

Trans-HHS Workshop: Diet, DNA Methylation Processes and Health

Diet, Methyl Donors and DNA Methylation: Interactions between Dietary Folate, Methionine and Choline^{1,2}

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ABSTRACT DNA methylation influences the expression of some genes and depends upon the availability of methyl groups from S-adenosylmethionine (SAM). Dietary methyl groups derive from foods that contain methionine, one-carbon units and choline (or the choline metabolite betaine). Humans ingest ~50 mmol of methyl groups per day; 60% of them are derived from choline. Transmethylation metabolic pathways closely interconnect choline, methionine, methyltetrahydrofolate (methyl-THF) and vitamins B-6 and B-12. The pathways intersect at the formation of methionine from homocysteine. Perturbing the metabolism of one of these pathways results in compensatory changes in the others. For example, methionine can be formed from homocysteine using methyl groups from methyl-THF, or using methyl groups from betaine that are derived from choline. Similarly, methyl-THF can be formed from one-carbon units derived from serine or from the methyl groups of choline via dimethylglycine, and choline can be synthesized de novo using methyl groups derived from methionine (via SAM). When animals and humans are deprived of choline, they use more methyl-THF to remethylate homocysteine in the liver and increase dietary folate requirements. Conversely, when they are deprived of folate, they use more methyl groups from choline, increasing the dietary requirement for choline. The availability of transgenic and knockout mice has made possible additional studies that demonstrate the interrelationship of these methyl sources. In summary, as we consider dietary requirements and possible effects on DNA methylation, it is important to realize that methionine, methyl-THF and choline can be fungible sources of methyl groups, and the design of our studies should reflect this. *J. Nutr.* 132: 2333S-2335S, 2002.

KEY WORDS: • methyl group • DNA methylation • choline • folate • methionine • diet

METHYL GROUPS PLAY A KEY ROLE IN GENE EXPRESSION

DNA-methylation, which can regulate tissue-specific expression of certain genes (1), is especially important during embryo-

genesis (1,2) and carcinogenesis (3,4). DNA-methylation is catalyzed by DNA methyltransferases (Dnmt1, Dnmt2, and Dnmt3; EC 2.1.1.37)⁴ that transfer methyl groups from S-adenosylmethionine (SAM) to cytosine (3). Cytosine guanine (CpG) dinucleotides often are located in the regions of DNA that regulate DNA expression (promoter regions), and DNA methylation is relatively specific for cytosine residues within these (3). In humans, 80% of CpG islands are methylated, whereas only 10% of cytosines in DNA are methylated (2). Methylations of CpG islands in the promoter region of a gene repress the expression of that gene (2,3). The mechanism that leads to gene repression remains unclear. It is suggested that methylated cytosines bind to a family of methyl cytosine-binding proteins (MeCP1, MeCP2, MBD1, MBD2, MBD3 and MBD4) that prevent the binding of transcription factors to methylated CpG sites from the promoter region (3,5). Another hypothesis proposes that the MeCP proteins bind MeCP-binding proteins and recruit histone deacetylases and other repressors that form stable complexes with the deacetylated histones that induce chromatin compaction and gene silencing (6). Whatever the proposed mechanism of action, the SAM needed for DNA methylation is derived in part from dietary methyl group intake (7-10).

DIET AND METHYL METABOLISM

For humans, the major sources of methyl groups in foods come from methionine (~10 mmol of methyl/d), one-carbon

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⁴ Abbreviations used: *c-fos*, *c-fos* proto-oncogene; *c-Ha-ras*, *c-Ha-ras* proto-oncogene; *c-myc*, *c-myc* oncogene; CpG, cytosine guanine; Dnmt1, Dnmt2, Dnmt3, DNA methyltransferases; *MAT1A*, methionine adenosyltransferase 1A gene; MBD, methyl-CpG-binding domain protein; MeCP1, methyl-CpG-binding protein 1; MeCP2, methyl-CpG-binding protein 2; methyl-THF, methyltetrahydrofolate; mRNA, messenger RNA; *raf*, *raf* oncogene; SAM, (S)-adenosylmethionine; THF, tetrahydrofolate; tRNA, transfer RNA.

metabolism via methylfolate (~5–10 mmol of methyl/d), and from choline [~ 30 mmoles methyl/d (11)] (Fig. 1). The Institute of Medicine, National Academy of Sciences, USA, recently made recommendations for choline intake in the diet (11). Aside from its role as a methyl donor, choline is needed for synthesis of the phospholipids in cell membranes, cholinergic neurotransmission, transmembrane signaling and lipid-cholesterol transport and metabolism (12). The tight interrelationship between these three dietary sources of methyl groups makes it important that all three be assessed when studying diet and DNA methylation.

Choline, methionine and folate metabolism interact at the point that homocysteine is converted to methionine. Betaine:homocysteine S-methyltransferase (EC 2.1.1.5) catalyzes the methylation of homocysteine using betaine as the methyl donor (13–15). In an alternative pathway, 5-methyltetrahydrofolate:homocysteine S-methyltransferase (EC 2.1.1.13) regenerates methionine using a methyl group derived de novo from the one-carbon pool (16,17). Methionine adenosyltransferase (EC 2.5.1.6) converts methionine to SAM (the active methylating agent for DNA methylation) (13).

Perturbing the metabolism of one of the methyl donors reveals the intermingling of these metabolic pathways. Total hepatic folate content decreased by 31–40% after 2 wk on a choline-deficient diet in rats (14,15). This effect was reversible by refeeding choline. Rats fed diets deficient in both methionine and choline for 5 wk had hepatic folate concentrations that were 50% of those in controls (16). Tetrahydrofolate deficiency, induced by treatment with methotrexate (17–21) or induced by dietary folate deficiency (22), resulted in diminished hepatic total choline, with the greatest decrease occurring in hepatic phosphocholine concentrations. During choline deficiency, hepatic SAM concentrations also decreased by as much as 50% (23–26). In rats, choline deficiency doubled plasma homocysteine levels (27).

The interrelationships between choline, methionine and folate are apparent when knockout mice are studied. Methylene tetrahydrofolate reductase (EC 1.5.1.20) knockout mice, which have impaired availability of methyl groups from methyltetrahydrofolate (methyl-THF), deplete choline and betaine so as to maintain homocysteine remethylation (Zeisel, S. H., unpublished data). Methionine adenosyltransferase knockout mice, which have impaired formation of SAM, activate the gene expressing betaine:homocysteine methyltransferase and

have increased dietary choline requirements (13). Further, cystathionine beta-synthase (EC 4.2.1.22) knockout mice, which accumulate homocysteine and must convert it to methionine to remove it, deplete choline and betaine pools in liver (Zeisel, S. H., unpublished data). Liver and kidney are the major tissues in which betaine:homocysteine methyltransferase is expressed (28); therefore, for other tissues to use choline-derived methyl groups, they must be exported from these organs.

DIET AND DNA METHYLATION

Animals fed diets deficient in methyl donors (choline and methionine) have hypomethylated DNA (29–31). These changes occur not only in global methylation (32), but also in the methylation of specific genes (33). Increases in levels of mRNA for *c-fos*, *c-Ha-ras* and *c-myc* were correlated with loss of methylation at specific sites within these genes as early as 1 wk after the start of feeding a methionine- and choline-deficient diet to rats (34,35). Mouse liver tumorigenesis induced by a choline-devoid, methionine-deficient diet was associated with the hypomethylation of *c-Ha-ras* and *raf* oncogenes (36). Surprisingly, the effects of methyl-deficient diets on DNA methylation occur rapidly (within 1 wk in Fischer rats) and before the tumor formation in their livers (30). One to two weeks after the restoration of an adequate diet, the overall extent of methylation of tRNA and DNA from livers of previously methyl-deficient rats returned to normal (37). It is interesting that DNA is not hypomethylated in methionine adenosyltransferase 1A gene (*MAT1A*) knockout animals (despite diminished SAM), unless the mice also are deprived of choline (13).

Folate deficiency also is associated with perturbed DNA methylation. DNA is hypomethylated in brains of rats fed a folate-deficient diet (38) or treated with methotrexate (7). A decrease in folic acid intake, and the subsequent DNA hypomethylation, may be involved in human gastric carcinogenesis (39). Postmenopausal women with modest dietary folate deficiency were observed to have hypomethylation of lymphocyte DNA (40). In healthy human females, both cervical tissue folate and serum folate levels were significantly correlated to cervical tissue DNA methylation (41). Thus, dietary status for choline and for folate can influence global DNA methylation.

DISCUSSION

As we consider dietary requirements and possible effects on DNA methylation, it is important to realize that methionine, methyl-THF and choline can be fungible sources of methyl groups. The importance of dietary choline and other methyl donors as factors that influence DNA methylation and gene expression has been evaluated in rodents and occasionally in humans.

Alterations in DNA methylation, with resulting changes in gene expression, can have important consequences for embryogenesis (1,42) and might explain our laboratory's observation that dietary choline availability during pregnancy influences the development of brain in the fetus via choline-mediated alterations in the birth, migration and death of cells in the hippocampus and septum (43,44). Diet-related changes in DNA methylation also may contribute to carcinogenesis that occurs in livers of methyl-deficient rats and mice (4,30,45).

Although we do not know whether there are significant numbers of humans who are choline deficient, there are many humans who are folate deficient (11), and 15–30% of the

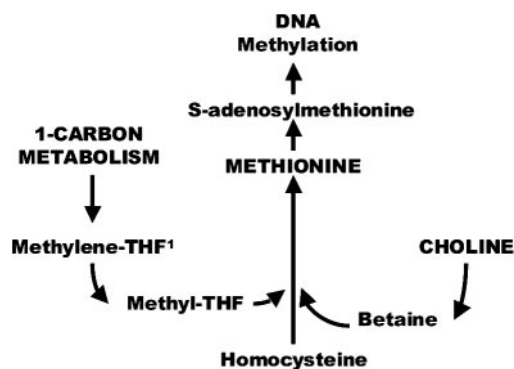


FIGURE 1 DNA methylation depends upon the availability of methyl groups from S-adenosylmethionine, which is derived from methionine. Transmethylation metabolic pathways closely interconnect choline, methionine and THF. The pathways intersect at the formation of methionine from homocysteine. Perturbing the metabolism of one of these pathways results in compensatory changes in the others. THF, tetrahydrofolate.

population may have increased dietary methyl requirements due to polymorphisms in genes involved in methyl metabolism (46). Therefore, it is likely that differences in DNA methylation, and resulting changes in gene expression, are due to dietary variations in humans. This promising new area of investigation promises to enhance our understanding of how nutrition modulates the milieu in which biochemical and genetic mechanisms operate.

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