Importance of methyl donors during reproduction

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ABSTRACT
Evidence is growing that optimal dietary intake of folate and choline (both involved in one-carbon transfer or methylation) is important for successful completion of fetal development. Significant portions of the population are eating diets low in one or both of these nutrients. Folates are important for normal neural tube closure in early gestation, and the efficacy of diet fortification with folic acid in reducing the incidence of neural tube defects is a major success story for public health nutrition. Similarly, maternal dietary choline is important for normal neural tube closure in the fetus and, later in gestation, for neurogenesis in the fetal hippocampus, with effects on memory performance. Although both folates and choline have many potentially independent mechanisms whereby they could influence fetal development, these 2 nutrients also have a common mechanism for action: altered methylation and related epigenetic effects on gene expression.

INTRODUCTION
Dietary intake of folates and choline can be marginal during pregnancy, and both nutrients have important effects on brain development. Although these nutrients participate in multiple different biochemical pathways (Figure 1), their metabolism intersects at an important step in one-carbon metabolism. This common pathway may explain why both nutrients are required during critical periods of neurogenesis in the brain and spinal cord.

FOLATE AND CHOLINE METABOLISM ARE DIFFERENT BUT RELATED
Dietary folates, in the form of tetrahydrofolates (THFs), are essential cofactors for several biochemical reactions that transfer one-carbon units (1). 10-FormylTHF (formed from formate and THF by the enzyme C1-THF synthase, the product of the MTHFD1 gene) is required for the biosynthesis of purines (1). 5,10-MethyleneTHF, derived from serine and THF, is required for thymidylate biosynthesis. In addition, 5,10-methyleneTHF can be reduced to 5-methylTHF (formed by methyleneTHF reductase, the product of the MTHFR gene), and this is needed for the biosynthesis of methionine from homocysteine, eventually influencing biosynthesis of S-adenosylmethionine (the most important methyl-group donor) (1). Thus, variation in dietary folate intake could influence fetal outcome by at least 3 distinct mechanisms: alteration of DNA biosynthesis, accumulation of toxic levels of homocysteine, and perturbation of methylation reactions.

Dietary choline can be acetylated to form acetylcholine, a neurotransmitter (2), or phosphorylated and then used as a precursor for the biosynthesis of phosphatidylcholine and sphingomyelin in mammalian membranes (3–5). Choline is committed to become a methyl donor after it is oxidized to form betaine in the inner mitochondrial membrane, catalyzed by choline dehydrogenase (the product of the CHDH gene) (6). In an alternative pathway to that previously described for 5-methylTHF, the methyl groups of betaine can be used for the synthesis of methionine from homocysteine, thereby influencing S-adenosylmethionine biosynthesis (7). Thus, variation in dietary choline intake could influence fetal outcome by 4 distinct mechanisms: perturbation of acetylcholine biosynthesis, changes in membrane synthesis, accumulation of toxic levels of homocysteine, and perturbation of methylation reactions.

The dietary requirements for choline and folate are interrelated because the folate and choline metabolic pathways intersect at the point that homocysteine is converted to methionine (8). These 2 pathways act in parallel, and both lower homocysteine concentrations (9). In the first pathway, vitamin B-12 and THF are required cofactors in a reaction catalyzed by methionine synthase (10). Deficiency of these nutrients (11, 12) or single nucleotide polymorphisms (SNPs) in the genes for the enzymes involved in this pathway (10, 12, 13) result in elevated plasma homocysteine concentrations.

The alternative choline-dependent pathway for the methylation of homocysteine to form methionine is catalyzed by betaine homocysteine methyltransferase (the product of the BHMT gene)

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673S
FIGURE 1. Choline and folate metabolic pathways intersect. Choline, via its metabolite betaine, is a methyl donor in the formation of methionine from homocysteine. In a parallel pathway, 5-methyl tetrahydrofolate serves as the methyl donor.

(14). Betaine, derived from dietary choline, is the methyl-group donor in this reaction, and supplemental oral betaine can lower plasma homocysteine concentrations (15, 16). After betaine donates a methyl group to homocysteine, the resulting methyl groups in dimethylglycine can be scavenged with THF as a cofactor (17). Because folate and choline are metabolically related, perturbing metabolism of one results in compensatory changes in metabolism of the other (18–20).

Rats treated with the antifolate methotrexate have diminished pools of choline metabolites in liver (19, 21). Conversely, rats ingesting a choline-deficient diet have diminished tissue concentrations of folate (20), methionine, and S-adenosylmethionine (22) and have elevated plasma homocysteine concentrations (23). Humans who are depleted of choline develop elevated homocysteine concentrations in plasma after a methionine loading test (24). These interactions between choline and folate metabolism are such that it is difficult to separate all of their effects on reproductive outcome.

FOLATE AND PREGNANCY

Dietary intake of folic acid can be marginal during pregnancy, resulting in decreased folate concentrations in serum and red cells to the point that some pregnant women can become clinically folate deficient (25, 26). Normally, the embryonic brain and spinal cord begin as a flat plate that must roll up and then join edges to form a tube. For some reason, this does not happen normally when folates are not available, resulting in a neural tube birth defect (NTD) in the fetus. This congenital malformation of the brain and spinal cord results from failure of normal developmental processes in the fetus that must occur during a critical window in time (21–28 d after conception in humans). Mothers with lower erythrocyte folate concentrations are more likely to have a baby with an NTD (27), and folic acid, administered to women who had previously had a child with an NTD, lowers the risk of recurrence by 72% (28). There is also an effect of folic acid in women who have never had a baby with an NTD, because rates of this birth defect fell by 26% in the United States after enriched cereal grains sold in the United States were fortified with 140 µg folic acid/100 g grain (29).

A similar fortification program prevented 47% of NTDs in Canada (30). Thus, it is apparent that folate availability is important during the first few weeks of pregnancy. Little thought has been given to folate nutrition during later pregnancy, but there are significant negative effects of folate deficiency in later gestation on neurogenesis in some areas of the brain related to memory function (31).

These observations have greater significance because genes of folate metabolism are polymorphic, variants are relatively common, and some can increase dietary requirements for folate. Although humans share the same genes, there are many individual variations (SNPs) in the codon sequences for these genes. In total, >10 million SNPs exist that occur in >1% of the population (32). Some common SNPs occur in >50% of the population. Most humans have ≥50,000 SNPs across their genes (33). Some fraction of these SNPs results either in alteration of regulation of gene expression or in changes in the gene product so that protein structure and function are altered, thereby altering metabolism and cell function.

As noted earlier, the product of the MTHFR gene commits folate one-carbon units to the biosynthesis of methionine from homocysteine. A variant of 5,10-methyleneTHF reductase (MTHFR 677C → T) occurs in as many as 8–15% of the population (34, 35). This SNP results in an alanine-to-valine substitution that produces a thermally unstable enzyme with a 50% reduction of enzymatic activity in homozgyous persons.

Risk of NTDs is elevated for both maternal (50% increase) and fetal (80% increase) TT genotypes (36). Another common SNP of this gene (MTHFR 1298A → C) also results in reduced enzymatic activity (37). Persons having both MTHFR polymorphisms have greater risk of NTDs than do persons that have either polymorphism alone (38). As noted earlier, MTHFD1 encodes for the enzyme C1-THF synthase, catalyzing the synthesis of 10-formylTHF and 5,10-methyleneTHF; the cofactors for de novo purine and thymidylate biosynthesis. A genetic variant of MTHFD1, 653R → Q is associated with increased maternal risk of NTDs (39). Thus, the interaction between dietary intake of folate and genetic predisposition clearly influences reproductive outcome.

CHOLINE AND REPRODUCTIVE OUTCOME

Although choline is found in a variety of foods, including eggs and meats (40) (see www.nal.usda.gov/fnic/foodcomp/Data/Choline/Choline.html), there is significant variation (likely 3–4-fold) in dietary intake of choline among different people. Choline intake on ad libitum diets for men and women averages 8.4 mg/kg and. 6.7 mg/kg of choline per day, respectively (41). However, in several studies in the United States, investigators observed intakes that were less than half this amount in 25% of the women studied (42–44). Choline is derived not only from the diet but also from de novo synthesis of phosphatidylcholine.
catalyzed by phosphatidylethanolamine N-methyltransferase (the product of the PEMT gene) in the liver (45).

When deprived of dietary choline, most men and postmenopausal women develop fatty liver or muscle damage (24, 46). However, only a portion (44%) of premenopausal women develop such problems when choline deficient. The difference in requirement occurs because estrogen induces the PEMT gene and allows premenopausal women to make more of their needed choline endogenously (47). During pregnancy, estrogen concentration rises from ~1 nmol/L to 60 nmol/L at term (48, 49), suggesting that capacity for endogenous synthesis of choline should be highest during the period when females need to support fetal development. This is fortunate because pregnancy and lactation are times when demand for choline is especially high because transport of choline from mother to fetus (50, 51) depletes maternal plasma choline in humans (52). Thus, despite enhanced capacity to synthesize choline, the demand for this nutrient is so high that stores are depleted. Pregnant rats had diminished total liver choline stores compared with nonmated controls and become as sensitive to choline-deficient diets as did male rats (53). Because milk contains a great deal of choline, lactation further increases maternal demand for choline, resulting in further depletions of tissue stores (53, 54). These observations suggest that women depend on high rates of endogenous biosynthesis of choline induced by estrogen (47) and dietary intake of choline to sustain normal pregnancy.

Feeding rodents more choline during a few days in pregnancy increases the rate of brain neurogenesis in the fetus; it also decreases apoptosis (cell suicide) rates in these cells (55, 56). Low maternal choline intake during days 11–17 of gestation resulted in half as much neural progenitor cell proliferation and twice as much progenitor cell apoptosis in the fetal hippocampus (memory center) compared with fetuses from mothers fed choline-adequate diets (56, 57). The offspring of choline-deficient dams had diminished visuospatial and auditory memory for the rest of their lives (58). Conversely, more choline (about 4 times normal dietary levels) fed to pregnant dams enhanced visuospatial and auditory memory in their offspring by as much as 30% throughout life (58–64). Indeed, adult rodents normally lose memory function as they age, and offspring exposed to extra choline in utero did not show this “senility” (61, 63).

It seems that the progenitor cells of the neural tube are affected by choline in the same way as are the hippocampal progenitor cells. In mice, choline is needed for normal neural tube closure in the fetus (65, 66), and, in humans, women who eat a relatively low-folate diet and are in the lowest quartile for dietary choline intake had 4 times the risk (compared with women in the highest quartile) of having a baby with an NTD (42). This observation supports the suggestion that the basic research in rodents will be applicable to the human condition. Of course, human and rat brains mature at different rates, with rat brain comparatively more mature at birth than is the human brain. In humans, the architecture of the hippocampus continues to develop after birth, and by 4 y of age it closely resembles adult structure (67). This area of brain is one of the few areas in which neurons continue to multiply slowly throughout life (68, 69).

The effects of dietary choline on fetal development have greater significance because genes of choline metabolism are polymorphic, variants are relatively common, and some can increase dietary requirements for choline. Among these functionally important SNPs, a number have been identified that explain differences in the risk of developing organ dysfunction or damage when humans are fed diets low in choline (24, 46, 70). As discussed earlier, the gene PEMT encodes for a protein responsible for endogenous formation of choline in the liver (71) and it is induced by estrogen (47). In studies of organ dysfunction after choline deficiency in humans, a SNP in the promoter region of the PEMT gene (rs12325817) was associated with greatly increased susceptibility to choline deficiency in women but not in men (70). This SNP was common, with 14% of a Chapel Hill, NC, population being homozygous for it, and 75% of the population having one allele (70). Two SNPs in the coding region of the choline dehydrogenase gene (CHDH) are common. One variant (rs9001; 13% of a Chapel Hill, NC, population has 1 allele) had a protective effect on susceptibility to choline deficiency, whereas a second variant (rs12676; 51% of a Chapel Hill, NC, population has 1 allele) was associated with increased susceptibility to choline deficiency (70).

Because choline and folate metabolism are intermingled, SNPs in one pathway can change the dietary requirement for the other nutrient. The MTHFD1 G1958A polymorphism affects the balance of flux between 5,10-methylene tetrahydrofolate and 10-formyl tetrahydrofolate and thereby reduces the availability of 5-methyltetrahydrofolate for homocysteine remethylation (72). Premenopausal women who were carriers of this common SNP (63% of the population has 1 allele) were >15 times as likely as were noncarriers to develop signs of choline deficiency on a low-choline diet (72). It is of interest that the risk of having a child with an NTD increases in mothers with this SNP (39).

DIET AND DNA METHYLATION

DNA can be methylated at cytosine bases that are followed by a guanosine (CpG islands) (73), and S-adenosylmethionine, derived from methionine, choline, or 5-methylTHF is the source of the methyl groups. Low dietary choline-folate intake not only depletes choline and folate metabolites but also decreases S-adenosylmethionine concentrations (22, 74), with resulting hypomethylation of DNA (75, 76). DNA methylation influences gene transcription and genomic stability (77–79); increased methylation is usually associated with gene silencing or reduced gene expression (80) because methylated CpG islands attract capping proteins that hinder access to the gene for the transcription factors that normally induce gene expression (81). Once CpG islands in genes are methylated, the methylation is reproduced every time the gene is copied. Thus, effects of methylation persist, perhaps throughout life.

Changes in dietary availability of methyl groups induces stable changes in gene methylation, altering gene expression and resulting phenotype (82, 83). For example, feeding pregnant pseudogouti Avy/a mouse dams a methyl-supplemented diet altered agouti gene expression in their offspring, as indicated by increased agouti/black motting of their coats (82, 84). In a similar study, maternal dietary intake of methyl groups influenced methylation of the gene axin fused that determined whether offspring had permanently kinked tails (85). Many of the changes in neurogenesis caused by altered availability of dietary choline or folate during pregnancy are probably mediated by altered DNA methylation. Decreased choline in diets of pregnant mice was associated with changes in DNA methylation in fetal brain that were specific to some CpG islands, and even to...
specific CpG sites, within genes that regulate cell cycling (86, 87). Methylation of the \(CDKN3\) gene promoter was decreased in fetal brain, resulting in overexpression of this gene that inhibits cell proliferation (86). It is clear that the dietary manipulation of methyl donors (either deficiency or supplementation) can have a profound effect on reproductive outcome through epigenetic mechanisms. For this reason, it is important that expert panels carefully consider recommendations for dietary intake of methyl donors during pregnancy. (Other articles in this supplement to the Journal include references 88–92.)

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676S ZEISEL


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