of malnutrition and, increasingly, chronic diseases (World Health Organisation, 2003). Maternal malnutrition and other insults in pregnancy reduce foetal growth and increase the likelihood of low birth weight. In addition to adverse effects on survival, growth and physical and mental capacity in childhood (de Onis *et al.* 1998), intrauterine growth retardation, especially when following by greater adiposity in childhood, increases the risk of several chronic diseases (Bhargava *et al.* 2004, Gluckman *et al.* 2008). At least

in an animal model, such prenatal under-nutrition in combination with an obesity-inducing diet post-weaning, reduces lifespan (Ozanne & Hales 2004). In addition, these dietary and other lifestyle factors interact with genetic susceptibility to determine health outcomes i.e. there is phenotypic plasticity in the context of a fixed genotype (Mathers 2002).

These observations demonstrate that dietary exposures can have long-term consequences for health and raise questions about the mechanisms through which early life exposures are ‘remembered’ over long-time periods and how they result in altered disease risk. Poor nutrition *in utero* may result in inadequate development of specific cells and tissues e.g. adverse intrauterine environments lead to the production of fewer nephrons and increase the risk of chronic kidney disease in later life (Vehaskari 2010). In addition, there is growing evidence that epigenetic mechanisms may mediate the effects of nutrition and may be causal for the development of common complex (or chronic) diseases (Petroinis 2010). We have developed the concept of the four ‘Rs’ of nutritional epigenomics to encapsulate the main events/processes linking dietary exposures to epigenetic marks and eventually to health outcomes (Mathers 2008) (see Fig. 1). In this model, nutritional and other exposures are **Received** and **Recorded** by the genome, evidence of these exposures is **Remembered** across successive cell generations and the consequences of these exposures are **Revealed** as altered gene expression, cell function and, ultimately, health (Mathers 2008).

Epigenetic marks

Epigenetics encompasses changes to marks on the genome (and associated cellular machinery) that are copied from one cell generation to the next, which may alter gene expression, but which do not involve changes in the primary DNA sequence. The totality of such marks on the genome is described as the epigenome and

comprises three distinct, but closely inter-acting, mechanisms including DNA methylation, histone modifications and non-coding microRNAs (miRNA) (Goldberg *et al.* 2007, Link *et al.* 2010). The combination of these marks and miRNA is responsible for regulating gene expression not only during cellular differentiation in embryonic and foetal development (Reik 2007) but also throughout the life-course.

DNA methylation

The vast majority of methylation marks on DNA are found on the 5′ position of cytosine residues where the cytosine is followed by a guanine residue in the 5′ to 3′ direction i.e. a so-called CpG dinucleotide. The human genome contains both CpG-rich and CpG-poor regions and these dinucleotides are the only ones that occur less frequently than expected in DNA (Jones & Liang 2009). Although the majority of cytosine residues within CpG dinucleotides are methylated some, particularly those in so-called CpG islands within the promoter regions of house-keeping genes, are normally unmethylated. There is growing evidence that DNA methylation patterns are tissue specific (Ollikainen *et al.* 2010, Schneider *et al.* 2010). DNA methylation is involved in several key physiological processes, including X chromosome inactivation, imprinting and the silencing of germline-specific genes and repetitive elements. These methylation marks are added to DNA by enzymes of the DNA methyltransferase (DNMT) family using S-adenosylmethionine (SAM) as the methyl donor. The conventional view has been that addition of methyl group *de novo* e.g. during embryogenesis is catalyzed by DNMT3A and DNMT3B whereas maintenance of DNA methylation patterns during cell replication uses DNMT1. A new model for maintenance of DNA methylation patterns proposes that, in addition to DNMT1 copying the bulk of methylation marks on the parental strand to the daughter strand,

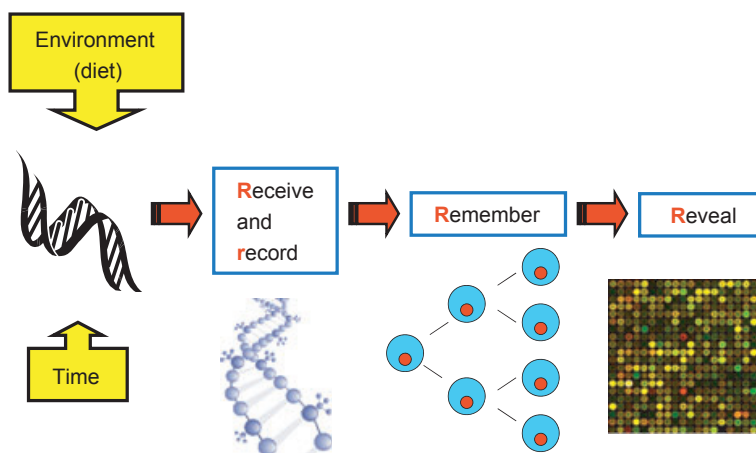


Figure 1 The four ‘Rs’ of nutritional epigenomics. This is a conceptual model of the key processes through which altered epigenomics markings as a result of dietary (and other environmental) exposures are Received, Recorded, Remembered and Revealed (from Mathers 2008).

soon after replication, DNMT3A and DNMT3B complete the methylation process and, in particular, correct errors left by DNMT1 (Jones & Liang 2009).

Recently, another methylation mark has been discovered on DNA viz 5-hydroxymethyl-2'-deoxycytidine (hmdC). hmdC may be particularly important in the brain where it has been reported to constitute 0.6% of total nucleotides in Purkinje cells (Kriaucionis & Heintz 2009). The origin of this hmdC is not known but (Kriaucionis & Heintz 2009) suggest that it may play a role in epigenetic control of neuronal function.

Histone modifications

Within the nucleus, DNA is packaged by sophisticated wrapping around an octet of globular proteins, known as histones, which contains two copies of each of the four core histones (H3, H4, H2A, H2B) (Kouzarides 2007). The tails of these histones which protrude from the globular cores host further epigenetic marks in the form of post-translational modification of specific amino acid residues, including acetylation and ubiquitination of lysine residues, phosphorylation of serines, and methylation of lysine and arginines (Berger 2007). There are more than 100 distinct post-translational modifications of histones (Kouzarides 2007). These individual histone modifications and/or patterns of modifications, described as 'histone decoration', are believed to constitute a histone code (Jenuwein & Allis 2001) which, in conjunction with DNA methylation status and the presence or absence of specific miRNA, regulates the expression of associated genes (Bernstein *et al.* 2007). This complex super-structure which overlays the primary genetic information in DNA allows sophisticated control of gene expression according to cell and tissue location, time and environment with major consequences for cell fate decisions, for both normal and pathological development (Jenuwein & Allis 2001) and for ageing (Mathers 2006).

MicroRNA

MicroRNA (miRNA) are a large family of small (≈ 22 nucleotides long) non-coding RNAs. There are at least 1046 miRNA in human genome and these are encoded on all human chromosomes ranging from 94 miRNA on chromosome X to 12 on chromosome 21 [data from miRBase (accessed 21 December 2010)] (Griffiths-Jones *et al.* 2008). These miRNA regulate transcription of $\approx 30\%$ of all protein-encoding genes through sequence-specific binding to RNA (Esquela-Kerscher & Slack 2006). Each miRNA binds to the 3'-untranslated region of the target mRNA and suppresses expression by affecting mRNA stability and/or targeting the mRNA for degradation (Esquela-Kerscher & Slack 2006). By

controlling the expression of about one-third of human mRNAs, miRNA may influence almost all genetic pathways by targeting transcription factors, secreted factors, receptors and transporters (Esquela-Kerscher & Slack 2006). Evidence is now emerging that a wide range of dietary factors ranging from macronutrients (fat, protein and alcohol) to micronutrients (vitamin E) alter expression of many miRNA in rodents and in humans [reviewed by (Mathers *et al.* 2010)]. It is now apparent that the genomic sequences encoding miRNA are polymorphic (Ryan *et al.* 2010) which adds a further layer of complexity to the interactions between the genome and the epigenetic machinery which regulate gene expression.

Epigenetic regulation of gene expression

To optimize function, cells need to express particular consortia of genes which are (i) characteristic of that cell lineage, (ii) appropriate for the developmental state of the organism and (iii) responsive to intrinsic and environmental signals (Jaenisch & Bird 2003). In addition, cells need to suppress viral genomes (Jaenisch & Bird 2003) and other potentially hazardous sequences which have become integrated into the human genome over evolutionary time. More than 3 decades ago, (Holliday & Pugh 1975) proposed that DNA methylation might be responsible for the stable maintenance of particular gene expression patterns and could explain how these patterns persist between one cell generation and the next i.e. how patterns of gene expression survive mitosis intact (Jaenisch & Bird 2003). It is now clear that epigenetic mechanisms (DNA methylation, histone modifications, miRNA and associated proteins) combine to provide a robust, yet responsive, system for controlling gene expression. In essence, DNA methylation and miRNA signal transcriptional silencing of associated genes whereas specific histone modifications and their associated proteins may be characteristic of either gene silencing or gene expression. The implications for transcription of many histone marks remain uncertain but there is good evidence that deacetylation of histones together with methylation of lysine residue 9 on histone H3 (H3K9) and of H3K27 are associated with silencing of gene expression (Delage & Dashwood 2008). In contrast, acetylation of histones and demethylation of H3K9 and H3K27 together with methylation of H3K4 is observed with active transcription of the associated gene (Delage & Dashwood 2008). Zaidi *et al.* (2010) have proposed the term 'architectural epigenetics' to describe the combination of mitotically inheritable epigenetic mechanisms which controls patterns of gene expression. This appears to have similarities with the concept of regulation of the chromatin landscape which is described by

Margueron & Reinberg (2010). Although there is now convincing proof of principle that epigenetic mechanisms are key regulators of gene transcription, with particular significance in responses to altered environmental signals (Jaenisch & Bird 2003), there are still considerable areas of ignorance about the roles of specific marks, proteins and their interactions.

Diet and epigenetic modifications

Over recent years there has been growing evidence that dietary factors may modify epigenetic marks (Davis & Uthus 2004, Mathers & Ford 2009). Whilst dietary habits in humans or strategies, such as feeding high fat, low protein or energy restricted diets in animal models have been shown to alter epigenetic marks (Hass *et al.* 1993, Miyamura *et al.* 1993, Rees *et al.* 2000, Lillycrop *et al.* 2005, 2008, Brait *et al.* 2009, van Straten *et al.* 2010, Widiker *et al.* 2010), much of the focus on effects of diet on epigenetic patterns has been restricted to specific nutrients, including micronutrients and to other non-nutrient dietary components such as genistein or polyphenols. Some of this work has been empirical – authors have investigated possible changes in epigenetic marks in response to, or in association with, diet – whilst in other cases specific nutrients have been

investigated to test hypothesized mechanisms through which the nutrient could affect epigenetic markings.

Gabory *et al.* (2009) propose that environmental, including dietary factors, may induce epigenetic changes via three possible mechanistic pathways. These are (i) activation/inhibition of chromatin machinery, (ii) activation of nuclear receptor by ligands and (iii) membrane receptor signalling cascades. The following section reviews known and proposed mechanisms through which nutrients may alter DNA methylation and histone modifications.

Mechanisms involved in nutritional modulation of DNA methylation

Broadly, there are two mechanisms through which nutritional and dietary factors have been postulated to effect DNA methylation. These are (i) changing the availability of methyl donors and (ii) altering the activity of enzymes involved in the process of DNA methylation, more specifically the DNMT enzymes. The most widely investigated of these is the influence of nutrients on methyl group supply for one-carbon metabolism (Fig. 2). One-carbon metabolism is central to the methylation of all biological molecules including DNA (Fig. 2), therefore any dietary factors influencing

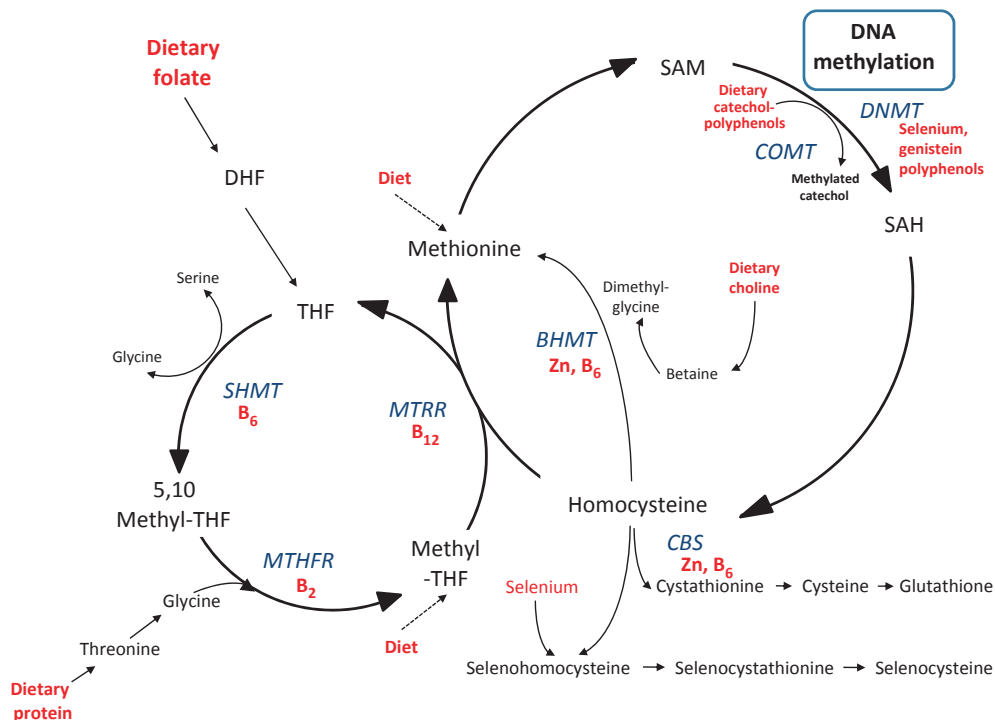


Figure 2 Summary diagram of the main pathways involved in cellular one-carbon metabolism and which includes the production of S-adenosylmethionine (SAM) for methylation of DNA. The nutrients, and other dietary components, which have potential to modulate epigenetic marks through affecting the availability of SAM are illustrated in red.

this pathway may affect DNA methylation. Strong evidence suggests that several nutrients can affect DNA methylation in this way (reviewed in Johnson & Belshaw 2008, Mathers & Ford 2009).

The nutrients implicated in this mechanism are the methyl donors and those micronutrients which are co-factors for enzymes involved in one-carbon metabolism including folate, vitamin B₆, vitamin B₁₂, choline and methionine. Indeed, all these nutrients are integral to the proper functioning of one-carbon metabolism such that, a deficiency or excess of any of these could alter the availability of S-adenosylmethionine (SAM) from the methionine cycle (Fig. 2). Aberrations in the availability of SAM could alter DNA methylation directly, as SAM is the methyl donor for DNA methylation.

However, other nutrients and other dietary components can affect one-carbon metabolism indirectly. For example, in rats, dietary selenium has been reported to cause an imbalance in the methylation cycle by decreasing homocysteine concentrations (Davis & Uthus 2003). The proposed mechanism is via the conversion of homocysteine to selenohomocysteine, cystathionine, selenocystathionine, glutathione and selenocysteine, thus reducing homocysteine availability for methionine

cycle and leading to reduced global DNA methylation (Davis *et al.* 2000) (Fig. 2). Furthermore, dietary protein can also affect the one-carbon pool by its impact on threonine concentrations (Fig. 2). This may alter the SAM:SAH ratio, via altered threonine>glycine>Methyl THF conversion (van Straten *et al.* 2010). Green tea polyphenols are also thought to affect DNA methylation by altering the SAM:SAH ratio. Catechol-containing dietary polyphenols such as epigallocatechin-3-O-gallate (EGCG) are excellent substrates for COMT-mediated O-methylation (Zhu *et al.* 1994, 2000, 2001, Zhu & Liehr 1996). COMT-mediated O-methylation also utilizes SAM as a substrate, therefore when O-methylation is increased due to increased catechol-containing dietary polyphenols, there is a subsequent decrease in available SAM for DNA methylation (Lee *et al.* 2005). In addition, dietary zinc intake may modulate DNA methylation via its impact upon the enzymes involved in the one-carbon metabolism. For example, betaine-homocysteine S-methyltransferase (BHMT) and cystathionine synthase (CBS) are zinc metalloenzymes, therefore changes in zinc availability may affect the activity of these enzymes and thus may alter homocysteine concentrations (Fig. 2)(Mathers & Ford 2009).

Table 1 Summary of effects of selected dietary factors on DNA methylation in human intervention studies

Nutrient/Dietary component	Observation	Reference
Folic acid	In patients with colon cancer and adenoma, 10 mg folic acid per day supplementation for 6 months increased rectal mucosa genomic DNA methylation compared with the placebo group	(Cravo <i>et al.</i> 1994)
	5 mg folic acid per day supplementation for 3 months increased rectal mucosa genomic DNA methylation significantly in patients with one adenoma, but not in those with multiple adenoma	(Cravo <i>et al.</i> 1998)
	5 mg folic acid per day supplementation for 6 months and 1 year increased genomic DNA methylation in the rectal mucosa in colonic adenoma patients, however placebo also increased methylation after 1 year	(Kim <i>et al.</i> 2001)
	400 µg per day folic acid supplementation for 10 weeks increased colonic mucosa and leucocyte genomic DNA methylation significantly in colonic adenoma patients	(Pufulete <i>et al.</i> 2005a)
	In post-menopausal women, moderate folate depletion induced by a low folate diet caused decrease genomic DNA methylation in lymphocytes. DNA hypomethylation was reversed with folate repletion	(Jacob <i>et al.</i> 1998)
	Moderate folate depletion by reduced folate intake in elderly women caused decreased genomic DNA methylation in leucocytes. No changes were observed in DNA methylation after folate repletion	(Rampersaud <i>et al.</i> 2000)
Folic acid and Vitamin B ₁₂	Increased methylation of promoters of a number of genes investigated in colorectal adenoma patients supplemented with 5 mg folic acid and 1.25 mg vitamin B ₁₂ for 6 months	(van den Donk <i>et al.</i> 2007)
Isoflavones	In premenopausal women, supplementary isoflavones induced dose-specific changes in mammary RARβ2 and CCND2 gene methylation, but not p16, RASSF1A, or ER	(Qin <i>et al.</i> 2009)

Altering the activity of the DNMT enzymes is the second mechanism by which dietary factors including selenium, genistein and green tea polyphenols have been postulated to alter DNA methylation. For example, DNMT1 normally binds to DNA at DNA-AP1 transcription factor complexes. However, selenite inhibits the binding of AP-1 to DNA, thus reducing the ability of DNMT1 to bind to DNA, which in turn could lead to reduced DNA methylation (Handel *et al.* 1995, Spyrou *et al.* 1995). Furthermore, selenium has also been reported to reduce DNMT expression, which would be expected to reduce the capacity for DNA methylation (Xiang *et al.* 2008). Genistein has been implicated in altering DNMT activity via its effect on oestrogen receptor-dependent processes (Fang *et al.* 2005). It has been hypothesized that genistein could trigger membrane mediated oestrogenic actions inducing c-fos to up-regulate DNMT1 transcription directly (Hyder *et al.* 1992, Bakin & Curran 1999) and therefore increase DNA methylation. Finally, there is evidence that EGCG can inhibit DNMT1 directly by fitting to the binding pocket of the DNMT1, thus reducing its ability to methylate DNA (Fang *et al.* 2003, Lee *et al.* 2005).

Dietary factors influencing DNA methylation.

Given the potential for dietary factors to alter DNA methylation through the mechanisms outlined above, there has been research into the effects of a range of nutrients and dietary compounds on DNA methylation and the outcomes are summarized in Tables 1–3. In human studies, the majority of the evidence that diet influences DNA methylation has focused around methyl donor intake, particularly in the context of colorectal cancer (Tables 1 and 2) whereby the majority of evidence from intervention studies indicates that methyl donor supplementation, in particular supplementation of folic acid, increases global DNA methylation. Much more information about the effects of nutrients and other dietary compounds on DNA methylation is available from work with animal models (Table 3) which have advantages in the greater ability to reduce confounding by controlling other environmental exposures whilst manipulating diet. Overall, DNA methylation at specific gene loci has been found to be correlated positively with supply of dietary methyl donors (Wolff *et al.* 1998, Waterland & Jirtle 2003,

Table 2 Summary of associations between selected dietary factors and DNA methylation in human observational studies

Nutrient/Dietary component	Observation	Reference
Early life exposure to famine	CRC patients exposed to severe famine during the Dutch Hunger Winter had a decreased risk of developing a tumour characterized by CpG Island methylator phenotype (CIMP) compared with those not exposed	(Hughes <i>et al.</i> , 2009)
Methyl donors	CIMP was not associated with methyl donor intake in CRC patients	(de Vogel <i>et al.</i> , 2010)
Folic acid	Colonic mucosal and leucocyte DNA methylation were negatively correlated with folate status, in patients with colorectal neoplasia	(Pufulete <i>et al.</i> 2003)
	Peripheral blood genomic DNA methylation correlated directly with folate status in healthy persons	(Friso <i>et al.</i> 2002)
	Serum and erythrocyte folate levels were inversely correlated with genomic DNA hypomethylation in colonic mucosa from healthy subjects	(Pufulete <i>et al.</i> 2005b)
	Periconceptional supplementation with 400 µg folic acid per day was associated with increased methylation at the <i>IGF2</i> loci in the offspring	(Steegers-Theunissen <i>et al.</i> 2009)
Folic acid and alcohol	Non-statistically significant increased promoter methylation of <i>APC</i> , <i>p14</i> , <i>bMLH1</i> , <i>O(6)-MGMT</i> and <i>RASSF1A</i> genes in sporadic colorectal cancers subjects with low folate/high alcohol intake	(van Engeland <i>et al.</i> 2003)
Folic acid and vitamin B ₁₂	Serum vitamin B ₁₂ was negatively associated with <i>ERα</i> , but not <i>MLH1</i> , promoter methylation in subjects with and without colorectal neoplasia. Serum and red cell folate concentrations did not correlate with promoter methylation	(Al-Ghnam <i>et al.</i> 2007)
Folic acid, vitamin B ₂ , vitamin B ₆ , methionine and alcohol	Vitamin B ₆ intake was positively associated with <i>MLH1</i> promoter methylation in tumours of male subjects. Folic acid, vitamin B ₂ , methionine and alcohol intake did not affect <i>MLH1</i> promoter methylation	(de Vogel <i>et al.</i> 2008)

Table 3 Summary of effects of selected dietary factors on DNA methylation in animal studies

Nutrient/Dietary component	Observation	Reference
Methyl donors	Progressive demethylation at certain CpG sites within hepatic <i>p53</i> gene of folate/methyl deficient Fisher 344 rats	(Pogribny <i>et al.</i> 1995)
	Hepatic global DNA hypomethylation together with hypermethylation in the brain of folate/methyl deficient rats	(Pogribny <i>et al.</i> 2008)
	Greater proportion of brown (pseudoagouti) coats rather than yellow coats in offspring born to methyl donor-supplemented dams which was accompanied by increased methylation at the <i>A^{vy}</i> locus	(Wolff <i>et al.</i> 1998, Waterland & Jirtle 2003)
	Methyl donor supplementation of dams increased DNA methylation at the <i>Axin(Fu)</i> locus in offspring and resulted in reduced tail kinking	(Waterland <i>et al.</i> 2006)
	Maternal supplementation with methyl donors reversed the effects of maternal bisphenol-A exposure during pregnancy on methylation at the <i>A^{vy}</i> of the offspring	(Dolinoy <i>et al.</i> 2007)
Folic acid	Methylation of the <i>p53</i> gene, but not genomic DNA methylation, was reduced within rats fed a folate deficient diet for 6 weeks	(Kim <i>et al.</i> 1997)
	Folate deficiency led to transient changes in <i>p53</i> , but not genomic DNA, methylation in the rat colon	(Sohn <i>et al.</i> 2003)
	In a folate deficient rat model, placental genomic DNA methylation correlated significantly with placental, maternal hepatic and maternal plasma folate concentrations	(Kim <i>et al.</i> 2009)
	Folate deficiency did not alter genomic DNA methylation in the liver of BALB/c mice	(Christensen <i>et al.</i> , 2011)
Choline	Choline deficiency was associated with genomic DNA and <i>Cdkn3</i> hypomethylation in murine foetal brain, but not methylation of <i>Cdkn2b</i> and <i>Calb2</i>	(Niculescu <i>et al.</i> 2006)
	Choline deficiency caused genomic and <i>Igf2</i> hypermethylation in the liver and brain of E17 rat embryos	(Kovacheva <i>et al.</i> 2007)
Selenium	Increased dietary selenium intake in rats was associated with increased DNA methylation in the colon, but not the liver	(Davis & Uthus 2003)
Genistein	Genistein supplementation (300 mg kg ⁻¹ diet) for 4 weeks caused increased methylation in the prostate, but not the liver, in rats	(Day <i>et al.</i> 2002)
	Maternal genistein supplementation during pregnancy increased offspring <i>A^{vy}</i> methylation in agouti mice with accompanying increase in the proportion of offspring with brown (rather than yellow) coats	(Dolinoy <i>et al.</i> 2006)
	Maternal supplementation with genistein reversed the effects of maternal bisphenol-A exposure during pregnancy on <i>A^{vy}</i> methylation of the offspring	(Dolinoy <i>et al.</i> 2007)
Genistein and daidzein	Feeding genistein and daidzein continuously pre-and post-weaning caused increased methylation at some CpG sites within <i>Act1</i> in the pancreas but not in the liver of mice. Diet did not influence methylation of <i>Erα</i> or <i>cfos</i>	(Guerrero-Bosagna <i>et al.</i> 2008)
ECGC	In female A/J mice given an intraperitoneal injection of NNK to induce lung tumourigenesis, lung genomic DNA methylation was not altered when green tea extract was added to drinking water	(Shi <i>et al.</i> 1994)
Protein	Hepatic DNA hypermethylation was observed in rat foetuses exposed to low maternal dietary protein supply during pregnancy	(Rees <i>et al.</i> 2000)
	Promoters of 204 genes were differentially methylated in murine foetal liver in response to low-protein feeding during pregnancy. The promoter of the liver X-receptor alpha was significantly hypermethylated by the protein restriction.	(van Straten <i>et al.</i> 2010)
Protein & folic acid	Maternal folate supplementation during pregnancy reversed the effects of protein restriction on methylation of the promoter regions of the glucocorticoid receptor and <i>PPARα</i> in livers of the offspring	(Lillicrop <i>et al.</i> 2005, 2008)

Waterland *et al.* 2006, Pogribny *et al.* 2008) but negatively associated with dietary protein intake (Rees *et al.* 2000, van Straten *et al.* 2010), whereas supplementing the diet with selenium or genistein is associated with tissue-specific increases in DNA methylation at selected loci (Day *et al.* 2002, Davis & Uthus 2003, Dolinoy *et al.* 2006, 2007, Guerrero-Bosagna *et al.* 2008). Despite the growing body of evidence indicating that several dietary factors can influence DNA methylation in both humans and animals, many questions remain.

Although we know that some nutrients and other food-derived compounds can alter DNA methylation patterns, little is known about the doses of dietary factors or the duration of exposure/depletion which are necessary to provoke changes in epigenetic marks. The data available to answer these questions are fragmentary and, in some case, appear contradictory. A good example is the question of effects of altering folate supply on genomic DNA methylation in humans. When patients with colon cancer and adenoma were supplemented with a very large dose of folic acid (10 mg folic acid per day) for 6 months, there was increased genomic methylation in DNA from the rectal mucosa and a lower dose (5 mg folic acid per day) for a shorter period of time (3 months) also significantly increased rectal mucosa genomic DNA methylation in patients with one adenoma (Cravo *et al.* 1994, 1998). However, those patients with multiple adenoma did not respond to the same supplementation regime in terms of DNA methylation, indicating that disease progression (or other environmental factors) may be more influential than folate supply in determining DNA methylation responses in those at higher risk of CRC. More recently, Pufulete *et al.* (2005a,b) observed that supplementation with a physiological dose of 400 µg folic acid per day for 10 weeks increased both colonic and leucocyte genomic DNA methylation significantly in colonic adenoma patients. These studies underscore the uncertainties about the effects of different doses and duration of exposure to alterations in the intake of a single nutrient (folate) on DNA methylation and highlight the need for more systematic studies using (where possible) similar protocols to provide more unequivocal findings. In addition to duration and dose of the dietary intervention, other factors that are likely to impact on changes in DNA methylation include the physiological state of the subject. For example, there is growing evidence that there may be critical time windows, such as *in utero*, during which nutritional insults can have particularly large impact upon health (reviewed in Burdge & Lillycrop 2010, Junien 2006). Given the major changes in epigenetic marks which accompany (and may be causally responsible for) cellular and tissue differentiation in early development, it has been sup-

posed that this is a period during which the epigenome may be especially plastic and may be susceptible to modification by dietary and other environmental factors. This has led to the hypothesis that altered epigenetic marks, and consequential changes in gene expression and cellular function, may be one of the mechanisms which explains developmental programming in early life by dietary (and other) environmental exposures. To date there is little evidence to support this hypothesis in humans. However, Heijmans *et al.* (2008) have reported that methylation of the imprinted IGF2 gene was lower in individuals who were prenatally exposed to famine during the Dutch Hunger Winter in 1944–1945 than in their unexposed, same-sex siblings when investigated 6 decades later. In addition, it has been shown that there was increased methylation at the IGF2 locus in very young children of mothers who had used periconceptional folic acid supplementation (400 µg per day) (Steegers-Theunissen *et al.* 2009). More recently we have found that higher maternal B₁₂ concentrations are associated with reduced DNA methylation in infant cord blood at birth (McKay JA, Mathers JC and colleagues, unpublished observations). Although these data suggest a role for maternal dietary methyl donor supply during embryonic or foetal development in shaping offspring DNA methylation, the available data from human studies are very limited and further research in this area is warranted not least because of the widespread use of folic acid supplements in pregnancy and the fortification (mandatory or voluntary) of staple foods with folic acid in many countries of the world.

However, there is greater evidence from animal models about the impact of maternal nutrition, or nutrition in early post-natal life, on epigenetic marks in the progeny. One of the first examples of this was in the agouti mouse, which carries the viable yellow agouti metastable epiallele, *A^{vy}*, which is epigenetically labile and is responsible for coat colour in these mice. Studies investigating this model observed that a greater proportion of the offspring born to methyl-supplemented dams had brown (pseudoagouti) rather than yellow coats – a phenotypic change which is due to increased methylation at the *A^{vy}* locus (Wolff *et al.* 1998, Waterland & Jirtle 2003). More recently, Dolinoy *et al.* (2006) have shown that maternal supplementation with genistein during pregnancy also produced a similar phenotypic change in agouti mice which was also due to increased methylation at the *A^{vy}* locus of agouti mice despite the fact that genistein is not a methyl donor. Furthermore, the axin fused [Axin(Fu)] metastable epiallele also exhibited epigenetic plasticity in response to differences in maternal methyl donor intake in mice. Dietary methyl donor supplementation of mouse dams increased DNA methylation at the Axin(Fu) locus of the

offspring, which resulted in reduced tail kinking (Waterland *et al.* 2006).

Recent studies have identified examples of gene promoters where the extent of methylation in the offspring can be increased or decreased in response to manipulations of the maternal diet. Using a rat model, Lillycrop *et al.* (2005, 2008) observed that protein restriction during pregnancy resulted in hypomethylation within the promoter regions of the hepatic PPAR γ and glucocorticoid receptor genes in rat offspring and these epigenetic changes were accompanied by the expected changes in gene transcription. However, these effects were negated when the maternal low-protein diet was supplemented with folic acid during pregnancy (Lillycrop *et al.* 2005, 2008). As yet, there is no established mechanistic explanation for these effects but, because both protein and folate can alter the one-carbon pool, supplementing a low-protein diet with folic acid may correct any methyl donor deficiency created by protein restriction. Furthermore, dietary factors can interact with other environmental influences in altering DNA methylation patterns. For example, Dolinoy *et al.* (2006) reported that supplementing the murine maternal diet with either methyl donors or genistein prevented bisphenol-A-induced DNA hypomethylation in the *A^{vy}* locus of the offspring. Interactions between dietary and other environmental stimuli that have the ability to alter DNA methylation are significant areas of research that remain largely to be explored.

A further consideration, adding to the complexity of the relationship between diet and DNA methylation, is the issue of tissue specificity. It is well documented that DNA methylation varies between tissue types (Illingworth *et al.* 2008, Christensen *et al.* 2009, Maegawa *et al.* 2010, Thompson *et al.* 2010a), and therefore it is plausible that modulation of DNA methylation by dietary factors might be tissue specific. For example, in folate/methyl deficient rats, hepatic global DNA hypomethylation and hypermethylation in the brain occur together (Pogribny *et al.* 2008), indicating differential tissue responsiveness to the same nutritional insult. Furthermore, Day *et al.* (2002) reported that feeding male mice a genistein supplemented diet (300 mg kg⁻¹ diet) for 4 weeks caused increased DNA methylation in the prostate but not the liver and that feeding mice a diet containing both genistein and daidzein caused increased methylation at some CpG sites within the pancreatic, but not the hepatic, *Act1* gene (Guerrero-Bosagna *et al.* 2008). It will be particularly important to characterize the relationship between methylation in specific tissue and that in DNA derived from blood because, in human studies, it is often difficult (or impossible) to collect DNA from the tissue of interest (e.g. bone, the brain or the liver) so that only blood derived DNA is available for methyl-

ation analysis. In most cases, the interpretation of such blood-based measurements remains uncertain with respect to other target tissues.

As discussed above, metastable epialleles are one example of genes or genomic regions that are epigenetically labile (epilabile). It is highly likely that there are other 'epilabile' regions within the genome whereas other DNA regions may be more 'epistable' in terms of susceptibility to alteration in DNA methylation. However, to date, relatively little is known about the characteristics of DNA domains which make them more or less epigenetically labile. Although human and mammalian genomes contain, typically, over 20 000 genes, researchers have tended to measure DNA methylation changes in response to altered dietary exposures either as overall genomic DNA methylation or methylation in one or a few candidate genes. Whilst these studies can provide evidence of a relationship between diet and DNA methylation, this is a snapshot only and may give a biased, and certainly incomplete, picture. For example, many studies have focused upon gene targets already known to display differences in DNA methylation in cancer or with ageing, which may not be relevant to the topic under investigation. A more objective perspective on the plasticity of epigenetic marks in response to diet may be obtained by taking whole genome scanning approach. For example, use of an array-based approach to investigate DNA methylation revealed that most tissue-specific DNA methylation was not at CpG islands but at regions of intermediate CpG density located up to 2 kb from the islands, and which were termed 'CpG island shores' (Irizarry *et al.* 2009). More recently, using a DNA methylation array approach, Thompson *et al.* (2010b) reported that intra-uterine growth restriction induced alterations in DNA methylation, particularly at intergenic regions in the pancreatic islets of 7-week-old rats. Such studies demonstrate the potential of using array-based approaches to identify novel epigenetic targets which are susceptible to modification by dietary influences. To date, there are only a few examples of such studies (Bouchard *et al.* 2010, van Straten *et al.* 2010, Thompson *et al.* 2010b), but data emerging from these array-based investigations will be critical not only in identifying genes or consensus sequences within the genome which are modifiable by individual nutrients, but also in identifying groups of nutrients that cause similar, or opposing, epigenetic modifications.

Although there is evidence from animal models to support the view that nutritionally induced epigenetic changes can lead to phenotypic consequences (Wolff *et al.* 1998, Waterland & Jirtle 2003, Dolinoy *et al.* 2006, Waterland *et al.* 2006), these examples are limited and the functional consequences of many of the epigenetic changes which have been reported remain

uncertain. Given the evidence for gene regulation via epigenetic mechanisms (see above), the most logical avenue of research would be to link changes in DNA methylation with gene expression. Although several publications report correlating changes in gene-specific methylation and in expression of the same gene in response to nutritional factors, (Lillicrop *et al.* 2005, van Straten *et al.* 2010, Uthus *et al.* 2010), to date, few have taken advantage of using both expression and methylation arrays together in investigating the functional consequences of changes in diet/nutrition (Bouchard *et al.* 2010, Thompson *et al.* 2010b). Further characterization of the effects that nutritionally mediated DNA methylation changes have on gene expression, phenotype and other epigenetic marks, will be essential in determining the overall impact dietary factors may have upon health.

Nutritional modulation of histone marks

Dietary factors can alter the post-translational modifications of histones which cause alterations to the

chromatin structure and thus influence transcription. As histone decoration is more diverse than DNA methylation, (histone tails can be modified by methylation, acetylation, phosphorylation, ribosylation, ubiquitination, sumolation and biotinylation) there are potentially many opportunities for nutrition to influence these histone marks. Overall there are two major ways in which these histone marks can be altered (i) altering the abundance and/or efficacy of the enzymes responsible for the modification and (ii) altering the availability of the enzyme substrate. Several enzymes known to be involved in the modification of histones; histone acetyltransferases (HATs) and histone methyltransferases (HMTs) which add acetyl and methyl groups to histones respectively, and the histone deacetylases (HDACs) and histone demethylases (HDMs) which remove acetyl and methyl groups respectively.

There is considerable potential for diet to influence histone marks and this topic has been reviewed elsewhere recently (Delage & Dashwood 2008, Link *et al.* 2010). However, some examples of the effects of diet upon modification of histone proteins are

Table 4 Summary of associations between selected dietary factors and histone modifications

Nutrient/Dietary component	Model	Observation	Reference
Butyrate	Human A375 & mouse S91 melanoma cell lines	Butyrate increased acetylation of histone H4	(Demary <i>et al.</i> 2001)
	EBC-1 human lung epithelium cells	Butyrate exposure increased acetylation of histones H3 & H4 associated with the promoter of the cathelicidin gene	(Kida <i>et al.</i> 2006)
	HepG2 human hepatocarcinoma & HT29 human colon adenocarcinoma cells	Overall acetylation of histones H3 & H4 increased in HepG2 cells, but deacetylation was observed at transcription start sites of multiple genes in cells treated with butyrate	(Rada-Iglesias <i>et al.</i> 2007)
Dietary polyphenols	Caco-2 human intestinal cells	Diallyl sulphide treatment reduced HDAC activity and increased H3 acetylation	(Druesne <i>et al.</i> 2004)
	HT29 human colon adenocarcinoma cells		
	LNCaP human prostate cancer cells	Increased H3 acetylation, increased H3K4 methylation and reduced H3K9 methylation in response to phenyl isothiocyanate	(Wang <i>et al.</i> 2007)
	Hep3B human hepatoma cells	Curcumin treatment decreased histone acetylation but did not affect HDAC activity	(Kang <i>et al.</i> 2005)
Copper	SCC-13 human skin cancer cells	Reduced cell survival associated with global reduction in H3K27 trimethylation in response to ECGC	(Balasubramanian <i>et al.</i> 2010)
	HL60 human leukaemia cells	Concentration and time-dependant decrease in histone acetylation in response to copper	(Lin <i>et al.</i> 2005)
Methyl donors	Rat liver	Methyl donor deficiency reduced H3K9 trimethylation and acetylation, reduced H4K20 trimethylation and increased H3S10 phosphorylation	(Pogribny <i>et al.</i> 2007)

summarized in Table 4. Several nutrients, and other dietary components, have been found to inhibit the enzymes which modify histones. Butyrate (a short-chain carboxylic acid produced in the colon by bacterial fermentation of carbohydrates) and dietary polyphenols (from e.g. garlic, soy and cinnamon) have been shown to be HDAC inhibitors (Demary *et al.* 2001, Druesne *et al.* 2004, Kida *et al.* 2006, Rada-Iglesias *et al.* 2007, Wang *et al.* 2007, Link *et al.* 2010), whereas, the HAT enzymes are inhibited by green tea polyphenols and copper (Kang *et al.* 2005, Lin *et al.* 2005, Choi *et al.* 2009). Less is known about the effects of dietary factors on activities of HMT and HDM enzymes, but it has been reported that EGCG from green tea inhibited HMT (Balasubramanian *et al.* 2010). Furthermore, HMT activity is known to be repressed by reduced availability of dietary methyl donors (Pogribny *et al.* 2007).

As histone modifications and DNA methylation have a combined role in transcriptional regulation, it is not surprising that dietary influences which alter one of these epigenetic marks may also affect the other. For example, a localized increase in DNA methylation in response to increased dietary methyl donors may attract HDAC enzymes to that genomic site, leading to secondary histone deacetylation. In future studies there may be profit in investigating the influence of dietary factors on both DNA methylation and histone modifications simultaneously. In the same way that use of DNA methylation arrays or next generation sequencing reveals new information about effects of nutritional exposures across the genome, so the combination of such data with those from ChIP-chip arrays on associated histone modifications is likely to advance understanding of the synchronization of these nutritionally orchestrated epigenetic events.

Implications for human health

There is growing support for the concept that chronic stresses which require significant cell renewal can lead to abnormal tissue homeostasis and become causal for the pathogenesis of common chronic diseases such as atherosclerosis, diabetes and cancers (Johnstone & Baylin 2010). These perturbations in cellular function may be mediated by epigenetic mechanisms (Johnstone & Baylin 2010, Petronis 2010). Given (i) the important contribution made by diet and other lifestyle factors in the aetiology of complex diseases, (ii) the accumulating evidence that diet, smoking, physical activity and other environmental exposures alter epigenetic marks and processes and (iii) the impact of nutrition and other lifestyle factors on the major cellular stressors (inflammation, metabolic stress and oxidative stress), there is reason for optimism that epigenetics-based studies will

be informative about the mechanisms through which dietary exposures influence human health over long-time periods and may offer novel opportunities for interventions to prevent, delay or treat common complex diseases. As an example, genome-wide analysis of DNA methylation marks which vary between individual and within individuals over a substantial time period (11 years) has revealed genetic loci which are associated with body mass index (a measure of adiposity) (Feinberg *et al.* 2010).

Although maternal under-nutrition remains a significant public health scourge with major adverse consequences for both the mother and her offspring, there is increasing concern about the adverse effects of maternal over-nutrition. A greater proportion of women are entering pregnancy with higher than optimum body fat and this maternal overweight and obesity is associated with several deleterious consequences for the mother and her baby (Stothard *et al.* 2009, Horvath *et al.* 2010). It is reasonable to suppose that the stresses associated with over-nutrition *in utero* may mark epigenetically the developing foetus with long-term consequences for patterns of gene expression, cell function and health. Of particular interest in the potential for differential regulation of genes involved in appetite and satiety which may contribute to enhanced obesity risk (Mathers 2010).

Research priorities

Although there is a growing body of information describing the effects of specific foods, nutrients or other food-derived substances on epigenetic marks and processes (reviewed above), much of this is phenomenological in nature. There is a need for more systematic research to identify those dietary factors which have the most influence on epigenetic marks and to develop an understanding of the mechanisms through which this occurs. Given that the preponderance of research to date has focussed on micronutrients, and other quantitatively small non-nutrient components of foods and diets, there may be particular scope for investigations of effects of macronutrients and of energy supply. In addition, much research on nutrition and epigenetics to date has taken a candidate gene approach so that relatively little is known about which epigenomic marks are most labile in response to dietary (and other environmental) exposures. A related research priority is identification of the stages in the life-course during which particular epigenetic loci are affected by diet. The majority of work to date has focussed on very early parts of the life-course (during development *in utero* or in early post-natal life) but it is possible that there is lifelong plasticity of the epigenome in response to dietary and other exposures. This hypothesis is

supported by greater diversity in epigenomics markings (and associated gene expression) with age in monozygotic twins (Fraga *et al.*, 2005). In addition, a recent study of factors contributing to colorectal cancer risk showed that individuals exposed to famine during the Dutch Hunger Winter of 1944–1945 had a significantly decreased risk of developing a tumour characterized by CpG island methylation phenotype (Hughes *et al.*, 2009). Dietary intervention studies aimed to enhance life-long health, or to reduce the risk of common complex diseases, need good biomarkers of the healthy phenotype for use as surrogate endpoints. Given the plasticity of epigenetic marks and their responsiveness to dietary factors, it will be important to discover whether epigenetic marks can be used as biomarkers of health in such intervention studies.

Conflicts of interest

The authors do not have any conflicts of interest in respect of this manuscript.

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