Review

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Folate and fetal programming: a play in epigenomics?

Jean-Louis Guéant[1,2], Fares Namour[1], Rosa-Maria Guéant-Rodriguez[1], and Jean-Luc Daval[1]

[1] Institut National de la Santé et de la Recherche Médicale (INSERM) Unité 954, Department of Nutrition-Genetics-Environmental Risk Exposure, University of Lorraine and University Hospital of Nancy, Vandoeuvre-lès-Nancy, France
[2] Instituto di Ricovero e Cura a Carattera Scientifico (IRCCS), Oasi Maria S.S. Institute for Research on Mental Retardation and Brain Aging, Troina (EN), Italy

Folate plays a key role in the interactions between nutrition, fetal programming, and epigenomics. Maternal folate status influences DNA methylation, inheritance of the agouti phenotype, expression of imprinting genes, and the effects of mycotoxin FB1 on heterochromatin assembly in rodent offspring. Deficiency in folate and other methyl donors increases birth defects and produces visceral manifestations of fetal programming, including liver and heart steatosis, through imbalanced methylation and acetylation of PGC1-α and decreased SIRT1 expression, and produces persistent cognitive and learning disabilities through impaired plasticity and hippocampal atrophy. Maternal folate supplementation also produces long-term epigenomic effects in offspring, some beneficial and others negative. Deciphering these mechanisms will help understanding the discordances between experimental models and population studies of folate deficiency and supplementation.

Role of folate during development, in susceptibility to disease in early life, and in aging

Folate is essential for human health and development. Its frequent deficiency during pregnancy produces adverse pregnancy outcomes, with impact upon public health worldwide [1]. Thus, there is a need to understand better the role of folate in normal physiology, during pregnancy, and in the long-term health of offspring. According to the developmental origins of health and disease (DOHaD) hypothesis (see Glossary) [2], increased susceptibility to disease is partly shaped during fetal programming by links between nutrition and epigenetic and epigenomic mechanisms [3,4]. Folate metabolism plays crucial role in some of these mechanisms because it determines the flux of monocarbons towards synthesis or methylation of DNA and RNA, and also governs the methylation of regulators of gene expression via *S*-adenosyl methionine (SAM). In this metabolic balance, 5-methyltetrahydrofolate (5-MeTHF) fuels the vitamin B12-dependent enzyme methionine synthase (MTR) that works at the crosspoint between

Glossary

Developmental origins of health and disease hypothesis (DOHaD): the hypothesis proposes that unfavorable intrauterine life, including IUGR, predicts the risk of postnatal complex diseases through a relationship that is further modified by postnatal environment.
DNA (cytosine-5)-methyltransferase 1 (DNMT1): the enzyme that propagates the CG methylation patterns through cell division, and which 'completes' hemimethylated but not unmethylated sites.
DNA methylation: the most characterized epigenetic modification in vertebrates is a covalent chemical modification of DNA occurring almost exclusively at cytosine residues in CpG dinucleotides. Methylated CG is symmetrically paired with the same sequence on the opposite DNA strand, thus after DNA replication these sites are transiently methylated on only one of the two strands. Although the distribution pattern of 5-methylcytosine in the genome of differentiated somatic cells is moderately stable, dynamic changes in methylation during early development constitute a form of epigenetic reprogramming. DNA methylation plays a crucial role in cell processes such as embryonic development, transcription, X-chromosome inactivation, and genomic imprinting.
Epigenetics: the study of heritable changes in gene expression that are caused by mechanisms other than changes in the underlying DNA sequence [114]. Examples are DNA methylation, histone acetylation/methylation, and synthesis and effects of miRNA.
Epigenomics: approaches for studying environmental or developmental epigenetic effects on gene functions. Epigenomics focuses on genes whose function is determined by external factors. Some epigenomic mechanisms are nonheritable and environmentally driven (such as by nutrition; nutritional epigenomics) changes in gene expression.
Post-translational covalent modifications of histones: these different classes of covalent modifications include acetylation, methylation, phosphorylation, sumoylation, and ubiquitination. Lysine methylation displays a high degree of complexity because each lysine residue can be mono-, di-, or tri-methylated, and each site of methylation can influence gene expression independently. Arginine residues can be mono-methylated or di-methylated symmetrically or asymmetrically. These modifications obey to inter- and trans-histone regulatory pathways that form a 'histone code'. Histone modifications are grouped into those which 'activate' and those which 'repress' transcription, and can influence DNA replication, repair, and condensation. The link between DNA methylation and gene silencing may involve covalent histone modifications, which serve as a bridge enabling the binding of chromatin remodeling factors.
Protein arginine methyl transferase 1 (PRMT1): the major arginine methyl transferase in mammals. It catalyzes the addition of two methyl groups to the same terminal guanidino nitrogen group of arginine of proteins, generating asymmetrically dimethylated NG,NG-dimethylarginine (ADMA). It methylates many categories of proteins, including histones, nuclear receptors, and coregulators of gene transcription.
***S*-adenosyl homocysteine (SAH):** the product of transmethylation reactions and also an inhibitor of these reactions. The ratio of SAM to SAH is an index of methylation status.
***S*-adenosyl methionine (SAM):** a common cosubstrate involved in methyl group transfers, mostly produced and consumed in the liver. SAM is made from ATP and methionine by the enzyme methionine adenosyltransferase. Some of the metabolic pathways that use SAM include transmethylation, transsulfuration, and aminopropylation.
Sirtuin1 (SIRT1): an NAD-dependent histone deacetylase (HDAC) that also deacetylates transcription factors and cofactors, and regulates numerous central metabolic pathways.

Corresponding author: Guéant, J.-L. (jean-louis.gueant@medecine.uhp-nancy.fr).
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the folate and methionine cycles [5]. Growing experimental and population-based evidence shows that folate influences the epigenetic and epigenomic mechanisms that underlie intrauterine growth retardation (IUGR), fetal programming, and embryo-fetal brain development [1,6]. This review focuses on these mechanisms and the subsequent early and late consequences as they relate to metabolic syndrome manifestations in the heart, gut and liver, and to brain disorders.

Influence of nutritional and genetic determinants on folate metabolism and methylation

Folate coenzymes are involved in the synthesis and exchange of mono-carbons

Folate represents a group of interconvertible coenzymes that differ in their oxidation states and in the number of glutamic acid moieties and one-carbon substitutions (Figure 1; Box 1). Folate metabolism displays complex biochemical regulation (Box 2) and participates in a network of interconnected pathways that are necessary for the synthesis of purine nucleotides, thymidylate, and amino acids [1]. Cellular methionine originates from the remethylation pathway and from the degradation of endogenous and food-derived proteins. A cellular deficit in 5-MeTHF leads to the decreased synthesis of methionine and the accumulation of homocysteine, which produces cellular stress by mechanisms previously described [7]. The homeostasis of methionine is crucial to the cell because it is the immediate precursor of SAM, the universal methyl donor in transmethylation reactions [1]. The consequences of folate deficiency on the cellular concentrations of SAM and *S*-adenosyl homocysteine (SAH), and on the SAM/SAH ratio, illustrate the importance of folate in maintaining adequate cellular SAM content [6]. A decreased SAM/SAH ratio impairs the ability of the cell to ensure the transmethylation reactions of DNA, RNA, histones, and coregulators of nuclear receptors, all of which play a key role in epigenetic and epigenomic mechanisms [6].

Methylenetetrahydrofolate reductase (MTHFR) 677 C>T polymorphism and folate status: an example of gene–nutrient interaction

Among single-nucleotide polymorphisms (SNPs) in the genes encoding folate and methionine cycle enzymes, the

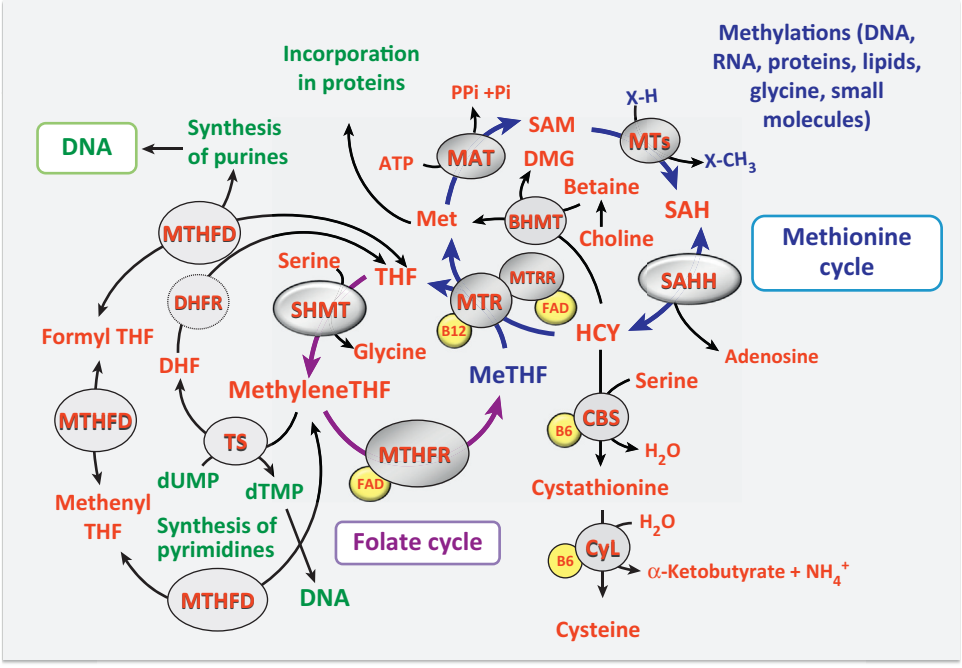

Figure 1. Folate and methionine cycles. In the folate cycle, the methyl donating 5-methyltetrahydrofolate (5-MeTHF) originates from 5,10-methyleneTHF by the flavin adenine dinucleotide (FAD)-dependent methylenetetrahydrofolate reductase (MTHFR). In addition to its conversion to 5-MeTHF by MTHFR, 5,10-methyleneTHF is also metabolized in several one-carbon transfer reactions during the synthesis of thymidylate [with methylation of deoxyuridine 5-monophosphate (dUMP) to deoxythymidine 5-monophosphate (dTMP)] as well as during the synthesis of purines [with conversion of 5,10-methyleneTHF into 5,10-methenyltetrahydrofolate (methenylTHF) and further into 10-formyltetrahydrofolate (formylTHF)]. After the release of their one-carbon units, all these substituted folates are converted to THF, which is finally recycled into methyleneTHF through the conversion of serine to glycine by the enzyme serine hydroxymethyltransferase (SHMT). This explains why MTHFR and methionine synthase (MTR) activities are pivotal in directing the reduced folate coenzyme pool towards either remethylation of homocysteine (HCY) or DNA/RNA synthesis [1]. In the so-called methionine cycle, HCY is remethylated into methionine (Met) by MTR with 5-MeTHF as a methyl donor and cobalamin (vitamin B12) as a cofactor. Alternatively, HCY can also be remethylated into methionine by betaine–homocysteine methyltransferase (BHMT), in which the methyl group provided by betaine is transformed into dimethylglycine. This alternative pathway seems to be limited to the liver. Met is further transformed into *S*-adenosylmethionine (SAM) by three isoforms of methionine *S*-adenosylmethionine transferase (MATI, II, and III). This synthesis is crucial for cell life because SAM is the universal methyl donor for methylation of nucleic acids, proteins, polysaccharides, phospholipids, glycine and many small molecules. MATII is expressed in extrahepatic tissues, and MATI and MATIII in liver, respectively. The switch of methionine metabolism to MATIII and glycine *N*-methyl transferase (GNMT), instead of to the MATI pathway, produces a bypass which appears to adapt liver transmethylation reactions to a narrow trigger zone of methionine concentration [115]. In transmethylation reactions, SAM is converted into *S*-adenosyl homocysteine (SAH), which is reversibly hydrolyzed into adenosine and HCY in a reaction catalyzed by *S*-adenosylhomocysteine hydrolase (SAHH, also known as *S*-adenosylhomocysteinase). Then, HCY is either remethylated or metabolized into cystathionine by the vitamin B6-dependent cystathionine β-synthase (CBS). This transsulfuration pathway is functional in liver, kidney, intestine, pancreas, and brain. Additional abbreviations: CyL, cystathionine lyase; DHF, dihydrofolate; DHFR, dihydrofolate reductase; MT, methyltransferase(s); MTHFD, 5,10-methylenetetrahydrofolate dehydrogenase; MTRR, methionine synthase reductase; TS, thymidylate synthase; X, substrate to be methylated.

most studied is the *MTHFR 677C to T* substitution which causes a 70% reduction in MTHFR activity in *TT* homozygotes (relative to *CC* homozygotes), and thus affects folate distribution [6]. *677TT* homozygosity leads to higher concentrations of 5,10-methylene THF and preferentially directs one-carbon units towards DNA synthesis instead of to homocysteine remethylation [6]. Conditions of impaired folate status thus lead to reduced genomic DNA methylation [8] but, in combination with positive folate balance, the *TT* genotype is associated with decreased risk of colorectal cancer. The *677T* allele has been associated with several diseases, including vascular diseases, schizophrenia, and neural tube defects [6]. High periconceptional folate intake increases the frequency of newborns bearing the *677T*

Box 1. Structure, absorption, metabolism, and functions of folate

The diet contains folate monoglutamates and polyglutamates (Figure I), which need to be hydrolyzed to monoglutamates by the glutamate carboxypeptidase II (GCPII), an exopeptidase of the intestinal apical brush-border membrane. Monoglutamates are subsequently absorbed through transmembrane transport by the proton-coupled folate transporter (PCFT1, encoded by the *SLC46A1* gene), which optimally functions at the acidic intraluminal pH of the upper intestinal epithelium (Figure II). Folic acid is the form of folate used in food fortification. It is more readily absorbed across the intestinal cell than dietary folate [116]. The reduced folate carrier (RFC, encoded by the *SLC19A1* gene) is a transmembrane protein, which can possibly transport folates at the more neutral intraluminal pH of the distal intestine. The monoglutamate form of 5-MeTHF is the predominant folate in the blood circulation, where it can be transported by soluble folate receptor. Folates are mainly stored in the liver, which plays a key role for their redistribution in the monoglutamate form of 5-MeTHF [117]. There are two main routes for the uptake of folate in peripheral tissues. The first route involves RFC, which is expressed in most tissues and functions as a bidirectional anion exchanger, with a high affinity for reduced folates but low affinity for folic acid. The second route of folate uptake involves the small family of folate receptors (FRs), FRα, FRβ, and FRγ, which are encoded by three distinct genes. FRα and FRβ are attached to cell membranes by a glycosylphosphatidylinositol (GPI)-anchor, whereas FRγ is a secreted protein. The membrane-attached FRs internalize folate by an endocytotic mechanism [117,118]. The expression of FRα, the most abundant isoform in adults, is restricted to few tissues and is a key mediator of folate uptake into the brain. It has a high affinity for folic acid and 5-MeTHF but a lower affinity for other reduced folates. The expression of FRα, the most abundant FR isoform in adults, is restricted to few tissues including the apical (luminal) surface of epithelial cells; FRα is a key mediator of folate uptake into the brain. PCFT1 is expressed in many tissues, including liver and adrenal glands, where its role is unclear because it functions optimally at acidic pH [119]. It plays a key role in the reabsorption of folate in the tubular cells of the kidney [119]. Folate isoforms are sequestered in the liver and peripheral tissues via their polyglutamylation by the folylpoly-γ-glutamate synthetase (FPGS). This enzyme modifies monoglutamates by adding 4–8 glutamates. A mitochondrial FPGS regulates the accumulation of folylpolyglutamates into mitochondria. Cellular sequestration can be reversed by hydrolysis of the terminal glutamate residues by γ-glutamyl hydrolase (GGH). Cellular efflux of folate isoforms involves several transporters of the ATP-binding cassette (ABC) superfamily (not shown in the figure) (Figure II).

The reactions in which folate is involved are essential for DNA and RNA synthesis, and for methylation reactions. Folate is also involved in methionine and glycine synthesis and in histidine catabolism. Folate-related defects in nucleotide synthesis lead to DNA instability and changes in the methylation of DNA and histones that play major roles in carcinogenesis. A recent review on the topic proposed that folate-related fetal programming is a potential contributing factor of cancer [120].

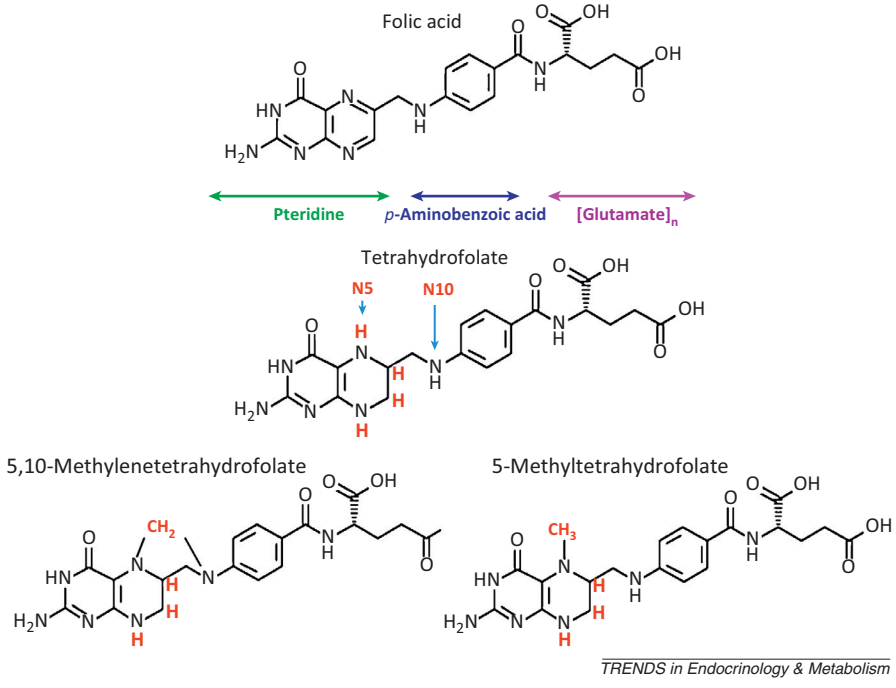

Figure I. Folate structure. Folate is a generic term for the water-soluble B9 vitamin, which is notably found in leafy vegetables, dried beans and peas, bakers yeast, and liver. Folate is a conjugated molecule consisting of a pteridine base attached to *p*-Aminobenzoic acid and glutamic acid residues. Dihydrofolate and tetrahydrofolate are the reduced from of folate (hydrogens in red). The function of tetrahydrofolate (THF) derivatives is to carry and transfer various forms of one-carbon units to other compounds. The single carbon groups can be carried on N5, N10 (see arrows) of reduced folate (see the example of 5-methyltetrahydrofolate) or bridged between both of these nitrogens (see the example of 5,10-methylenetetrahydrofolate). The one-carbon units are methyl (–CH_3), methylene (–CH_2–), methenyl (=CH–), formyl (–CHO–), or formimino groups (–CH=NH).

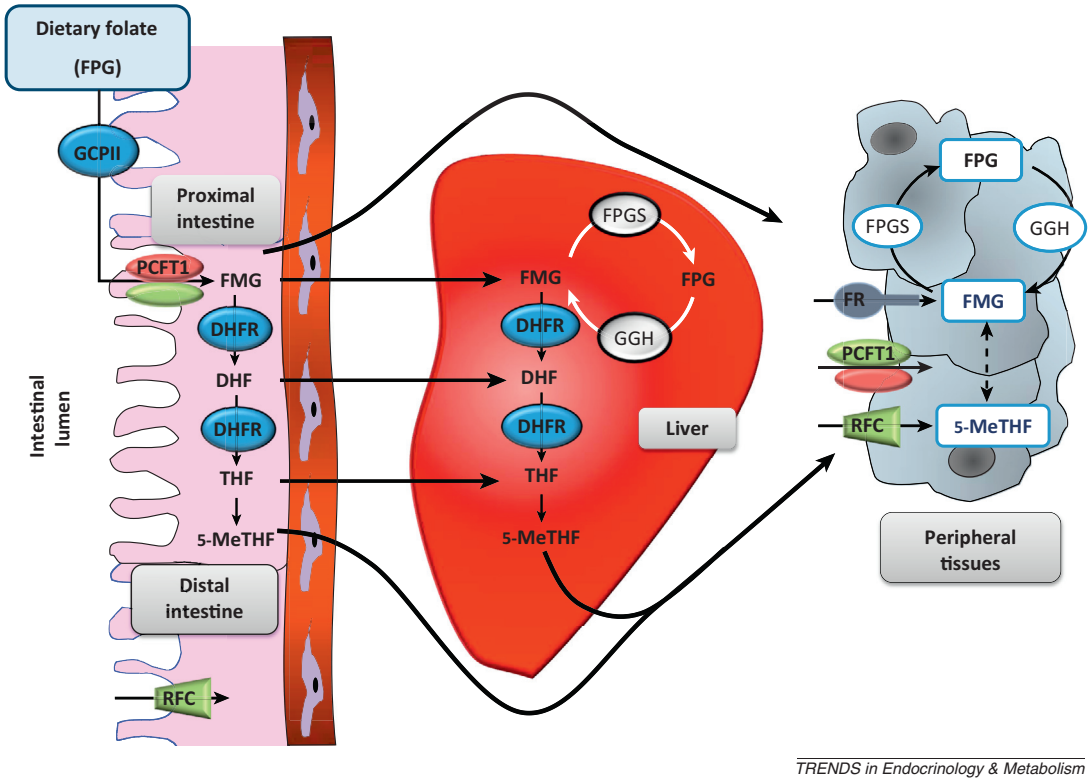

Figure II. Folate absorption and distribution in liver and peripheral tissues. Folate polyglutamates (FPG) are hydrolyzed to folate monoglutamates (FMG) by the glutamate carboxypeptidase II (GCPII). Monoglutamates are absorbed by the proton-coupled folate transporter (PCFT1). Three systems of folate transport proteins are involved in folate uptake in liver and peripheral tissues: reduced folate carrier (RFC), folate receptors (FRs), and PCFT1. Folate polyglutamylation is catalyzed by the folylpoly-γ-glutamate synthetase (FPGS). Conversely, FPG can be hydrolyzed in FMG by γ-glutamyl hydrolase (GGH).

allele [9]. These data and the comparison of folate status and *677T* allele distribution worldwide suggest a selective advantage of the *TT* homozygous genotype when dietary intake of folate is adequate [8,10,11].

Influence of folate on epigenetic and epigenomic mechanisms of embryo-fetal development

Folate, reproduction, and pregnancy outcomes

Fertility is influenced by folate in both males and females. Folate deficiency is associated with altered spermatogenesis and impaired ovarian reserve through reduced cell division, inflammatory cytokine production, altered nitric oxide (NO) metabolism, oxidative stress, apoptosis, and defective methylation reactions [1,12]. Preimplantation embryos express almost all enzymes that participate in one-carbon metabolism [13], and the role of exogenous folate on their development is under debate [14]. Embryo implantation is not affected by folate deficiency, suggesting that abnormalities might start later in life [15]. By contrast, folate is crucial for proper fetal development [12,16,17], and its deficiency is associated with IUGR, preterm or fetal death, Down syndrome, neural crest-related birth defects including neural tube defects, conotroncal cardiopathies, and oro-facial clefting [18–20]. Periconceptional folate supply alone or in combination with other B vitamins prevents neural tube defects, but its effects are less clear on oro-facial clefting and congenital heart defects [21]. The effect of folate supplementation throughout pregnancy remains controversial, compared to the well-established protective influence of periconceptional folate [22]. A recent meta-analysis concluded that increased folate intake is associated with higher birth weight after the first trimester, but has no effect on length of gestation [22].

DNA and RNA methylation in embryo-fetal development

The genome of the very early mouse embryo is substantially methylated, but during the preimplantation stage there is a wave of genome-wide demethylation followed by *de novo* methylation in somatic and extraembryonic tissues [3,4]. This demethylation is less prominent in other species. As differentiation progresses, methylation patterns are reset in a lineage-specific fashion. During fetal development, a second wave of epigenetic erasure and re-establishment takes place, specifically in primordial germ cells, and involves imprinted genes [23]. Folic acid supplementation during pregnancy produces epigenomic changes in cord blood DNA, favoring CpG methylation patterns that are associated with increased methylation levels of long interspersed nuclear elements (LINE-1) and with birth weight [24]. A diet deficient in folate, choline, and methionine results in upregulated expression of DNA methyltransferase 1 (Dnmt1) in rat liver [25]. Folic acid supplementation of a protein-restricted maternal diet prevents both a decrease in promoter CpG methylation and increased expression of hepatic genes, including peroxisome proliferator-activated receptor α (PPAR-α) and glucocorticoid receptor (GR), in the offspring [26]. Beside its effects on DNA methylation, folate deficiency in rodents may also inhibit the expression of miRNAs involved in the

Box 2. Biochemical mechanisms regulating folate metabolism

The complex biochemical mechanisms that regulate intracellular folate metabolism can be summarized in five interdependent aspects.

(i) The intracellular concentration of folate isoforms is lower than the Michaelis constant of the enzymes using folate and which are involved in the methionine cycle [118]. Consequently, the rate of the reactions can shift dramatically with slight changes in folate concentrations.

(ii) The function of intracellular folylpolyglutamates is to increase affinity of folate for enzymes that interconvert two folate substrates, to inhibit folate dependent enzymes for which they are not substrates, and to retain folate derivatives in cells [121].

(iii) Excess folate inhibits enzymes [122,123]. For example, dihydrofolate inhibits MTHFR whereas dihydrofolate in its polyglutamate form inhibits thymidilate synthase, thus hampering DNA synthesis.

(iv) An additional feature of the biochemistry of folate and methionine cycles is that SAM and 5-MeTHF exert allosteric effects in enzymes of the one-carbon metabolism; SAM inhibits MTHFR and betaine homocysteine methyltransferase (BHMT) and activates cystathionine β-synthase (CBS), whereas 5-MeTHF inhibits glycine *N*-methyltransferase (GNMT), which diverts SAM away from the methylation reactions by retrieving a methyl group from SAM and adding it to glycine, thus generating sarcosine. Folate found in food can in theory modulate the availability of SAM, influence the methionine cycle, and affect DNA methylation. In a mathematical model of one-carbon metabolism, low 5-MeTHF levels produce increased homocysteine, decreased SAM and THF, and two oscillations of lower amplitude of DNA methylation, compared to a normal supply of 5-MeTHF [124]. Consistently, another mathematical model suggests that allosteric regulation stabilizes methylation rate at low methionine input [125]. This suggests that long-range regulatory mechanisms connecting the folate and methionine cycles have evolved to preserve methylation reactions and protect ancient populations during periods of protein starvation.

control of apoptosis and proliferation [27]. Therefore, methyl-donor status during pregnancy produces persistent epigenomic changes related to fetal development and metabolic homeostasis.

Postnatal phenotypes are influenced by epigenetic changes through folate status during pregnancy

Rodents on a methyl-deficient diet provide a suitable model to investigate promoter and histone demethylation, gene expression, and tissue-specific effects [28,29]. For example, maternal dietary folate supplementation produces epigenetic effects in the progeny of the agouti mice model [30,31]. The agouti gene (termed *A*) controls the differential production of melanin pigments in the coat of mice that gives rise to their wild type brown coat color. A mutation designed A^{vy} is caused by the retrotransposition of a CpG-containing intracisternal A particle (IAP) upstream of the transcription start-site of the wild type *agouti* gene. Hypomethylation turns IAP into an ectopic promoter resulting in a yellow coat, whereas hypermethylated IAP maintains the agouti brown coat. DNA methylation and histone modifications cooperate because yellow mice display histone marks associated with transcriptional activity (H3 and H4 diacetylation) [30,31]. 'Viable yellow' mice A^{vy}/a (*a* = *nonagouti* loss-of-function allele) display a tendency to obesity, diabetes, and shorter lifespan [32]. The locus displays maternal epigenetic inheritance [32]. Maternal methyl-supplemented diet alters agouti expression in the direction of pseudo-agouti phenotype, with leaner and healthier offspring [32], increased DNA methylation at the A^{vy} locus, and epigenetic metastability of the A^{vy} IAP [33]. The diet-induced epigenetic phenotype change resulting from methyl-donor supplementation in the F0 generation is passed to the F2 generation [34], suggesting that F1 offspring exposed to methyl-donor deficiency produce the gametes that will give rise to the F2 generation *in utero* [35]. However, other reports showed no cumulative increase in pseudo-agouti color across three generations (F1, F2, and F3) in methyl-supplemented yellow dams [36], and no inherited mark on A^{vy} DNA methylation [37]. Feeding pregnant A^{vy} obese mice with a diet enriched in methyl donors decreases body weight in the F3 generation. However, the diet produces no association between body weight and coat color, suggesting that epigenomic changes in genes other than A^{vy} might affect body-weight regulation [38]. The influence of dietary folate during pregnancy and lactation has been also observed for $Axin^{Fu}$, a gene involved in embryonic axis formation, with increased locus-specific CpG methylation that influenced the incidence of tail kinking in offspring [39]. In other studies, maternal folic acid supplementation in rodents on a protein-restricted diet produced epigenomic changes affecting hepatic metabolism and vascular function, and negative effects such as increased mammary tumorigenesis and multigenerational respiratory impairment, suggesting that carefully consideration of the long-term effects related to the epigenome and epigenetics is warranted [40].

Toxic dietary factors also influence the epigenetic hallmarks related to folate status. Maternal exposure to bisphenol A, an estrogenic xenobiotic, decreases DNA methylation in the retrotransposon upstream of the agouti gene and shifts coat-color distribution in the offspring. This effect is neutralized by maternal supplementation with folic acid, betaine, and choline [41]. Fumonisin, a fungus-derived mycotoxin rich in maize-based diets, is associated with growth retardation in exposed infants in Tanzania [42], whereas maternal intake of fumonisin from maize-based food products increases the risk of neural tube defects in offspring on the Texas–Mexico border [43]. Fumonisin FB1 inhibits folate receptor expression [44,45], and methyl-donor deficiency and fumonisin FB1 synergistically increase DNA instability through alteration in heterochromatin assembly [46]. This suggests that part of FB1 toxicity is related to epigenetic alterations produced by impaired folate metabolism.

Folate and genomic imprinting

Methyl group donors in the diet of pregnant mothers can influence the development and health of their offspring through altered expression of imprinted genes. Imprinted genes are organized in clusters and are expressed from only one of the parental alleles. In the *H19*/*Igf2* cluster, *H19* encodes a non-coding RNA, whereas the neighboring *Igf2* gene influences embryonic and placental development through the expression of insulin-like growth factor 2 (IGF2) [47]. *Igf2* is expressed almost exclusively from the paternal allele, and the tightly linked *H19* from the

maternal allele, through a complex regulation that involves differentially methylated regions (DMR), also known as the 'imprinting center' in human genome, located between the two genes. In proper imprinting of H19 and Igf2, the imprinting center is methylated on the paternal allele and unmethylated on the maternal allele [47]. A methyl donor-deficient diet administered to mice for 60 days post-weaning causes loss of imprinting of *Igf2* independently of DNA methylation, and this persists during the subsequent 100 days on a 'natural' diet, demonstrating a permanent post-weaning effect on *Igf2* expression [48]. Increased maternal intake of folic acid before and during pregnancy produces loss of *H19* and *Igf2* imprinting in human umbilical cord blood leukocyte DNA, with the most pronounced effect in male infants [49]. Patients heterozygous for a *H19* polymorphism and presenting with high homocysteine concentrations due to renal failure (60 μmol/l) display bi-allelic expression of *H19* that correlates with decreased DNA methylation, and thus increased H19 expression, a phenotype that reverts to mono-allelic expression and increased *Igf2* expression after treatment with 5-MeTHF [50]. These results show that the epigenomic influence of folate on *Igf2* expression is effective in the intrauterine and postnatal periods in humans.

Consequences of folate deficiency during pregnancy on postnatal and adult gut, liver, and heart physiology

Effects of folate deficiency on functional organization and epigenomic changes in the digestive mucosa

Deficiency in folate and vitamin B12 produces a dramatic injury in the digestive mucosa that may participate to the patho-mechanisms of IUGR and low birth weight [51–54]. Ghrelin is a gastric peptide involved in fetal growth through its dual role as growth hormone-releasing factor and appetite stimulant [52]. Methyl-donor deficiency produces aberrant localization of ghrelin cells in the pit region of oxyntic glands and impairs the release of ghrelin into the blood [51]. It also results in overexpression of the cyclooxygenase 2 (Cox-2), phospholipase A2 (PLA2), and tumor necrosis factor-α (TNF-α) proinflammatory pathways in gastric and intestine mucosa, and alters mucosal differentiation and barrier function, in rat pups from dams subjected to folate deficiency during gestation and lactation [53,54]. Addition of folic acid to the maternal or post-weaning diets of pups induces specific changes in the methylation of individual CpG islands in the phosphoenolpyruvate carboxykinase (*pepck*) gene in females [55]. In addition, low folate during pregnancy reduces global DNA methylation in the murine small intestine of adult offspring, without subsequent influence of post-weaning folate supply [56]. It also reduces methylation of the solute carrier family 39 member 4 gene (*slc394a*) that encodes a renal- and intestine-specific transmembrane zinc transporter protein, and of the estrogen receptor gene (*Esr1*) in the fetal gut, with a higher effect in males [57]. Epigenetics may mediate also some of the effects of environment, genetics, and intestinal microbiota in the pathogenesis of inflammatory bowel diseases [58]. These consequences of methyl-donor deficiency illustrate the crucial role of fetal programming and folate-related epigenomic effects in (patho)physiology.

Folate deficiency produces liver and heart steatosis through epigenomic dysregulation of mitochondrial energy metabolism

The mother–progeny rat model of folate and vitamin B12 deficiency aids our understanding of the complex link between metabolic syndrome and birth weight

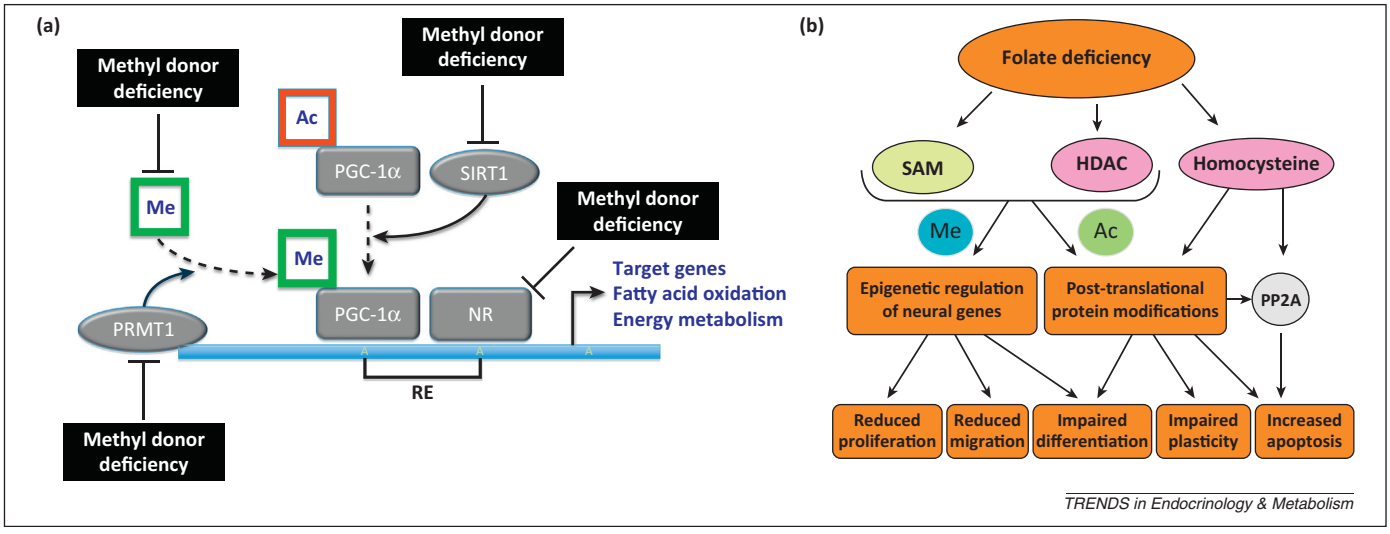

Figure 2. Molecular mechanisms explaining the link between methyl-donor deficiency, energy metabolism in heart and liver, and differentiation/plasticity in neurons. **(a)** The deficiency in folate and vitamin B12 of the rat 'dam–progeny' model impairs fatty acid oxidation and the function of the mitochondrion respiratory chain through decreased expression of PPARα, ERRα, and HNF-4, and impaired coactivation of these nuclear receptors by PGC-1α, a result of decreased methylation and/or increased acetylation of PGC-1α. The imbalanced methylation/acetylation is a consequence of the decreased expression of PRMT1 arginine methyltransferase, increased cellular concentration of *S*-adenosyl homocysteine (SAH) (a potent inhibitor of PRMT1 activity), and decreased expression of the deacetylase SIRT1 (adapted from [60,62,67]). **(b)** Suggested consequences of folate deficiency in neuron progenitors involve sequential events related to increases of histone deacetylase (HDAC) and homocysteine (in pink) and decrease in *S*-adenosyl homocysteine (SAM) (in green). Folate deprivation reduced proliferation and sensitized progenitors to differentiation-associated apoptosis through increased homocysteine and decreased protein phosphatase 2A (PP2A). Decreased production of *S*-adenosylmethionine and altered HDAC expression led to epigenomic dysregulation of the differentiation neuronal program that impairs neurite outgrowth. Vesicular transport and synaptic plasticity are dramatically affected, with alterations of major cytoskeleton proteins. Increased homocysteine produced homocysteinylation of actin and kinesin and subsequent formation of protein aggregates. Figure adapted from [88].

[59–62]. In this model, fatty liver and fatty heart can be regarded as two early manifestations of metabolic syndrome [60–64].

Methyl-donor deficiency during pregnancy and lactation induces heart hypertrophy, impairs mitochondrial fatty acid oxidation, and decreases the activity of complexes I and II of the respiratory chain in rat pups [60]. These changes relate to decreased expression of PPAR-α and estrogen-related receptor α (ERR-α), and hypomethylation/hyperacetylation of the peroxisome proliferator-activated receptor γ coactivator 1-α (PGC-1α) protein through decreased expression and activity of arginine methyltransferase 1 (PRMT1) and sirtuin 1 (SIRT1) [60,65–67] (Figure 2). The link between the one-carbon metabolism and impaired mitochondrial fatty acid oxidation has been confirmed in patients undergoing coronarography and in ambulatory elderly subjects [68], suggesting an association between homocysteine and vitamin B12 levels and left ventricular mass and systolic dysfunction [69,70].

Experimental rodent and pig models have established a link between one-carbon metabolism and non-alcoholic fatty liver disease (NAFLD). Mice fed a methionine- and choline-deficient diet develop steatohepatitis [71], and folate and vitamin B12 deficiency yields microvesicular liver steatosis in rat pups from dams with methyl-donor deficiency during pregnancy and lactation [72]. The steatosis results from a deficit in carnitine synthesis and epigenomic dysregulation, with hypomethylation and decreased expression of PPAR-α, ERR-α, estrogen receptor α (ER-α) and hepatocyte nuclear factor 4 (HNF-4α), and hypomethylation of PGC1-α, through decreased activity of PRMT1 [62]. As shown in the hearts of rat pups, hypomethylation of the PPAR-α, ERR-α, and HNF-4α coactivator PGC1-α proteins in the liver affects nuclear receptor activity and results in the dysregulation of mitochondrial fatty acid β-oxidation (Figure 2a) [60,62]. Consistently, lack of PPAR-α enhances steatohepatitis, whereas PPAR-α agonism has the opposite effect, in mice fed a methionine- and choline-deficient diet [73]. In addition, methyl-donor deficiency produces decreased SIRT1 expression, which is also observed in animals on a high-fat diet [74]. These data show that the epigenomic effects of methyl-donor deficiency produce a deregulation of energy metabolism that could potentially contribute to the persistent visceral manifestations of fetal programming.

Folate and metabolic manifestations of fetal programming: population-based studies

The link between methyl-donor deficiency, fetal programming, and subsequent manifestations of metabolic syndrome has produced contrasting results in population studies, with a higher influence of vitamin B12 than folate [75–77]. In India, there is a higher prevalence of low maternal serum vitamin B12, folate deficiency, and IUGR compared to Europe [75]. In a rural area of South India, the most insulin-resistant children were born to mothers with the lowest serum vitamin B12 and the highest erythrocyte folate concentrations [76]. Consistently, studies performed in rural Nepal showed that low maternal vitamin B12 blood levels in early pregnancy predicted an elevated risk of insulin resistance in school-aged offspring, and that maternal supplementation with folic acid had a weak protective effect on metabolic syndrome in offspring [77]. Folate maternal intake and *MTHFR 677 C>T* seem to have a limited effect on the metabolic consequences of fetal programming, body composition, and obesity in infants in the UK, Denmark, and Italy [78–80]. By contrast, both factors were associated with birth weight and insulin resistance in a French study of obese adolescents [81]. The respective influences of vitamin B12 and folate, and the existence of the epigenomic deregulation as found in experimental rodent models, need to be investigated further in humans.

Folate and neural and brain disorders

Neural tube defects

Folate supplementation exerts significant protective effect in preventing neural tube defects (NTDs), including anencephaly and spina bifida, whereas folate deficiency increases the risk through unclear mechanisms that could involve the decreased availability of methyl groups [20,21]. Impaired thymidylate synthesis is another possible mechanism in $Shmt1^{+/-}$ and $Shmt1^{-/-}$ embryos from mice subjected to folate and choline deficiency [82]. However, it is important to point out that many rodent models do not recapitulate the NTDs seen in human populations. Interestingly, a link involving epigenetic mechanisms has been found between paternal exposure to dioxin and spermatozoid folate deficiency, which could increase the risk of spina bifida [83]. Consistently, paternal folate deficiency could be one of the factors explaining the incomplete success of recommended folate supplementation in the prevention of NTDs [84].

Folate, brain development, neuroplasticity, and postnatal cognitive functions

In addition to metabolic diseases, the importance of fetal programming can now be extended to brain disorders. Folate deprivation markedly affects neural cell proliferation, migration, differentiation, survival, vesicular transport, and synaptic plasticity through epigenomic effects and dramatic increases in *N*-homocysteinylation of neuronal proteins (Figure 2b) [85–88]. Conversely, folate supplementation promotes neurogenesis by stimulating Erk 1/2 phosphorylation and Notch signaling [89,90], and produces axonal pro-regenerative effects in the rodent central nervous system via DNA methylation [91]. Administration of folic acid may protect, at least partly, against functional impairments in animal models of brain hypoxia–ischemia [86,92]. However, few experimental and epidemiological studies have investigated whether early-life folate status, acting via epigenomic mechanisms, can influence neuroplasticity and cognitive functions later in life.

The strong link between folate and fetal programming is highlighted by the detrimental consequences of folate deficiency on brain development during the periconceptional period, in pregnancy, and in early childhood [93]. Rat pups from dams fed a diet lacking vitamins B during gestation and lactation display delayed onset of cognitive and learning abilities, and poorer locomotor coordination, in relation to homocysteine accumulation, decreased SAM/SAH ratio,

and increased apoptosis in particular brain structures such as the hippocampus, cerebellum, and the neurogenic subventricular zone lining the lateral ventricle [72,94]. Early vitamin B deprivation is also associated with long-lasting disabilities in learning and memory at 80 days of age, together with a marked reduction in the thickness of the hippocampus CA1 region, long after a switch to normal food. However, it is not known whether high concentrations of folate might influence brain development positively [95]. Maternal folic acid supplementation in conjunction with a B12-deficient diet in rats increases oxidative stress and the risk of neurodevelopmental disorders in both the mothers and in their pups at 20 days of age [96]. Epidemiological data also support an early role of folate on cognitive function. Higher maternal folate concentrations during pregnancy predict better cognitive ability in children aged 9–10 years in South India [97]. Furthermore, Nilsson *et al.* [98] reported that folate intake associates positively with academic achievement in Swedish adolescents aged 15 years. Collectively, the data in rodents and humans suggest that early exposure to methyl-donor deficiency produces long-term effects on cognition and learning abilities through mechanisms that are not fully elucidated.

Cerebral folate deficiency (CFD) syndrome

CFD syndrome is characterized by low levels of 5-methyltetrahydrofolate (5-MeTHF) in the cerebrospinal fluid, despite normal folate levels in plasma and red blood cells [99]. This syndrome is related to a specific inability to transport 5-MTHF across the blood–brain barrier via folate receptor-α. The syndrome is associated with altered brain myelination, delayed motor and cognitive development, cerebellar ataxia, speech difficulties, visual and hearing impairment, dyskinesia, spasticity, and epilepsy [99]. Two causes are mutations in the folate receptor 1 (*FOLR1*) gene encoding folate receptor-α (Box 1), and the presence of autoantibodies against folate receptor [99–101]. Cross-reactivity of these autoantibodies with animal milk proteins homologous to folate receptor suggests an autoimmune mechanism associated with animal milk consumption [102]. CFD has been also reported in Aicardi–Goutières and Rett syndromes as well as in ATP-related mitochondrial respiratory chain diseases [99]. Therapy with folinic acid (5-formyltetrahydrofolate) seems to be more effective than folic acid [99].

Neuropsychiatric disorders

Folate has been linked to various neuropsychiatric diseases, but whether this link involves epigenetic mechanisms remains an open question [103]. Causes of autism are poorly understood, but an association between maternal periconceptional folate intake and risk of autistic symptoms has been reported, especially in individuals carrying the *MTHFR 677 C>T* variant [104]. The data suggest that perinatal folate supplementation can reduce the risk of autism in offspring. As with cerebral folate deficiency, folate receptor autoantibodies are also implicated in some autism spectrum disorders [105]. Kirkbride *et al.* [106] postulated that prenatal nutrition influences the risk of schizophrenia in offspring via epigenetic effects. The authors reported a relationship with one-carbon metabolism by employing a Mendelian randomization method – using one or more genetic variants (here the maternal *MTHFR* genotype) as proxies for an environmentally modifiable exposure (dietary folate) – to establish whether an exposure is causally related to disease (schizophrenia).

Folate and the aging brain

Despite a clear relationship between vitamins B, neurodegenerative disease, and age-related cognitive decline, current interventional and experimental studies remain globally discordant as to the utility of folate as a cognition-protective agent [107]. At the tissue level, vitamins B can slow the rate of brain atrophy in patients with mild cognitive impairment [108]. In the rat, folate and vitamin B12 deficiency during pregnancy and lactation produces irreversible hippocampal atrophy in offspring [94]. An active role of fetal programming is hypothesized, and this is supported by the epigenomic dysregulation observed in these age-related pathologies [109,110]. SIRT1 prevents key mechanisms driving neurodegenerative disorders and brain aging: it reduces the load of β-amyloid peptide and tangle formation and deacetylates tau protein [111]. Reduced SIRT1 expression triggers endoplasmic reticulum stress through deacetylation of HSF1 in neuroblastoma cells with impaired intracellular availability of vitamin B12, and independently of homocysteine and Herp [111–113]. The reduced expression and activity of SIRT1 in rat pups from dams subjected to folate deficiency during gestation and lactation [60] should therefore be regarded as a potential epigenomic link between methyl-donor deficiency and abnormal brain aging.

Concluding remarks

In addition to the well-known role of protein and energy restriction in fetal programming, increasing data now highlight the *in utero* influence of folate and other methyl donors on heritable and non-heritable postnatal epigenomic changes. These data are still fragmentary and some are conflicting. The deficiency in methyl donors produces epigenomic mechanisms that lead to IUGR, abnormal fetal development, impaired fatty acid oxidation and mitochondrial energy production, and visceral steatosis. These data are consistent with the potential participation of folate-related epigenomics in visceral manifestations of metabolic syndrome. Folate maternal supplementation in rodents and humans also produces long-term effects, some beneficial and other negative, including increased mammary tumorigenesis and multigenerational respiratory impairment. Improving our understanding of the genomic, epigenomic, and epigenetic effects of folate deficiency and supplementation during pregnancy and postnatal life, taking into consideration interactions with maternal protein restriction, overnutrition, and exposure to toxic dietary factors, should be therefore considered a priority in the context of the high prevalence of folate deficiency in some countries and of folate fortification in others (Box 3). These studies would also help to understand the discordances between experimental models and population studies regarding the effects of folate deficiency and folate supplementation.

Box 3. Outstanding questions

The role of folate in linking fetal programming and epigenomics is an emerging field of interest, in which many questions on its relevance in mechanisms of complex diseases remain open, and deserve further attention:

- What is the contribution of nutritional folate deficiency to fetal programming, as compared to that of other methyl donors such as vitamin B12 and choline? What are their heritable/non-heritable epigenomic consequences? Do these consequences involve disruption of PGC-1α interaction with the transcription factors nuclear respiratory factors (NRFs) and forkhead box class-O (FOXO), or with nuclear receptors (PPAR, ERR, HNF-4, estrogen, glucocorticoid, vitamin D, retinoid, and thyroid receptors)?
- Does the decreased expression and activity of SIRT1 play a role in the effects of folate and other methyl donors on fetal programming, in the brain, on vascular aging or visceral manifestations of metabolic syndrome?
- Does 'excess fuel' contribute to the mechanisms of insulin-resistance mechanisms through additive and/or consecutive influence of folate-related disruption of mitochondrion fatty acid oxidation and overnutrition?
- Do folate-related epigenomic mechanisms contribute to the patho-mechanisms of folate deficiency during brain development and subsequent brain disorders?
- How do folate-related epigenomic mechanisms in prenatal and early postnatal life, or folate supplementation later in life, affect age-related complex diseases associated with low folate?

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