Importance of methyl donors during reproduction[1–4]

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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 that persist in adult offspring; higher choline intake is associated with enhanced 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. *Am J Clin Nutr* 2009;89(suppl): 673S–7S.

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 C^1-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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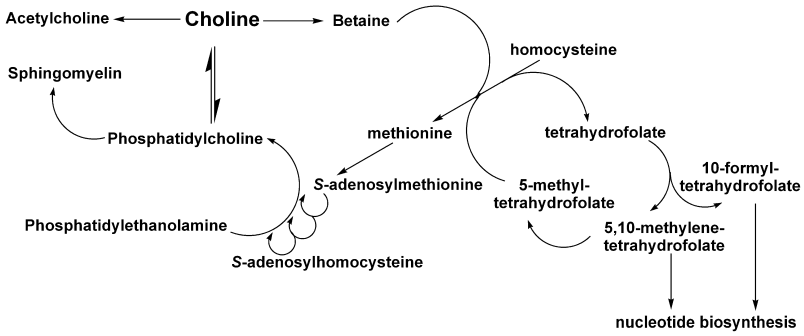

FIGURE 1. Choline and folate metabolic pathways intersect. Choline, via its metabolite betaine, is a methyl donor in the formation of methionine from homocysteine. In a parallel pathway, 5-methyl tetrahydrofolate serves as the methyl donor.

(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 $\geq$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$\rightarrow$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 homozygous 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$\rightarrow$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 C^1-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$\rightarrow$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 depletion 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-methyl tetrahydrofolate 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 pseudoagouti Avy/a mouse dams a methyl-supplemented diet altered agouti gene expression in their offspring, as indicated by increased agouti/black mottling 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 (867, 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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REFERENCES

1. Beaudin AE, Stover PJ. Folate-mediated one-carbon metabolism and neural tube defects: balancing genome synthesis and gene expression. Birth Defects Res C Embryo Today. 2007;81:183–203.
2. Blusztajn JK, Wurtman RJ. Choline and cholinergic neurons. Science 1983;221:614–20.
3. Vance DE. Boehringer Mannheim Award lecture. Phosphatidylcholine metabolism: masochistic enzymology, metabolic regulation, and lipoprotein assembly. Biochem Cell Biol 1990;68:1151–65.
4. Kent C. Regulation of phosphatidylcholine biosynthesis. Prog Lipid Res 1990;29:87–105.
5. Hanada K, Horii M, Akamatsu Y. Functional reconstitution of sphingomyelin synthase in chinese hamster ovary cell membranes. Biochim Biophys Acta 1991;1086:151–6.
6. Lin CS, Wu RD. Choline oxidation and choline dehydrogenase. J Protein Chem 1986;5:193–200.
7. Niculescu MD, Zeisel SH. Diet, methyl donors and DNA methylation: interactions between dietary folate, methionine and choline. J Nutr 2002;132:2333S–5S.
8. Finkelstein JD. Pathways and regulation of homocysteine metabolism in mammals. Semin Thromb Hemost 2000;26:219–25.
9. Olthof MR, van Vliet T, Boelsma E, Verhoef P. Low dose betaine supplementation leads to immediate and long term lowering of plasma homocysteine in healthy men and women. J Nutr 2003;133:4135–8.
10. Weisberg IS, Jacques PF, Selhub J, et al. The 1298A→C polymorphism in methylenetetrahydrofolate reductase (MTHFR): in vitro expression and association with homocysteine. Atherosclerosis 2001;156:409–15.
11. Shelnutt KP, Kauwell GP, Chapman CM, et al. Folate status response to controlled folate intake is affected by the methylenetetrahydrofolate reductase 677C→T polymorphism in young women. J Nutr 2003;133: 4107–11.
12. Jacques PF, Bostom AG, Wilson PW, Rich S, Rosenberg IH, Selhub J. Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort. Am J Clin Nutr 2001;73:613–21.
13. Watkins D, Ru M, Hwang HY, et al. Hyperhomocysteinemia due to methionine synthase deficiency, cblG: structure of the MTR gene, genotype diversity, and recognition of a common mutation, P1173L. Am J Hum Genet 2002;71:143–53.
14. Sunden SL, Renduchintala MS, Park EI, Miklasz SD, Garrow TA. Betaine-homocysteine methyltransferase expression in porcine and human tissues and chromosomal localization of the human gene. Arch Biochem Biophys 1997;345:171–4.
15. Steenge GR, Verhoef P, Katan MB. Betaine supplementation lowers plasma homocysteine in healthy men and women. J Nutr 2003;133: 1291–5.
16. Wendel U, Bremer H. Betaine in the treatment of homocystinuria due to 5,10-methylenetetrahydrofolate reductase deficiency. Eur J Pediatr 1984;142:147–50.
17. Mudd SH, Ebert MH, Scriver CR. Labile methyl group balances in the human: the role of sarcosine. Metabolism 1980;29:707–20.
18. Kim YI, Miller JW, da Costa K-A, et al. Severe folate deficiency causes secondary depletion of choline and phosphocholine in liver. J Nutr 1994; 124:2197–203.
19. Selhub J, Seyoum E, Pomfret EA, Zeisel SH. Effects of choline deficiency and methotrexate treatment upon liver folate content and distribution. Cancer Res 1991;51:16–21.
20. Varela-Moreiras G, Selhub J, da Costa K, Zeisel SH. Effect of chronic choline deficiency in rats on liver folate content and distribution. J Nutr Biochem 1992;3:519–22.
21. Pomfret EA, da Costa K, Zeisel SH. Effects of choline deficiency and methotrexate treatment upon rat liver. J Nutr Biochem 1990;1:533–41.
22. Zeisel SH, Zola T, daCosta K, Pomfret EA. Effect of choline deficiency on S-adenosylmethionine and methionine concentrations in rat liver. Biochem J 1989;259:725–9.
23. Varela-Moreiras G, Ragel C, Perez de Miguelsanz J. Choline deficiency and methotrexate treatment induces marked but reversible changes in hepatic folate concentrations, serum homocysteine and DNA methylation rates in rats. J Am Coll Nutr 1995;14:480–5.
24. da Costa KA, Gaffney CE, Fischer LM, Zeisel SH. Choline deficiency in mice and humans is associated with increased plasma homocysteine concentration after a methionine load. Am J Clin Nutr 2005;81:440–4.
25. Willoughby ML, Jewell FG. Folate status throughout pregnancy and in postpartum period. BMJ 1968;4:356–60.
26. Qvist I, Abdulla M, Jagerstad M, Svensson S. Iron, zinc and folate status during pregnancy and two months after delivery. Acta Obstet Gynecol Scand 1986;65:15–22.
27. Smithells RW, Sheppard S, Schorah CJ. Vitamin dificiencies and neural tube defects. Arch Dis Child 1976;51:944–50.
28. MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the medical Research Council Vitamin Study. Lancet 1991; 338:131–7.
29. Centers for Disease Control and Prevention. Spina bifida and anencephaly before and after folic acid mandate–United States, 1995–1996 and 1999–2000. MMWR Morb Mortal Wkly Rep 2004;53:362–5.
30. Persad VL, Van den Hof MC, Dube JM, Zimmer P. Incidence of open neural tube defects in Nova Scotia after folic acid fortification. Can Med Assoc J 2002;167:241–5.
31. Craciunescu CN, Brown EC, Mar MH, Albright CD, Nadeau MR, Zeisel SH. Folic acid deficiency during late gestation decreases progenitor cell proliferation and increases apoptosis in fetal mouse brain. J Nutr 2004; 134:162–6.
32. McVean G, Spencer CC, Chaix R. Perspectives on human genetic variation from the HapMap Project. PLoS Genet. 2005;1:e54.
33. Hinds DA, Stuve LL, Nilsen GB, et al. Whole-genome patterns of common DNA variation in three human populations. Science 2005;307: 1072–9.
34. Motulsky AG. Nutritional ecogenetics: homocysteine-related arteriosclerotic vascular disease, neural tube defects, and folic acid. Am J Hum Genet 1996;58:17–20.
35. van der Put NM, Steegers-Theunissen RP, Frosst P, et al. Mutated methylene tetrahydrofolate reductase as a risk factor for spinal bifida. Lancet 1995;346:1070–1.
36. Blom HJ, Shaw GM, den Heijer M, Finnell RH. Neural tube defects and folate: case far from closed. Nat Rev Neurosci 2006;7:724–31.
37. Weisberg I, Tran P, Christensen B, Sibani S, Rozen R. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Mol Genet Metab 1998;64: 169–72.
38. Relton CL, Wilding CS, Laffling AJ, et al. Low erythrocyte folate status and polymorphic variation in folate-related genes are associated with risk of neural tube defect pregnancy. Mol Genet Metab 2004;81:273–81.
39. Brody LC, Conley M, Cox C, et al. A polymorphism, R653Q, in the trifunctional enzyme methylenetetrahydrofolate dehydrogenase/ methenyltetrahydrofolate cyclohydrolase/formyltetrahydrofolate synthetase is a maternal genetic risk factor for neural tube defects: report of the Birth Defects Research Group. Am J Hum Genet 2002;71:1207–15.
40. Zeisel SH, Mar MH, Howe JC, Holden JM. Concentrations of choline-containing compounds and betaine in common foods. J Nutr 2003;133: 1302–7.
41. Fischer LM, Scearce JA, Mar MH, et al. Ad libitum choline intake in healthy individuals meets or exceeds the proposed adequate intake level. J Nutr 2005;135:826–9.
42. Shaw GM, Carmichael SL, Yang W, Selvin S, Schaffer DM. Periconceptional dietary intake of choline and betaine and neural tube defects in offspring. Am J Epidemiol 2004;160:102–9.

43. Cho E, Willett WC, Colditz GA, et al. Dietary choline and betaine and the risk of distal colorectal adenoma in women. J Natl Cancer Inst 2007; 99:1224–31.
44. Bidulescu A, Chambless LE, Siega-Riz AM, Zeisel SH, Heiss G. Usual choline and betaine dietary intake and incident coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) Study. BMC Cardiovasc Disord 2007;7:20.
45. Zhu X, Mar MH, Song J, Zeisel SH. Deletion of the Pemt gene increases progenitor cell mitosis, DNA and protein methylation and decreases calretinin expression in embryonic day 17 mouse hippocampus. Brain Res Dev Brain Res 2004;149:121–9.
46. da Costa KA, Badea M, Fischer LM, Zeisel SH. Elevated serum creatine phosphokinase in choline-deficient humans: mechanistic studies in C2C12 mouse myoblasts. Am J Clin Nutr 2004;80:163–70.
47. Resseguie M, Song J, Niculescu MD, da Costa KA, Randall TA, Zeisel SH. Phosphatidylethanolamine N-methyltransferase (PEMT) gene expression is induced by estrogen in human and mouse primary hepatocytes. FASEB J 2007;21:2622–32.
48. Sarda IR, Gorwill RH. Hormonal studies in pregnancy. I. Total unconjugated estrogens in maternal peripheral vein, cord vein, and cord artery serum at delivery. Am J Obstet Gynecol 1976;124:234–8.
49. Adeyemo O, Jeyakumar H. Plasma progesterone, estradiol-17 beta and testosterone in maternal and cord blood, and maternal human chorionic gonadotropin at parturition. Afr J Med Med Sci 1993;22:55–60.
50. Sweiry JH, Yudilevich DL. Characterization of choline transport at maternal and fetal interfaces of the perfused guinea-pig placenta. J Physiol 1985;366:251–66.
51. Sweiry JH, Page KR, Dacke CG, Abramovich DR, Yudilevich DL. Evidence of saturable uptake mechanisms at maternal and fetal sides of the perfused human placenta by rapid paired-tracer dilution: studies with calcium and choline. J Dev Physiol 1986;8:435–45.
52. McMahon KE, Farrell PM. Measurement of free choline concentrations in maternal and neonatal blood by micropyrolysis gas chromatography. Clin Chim Acta 1985;149:1–12.
53. Zeisel SH, Mar M-H, Zhou Z-W, da Costa K-A. Pregnancy and lactation are associated with diminished concentrations of choline and its metabolites in rat liver. J Nutr 1995;125:3049–54.
54. Holmes-McNary MQ, Cheng WL, Mar MH, Fussell S, Zeisel SH. Choline and choline esters in human and rat milk and infant formulas. Am J Clin Nutr 1996;64:572–6.
55. Craciunescu CN, Albright CD, Mar MH, Song J, Zeisel SH. Choline availability during embryonic development alters progenitor cell mitosis in developing mouse hippocampus. J Nutr 2003;133:3614–8.
56. Albright CD, Tsai AY, Friedrich CB, Mar MH, Zeisel SH. Choline availability alters embryonic development of the hippocampus and septum in the rat. Brain Res Dev Brain Res 1999;113:13–20.
57. Albright CD, Friedrich CB, Brown EC, Mar MH, Zeisel SH. Maternal dietary choline availability alters mitosis, apoptosis and the localization of TOAD-64 protein in the developing fetal rat septum. Brain Res Dev Brain Res 1999;115:123–9.
58. Meck WH, Williams CL. Choline supplementation during prenatal development reduces proactive interference in spatial memory. Brain Res Dev Brain Res 1999;118:51–9.
59. Meck WH, Williams CL. Perinatal choline supplementation increases the threshold for chunking in spatial memory. Neuroreport 1997;8: 3053–9.
60. Meck WH, Williams CL. Characterization of the facilitative effects of perinatal choline supplementation on timing and temporal memory. Neuroreport 1997;8:2831–5.
61. Meck WH, Williams CL. Simultaneous temporal processing is sensitive to prenatal choline availability in mature and aged rats. Neuroreport 1997;8:3045–51.
62. Meck WH, Smith RA, Williams CL. Pre- and postnatal choline supplementation produces long-term facilitation of spatial memory. Dev Psychobiol 1988;21:339–53.
63. Meck WH, Williams CL. Metabolic imprinting of choline by its availability during gestation: implications for memory and attentional processing across the lifespan. Neurosci Biobehav Rev 2003;27:385–99.
64. Williams CL, Meck WH, Heyer DD, Loy R. Hypertrophy of basal forebrain neurons and enhanced visuospatial memory in perinatally choline-supplemented rats. Brain Res 1998;794:225–38.
65. Fisher MC, Zeisel SH, Mar MH, Sadler TW. Inhibitors of choline uptake and metabolism cause developmental abnormalities in neurulating mouse embryos. Teratology 2001;64:114–22.
66. Fisher MC, Zeisel SH, Mar MH, Sadler TW. Perturbations in choline metabolism cause neural tube defects in mouse embryos in vitro. FASEB J 2002;16:619–21.
67. Dani S, Hori A, Walter G, eds. Principals of neural aging. Amsterdam, Netherlands: Elsevier, 1997.
68. van Praag H, Kempermann G, Gage FH. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat Neurosci 1999;2:266–70.
69. Markakis EA, Gage FH. Adult-generated neurons in the dentate gyrus send axonal projections to field CA3 and are surrounded by synaptic vesicles. J Comp Neurol 1999;406:449–60.
70. da Costa KA, Kozyreva OG, Song J, Galanko JA, Fischer LM, Zeisel SH. Common genetic polymorphisms affect the human requirement for the nutrient choline. FASEB J 2006;20:1336–44.
71. Vance DE, Walkey CJ, Cui Z. Phosphatidylethanolamine N-methyltransferase from liver. Biochim Biophys Acta 1997;1348:142–50.
72. Kohlmeier M, da Costa KA, Fischer LM, Zeisel SH. Genetic variation of folate-mediated one-carbon transfer pathway predicts susceptibility to choline deficiency in humans. Proc Natl Acad Sci USA 2005;102: 16025–30.
73. Holliday R, Grigg GW. DNA methylation and mutation. Mutat Res 1993;285:61–7.
74. Shivapurkar N, Poirier LA. Tissue levels of S-adenosylmethionine and S-adenosylhomocysteine in rats fed methyl-deficient, amino acid-defined diets for one to five weeks. Carcinogenesis 1983;4:1051–7.
75. Locker J, Reddy TV, Lombardi B. DNA methylation and hepatocarcinogenesis in rats fed a choline devoid diet. Carcinogenesis 1986;7: 1309–12.
76. Tsujiuchi T, Tsutsumi M, Sasaki Y, Takahama M, Konishi Y. Hypomethylation of CpG sites and c-myc gene overexpression in hepatocellular carcinomas, but not hyperplastic nodules, induced by a choline-deficient L-amino acid-defined diet in rats. Jpn J Cancer Res 1999;90:909–13.
77. Jaenisch R. DNA methylation and imprinting: why bother? Trends Genet 1997;13:323–9.
78. Jones PA, Gonzalgo ML. Altered DNA methylation and genome instability: a new pathway to cancer? Proc Natl Acad Sci USA 1997;94:2103–5.
79. Robertson KD, Wolffe AP. DNA methylation in health and disease. Nat Rev Genet 2000;1:11–9.
80. Jeltsch A. Beyond Watson and Crick: DNA methylation and molecular enzymology of DNA methyltransferases. ChemBioChem 2002;3:274–93.
81. Fan G, Hutnick L. Methyl-CpG binding proteins in the nervous system. Cell Res 2005;15:255–61.
82. Cooney CA, Dave AA, Wolff GL. Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. J Nutr 2002;132:2393S–400S.
83. Waterland RA, Jirtle RL. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. Mol Cell Biol 2003;23: 5293–300.
84. Wolff GL, Kodell RL, Moore SR, Cooney CA. Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. FASEB J 1998;12:949–57.
85. Waterland RA, Dolinoy DC, Lin JR, Smith CA, Shi X, Tahiliani KG. Maternal methyl supplements increase offspring DNA methylation at Axin fused. Genesis 2006;44:401–6.
86. Niculescu MD, Craciunescu CN, Zeisel SH. Dietary choline deficiency alters global and gene-specific DNA methylation in the developing hippocampus of mouse fetal brains. FASEB J 2006;20:43–9.
87. Niculescu MD, Yamamuro Y, Zeisel SH. Choline availability modulates human neuroblastoma cell proliferation and alters the methylation of the promoter region of the cyclin-dependent kinase inhibitor 3 gene. J Neurochem 2004;89:1252–9.
88. Greer FR. Introduction. Am J Clin Nutr 2009;89(suppl):661S–2S.
89. Picciano MF, McGuire MK. Use of dietary supplements by pregnant and lactating women in North America. Am J Clin Nutr 2009;89(suppl): 663S–7S.
90. Zimmermann MB. Iodine deficiency in pregnancy and the effects of maternal iodine supplementation on the offspring: a review. Am J Clin Nutr 2009;89(suppl):668S–72S.
91. Carlson SE. Docosahexaenoic acid supplementation in pregnancy and lactation. Am J Clin Nutr 2009;89(suppl):678S–84S.
92. Zeisel SH. Is maternal diet supplementation beneficial? Optimal development of infant depends on mother's diet. Am J Clin Nutr 2009; 89(suppl):685S–7S.