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REVIEWS: CURRENT TOPICS

Nutrition and epigenetics: an interplay of dietary methyl donors, one-carbon metabolism and DNA methylation☆,☆☆

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Abstract

DNA methylation is the most extensively studied mechanism of epigenetic gene regulation. Increasing evidence indicates that DNA methylation is labile in response to nutritional and environmental influences. Alterations in DNA methylation profiles can lead to changes in gene expression, resulting in diverse phenotypes with the potential for increased disease risk. The primary methyl donor for DNA methylation is *S*-adenosylmethionine (SAM), a species generated in the cyclical cellular process called one-carbon metabolism. One-carbon metabolism is catalyzed by several enzymes in the presence of dietary micronutrients, including folate, choline, betaine and other B vitamins. For this reason, nutrition status, particularly micronutrient intake, has been a focal point when investigating epigenetic mechanisms. Although animal evidence linking nutrition and DNA methylation is fairly extensive, epidemiological evidence is less comprehensive. This review serves to integrate studies of the animal *in vivo* with human epidemiological data pertaining to nutritional regulation of DNA methylation and to further identify areas in which current knowledge is limited.

Keywords: Epigenetics; Nutri-epigenomics; One-carbon metabolism; Dietary methyl donors; DNA methylation

1. Examining DNA methylation in the context of diet

Epigenetics is the study of mitotically heritable yet potentially reversible, molecular modifications to DNA and chromatin without alteration to the underlying DNA sequence [1,2]. Increasingly, it is recognized that epigenetic marks provide a mechanistic link between environment, nutrition and disease. Although DNA sequence is fairly permanent, epigenetic modifications are dynamic throughout the life course and can be heavily influenced by external factors [2]. Thus, external effects on the epigenome may alter gene expression, potentially giving rise to phenotypic disparity including disease formation. Epigenetic modifications include chromatin remodeling, histone tail modifications, DNA methylation and, more recently, have expanded to include non-coding RNA and microRNA gene regulation [3].

DNA methylation is the most widely studied form of epigenetic modification and occurs within the one-carbon metabolism pathway, which is dependent upon several enzymes in the presence of dietary micronutrients as cofactors, including the availability of folate, choline and betaine through the diet (Fig. 1). Through an ATP-driven reaction, methionine is converted into *S*-adenosylmethionine (SAM), the universal cellular methyl donor [4]. DNA methyltransferases (DNMTs) covalently attach methyl groups from SAM to the carbon-5 position of cytosine bases, generating 5-methylcytosine thus methylating DNA.

In mammals, DNA methylation is primarily a stable repressive mark found at cytosines in CpG dinucleotides; however, its regulation is more dynamic than previously believed [5]. These regulatory events may occur in a gene-specific and global manner. Passive demethylation of DNA from tissues is widely accepted, although evidence for active demethylation is mostly limited to various stages of development [6]. Passive demethylation typically occurs when DNMT activity is reduced or inhibited at the time that DNA replication occurs. Simply, there is no addition of methyl groups to newly synthesized strands of DNA during cell division. Active demethylation, or the enzymatic conversion of 5-methylcytosine to cytosine, is fairly controversial, and the mechanisms by which it occurs still remain to be elucidated. Enzymatic conversion of 5-methylcytosine has produced three additional bases, 5-hydroxylmethylcytosine, 5-formylcytosine and 5-carboxylcytosine, yet their potential roles in epigenetic regulation have yet to be characterized [7]. In addition, Lister et al. [8] provided evidence for methylation of non-CpG cytosines in human embryonic

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stem cells, suggesting that methylation at non-CpG sites may be important to developmental homeostasis. The distribution of CpG sequences in mammalian genomes is non-random [9]. CpG dinucleotides are greatly underrepresented in the mammalian genome due to evolutionary spontaneous deamination of 5-methylcytosine to thymine [10]. The majority of unmethylated CpG sites occur within CpG islands, defined as discreet regions containing a preponderance of CpG content [11]. Normally, CpG islands are located within or near gene promoters or in the first exons of housekeeping genes. In contrast, the promoter and regulatory regions of repetitive DNA sequences, such as transposable elements, are methylated, consequently inhibiting the parasitic transposable and repetitive elements from replicating.

DNA methylation patterns are prone to change throughout the life course especially during reprogramming events associated with normal development and aging [12–14]. For example, the epigenome is particularly dynamic during embryogenesis because of extensive DNA synthesis, and the elaborate DNA methylation patterning required for normal tissue development is established during early development [15]. As individuals age, gradual DNA hypomethylation occurs at the genome-wide level, concurrent with locus-specific promoter hypermethylation at normally unmethylated CpG islands, leading, for example, to genome instability or gene-specific suppression, respectively [16]. Additionally, cancer is often associated with hypomethylated DNA and notable hypermethylation of tumor suppressor genes, as compared to normal tissue [17]. These reprogramming events throughout the life course result in tissue-specific DNA methylation patterning [2,13]. Differences in these epigenetic patterns are important to cellular differentiation and tissue homeostasis.

In the context of nutritional biochemistry, it is significant that the one-carbon metabolism pathway is cyclical and is regenerated via dietary micronutrients. Nutri-epigenomics is an emerging discipline examining the role of dietary influences on gene expression. Ultimately, DNA methylation events, and dietary practices, particularly micronutrient intake, may influence disease phenotypes. The study of nutri-epigenomics is particularly timely in the context of the developmental origins of health and disease (DOHaD) hypothesis [18], which posits that increased susceptibility to disease following early life experiences is shaped by epigenetic modifications such as DNA methylation and chromatin modifications [19]. In this review, we take an interspecies approach to synthesize the existing nutri-epigenomic literature in order to identify sensitive periods throughout the life course where diet may substantially alter DNA methylation. By investigating varying levels of nutrient exposure during vulnerable time points, researchers can grasp the magnitude and degree of impact that each nutrient has on one-carbon metabolism and, subsequently, DNA methylation. Throughout the review, careful attention is given to areas in which further research is needed to understand the link between dietary micronutrients and DNA methylation, and life course health and disease.

Nutri-epigenomics studies have utilized a combination of global, candidate gene, and, to a lesser extent, genome-wide approaches to examine the influence of methyl donors on DNA methylation. Until recently, most attempts to elucidate the effects of nutritional status on the epigenome were either (1) candidate gene driven or based on epigenetic techniques with limited genome coverage/sensitivity, (2) restricted in dose–response assessment, or (3) confined to animal models. Emerging advances in epigenomic and high-throughput quantitative epigenetic technologies now allow for the identification of the constellation of genomic loci with altered epigenetic status following dose-dependent exposures. Epigenetic epidemiology approaches facilitate the identification of epigenetically modified regions of the genome in human cells. Thus, identifying epigenetic biomarkers will enable clinicians to identify at-risk individuals prior to disease onset. Furthermore, unlike genetic mutations, epigenetic marks are potentially reversible. Therefore, epigenetic approaches for prevention and treatment, such as nutritional supplementation and/or pharmaceutical therapies, may be developed to counteract negative epigenomic profiles.

2. Studies of combined methyl donor supplementation

Novel experiments investigating maternal methyl donor supplementation *in utero* clearly demonstrate the impact early nutrition has in shaping the epigenome. The *axin fused* (*Axin*^{Fu}) and *viable yellow agouti* (*A*^{vy}) murine models contain metastable epialleles, loci variably expressed in isogenic individuals due to the establishment of stochastic epigenetic modifications early in development [20]. Variable DNA methylation of an intracisternal A particle (IAP)

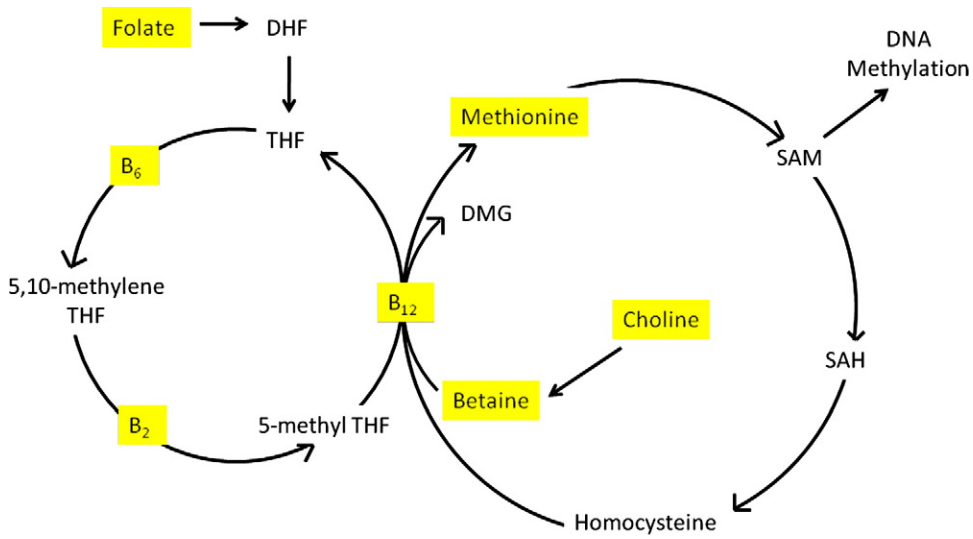

Fig. 1. Involvement of dietary micronutrients in one-carbon metabolism. Substrates obtained via diet are highlighted in yellow. (1) Vitamin B_6 is a cofactor to serine hydroxymethyltransferase in the conversion of tetrahydrofolate (THF) to 5,10-methylene THF. (2) Vitamin B_2 is a precursor to FAD, which is a cofactor to methylenetetrahydrofolate reductase (MTHFR) in the conversion of 5,10-methylene THF to 5-methyl THF. (3) Vitamin B_{12} is a precursor to methionine synthase, involved in the production of methionine from homocysteine and betaine. DHF, Dihydrofolate; FAD, flavin adenine dinucleotide; DMG, dimethyl glycine; MTHFR, methylenetetrahydrofolate reductase; SAH, *S*-adenosylhomocysteine; THF, tetrahydrofolate.

retroelement in intron 6 of the *Axin* gene results in distinct kinky tail phenotypes, from very kinky (hypomethylated) to no tail kink (hypermethylated) among genetically identical $Axin^{Fu}$ mice [21]. Waterland et al. [22] demonstrated that maternal diet supplemented with methyl donors including folic acid, vitamin B_{12}, betaine and choline resulted in offspring displaying less kinky tail phenotype and, accordingly, hypermethylation of the $Axin^{Fu}$ metastable epiallele. Similarly, the coat color of isogenic A^{vy} mice correlates to stochastic methylation of an IAP upstream of the *Agouti* gene promoter, resulting in phenotypes ranging from yellow (hypomethylated) to brown (hypermethylated) fur [23]. A methyl donor-supplemented maternal diet shifted the distribution of coat color phenotype towards brown in comparison to unsupplemented mothers [24]. The shift towards the brown phenotype was concomitant to hypermethylation at A^{vy} IAP. Furthermore, using the A^{vy} model, we have demonstrated that maternal diet supplemented with methyl donors negated a shift towards hypomethylation due to bisphenol A exposure, restoring normal stochastic methylation in offspring [25].

The $Axin^{Fu}$ and A^{vy} models serve as informative visual epigenetic biosensors for maternal nutritional status, particularly methyl donors, needed for the maintenance of one-carbon metabolism and DNA methylation. Additional *in vivo* rodent studies have relied upon wild-type strains of rodents to investigate the effects of maternal methyl donor supplementation in altering phenotypes, especially in the exacerbation of disease. For example, modulation of allergic airway disease risk was explored using C57BL/6 mice [26]. Hypermethylation of a gene crucial in lymphocyte regulation, *Runx3*, arose in offspring exposed to methyl donors *in utero* resulting in increased allergic airway disease development in conjunction with amplified symptom severity. Furthermore, C57BL/6 mice exposed to methyl donor supplementation *in utero* exhibited enhanced colitis susceptibility, which was also associated with aberrant DNA methylation among genes associated with immunologic processes [27].

Methylenetetrahydrofolate reductase (*MTHFR*) is crucial to one-carbon metabolism, catalyzing the conversion of homocysteine to methionine and generating 5-methyltetrahydrofolate [28]. Because of this relationship, the role of *MTHFR* genotype in DNA methylation has been investigated. An extensive study looking at *MTHFR* variants was conducted in healthy women [29]. Over 14 weeks, participants were randomly assigned to four study diets consisting of established concentrations of folate, betaine, choline phosphatidylcholine, sphingomyelin and various other choline sources within the cell. A cytosine extension assay was used to determine global percent methylation, but neither diet nor *MTHFR* covariate showed a significant effect on leukocyte DNA methylation. In another study, reduced dietary choline and folate intake by women ages 20–30 years was associated with decreased global methylation of leukocyte DNA [30]. Moreover, dietary resupplementation with folate resulted in DNA remethylation on the global scale. This strength of the association was more robust in individuals with *MTHFR* mutations, suggesting that folate supplementation may be crucial for methylation maintenance in individuals with *MTHFR* polymorphisms.

3. Studies of individual methyl donors

3.1. Folate

Dietary folate is the most extensively studied micronutrient in animal and epidemiological DNA methylation research (Table 1). Folate is reduced to dihydrofolate (DHF) and subsequently to tetrahydrofolate (THF), serving as a single carbon donor in the form of 5-methyl THF (Fig. 1). Consequently, 5-methyl THF feeds into the one-carbon metabolism cycle by donating its methyl group to homocysteine converting it to methionine. Cofactor B vitamins provide the enzymatic support necessary for these transformations, making it possible for dietary folate to feed into the one-carbon metabolism cycle to replenish cellular SAM. For this reason, folate supplementation has generally been associated with increased DNA methylation and vice versa for folate restriction. As underscored below, however, conflicting evidence has emerged, suggesting the mechanisms associated with micronutrient influence on DNA methylation are more complex than previously understood.

Three notable animal studies have evaluated the global methylation effects of maternal folate status. First, murine offspring exposed to a low-folate diet during gestation and lactation exhibited decreased global methylation in small intestinal tissue as adults [31], as measured by a cytosine extension technique quantifying unmethylated cytosines within CpG islands. In contrast, the measurement of global methylation by methyl acceptance through the incorporation of ^{3}H-methyl SAM following *in utero* folate supplementation was associated with CpG hypomethylation of the mammary tissue among rat offspring 28 weeks of age [32]. Finally, post-weaning folate supplementation equivalent to recommended intake levels for US women of child-bearing age resulted in decreased global methylation levels in murine colorectal tissue, as measured by liquid chromatography–mass spectrometry [33]. Thus, these *in vivo* animal data support a role for global DNA hypomethylation in both folate restriction and folate supplementation.

A number of human epidemiological studies have also measured global DNA methylation indirectly through the incorporation of ^{3}H-methyl SAM, looking at the correlation between folate and DNA methylation in colorectal cancer (CRC) and in postmenopausal women [30,34–36]. First, two studies were conducted on patients admitted for colonoscopies. In patients who tested negative for CRC, colonic mucosa and lymphocyte DNA methylation were found to be weakly associated with serum folate concentrations and dietary folate as measured using a food frequency questionnaire [35]. In patients diagnosed with CRC, folate supplementation after diagnosis was correlated with an increase in global DNA methylation [34].

Table 1
Studies providing for folate impacts on DNA methylation

Authors	Study population/tissue	Methylation measure
Human		
Ba et al. (2011) [47]	Pregnant women, maternal blood and cord blood	Gene-specific
Christensen et al. (2011) [50]	Primary breast tumors	Epigenome-wide
Hoyo et al. (2011) [49]	Pregnant women, cord blood leukocytes	Gene-specific
Vineis et al. (2011) [46]	Lung cancer cases and controls, leukocytes	Gene-specific
Stidley et al. (2010) [45]	Smokers, sputum	Gene-specific
Hervouet et al. (2009) [37]	Cells cultured from glioblastomas	Global, Gene-specific
van den Donk et al. (2007) [43]	Colon tumors from MTHFR variants	Gene-specific
Pufulete et al. (2005) [35]	Healthy adult colonic mucosa	Global
Pufulete et al. (2005) [34]	Adults with CRC, leukocytes and colonic mucosa	Global
Shelnutt et al. (2004) [30]	Adult women, leukocytes	Global
van Engeland et al. (2003) [44]	Colon tumors	Gene-specific
Rampersaud et al. (2000) [36]	Postmenopausal women, leukocytes	Global
Mouse		
McKay et al. (2011) [42]	Various adult tissues	Gene-specific
McKay et al. (2011) [31]	Adult small intestine	Global
Wakefield et al. (2010) [38]	Various adult tissues	Gene-specific
Rat		
Ly et al. (2011) [32]	Adult mammary tissue	Global
McKay et al. (2011) [41]	Adult small intestine	Gene-specific
Sie et al. (2011) [33]	Adult colonic mucosa	Global
Burdge et al. (2009) [40]	Adult liver and adipose tissue	Gene-specific
Kim et al. (1997) [39]	Adult liver and blood	Global, gene-specific

Second, among postmenopausal women, dietary folate restriction and corresponding decreased serum folate concentration were associated with decreased DNA methylation in leukocytes in a dose-dependent manner [36]. Moreover, folate supplementation to cells cultured from human glioblastomas methylates DNA that is globally hypomethylated in glioblastoma [37]. Therefore, human evidence suggests that folate intake and global DNA methylation are positively correlated.

Numerous studies utilize a candidate gene-specific approach to investigate the effects of folate on DNA methylation. Genomic loci selected are often associated with a disease outcome of interest, such as cancer or diabetes, for the prospect of future preventative or therapeutic strategies through diet targeting the epigenome. For example, *N-acetyltransferase 2* (*Nat2*), a gene whose human homologue, *NAT1*, is associated with cancer development including breast carcinogenesis when overexpressed, displayed increased methylation after adult rats were exposed to a high-folate diet [38]. Additionally, the effect of folate deficiency on the methylation of *p53*, an important tumor suppressor gene, was evaluated [39]. Rats fed low-folate diets exhibited hepatic hypomethylation within two exon regions of *p53*, further suggesting that methylation status can play a role in the molecular underpinnings of carcinogenesis. Folic acid supplementation during the rat juvenile–pubertal period increased methylation in the promoter regions of *insulin receptor*, *PPAR-α* and *glucocorticoid receptor*, genes involved in metabolic homeostasis, compared to offspring exposed to the same *in utero* environment with the exception of a folate-adequate diet during puberty [40]. Wild-type (WT) and $Apc^{+/Min}$ rats exhibited distinct alterations in DNA methylation measured at several loci involved in CRC at weaning and as adults after exposure to folate depletion during gestation and lactation [41]. Importantly, the results of this experiment indicate that the same folate exposure alters DNA methylation in a gene- and tissue-specific manner at different life stages. Additionally, differences in methylation were dependent on sex and genotype.

The aforementioned *in vivo* candidate gene–driven studies explored folate's role in methylation of target tissue types. Using blood as a surrogate biomarker for methylation in targeted tissues will permit thorough human epigenetic research and the development of noninvasive methylation measurements for epigenetic epidemiology studies. Mice deprived of folate *in utero* showed hypomethylation at the differentially methylated region (DMR) 1 of the imprinted locus *insulin-like growth factor 2* (*Igf2*) and at *Slc389a4*CGl1 across blood, liver and kidney tissue. Conversely, *Igf2* DMR 2 showed significant methylation differences in blood vs. kidney and liver [42], providing further evidence that methylation is gene and tissue specific.

There is growing investigation of folate's role in methylation status of select genes known to alter disease susceptibility in humans. Evidence obtained via food frequency questionnaire suggests that folate intake did not have any significant effect on CRC-associated gene-specific methylation of white blood cells [43]. Likewise, low-folate intake combined with high alcohol consumption demonstrated non-significant changes in gene promoter methylation in blood from CRC patients [44]. Another study examined serum folate concentrations among smokers to detect methylation in eight genes that have previously been hypermethylated in lung cancer [45]. Interestingly, increased serum folate was associated with decreased methylation among smokers in five of the eight genes examined. Alternatively, a positive correlation between serum folate concentrations and promoter methylation was identified in *RASSF1A,* a gene whose increased expression is implicated in multiple diseases, such as breast and lung cancer [46]. Although gene expression was not examined in this study, increased promoter methylation may repress *RASSF1A* expression subsequently decreasing cancer risk. Together, these data suggest that folate may be associated with protection against some cancers.

Human studies have also investigated the role of maternal folate status and offspring methylation. A cross-sectional study of pregnant women at the time of parturition looked at the imprinted gene *IGF2* [47]. Imprinted genes are monoallelically expressed due to allelic repression from epigenetic modifications such as DNA methylation. Because imprinted genes are functionally haploid, they are more prone to deregulating events and have been investigated in methylation-based studies [48]. Cord blood and maternal blood were collected at time of delivery, and serum folate concentrations were determined for both specimens [47]. Methylation-specific PCR determined that maternal and cord blood folate concentrations were not associated with methylation outcomes in the P2 and P3 promoters of *IGF2*. Additionally, hypomethylation within the *IGF2* promoter of umbilical cord blood leukocytes was inversely associated with maternal report of folate supplementation during pregnancy [49].

One study has looked for correlations between diet and methylation on an epigenome-wide scale. The Illumina GoldenGate bead array was used to conduct an epigenome-wide study examining connections between human folate intake and breast cancer [50]. Dietary folate, as determined by food frequency questionnaires, was strongly associated on a genome-wide scale with hypermethylation of CpG sites in cancer-related gene promoters. More epigenome-wide scale studies are necessary in discovering candidate loci with variable methylation that have the potential to increase individual or population susceptibility to disease.

3.2. Choline and betaine

Choline is an indirect methyl group donor for one-carbon metabolism. Within this pathway, dietary choline is oxidized to betaine. Betaine then contributes to methionine homeostasis through the donation of a methyl group to homocysteine, resulting in homocysteine's conversion to methionine (Fig. 1) [51]. Thus, a number of animal *in vivo* and, to a lesser extent, human studies have investigated the role of dietary choline and/or betaine and their impact on global and candidate gene DNA methylation (Table 2).

Choline deficiency is associated with altered neurogenesis followed by declined memory function [52,53]. Since choline is crucial for maintenance of one-carbon metabolism, several studies have investigated choline deficiency and its influence on global and candidate gene methylation of fetal brain as well as other tissues. Global methylation analysis of Embryonic Day (ED) 17 mouse fetal brain displayed a significant shift towards hypomethylation [54]. Three candidate genes were also analyzed for DNA methylation status including *Cdkn3, Cdkn2b* and *Calb2*. Of these candidate genes, *Cdkn3* showed hypomethylation in its promoter region in offspring exposed to a maternal choline-deficient diet. Additionally, choline deficiency during Gestational Days 12–17 decreased methylation within a CpG site located in the *Calb1* promoter of the fetal hippocampus [55]. Further examination of early choline exposure on fetal brain gene methylation was performed on *Vegfc* and *Angpt2* [53]. Both candidate loci displayed hypomethylation when exposed to a maternal choline-deficient diet with correlating increased expression of their encoded proteins, VEGF-C and angiopoietin 2, respectively.

Global methylation of rat brain and liver tissue was analyzed following exposure to a choline-deficient diet from EDs 11–17 [56]. Interestingly, global hypomethylation occurred in the brain, while global hypermethylation was observed in liver tissue. Furthermore, hypermethylation at the DMR 2 of *Igf2* within the liver was correlated with hypomethylation of CpG sites within *Dnmt1*, the gene encoding DNA methyltransferase. Thus, these data suggest that early choline deficiency has the ability to deprive DNA

Table 2
Studies providing for choline, betaine, vitamin B and methionine impacts on DNA methylation

Authors	Study population/tissue	Methylation measure
Choline and betaine		
Xing et al. (2011) [58]	Chicken adipocytes	Gene-specific
Mehedint et al. (2010) [53]	Mouse fetal brain	Gene-specific
Mehedint et al. (2010) [55]	Mouse fetal brain	Gene-specific
Du et al. (2009) [57]	Rat adult liver	Gene-specific
Kovacheva et al. (2007) [56]	Rat fetal liver and brain	Global, gene-specific
Niculescu et al. (2006) [54]	Mouse fetal brain	Global, gene-specific
Vitamin B		
Ba et al. (2011) [47]	Pregnant women, maternal blood and cord blood	Gene-specific
Kulkarni et al. (2011) [61]	Rat placenta	Global
Vineis et al. (2011) [46]	Lung cancer cases and controls, leukocytes	Gene-specific
Methionine		
Amaral et al. (2011) [62]	Rat adult kidney	Gene-specific
Vineis et al. (2011) [46]	Lung cancer cases and controls, leukocytes	Gene-specific

methylation machinery of proper substrates of one-carbon metabolism leading to increased expression of methyltransferases and, subsequently, to increased methylation at specific loci, in this case the imprinted gene *Igf2*. This is a complex concept that must be explored further to understand wholly the mechanisms involved in gene-specific methylation.

Betaine supplementation has also been explored for its role in carcinogenesis. Adult rats were exposed to three varying levels of betaine through the diet [57]. Hypermethylation of the tumor suppressor gene *p16* was displayed in liver tissue of the rats exposed to all three doses when compared to the control group. Hypermethylation of *p16* was concomitantly correlated to decreased mRNA expression of *p16*. In chickens, betaine supplementation was associated with modest positional effects in the *lipoprotein lipase* (*LPL*) gene promoter, as measured in adipocytes [58]. While most of the promoter was highly hypomethylated, several CpG sites were found to be hypermethylated with betaine supplementation. Interestingly, *LPL* gene expression was significantly decreased with betaine supplementation, but the influence of DNA methylation on this change is unknown.

3.3. Vitamins B_2, B_6 and B_{12}

The water-soluble vitamins B_2, B_6 and B_{12} have an important catalytic role in folate and one-carbon metabolism (Fig. 1). Vitamin B_6 serves as a coenzyme to serine hydroxymethyltransferase, the key enzyme in the folate cycle converting THF to 5,10-methylene THF [59]. Riboflavin, or vitamin B_2, is a precursor for flavin adenine dinucleotides, which is a cofactor to MTHFR, the enzyme responsible in the reduction of 5,10-methylene THF to 5-methyl THF [60]. Vitamin B_{12} is the coenzyme of methionine synthase, which catalyzes the reaction of homocysteine, the by-product of SAM, to methionine. Thus, dietary consumption of these water-soluble B vitamins has the potential to affect the efficiency of the one-carbon metabolism pathway.

Both animal and human studies have evaluated the role of vitamin B_{12} in DNA methylation profiles (Table 2). An *in vivo* rat model showed that deprivation of vitamin B_{12} in addition to a diet supplemented with folate enhanced placental global hypomethylation compared to a diet exclusively supplemented with folate, suggesting that the interaction between micronutrients can alter methylation patterns more profoundly than excess or deprivation of just one micronutrient [61]. Two human epidemiological studies have examined the role of vitamin B_{12} in the regulation of DNA methylation. Using a cross-sectional study design, Ba et al. [47] assessed vitamin B_{12} and folate status in pregnant women at the time of parturition. Cord blood *IGF2* methylation at the P3 promoter was inversely correlated with maternal blood serum B_{12}. Additionally, maternal blood methylation in the P2 promoter was inversely correlated with maternal serum B_{12}. A second study examined the relationship among plasma vitamin B_2, B_6 and B_{12} concentrations, DNA methylation and smoking status [46]. Among former smokers, an increase in serum vitamin B_{12} concentration was associated with a decrease in methylation for several multi-disease–related gene promoters. A similar decrease in methylation was associated with increased serum vitamin B_6 concentration for one of the disease-linked genes.

3.4. Methionine

Methionine is an essential amino acid that is continuously regenerated from homocysteine in one-carbon metabolism to serve as the precursor to SAM (Fig. 1). Thus, fluctuation of methionine in the diet has potential effects on DNA methylation. Although methionine has an immense role as a methyl donor, there is limited evidence when evaluating methionine and DNA methylation (Table 2). Rats fed a methionine-supplemented diet had no change in *p53* promoter region methylation [62]. Additionally, plasma methionine concentrations were inversely correlated to DNA methylation in the promoters of several multi-disease–related genes among former and current smokers [46].

4. Discussion

The role of nutrition in one-carbon metabolism and DNA methylation has been more extensively studied in animal models than in humans; however, this review reveals that the epidemiologic data are becoming increasingly robust. Herein, we have highlighted data in which altered consumption of folate, choline, betaine, B vitamins and methionine acts to modify methylation both globally and in the promoters of disease-related genes in animals and humans. Thus, nutri-epigenetics approaches provide a molecular foundation for understanding the role of diet throughout the life course and its prospective role in disease prevention and/or therapy.

There are a limited number of human studies on micronutrient intake and methylation; nonetheless, multiple laboratories have established a solid foundation using animal *in vivo* models for future studies in this discipline. This body of literature indicates the importance of exposure timing, genotype, and tissue- and gene-specific DNA methylation and the interpretation of results. Because methylation is cell type dependent, a comprehensive epigenetic analysis remains an optimal approach. Future studies should continue to focus on tissue specificity in DNA methylation investigation, as this will likely most significantly influence varying disease states. In addition to examining tissue-specific methylation, data surrounding methyl donor exposure during windows of susceptibility throughout the life course such as embryogenesis, fertilization, neonatal, puberty and aging should be thoroughly investigated. Vulnerable epigenetic states combined with tissue-specific outcomes can shape the knowledge of disease etiology and, possibly, preventive and therapeutic approaches to disease.

As reviewed above, a vast array of methodologies have been used to detect DNA methylation in nutri-epigenetics studies. Even over the past decade, epigenetic technologies have evolved from once traditional methods using restriction enzymes or focusing on candidate genes to modern technologies allowing for unbiased epigenome-wide investigation across tissues and species. Since a main goal of nutri-epigenetics is to better understand the role of diet in disease, epigenome-wide investigations involving deep sequencing and tiling array technologies will be valuable in future studies, as

they apply an integrative approach and can identify key regulatory pathways and interactions to which diet is a modifier. Other epigenetic mechanisms such as histone modifications and chromatin remodeling complexes should also be considered [63]. Additionally, investigating the interactions among micronutrients required in one-carbon metabolism and those that may indirectly affect their supply to maintain cycle efficiency can be implicit by integrating a genome-wide application.

As the field of nutri-epigenetics continues to emerge, it will enable clinical and public health practices to apply epigenetically driven therapeutic and preventative strategies when evaluating a population or individuals in a certain disease state. For example, registered dietitians can make nutritional recommendations based upon individual epigenetic profiles. Because the epigenome is particularly sensitive at various time points throughout the life course, targeted nutri-epigenomic population interventions may be efficient and cost-effective. Continued animal nutri-epigenomic models translated into human research will strengthen our understanding in the biological pathways associated with diet and human health.

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