**FATTY ACIDS AND EPIGENETICS**

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**Purpose of review**

The purpose of this review is to assess the findings of recent studies on the effects of fatty acids on epigenetic process and the role of epigenetics in regulating fatty acid metabolism.

**Recent findings**

The DNA methylation status of the Fads2 promoter was increased in the liver of the offspring of mice fed an α-linolenic acid-enriched diet during pregnancy. In rats, increasing total maternal fat intake during pregnancy and lactation induced persistent hypermethylation of the Fads2 promoter in the liver and aortae of their offspring. However, increased fish oil intake in adult rats induced transient, reversible hypermethylation of Fads2. High fat feeding in rodents also altered the levels of histone methylation in placentae and in adipose tissue. Dietary docosahexaenoic acid supplementation in pregnant women induced marginal changes in global DNA methylation in cord blood leukocytes. A high fat diet altered the DNA methylation status of specific genes in skeletal muscle in young men.

**Summary**

There are emerging findings that support the suggestion that fatty acids, in particular polyunsaturated fatty acids, can modify the epigenome. However, there is a need for rigorous investigations that assess directly the effect epigenetic modifications induced by fatty acids on gene function and metabolism.

**Key words**

Nutriepigenomics, epigenetics, polyunsaturated fatty acids

**INTRODUCTION**

Nutrigenomics is now a well-established area of research in nutritional science. Polymorphisms have been identified in genes involved in a wide range of pathways that alter nutrient metabolism and modify disease risk. However, such polymorphisms are fixed throughout the life course and their effects need to accommodated within the diet and lifestyle of an individual in order to maintain optimal health. Nutriepigenomics is an emerging field of research that is focussed on the interaction between nutrition and the epigenome. Epigenetic marks can exhibit plasticity throughout the life course, albeit to varying degrees, and can be modified by environmental factors including diet [1]. This suggests that while variations in the epigenetic control of genes involved in nutrient metabolism may contribute to differences between individuals in their nutrient requirements and susceptibility to disease, nutritional interventions or dietary choices may modify the epigenome. One implication of this bidirectional interaction between the diet and the epigenome is that it may be possible to reprogramme epigenetic marks that are associated with increased disease risk by nutritional or lifestyle interventions.

 The induction and maintenance of epigenetic marks, in particular DNA methylation and some covalent modifications of histones, are associated intimately with 1-carbon metabolism and hence there is a substantial body of evidence which shows that dietary intakes of nutrients involved in this pathway alter the epigenetic regulation of genes [1]. However, there is emerging evidence that fatty acids and dietary fats can also alter the epigenome. The purpose of this review is to discuss recent studies that have investigated the interaction between fatty acids and the epigenome and to consider the implications of the findings for human health.

**AN OVERVIEW OF EPIGENETICS**

Epigenetics refers to a group of heterogeneous processes that regulate transcription without changing the DNA coding sequence. These are the methylation at the 5’ position of cytosine bases in CpG dinucleotide pairs, covalent modifications of histones, principally acetylation and methylation of lysine residues but also phosphorylation and ubiquitination, and the activities of non-coding RNA species [2]. Such epigenetic marks confer transcriptional regulation over times scales that vary between minutes, particularly in the case of histone modifications, to the induction of gene silencing by the methylation of CpG loci within gene promoters during cell differentiation in the early embryo which is maintained throughout the whole life course [3]. Transcriptional silencing by DNA methylation involves recruitment of, DNA methyltransferases, methyl cytosine binding proteins, histone deacetylases and histone methyltransferases which block the activity of the RNA polymerase complex by causing condensation of chromatin and inhibition of transcription factor binding [4-8]. However, transcriptional activity can also be modified by methylation of one or more CpG dinucleotides within a promoter leading to differential binding or blocking of transcription factors. Although DNA methylation is considered generally to be a repressive epigenetic mark [9], there is some evidence that methylation at specific CpG loci may increase transcriptional activity, for example by blocking the binding of a repressive transcription factor [10]. Histone acetylation, which is induced by histone acetyltransferases maintains an open potentially transcriptionally active chromatin structure.

 The level of plasticity of the DNA methylome during different stage of the life course is understood poorly, except during gamete maturation and embryogenesis. It has been proposed that the prenatal and neonatal periods, puberty and aging represent time of increased epigenetic plasticity [11]. For example, the DNA methylome of centenarian differs markedly from that of newborns [12]. However, there is little information in humans about plasticity during the intervening years, particularly during childhood. Understanding the extent of epigenetic plasticity throughout the life course has important implications for dietary choices in relation to health outcomes.

**THE INTERACTION BETWEEN FATTY ACIDS AND THE EPIGENOME**

The review will focus on recent reports that focus specifically on the interaction between fatty acids and the epigenome. We have excluded studies of obesity and the epigenome because the multifactorial nature of obesity may confound any attempt to assign a specific role of fatty acids. Recent studies of the interaction between fatty acids and the epigenome encompass two general areas of research; the effect of maternal fatty acid intake on the epigenome of the offspring and the effect of manipulating the adult diet and on epigenetic outcomes.

**The effects of maternal fatty acid intake on the epigenome of the offspring**

Three articles have reported the effects of modifying the amount of n-3 polyunsaturated fatty acids (PUFA) in the maternal diet on epigenetic outcomes in the offspring. Niculescu et al. investigated the effect of feeding pregnant and lactating mice diets with different ratios of linoleic acid (LA) to α-linolenic acid (ALNA) on DNA methylation in the liver of the offspring at weaning[13]. Female mice were fed diets with LA : ALNA ratios of 8:1 (control) to 55:1 (ALNA deficient) for 30 days before pregnancy and then until delivery when lactating dams were switched to either the control or a ALNA-supplemented (LA : ALNA ratio 0.3:1) diet until weaning [14]. The average methylation of the Fads2 promoter was found to be 1% to 2% higher in the liver of dams fed the ALNA supplemented diet, while methylation of intron 1 was not affected. Although the level of methylation of the Fads2 promoter showed a negative statistical association with mRNA expression, the biological significance of these small differences is difficult to deduce because the study did not include measures of PUFA status or biosynthesis.

 Two reports based on the same study design have investigated the effect of the amount and type of maternal fatty acids consumed before pregnancy, and throughout pregnancy and lactation on the epigenetic regulation of PUFA biosynthesis in rats. In one study, female rats were fed diets containing either 3.5% (low fat), 7% (adequate fat) or 21% (high fat) fat (w/w) enriched in either saturated and monounsaturated fatty acids derived from butter or in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) derived from fish oil [15]. The offspring were weaned on to a diet containing 4% (w/w) soybean oil. The proportions of arachidonic acid (AA) and DHA were significantly lower in liver phosphatidylcholine (PC) and phosphatidylethanolamine, and in plasma PC of the adult (postnatal day 77) male and female offspring of dams fed 21% fat compared to those fed the lower fat diets irrespective of the type of fat consumed. There was no effect of maternal fat intake on Fads1 promoter methylation or its mRNA expression in the offspring liver. Fads2 mRNA expression in offspring was decreased significantly with increasing maternal dietary fat intake and was associated with increased methylation (up to 20%) of specific CpG loci in the Fads2 promoter between offspring of dams fed 3.5% or 21% fat. The magnitude of this effect differed slightly between types of maternal dietary fat. These findings indicated that the amount of maternal fat consumed during pregnancy and lactation influenced the capacity of the offspring to synthesise PUFA via changes in the epigenetic regulation of Fads2. Furthermore, the effect of maternal fat intake on the epigenetic regulation of Fads2 in the offspring persisted into adulthood. Feeding adult, non-pregnant female rats a fish oil enriched diet for 9 weeks induced reduced Fads2 mRNA expression and increased methylation of specific CpG loci in the Fads2 promoter compared to those fed soy bean oil [15]. However, these changes in the epigenetic regulation of Fads2 were lost when the diet was switched from fish oil to soybean oil which suggests that the nature of the effect of fatty acid intake on the epigenetic regulation of genes by DNA methylation is contingent on the timing of the dietary change during the life course.

 The second study extended the above design to investigate the effects of feeding dams diets containing 7% and 21% (w/w) safflower oil, enriched in LA, or hydrogenated soybean oil, enriched in *trans* fatty acids [16]. The findings showed that proportion of AA, but not DHA, was reduced in the aorta of the offspring of dams fed 21% fat compared to those of dams fed 7% fat irrespective of the fatty acid composition. This was associated with increased mRNA expression of Fads1, but lower expression of Fads2. Analysis of the methylation of individual CpG loci showed that there was no significant effect of maternal fat intake on the methylation status of the Fads1 promoter. However, specific CpG loci were hypermethylated in the Fads2 promoter. Mutation of one CpG locus that was hypermethylated in the aortae of offspring of dams fed 21% fat and, which is located within an estrogen receptor response element, decreased the activity of the promoter. This indicates that at least some of the hypermethylated loci were involved directly in the regulation of Fads2 transcription. These findings support the concept that fatty acid exposures during development induce persistent changes in the epigenetic regulation of PUFA biosynthesis, but that the precise nature of such effects differs between tissues. Furthermore, the amount of maternal dietary fat appears to exert a greater influence on the DNA methylome of the offspring than the fatty acid composition of the diet.

 One important consideration in the design of studies to investigate the effect of maternal diet on the epigenetic regulation of Fads2 is the choice of animal species. The Fads2 promoter in mouse liver appears to have low levels of methylation, typically less than 10% [13, 17], while in the rat the level of methylation is between 30% and 90% depending on the CpG locus [15]. The level of methylation of the Fads2 promoter in human liver has yet to be reported and so it is not known which animal model reflects humans most closely. However, another consideration is that methylation levels of 5% or less are close to or below the detection limit of the sequencing technology [18] and hence reports of methylation levels in this range need to be interpreted cautiously.

 Feeding female mice a diet containing either 12% energy or 62% energy derived from fat (fatty acid composition not disclosed) for 28 before mating and throughout pregnancy and lactation induced in the adult offspring, which were fed the 12% fat diet, hypertension, hypertriglycerideamia, hyperleptinaemia, insulin resistance and lower adiponectin concentration [19]. Adiponectin mRNA expression was lower and leptin expression higher in white adipose tissue from the offspring of dams fed the high fat diet compared to those of dams fed the control fat diet. This suggests that the differences between groups in the circulating levels of these adipokines reflected changes in the transcriptional activity of these genes. The transcriptionally permissive histone modification H3K9 acetylation was lower and the repressive histone mark dimethyl H3K9, but not methyl H4K20, was higher in the offspring of dams fed the high fat diet compared to those of dams fed the control diet. Conversely, H3K9 acetylation and dimethyl H3K9 were unchanged at the leptin promoter, while methyl H4K20 was increased in the offspring of dams fed the high fat diet compared to controls. Histone modifications induced in the adiponectin gene are consistent with the pattern of mRNA expression. However, although H4K20 is generally regarded as a repressive histone mark [20], there is evidence that suggests that is can also be associated with transcriptionally active promoters [21]. This study provides the first evidence that maternal fat intake can alter transcription in gene specific manner by inducing persistent alterations in histone modifications.

 One study has investigated the effect of a maternal high fat diet on the expression and epigenetic regulation by histone modifications of placental genes in mice [22]. Female mice were fed ether a control (105 energy from fat) or high fat (60% energy from fat) diet with undisclosed fatty acid composition for 15 days prior to mating and then during pregnancy until they were killed at day 15.5 (study design reported in [23]). The high fat diet induced altered expression of 164 genes (99 up-regulated) in placentae from female conspectuses and of 187 genes male placentae (86 up-regulated) [22]. These included histone methyl transferases and demethylases. The changes in the mRNA expression of these genes by the maternal high fat diet were associated with altered global alterations in the levels of tri-methyl H3K4 and tri-methyl H3K9.

 Lee et al. investigated the effect of supplementing the diet of pregnant women with 400mg DHA per day or a placebo containing olive oil from 18 – 22 weeks gestation on the methylation of the promoters of genes involved in immune function, including IFNγ, TNF-α, GATA3 and STAT3, in leukocytes isolated from umbilical cord blood [24]. There were no significant differences in the level of average methylation in any of the genes measured between women who took DHA and the placebo group even when the groups were sub-divided for smoking and irrespective of whether the findings were adjusted for sex, duration of gestation, BMI and batch of analysis. However, measurement of the average methylation of long interspersed repetitive sequence element (LINE)-1 showed slightly higher methylation (1%) in women in the DHA group who smoked compared to placebo group, although this difference became non-significant when the data were adjusted as described above. Thus the author’s conclusion that ‘…maternal supplementation with omega-3 PUFA during pregnancy may modulate global methylation levels and the Th1/Th2 balance in infants.’ does not appear to be supported by their findings.

**The effects of fatty acid intake on the epigenome in adult animal models**

Feeding adult male mice either a high fat (35% (w/w)) or control fat (4% (w/w)) diets for 18 weeks decreased the mRNA expression of stearoyl-CoA-desaturase (Scd)-1 in the liver [25]. Two CpG loci at -838 and -833 base pairs relative to the transcription start site showed higher methylation in mice fed the high fat diet compared to those fed the control diet, and were associated negatively with Scd-1 mRNA expression in liver. A third CpG locus at +384 base pairs was also hypermethylated in mice fed the high fat diet compared to those fed the control diet, but did not show a significant association with mRNA expression. Methylation of these three CpG loci was associated positively with body lean and fat mass. Methylation at CpGs -838 and -833 was also associated positively with plasma leptin and insulin concentrations, but associated negatively with circulating ghrelin levels. One implication of these findings is that long-term consumption of high fat diets induces changes in the control of a key regulator of hepatic fatty acid metabolism. Unfortunately, the study did not test whether such effects persisted beyond the period of feeding the high fat diet.

 Adult mice carry the human APOE2 gene showed marked hepatic lipid accumulation and altered expression of 70% of the genes measured in a transcriptome-wide analysis when fed a high fat diet (60% energy from fat) compared to a control diet (12% energy from fat) [26]. This was associated with increased levels of tri-methyl H3K9 and tri-methyl H3K4 in genes in the PPARα network which is consistent with down-regulation of fatty acid β-oxidation and hepatic lipid accumulation.

**The effects of fatty acid intake on the epigenome in adult humans**

One recent study investigated the effects of feeding young men either a high fat (60% of energy from fat composed of 33% each of monounsatured fatty acids, PUFA and saturated fatty acids, although the detailed fatty acid composition was not disclosed [27]) compared to a control diet in which 35% of the energy was derived from fat for 5 days in a randomised, cross-over study. 27,578 CpG loci in 14,475 genes were measured at the end of the interventions [28]. The methylation status of 7,909 CpG loci in 6,58 genes was altered in skeletal muscle after consuming the high fat diet compared to the control diet. The distribution of loci that showed increased methylation was skewed towards the lower end of the range such the that median difference was 2 – 3% (1,979 loci) and only 8 loci showed a difference in methylation of greater than 10%. Loci that showed lower methylation after feeding the high fat diet approximated a Gaussian distribution with a median difference of 4 - 5% (214 loci). Pathway analysis showed that loci that exhibited increased methylation to be associated with cancer, reproductive system disease and gastrointestinal disease, while the loci that showed lower methylation in response to the high fat diet were associated with inflammatory disease, inflammatory response and ophthalmic disease. However, the significance of changes in the methylation of genes involved in these pathways to muscle function is unclear. The differences in methylation between the dietary interventions were associated poorly with mRNA expression and, unfortunately, the effect of differential methylation on gene function was not tested directly.

**The effects of treatment of cells with fatty acids *in vitro***

Addition of 10μmol/l DHA to M17 neuoblastoma cells for 48 hours induced increased global acetyl H3K9 and decreased levels of histone deacetylases 1, 2 and 3 [29]. DHA also decreased global levels of dimethyl H3K4, dimethyl H3K9, dimethyl H3K27, dimethyl H3K36 and dimethyl H3K 79. DHA treatment also increased Bcl-2 expression and decreased caspase-3 expression. Overall, treatment with DHA tended to induce histone changes that are consistent with active transcription and modifications to gene expression that suggest reduced apoptosis. Although these effects suggest that DHA may protect against neurodegeneration through epigenetic changes, such effects may be harmful in neurological cancers including neuoblastoma. Unfortunately, this study did not test whether such effects were specific to DHA nor whether they were dependent on the concentration of the fatty acid. However, an earlier study has shown differential effects of treatment with EPA compared to a range of other fatty acids on U937 leukaemic cells that included increased the mRNA expression of CCAAT/enhancer-binding proteins C/EBP-β and C/EBP-δ, PU.1 and c-Jun which was accompanied by locus-specific demethylation of the C/EBP-δ promoter [30].

**Mechanisms**

 It is not known at present how dietary fatty acids modify the epigenome. Short chain fatty acids can inhibit histone deactylase activity (butyric acid > valeric acid > propionic acid > caproic acid > acetic acid) [31]. Variation in energy intake leading to changes in cellular NAD+/NADH may alter histone acetylation by modulating the activities of the histone deaceylases; sirtuins [32]. It is possible that these processes may contribute to epigenetic changes induced by high fat feeding, but cannot explain the effects on individual fatty acid species on epigenetic marks. Feeding mice a high fat diet during pregnancy and lactation reduced the expression of a number of non-coding RNAs in the adult offspring [33]. Non-coding RNAs have been shown to modify DNA methylation by altering DNA methyltransferase 3A and 3B activities[34], and to change chromatin structure by modulation of MeCP2 translation [35] and expression of the histone methyltransferase EZH2 [36]. Thus the observation that exposure to a high fat diet during development change selectively the expression of non-coding RNA species suggests a putative mechanism by which fatty acid intake may alter specific epigenetic regulatory processes. Whether individual fatty acids can alter the activities of different non-coding RNAs remains to be investigated. Furthermore, it is still not known why fat intake during pregnancy induces persistent changes in the epigenome of the offspring, while at least in some systems the effects of feeding diets with different fatty acid contents only induce transient epigenetic changes in adults.

**CONCLUSION**

There are emerging findings that support the idea that dietary fatty acids can modify the epigenome. However, the specificity of such effects is not clear due, in part, to the limited characterisation of the diets used in a number of studies. Progress in this area is also limited by the lack of experimental to shown whether differential epigenetic changes that are induced by dietary fatty acids change gene function. Nevertheless, this area of research has the potential to provide new insights into the effects of dietary fatty acids on development, metabolism and risk of disease.

**KEY POINTS**

* Altered maternal fat intake during pregnancy and lactation can induce persistent changes in the epigenetic regulation by DNA methylation and histone modifications of genes in the tissues of the offspring.
* Altered dietary fat in adults can induce altered epigenetic regulation of specific genes, although this may be transient and reversible.
* Induced changes in the epigenetic regulation of genes by dietary fatty acid intake is potentially an important mechanism in metabolic control and in disease risk, but there is a substantial need for studies that demonstrate a direct effect of epigenetic changes on transcription and metabolism.

**Conflicts of interest**

*There are no conflicts of interest*

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\*of special interest

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