

Minireview

Polymorphisms in 1-Carbon Metabolism, Epigenetics and Folate-Related Pathologies

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Key Words

Epigenetics · Folate · Genetic variation · Methylation · One-carbon metabolism

Abstract

Folate-mediated 1-carbon metabolism is a network of interconnected metabolic pathways necessary for the synthesis of purine nucleotides, thymidylate and the remethylation of homocysteine to methionine. Disruptions in this pathway influence both DNA synthesis and stability and chromatin methylation, and result from nutritional deficiencies and common gene variants. The mechanisms underlying folate-associated pathologies and developmental anomalies have yet to be established. This review focuses on the relationships among folate-mediated 1-carbon metabolism, chromatin methylation and human disease, and the role of gene-nutrient interactions in modifying epigenetic processes.

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Introduction

Epigenetics refers to the inheritance and/or self-propagation of gene expression potential that is independent of the primary DNA sequence [1, 2]. Epigenetics includes biological processes that are hardwired and mostly genetically determined, such as *genomic imprinting* [3], whereby genes exhibit parent-of-origin-specific allelic expression [4]. The term also includes more plastic processes including chromatin modifications resulting from metabolic, nutri-

tional and other environmental queues [5]. Environmentally responsive chromatin modifications can be reversible, transient and enable adaptation, or in other cases persist throughout the lifetime of the organism. The latter is referred to as *metabolic imprinting*, and includes the influence of maternal nutrition during gestation and the suckling period on chromatin modifications and gene expression profiles that influence lifelong risk of chronic disease [6].

Chromatin methylation, which includes methylation of cytosine bases within DNA CpG islands [7] and methylation of histones on lysine and arginine residues [8], directly affects the activity and function of DNA binding proteins, regulates gene expression and can influence genome stability. Histone and DNA methylation are linked and coordinately regulated [9], can affect gene expression patterns, and are a primary molecular mechanism associated with epigenetic signatures and inheritance [10].

DNA and histone protein methylation are linked by their common dependence upon S-adenosylmethionine (AdoMet) as a methyl donor. All DNA and histone methyltransferases require this cofactor for both cytosine as well as lysine and arginine histone modifications [11, 12]. AdoMet synthesis occurs through a metabolic network known as folate-mediated 1-carbon metabolism, which requires folate, vitamin B₁₂ and other water-soluble B vitamins [13]. The dependency of cellular methylation reactions on AdoMet synthesis and availability enables the cell to sense cellular metabolism and nutritional state, and respond and adapt by altering genome-wide expression patterns [14]. This review focuses on the genetic and nutritional factors that influence the synthesis and accumulation of AdoMet, and its influence on chromatin methylation and pathologies associated with altered folate-mediated 1-carbon metabolism (fig. 1).

One-Carbon Metabolism

Folate-mediated 1-carbon metabolism is a metabolic network present in the cytoplasm, mitochondria and nucleus of cells and functions to generate and transfer 1-carbons for the de novo synthesis of purines, thymidylate and methionine [13]. These biosynthetic pathways all utilize tetrahydrofolate polyglutamates (THF) as cofactors. THF is present in cells as family of 1-carbon substituted cofactors that carry and chemically activate single carbons for 1-carbon transfer reactions. Because folate is a vitamin, it is acquired from the diet from either natural food or enriched grain products and dietary supplements which contain a synthetic form of the vitamin referred to as folic acid [15].

One-carbon metabolism in mitochondria utilizes THF cofactors to generate the 1-carbon unit formate from the amino acids serine, glycine, and from sarcosine and dimethylglycine which are derived from choline degradation [13, 16]. Once generated, formate is transported into the cytoplasm where it condenses with THF in an ATP-dependent reaction to form 10-formyl-THF (FTHF; fig. 1). Formate is the primary source of 1-carbons for de novo purine biosynthesis and for homocysteine remethylation to methionine, and therefore formate generation is essential for AdoMet-dependent methylation reactions, including chromatin methylation [17, 18]. The de novo synthesis of thymidylate from uridylate occurs in both the mitochondria and nucleus [19].

The remethylation of homocysteine to methionine and subsequent conversion of methionine to AdoMet occurs exclusively in the cytoplasm [20] (fig. 1). Mammalian cells contain numerous AdoMet-dependent methyltransferases involved in many cellular processes including chromatin remodeling, regulatory functions including gene transcription [21], protein localization [22], and the biosynthesis and catabolism of small molecules including neurotransmitters [23]. These reactions occur in the cytoplasm, nucleus and mitochondria, and generate S-adenosylhomocysteine (AdoHcy), which is the product of AdoMet-depen-

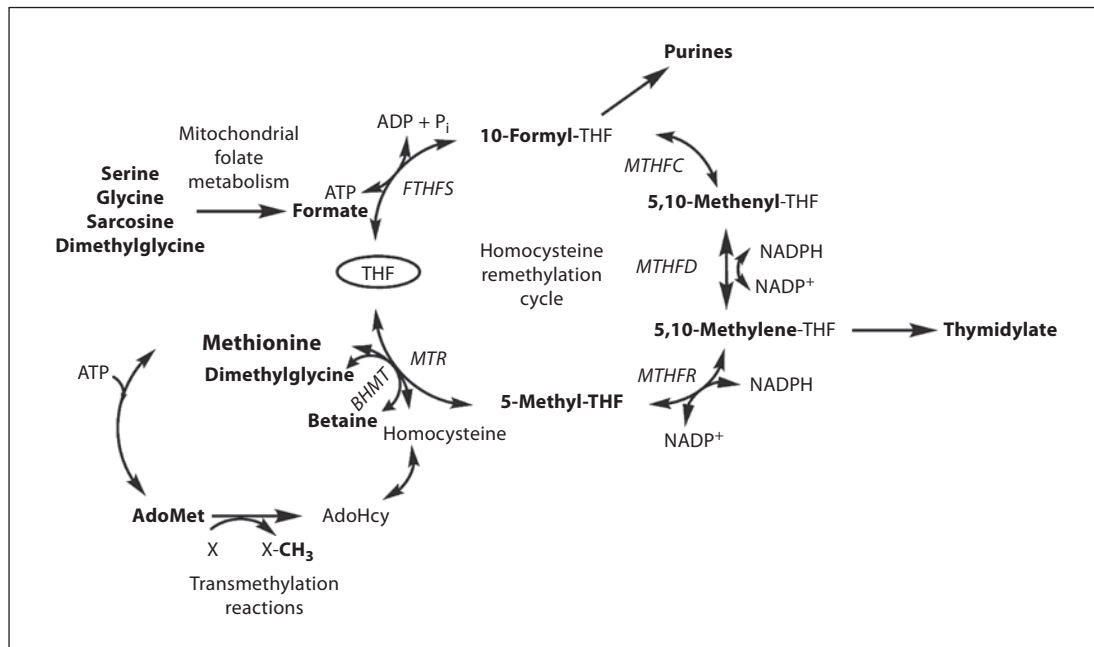


Fig. 1. Folate-dependent homocysteine remethylation and its interaction with AdoMet-dependent transmethylation reactions. One-carbon metabolism in the cytoplasm generates folate-activated 1-carbon units for remethylation of homocysteine to methionine. One carbon metabolism in the mitochondria generates formate for 1-carbon metabolism in the cytoplasm. The 1-carbon unit is shown in bold. BHMT = Betaine-homocysteine methyltransferase; X = a substrate that serves as a methyl group acceptor.

dent transmethylation reactions. AdoHcy is hydrolyzed to adenosine and homocysteine to complete the homocysteine remethylation cycle in the cytoplasm (fig. 1).

One-carbon metabolism is highly sensitive to vitamin deficiency [17], and its impairment impacts each of the folate-dependent pathways resulting in decreased rates of purine and thymidylate synthesis, elevated levels of homocysteine and AdoHcy and depleted levels of AdoMet [24]. These metabolic disruptions affect DNA synthesis and stability and impair cellular methylation reactions [24, 25]. Vitamin B₁₂ deficiency can also disrupt 1-carbon metabolism. Methionine synthase (MTR), which catalyzes the remethylation of homocysteine to methionine, is vitamin B₁₂ dependent [26]. It has been estimated that 38% of older adults may exhibit mild vitamin B₁₂ deficiency [27]. Both folate and vitamin B₁₂ deficiency elevate plasma homocysteine and AdoHcy [27], and homocysteine is a biomarker for folate and vitamin B₁₂ deficiency [28].

One-Carbon Metabolism and Human Pathology

Population studies and randomized clinical trials implicate impaired 1-carbon metabolism in several pathologies and developmental anomalies, e.g. neural tube defects [29, 30], cardiovascular disease [31–33] and cancer [34–38]. Elevated plasma homocysteine, resulting from folate and/or vitamin B₁₂ deficiency, is a risk factor for certain cancers [39], cardiovascular disease [40], neural tube defects [41], and Alzheimer's disease [42]. These folate-related pathologies are complex traits resulting from deleterious gene-environment interactions

[14]. Although nutritional deficiencies in folate and vitamin B₁₂ can impair 1-carbon metabolism, the degree of the metabolic impairment and its relationship to pathology depends on the genetic background of the individual. Many common, penetrant genetic mutations and polymorphisms have been identified in 1-carbon metabolism that are associated with pathology and interact with and/or can cause nutritional deficiencies [29, 43–45]. The molecular mechanisms and causal pathways underlying the gene-nutrient interactions that contribute to the initiation and/or progression of folate-associated pathologies have yet to be established, but are assumed to result from impairments in DNA synthesis and repair, and/or changes in chromatin methylation that alter genome expression and stability [24].

The Cellular Methylation Potential Links 1-Carbon Metabolism and Transmethylation Reactions

The ratio of AdoMet to AdoHcy levels in the cell is often referred to as the methylation potential [46]. AdoMet and AdoHcy both affect the activity of AdoMet-dependent methyltransferases. AdoMet serves as the substrate for transmethylation reactions including DNA and histone methyltransferases, whereas the product, AdoHcy, binds tightly to these enzymes and inhibits their activity through product inhibition and therefore is a physiologically relevant inhibitor of chromatin methylation. AdoHcy accumulates in cells when homocysteine remethylation to methionine is inhibited, because homocysteine is in equilibrium with AdoHcy (fig. 1). Therefore, metabolic disruptions in 1-carbon metabolism that impair homocysteine remethylation are sensed by the genome through changes in chromatin methylation, and elicit alterations in gene expression [47].

Nutrient-Induced Changes in the Cellular Methylation Potential and Chromatin Methylation

Changes in the AdoMet/AdoHcy ratio influence the cellular activity of DNA and histone methyltransferases, and different regions of DNA may be more sensitive than others to changes in the cellular methylation potential [48]. Subcutaneous methionine treatment (5 mmol/kg twice a day for 3 days) induces GAD67 promoter hypermethylation in mice through elevations in AdoMet levels [49]. In human lymphocytes, AdoHcy is a more important determinant of cellular methylation capacity and global DNA methylation levels compared to AdoMet [50]. In mice with elevated homocysteine, AdoHcy but not AdoMet predicts global DNA hypomethylation [51]. Other studies demonstrated that mice maintained on a folate-deficient diet (<0.05 ppm folic acid) for 32 weeks exhibited elevated serum homocysteine and global DNA hypomethylation in splenocytes (reduced 9.1%) and colonic epithelial cells (reduced 7.2%), without changes in allele-specific methylation at the mouse B1 element, *H19* or *Oct4* loci [25]. Endothelial cells exposed to homocysteine exhibited 30% reduced DNA methyltransferase 1 (DNMT1) activity without changes in DNMT1 protein levels, and reduced CpG methylation of the cyclin A promoter, leading to depressed *cyclin A* transcription. These studies demonstrate that homocysteine-induced elevations in AdoHcy repress DNMT1 activity and chromatin methylation [52].

AdoHcy elevations have also been demonstrated to affect the expression of genetically imprinted genes. Elevations in plasma homocysteine at levels observed in patients with homocystinuria resulted in DNA CpG hypomethylation and biallelic expression of *H19* and other genomically imprinted genes. Folic acid administration, in the form of 15 mg oral methyl-THF a day for 8 weeks, corrected the DNA hypomethylation and restored monoal-

lelic gene expression patterns [53]. Diet-induced changes in the cellular methylation potential also influence imprinted gene expression in animal models. Mice lacking dietary choline and methionine exhibited elevated *Igf2* and *H19* expression in the prostate without changes in promoter methylation or imprinting, but did exhibit lower levels of repressive histone modifications (dimethyl-H3K9). These effects were reversible by feeding mice a nutrient-sufficient diet [54]. In non-small cell lung cancers, folate levels correlated with global methylation when LINE-1 methylation is used as a surrogate, as well as allelic-specific methylation at the promoters of CDH13, RUNX3, but not MYOD1, RASSF1P16, APC, and RARB. Therefore, folate levels influence both global and allele-specific methylation in transformed cells [55]. Vitamin B₁₂ deficiency, induced in gastrectomized rats which have reduced capacity to absorb vitamin B₁₂, also causes elevated plasma homocysteine and DNA hypomethylation in rodents [56]. However, the relationship between diet and DNA methylation may be most pronounced in nutrient deficiency, in cancers and in severe inborn errors of metabolism that elevate homocysteine. Folate nutrition and global DNA CpG methylation do not always correlate; folate supplementation of human subjects at 1 mg/day did not alter LINE-1 methylation density, which is a proxy for DNA global methylation, in normal colonic mucosa cells [57].

One-Carbon Metabolism Regulates the Activity of DNA Methyltransferases

Changes in DNMT1 expression correlate with CpG methylation levels in nuclear DNA in tissues and transgenic animals [58], indicating that methyltransferase levels, like changes in the AdoMet/AdoHcy ratio, affect cellular methylation capacity. DNMT1 ensures that DNA methylation patterns are reestablished during DNA replication and that global DNA methylation levels are maintained.

One-carbon metabolism also regulates the expression of methyltransferases, in addition to maintaining the AdoMet/AdoHcy ratio. DNMT1 levels are increased in choline-deficient embryos leading to increased global DNA methylation, including *Igf2* methylation. Choline degradation is a source of formate from mitochondrial 1-carbon metabolism, and provides an alternative pathway for homocysteine remethylation independent of folate metabolism through the enzyme betaine-homocysteine methyltransferase [59]. Choline deficiency induces hypomethylation of the *Dnmt1* promoter and increased *Dnmt1* expression, indicating that *Dnmt1* promoter hypomethylation is a compensatory mechanism that maintains methylation capacity when the AdoMet/AdoHcy ratio decreases [60]. mRNA levels of *Dnmt3a*, a de novo DNA methyltransferase, also inversely correlate with maternal choline intake [60].

Genetic Variation in 1-Carbon Metabolism and Its Impact on Cellular Methylation

Genetic variation in the genes that encode the enzymes that constitute folate-mediated 1-carbon metabolism is associated with changes in cellular metabolism, genome methylation and risk for human pathologies. This section reviews common genetic variants in this metabolic network and their impact on 1-carbon metabolism, epigenetic processes and human disease.

Methylenetetrahydrofolate Dehydrogenase 1

The mammalian *MTHFD1* gene encodes C₁-THF synthase, a trifunctional enzyme that contains FTHF synthetase (FTHFS) activity on the C-terminal domain, and 5,10-methenyl-THF cyclohydrolase (MTHFC) and dehydrogenase (MTHFD) activities on the N-terminal

domain [61, 62] (fig. 1). FTHFS functions as a formate-activating enzyme by catalyzing the ATP-dependent conversion of THF and formate to FTHF, ADP, and inorganic phosphate [16]. FTHF is used as cofactor for purine biosynthesis [63], or the 1-carbon can be reduced by MTHFC/MTHFD for use in the biosynthesis of thymidylate and methionine (fig. 1). MTHFC catalyzes the reversible interconversion of FTHF and 5,10-methenyl-THF, whereas MTHFD catalyzes the NADPH-dependent and reversible reduction of 5,10-methenyl-THF to 5,10-methylene-THF. Disruption of a single *Mthfd1* allele in mice lowers hepatic AdoMet levels, consistent with formate serving as a source of 1-carbons for cellular methylation reactions [64] (fig. 1).

A common single nucleotide variant in human *Mthfd1*, G1958A, results in the substitution of glutamine for arginine at position 653 in the 10-FTHFS domain of MTHFD1. The variant enzyme is thermolabile with 25% reduced activity in cultured cells [65]. In humans, the R653Q variant does not influence levels of homocysteine, plasma folate or red blood cell folate [66], but was found to increase a mother's risk of having a child with a neural tube defect in several populations [66–68], to increase risk for intrauterine growth restriction [69], to increase risk for congenital heart defects [65], and to increase risk for non-Hodgkin's lymphoma [70]. This polymorphism is also a maternal risk factor for severe placental abruption and unexplained second trimester loss [71, 72], and has been demonstrated to be a maternal and fetal risk factor for cleft lip and cleft palate in an Irish population [73], but not in an Italian population [74]. There is also a common variant in the MTHFD/MTHFC domain (R134K), but the functional significance of the variant domain is not known. It has been associated with a significant increase in the risk for postmenopausal breast cancer [75].

FTHF Dehydrogenase (ALDH1L1)

ALDH1L1 catalyzes the irreversible and NADP⁺-dependent oxidation of FTHF to THF and CO₂ [76, 77]. It is one of the most abundant folate enzymes in the liver and plays several roles in regulating folate metabolism, including the removal of excess FTHF in the form of CO₂. ALDH1L1 has been shown to regulate cellular concentrations of FTHF and the homocysteine remethylation cycle in cultured neuroblastoma cells [78] by limiting the supply of folate-activated 1-carbon units. Interestingly, ALDH1L1 gene expression is epigenetically silenced in cancers [79]. Two ALDH1L1 gene variants have been shown to alter the risk of developing postmenopausal breast cancer. The variant [rs2276731 (T/C)] is associated with an increased risk, while [rs2002287 (T/C)] is associated with a decreased risk. Both single nucleotide polymorphisms are intronic, and may exist in linkage disequilibrium with coding variants including V812I, G481S, or F330V [75].

5,10-Methylene-THF Reductase

Methylene-THF reductase (MTHFR) is a flavoprotein that catalyzes the NADPH-dependent reduction in 5,10-methylene-THF to 5-methyl-THF, which is an essential folate cofactor for the remethylation of homocysteine to methionine (fig. 1). The MTHFR reaction is irreversible in vivo and thereby commits folate cofactors to the homocysteine remethylation pathway [16, 80]. MTHFR activity is inhibited by AdoMet, providing feedback inhibition and limiting AdoMet synthesis and accumulation [81].

MTFHR is expressed ubiquitously, with the highest mRNA levels observed in the testis where DNA methylation is critical for germ cell maturation and genomic imprinting [82]. Mild MTHFR deficiency, defined as 35–45% residual activity, is the most common inborn error of folate metabolism and affects 5–20% of North Americans and Europeans [83]. The most common cause is a C to T substitution at nucleotide position 677, which results in the amino acid change (A222V) in the catalytic domain of the protein [84]. The C677T variant does not exhibit altered kinetic properties compared to the more common allele, but rather

exhibits enhanced loss of the FAD cofactor [83, 85, 86] creating a thermolabile protein [87]. The C677T variant is associated with mild hyperhomocysteinemia, especially in those with low folate concentrations [88], and lower plasma and red cell folate levels [89, 90]. The C677T variant has been demonstrated to effect the cellular methylation potential and is associated with DNA hypomethylation in lymphocytes [91] and increased tumor suppressor gene CDKN2A promoter methylation in colon cancer [92].

The C677T MTHFR variant has been shown to modify risk for several clinical outcomes, especially outcomes related to reproduction and cancer, as well as chronic disease. The variant has been shown to be associated with an increased risk for cardiovascular disease [93–95], neural tube defects [96–98], cleft lip and palate [41, 99], thrombosis [100–102] and schizophrenia [103–106]. It has also been shown to be protective against several types of cancers, including acute lymphoblastic leukemia [107], childhood acute leukemia [108], and colorectal cancer [109, 110]. It is not known if the association of the MTHFR C677T variant with pathology results from its effect on the homocysteine remethylation pathway and cellular methylation, or from its effect on lowering cellular folate levels.

Another common coding MTHFR single nucleotide polymorphism, A1298C (E429A), exists in strong linkage disequilibrium with C677T [111]. Unlike C677T which affects the active site of the protein, A1298C is located in the regulatory domain of the protein and is catalytically indistinguishable from the wild-type MTHFR [86]. The A1298C polymorphism is associated with increased red cell folate levels and does not affect homocysteine levels [90]. This polymorphism was shown to be associated with a decreased risk for acute lymphoblastic leukemia [107] and childhood acute leukemia [108].

Methionine Synthase

MTR is a cobalamin (vitamin B₁₂)-dependent enzyme that catalyzes the 5-methyl-THF-dependent remethylation of homocysteine to methionine (fig. 1). MTR activity is essential to supply methionine for AdoMet synthesis and the transmethylation reactions (fig. 1), and to prevent the accumulation of homocysteine and its conversion to AdoHcy. MTR is an essential enzyme in mice; mice lacking MTR exhibit embryonic lethality [112]. Although MTR activity is redundant with betaine-homocysteine methyltransferase which can also remethylate homocysteine to form methionine, its expression is limited to the liver and kidney, whereas methionine synthetase displays ubiquitous expression [113].

Inborn errors of metabolism associated with MTR, including the P1173L mutation, result in an autosomal recessive disease characterized by homocysteinemia, homocystinuria, hypomethioninemia, megaloblastic anemia, neural dysfunction, and mental retardation [114]. The common MTR polymorphic variant, A2756G, which affects the domain involved in methylation and activation of the vitamin B₁₂ cofactor [115], is associated with more subtle clinical outcomes; including decreased plasma homocysteine levels [116] and aberrant methylation in patients with colorectal, breast, and lung tumors [117], and is a risk factor for systemic lupus erythematosus [118], bipolar disorder, schizophrenia [119], spina bifida [120], orofacial clefts [121], nonsyndromic cleft lip and palate [122] and Down's syndrome [123]. This variant was also shown to reduce *MLH1* promoter hypermethylation in colorectal cancer [124].

Glycine N-Methyltransferase

Glycine N-methyltransferase (GNMT) is a methyltransferase that catalyzes the AdoMet-dependent methylation of glycine to sarcosine. The primary function of this reaction is to regulate and buffer the AdoMet/AdoHcy ratio and prevent AdoMet accumulation. Mice lacking GNMT exhibit fatty liver with a 36-fold elevation in AdoMet concentrations and a 100-fold increase in the AdoMet/AdoHcy ratio [125]. Humans with loss-of-function GNMT

mutations present with similar metabolic disruptions [125]. GNMT expression is regulated by other nutrients, including retinoic acid [126] and glucocorticoids [127]; this regulation of GNMT expression provides a mechanism whereby nutrients unrelated to 1-carbon metabolism signal influence chromatin methylation and epigenetic processes [12]. The GNMT C1289T variant was shown to be associated with elevated plasma total homocysteine concentrations in women with the MTHFR C677T genotype [128].

One-Carbon Metabolism in Mitochondria

Mitochondria play an important role in generating formate for AdoMet synthesis in the cytoplasm, but our understanding of the pathway and its regulation is incomplete, including its role in folate-associated pathologies and cellular methylation reactions. A connection between mitochondrial 1-carbon metabolism and homocysteine has been established in patients with nonketotic hyperglycinemia, an autosomal recessive inborn error of metabolism caused by mutations in the P-protein or T-protein of the glycine cleavage system [129]. This system accounts for nearly 40% of overall glycine flux in humans and the formate produced from glycine catabolism makes major contributions to 1-carbon flux through cytoplasmic 1-carbon metabolism [130]. Mutations in the glycine cleavage system are associated with elevated homocysteine levels in cerebrospinal fluid, severe mental retardation, seizures, apnea, and hypotonia, but their impact on cellular methylation has yet to be established [131].

Concluding Remarks

Folate-mediated 1-carbon metabolism is a conduit that links cellular metabolism to the epigenetic machinery through the common molecule, AdoMet. There is strong evidence that changes in the cellular methylation potential (AdoMet/AdoHcy ratio), induced by changes in cellular metabolism, can influence the activity of both DNA and histone methyltransferases and the expression of DNA methyltransferases, and thereby alter chromatin methylation patterns. These changes in chromatin methylation can be global and/or allele specific. It remains to be established how changes in the cellular methylation potential can induce allele-specific alterations in genome methylation, including the mechanisms underlying the targeting of specific alleles. Likewise, the establishment of pathways that link B-vitamin nutrition, cellular methylation and downstream effects on gene expression is needed to elucidate mechanisms underlying B-vitamin-associated pathologies. Finally, understanding the relative contributions of genetic variation and environment (including nutrition) to epigenetic processes is essential to the design of nutritional and/or pharmaceutical approaches for the prevention and management of chronic disease.

Acknowledgments

This work was supported by PHS DK58144 to P.J.S

Disclosure Statement

The author has no conflict of interest.

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