

Review

Nutritional Importance of Choline for Brain Development

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Choline is a dietary component essential for normal function of all cells. In 1998 the National Academy of Sciences, USA, issued a report identifying choline as a required nutrient for humans and recommended daily intake amounts. In ongoing studies we are finding that men have a higher requirement than do postmenopausal women, who in turn need more than premenopausal women. Pregnancy and lactation are periods when maternal reserves of choline are depleted. At the same time, the availability of choline for normal development of brain is critical. When rat pups received choline supplements (*in utero* or during the second week of life), their brain function is changed, resulting in lifelong memory enhancement. This change in memory function appears to be due to changes in the development of the memory center (hippocampus) in brain. These changes are so important that investigators can pick out the groups of animals whose mothers had extra choline even when these animals are elderly. Thus, memory function in the aged is, in part, determined by what mother ate. Foods highest in total choline concentrations per 100g were beef liver (418 mg), chicken liver (290 mg), and eggs (251 mg). We suggest that choline-rich foods are an important component of the diet and that especially during pregnancy it would be prudent to include them as part of a healthy diet.

Key teaching points:

- Choline is an essential nutrient that plays a critical role in the normal development of the brain, especially the memory center (hippocampus) of the brain.
- Studies show that during pregnancy and lactation, maternal reserves of choline are depleted.
- Lack of choline in a mother's diet during pregnancy and lactation may have life-long effects on their child.
- The Institute of Medicine (IOM) of the National Academy of Sciences set an adequate intake (AI) level for choline of 550 mg/day for men and 425 mg/day for women.
- Foods rich in total choline include beef and chicken liver, eggs, wheat germ, bacon, dried soybeans and pork.

Nutritional Importance of Choline

Does nutrient availability during the perinatal period influence the development of critical areas in the brain and impact on brain function later in life? We will discuss how diet-induced changes in mitosis, migration, apoptosis and differentiation within critical areas of brain are the earliest events that eventually result in changes in structure, electrophysiology and function of brain that results in changes in memory function in adult and aged rats and mice. Studies are ongoing to determine whether this effect of choline occurs in humans.

Choline, or its metabolites, are needed for the structural integrity and signaling functions of cell membranes; it is the major source of methyl-groups in the diet (one of choline's metabolites, betaine, participates in the methylation of homocysteine to form methionine), and it directly affects cholinergic neurotransmission, transmembrane signaling and lipid transport/metabolism [1]. Availability of dietary choline influences neural tube closure and hippocampal development, apoptotic signaling in neurons and liver cells, hepatic transport of lipoproteins, and hepatic carcinogenesis [2].

The Institute of Medicine (IOM) of the National Academy

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of Sciences, USA, set an adequate intake level for choline of 550 mg/day for men and 425 mg/day for women [3]. We are currently funded by the NIH to refine these estimates of choline requirements in adult humans, and our preliminary data suggest that the dietary choline requirement is greatest in men > postmenopausal women > premenopausal women who are not pregnant. Based on our studies in rats, we predict that pregnant women will be the most sensitive to availability of dietary choline because of the greatly increased need for choline to form the fetus [4]. Also, in our ongoing studies, we observe that some individuals rapidly deplete when deprived of choline (days) while others take much longer to become depleted (weeks). We are investigating whether these differences are due to single nucleotide polymorphisms in the human PEMT gene (responsible for endogenous synthesis of choline in the liver) that apparently are very common [5].

During development, there is a progressive decline in blood choline concentration that begins *in utero*. In fact, plasma or serum choline concentrations are 6 or 7-fold higher in the fetus and neonate than they are in the adult [6–8]. High levels of choline circulating in the fetus presumably ensure enhanced availability of choline to tissues. Neonatal rat brain efficiently extracts choline from blood [9,10]. Supplementing choline during the perinatal period further increases blood and brain choline metabolite concentrations [11]. In lactating women eating a low-choline diet, milk choline content is lower than those eating a more adequate diet [12]. Dietary variation causes a 4-fold change (comparing the choline-deficient and choline-supplemented diets) in milk phosphocholine content [13,14]. The free choline content of human milk is very high at the start of lactation, and diminishes to quantities similar to that in commercial formulas by 30 days postpartum [13]. Ensured availability of choline appears to be important to infants because organ growth—extremely rapid in the neonate—requires large amounts of choline for membrane biosynthesis [6,15].

Choline and Brain Development

Choline is particularly important during the neonatal period because it changes brain development. When rat pups received choline supplements (*in utero* during days 12–17 of gestation (E12–17)), their brain function changed, resulting in lifelong enhancement of memory and attention [16–21]. The effects of perinatal choline supplementation on memory were initially found using radial-arm maze tasks and the Sprague-Dawley rat strain, but other laboratories have found similar results using other spatial memory tasks, such as the Morris water maze [22,23] and using other strains of rats such as Long-Evans [24–26], as well as using mice [27]. The underlying physiological and biochemical basis for these lifelong alterations is just beginning to be understood. Certain anatomical regions of hippocampus (e.g., dentate gyrus) play critical roles in learning

and acquisition of memory. In rats, prenatal choline supplementation increased the sensitivity of CA1 hippocampal neurons to stimulation of long-term potentiation (LTP) [28], and increased working spatial memory [18,20,21,24,25], whereas prenatal choline deficiency increased the threshold for long term potentiation (LTP), and retarded temporal processing [19]. We previously reported that, in the rat and mouse, these physiological and behavioral changes may be related to neuroanatomical changes in regions of fetal brain hippocampus and basal forebrain that are known to regulate memory [29–32].

Whether these findings in rodents apply as well to humans is not known. 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 years of age it closely resembles adult structure [33]. This area of brain is one of the few areas in which nerve cells continue to multiply slowly throughout life [34,35]. Should there be a recommended intake for choline in pregnant women? Are we varying the availability of choline when we feed infant formulas instead of milk? Does the form and amount of choline ingested contribute to variations in memory observed between humans? All are good questions that are worthy of additional research.

Choline and Genes Regulating Cell Division, Migration, Apoptosis and Differentiation

During embryogenesis progenitors of neurons and glia divide, many migrate to new locations, and unnecessary cells die by apoptosis [46–48]. We found that choline deficiency decreased, and choline supplementation increased, the rate of cell division in the neuroepithelial layer of E18 hippocampus and septum [29–31]. We reported that the migration of cells from the hippocampal neuroepithelium to the dentate gyrus was perturbed in choline deficient rats [29,30], and we described choline-mediated changes in the proteins that regulate cell cycling [31]. Apoptosis is a regulated form of cell suicide [49] and in neurons is modulated by survival factors such as neurotrophins, sex hormones, and neuronal activity [50,51], and by choline availability [52–55]. Apoptosis plays a critical role in determining the size of neuronal subpopulations, and thus, morphogenesis, in developing brain regions [56]. Studies in developing rodent brains demonstrated that in infants from mothers consuming low choline diets there was increased, whereas in infants from mothers consuming high choline diets there was decreased apoptosis in the hippocampus of the fetus [30,32].

The effects of choline availability on embryogenesis are not limited to middle-late gestation. We described the development of neural tube defects in mouse embryos where choline availability was manipulated at the beginning of gestation [57,58]. These observations suggest that the behavioral effects of dietary choline could be due to the relative changes in the birth, migration, survival and differentiation of brain cells at a critical period in brain development.

Choline, Folate and Homocysteine Are Inter-Related Nutrients

The metabolism of choline, homocysteine and methyl-folate are closely interrelated (Fig. 1). Perturbing metabolism of one of the methyl-donors results in compensatory changes in the other methyl-donors due to the intermingling of these metabolic pathways [36–38]. Rats ingesting a choline-deficient diet had diminished tissue concentrations of methionine and S-adenosylmethionine (SAM) [39] and of total folate [36,37]. Treatment with betaine [40] or choline [41], lowers elevated plasma homocysteine in humans. Rats treated with the antifolate, methotrexate, have diminished pools of choline metabolites in liver [36,42]. Diminished phosphatidylcholine (Ptd-Cho) concentrations are observed in brains from folate deficient adult rats [43]. Thus, the vulnerability to choline deficiency that we have described during pregnancy and lactation may have important effects on folate and homocysteine metabolism. We find that dietary folic acid availability also influences progenitor cell mitosis and apoptosis in the fetal mouse telencephalic portion of the forebrain (submitted). We discussed earlier that choline nutrition is marginal during pregnancy; folate supply is also limiting. Folate concentrations in serum and red cells decline during pregnancy to the point that pregnant women can become clinically folate deficient [44,45]. That both choline and folate exert similar effects on brain development suggests that the methyl-donation capacity that both pathways share may be involved in the mechanism explaining these effects.

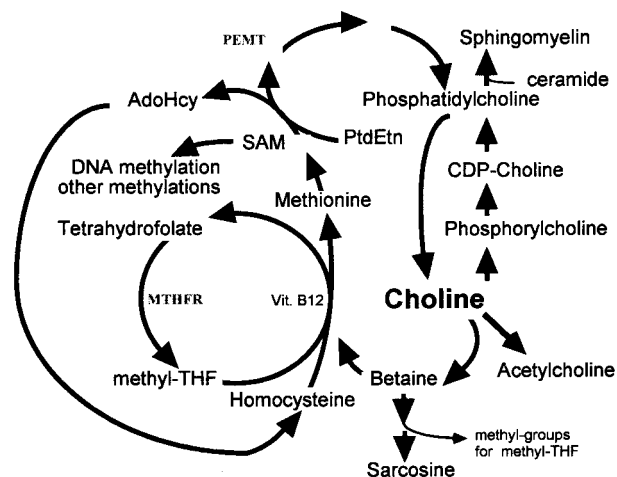


Fig. 1. Choline, folate and homocysteine metabolism are closely inter-related. The pathways for the metabolism of these three nutrients intersect at the formation of methionine from homocysteine. PtdEtn = phosphatidylethanolamine, AdoHcy = S-adenosylhomocysteine, SAM = S-adenosylmethionine, B12 = vitamin B12, PEMT = phosphatidylethanolamine methyltransferase, MTHFR = methyltetrahydrofolate reductase.

How Might Choline Influence Cell Proliferation and Apoptosis?

We hypothesize that some of the regulators of cell cycling are, in turn, regulated by epigenetic events that are modulated by choline. Specifically, DNA and histone methylation regulate key genes regulating cell cycling. DNA and histone lysine methylation are interrelated epigenetic events that are required for a proper gene regulation during neurogenesis [59,60]. DNA methylation, especially at the CG sites, is associated with a reduction of gene expression [59,61,62], perhaps because 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 the promoter region [63,64]. The progressive neurodevelopmental disorder, Rett syndrome, is caused by mutations in the gene for methyl cytosine-binding protein (MeCP2) [65]. Other functions for DNA methylation are: protection against DNA fragmentation and alteration in chromatin compaction and chromosome stability (in association with histone modifications) [59].

Cell proliferation is regulated by a complex interaction of signals, some of which are inhibitory signals mediated by cyclin dependent kinase inhibitors. We found that these inhibitors were lower in fetal hippocampus from pups of choline-supplemented dams [31]. One of these inhibitors changed by choline availability is p15; expression of this protein is methylation-dependent [66,67]. We were able to demonstrate that a gene for a cyclin kinase inhibitor (CDKN3, inhibits cyclin kinase 2) was hypomethylated. This change was associated with increased expression of CDKN3, and increased levels of its gene product, kinase-associated phosphatase (KAP, inhibits the G₁/S transition of the cell cycle by dephosphorylating cyclin-dependent kinases) (submitted).

There is good reason to propose that choline effects on brain development might be mediated by DNA methylation. In brain and other tissues, a choline-methyl deficient diet directly alters gene methylation; specifically in CpG islands within specific genes: global DNA was significantly undermethylated in brains of choline-methyl deficient rats [68]. In choline deficient liver there is hypomethylation of specific CCGG sites within several genes for which mRNA levels were increased including c-myc, c-fos and c-Ha-ras [69]. Hypomethylation of CpG sites and c-myc gene overexpression occurs in hepatocellular carcinomas induced by a choline-deficient diet in rats [70]. Also, it is reasonable that maternal diet during pregnancy could alter the methylation status of fetal genes. Feeding pregnant Pseudoagouti Avy/a mouse dams a choline methyl-supplemented diet altered epigenetic regulation of agouti expression in their offspring, as indicated by increased agouti/black mottling of their coats [71,72].

Choline in Foods

We estimate that choline intake is >550 mg/day in adult humans eating normal diets. Choline, choline esters and betaine

can be found in significant amounts in many foods consumed by humans [73]; some of the choline and betaine is added during processing (especially in the preparation of infant formula). Though the different esters of choline have different bioavailability, it is likely that choline in all forms is fungible; therefore total choline content is probably the best indicator of food choline content. Foods highest in total choline concentrations per 100g were: beef liver (418 mg), chicken liver (290 mg), eggs (251 mg), wheat germ (152 mg), bacon (125 mg), dried soybeans (116 mg), and pork (103 mg). Betaine should also be considered, as it spares the use of choline for methyl donation. Foods with the highest betaine concentrations per 100g were: wheat bran (1506), wheat germ (1395), and spinach (725).

We suggest that choline-rich foods are an important component of the diet and that especially during pregnancy it would be prudent to include them as part of a healthy diet.

REFERENCES

1. Zeisel SH, Blusztajn JK: Choline and human nutrition. *Ann Rev Nutr* 14:269–296, 1994.
2. Zeisel SH: Choline: an essential nutrient for humans. *Nutrition* 16:669–671, 2000.
3. Institute of Medicine and National Academy of Sciences USA: “Dietary reference intakes for folate, thiamin, riboflavin, niacin, vitamin B₁₂, panthothenic acid, biotin, and choline” Vol. 1. Washington DC: National Academy Press, 1998.
4. 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* 125:3049–3054, 1995.
5. Saito S, Iida A, Sekine A, Miura Y, Sakamoto T, Ogawa C, Kawauchi S, Higuchi S, Nakamura Y: Identification of 197 genetic variations in six human methyltransferase genes in the Japanese population. *J Hum Genet* 46:529–537, 2001.
6. Zeisel SH, Wurtman RJ: Developmental changes in rat blood choline concentration. *Biochem J* 198:565–570, 1981.
7. Zeisel SH, Epstein MF, Wurtman RJ: Elevated choline concentration in neonatal plasma. *Life Sci* 26:1827–1831, 1980.
8. McMahon KE, Farrell PM: Measurement of free choline concentrations in maternal and neonatal blood by micropyrolysis gas chromatography. *Clin Chim Acta* 149:1–12, 1985.
9. Braun LD, Cornford EM, Oldendorf WH: Newborn rabbit blood-brain barrier is selectively permeable and differs substantially from the adult. *J Neurochem* 34:147–152, 1980.
10. Cornford EM, Braun LD, Oldendorf WH: Developmental modulations of blood-brain barrier permeability as an indicator of changing nutritional requirements in the brain. *Pediatr Res* 16:324–328, 1982.
11. Garner SC, Mar M-H, Zeisel SH: Choline distribution and metabolism in pregnant rats and fetuses are influenced by the choline content of the maternal diet. *J Nutr* 125:2851–2858, 1995.
12. Zeisel SH, Stanbury JB, Wurtman RJ, Brigida M, Fierro BR: Choline content of mothers’ milk in Ecuador and Boston. *New Engl J Med* 306:175–176, 1982.
13. Zeisel SH, Char D, Sheard NF: Choline, phosphatidylcholine and sphingomyelin in human and bovine milk and infant formulas. *J Nutr* 116:50–58, 1986.
14. Holmes-McNary M, Cheng WL, Mar MH, Fussell S, Zeisel SH: Choline and choline esters in human and rat milk and infant formulas. *Am J Clin Nutr* 64:572–576, 1996.
15. Zeisel SH: Choline: an important nutrient in brain development, liver function and carcinogenesis. *J Am Coll Nutr* 11:473–481, 1992.
16. Loy R, Heyer D, Williams CL, Meck WH: Choline-induced spatial memory facilitation correlates with altered distribution and morphology of septal neurons. *Adv Exp Med Biol* 295:373–382, 1991.
17. Meck WH, Smith RA, Williams CL: Pre- and postnatal choline supplementation produces long-term facilitation of spatial memory. *Dev Psychobiol* 21:339–353, 1988.
18. Meck WH, Smith RA, Williams CL: Organizational changes in cholinergic activity and enhanced visuospatial memory as a function of choline administered prenatally or postnatally or both. *Behav Neurosci* 103:1234–1241, 1989.
19. Meck W, Williams C: Simultaneous temporal processing is sensitive to prenatal choline availability in mature and aged rats. *Neuroreport* 8:3045–3051, 1997.
20. Meck W, Williams C: Characterization of the facilitative effects of perinatal choline supplementation on timing and temporal memory. *Neuroreport* 8:2831–2835, 1997.
21. Meck W, Williams C: Perinatal choline supplementation increases the threshold for chunking in spatial memory. *Neuroreport* 8:3053–3059, 1997.
22. Schenk F, Brandner C: Indirect effects of peri- and postnatal choline treatment on place-learning abilities in rat. *Psychobiology* 23:302–313, 1995.
23. Brandner C: Perinatal choline treatment modifies the effects of a visuo-spatial attractive cue upon spatial memory in naive adult rats. *Brain Res* 928:85–95, 2002.
24. Tees RC: The influences of rearing environment and neonatal choline dietary supplementation on spatial learning and memory in adult rats. *Behav Brain Res* 105:173–188, 1999.
25. Tees RC: The influences of sex, rearing environment, and neonatal choline dietary supplementation on spatial and nonspatial learning and memory in adult rats. *Dev Psychobiol* 35:328–342, 1999.
26. Tees RC, Mohammadi E, Adam TJ: Altering the impact of early rearing on the rat’s spatial memory with pre- and postnatal choline supplementation. *Soc Neurosci Abstr* 17:1401, 1999.
27. Ricceri L, Berger-Sweeney J: Postnatal choline supplementation in preweanling mice: sexually dimorphic behavioral and neurochemical effects. *Behav Neurosci* 112:1387–1392, 1998.
28. Jones JP, Meck W, Williams CL, Wilson WA, Swartzwelder HS: Choline availability to the developing rat fetus alters adult hippocampal long-term potentiation. *Brain Res Dev Brain Res* 118: 159–167, 1999.
29. 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* 113:13–20, 1999.
30. 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* 115:123–129, 1999.

31. Albright CD, Mar MH, Friedrich CB, Brown EC, Zeisel SH: Maternal choline availability alters the localization of p15Ink4B and p27Kip1 cyclin-dependent kinase inhibitors in the developing fetal rat brain hippocampus. *Dev Neurosci* 23:100–106, 2001.
32. Craciunescu C, Albright C, Mar M-H, Song J, Zeisel S: Choline availability during embryonic development alters progenitor cell mitosis in developing mouse hippocampus. *J Nutr* 133:3614–3618, 2003.
33. Dani S, Hori A, Walter G (eds): “Principles of Neural Aging.” Amsterdam: Elsevier, 1997.
34. van Praag H, Kempermann G, Gage FH: Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus [see comments]. *Nat Neurosci* 2:266–270, 1999.
35. 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* 406:449–460, 1999.
36. Selhub J, Seyoum E, Pomfret EA, Zeisel SH: Effects of choline deficiency and methotrexate treatment upon liver folate content and distribution. *Cancer Res* 51:16–21, 1991.
37. 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* 3:519–522, 1992.
38. Kim Y-I, Miller JW, da Costa K-A, Nadeau M, Smith D, Selhub J, Zeisel SH, Mason JB: Folate deficiency causes secondary depletion of choline and phosphocholine in liver. *J Nutr* 124:2197–2203, 1995.
39. Zeisel SH, Zola T, daCosta K, Pomfret EA: Effect of choline deficiency on S-adenosylmethionine and methionine concentrations in rat liver. *Biochem J* 259:725–729, 1989.
40. Anonymous: Betaine for homocystinuria. *Med Lett Drug Therap* 39:12:1997.
41. Kang S: Treatment of hyperhomocyst(e)inemia: physiological basis. *J Nutr* 126:1273S–1275S, 1996.
42. Pomfret EA, da Costa K, Zeisel SH: Effects of choline deficiency and methotrexate treatment upon rat liver. *J Nutr Biochem* 1:533–541, 1990.
43. Akesson B, Fehling C, Jagerstadt M, et al.: Effect of experimental folate deficiency on lipid metabolism in liver and brain. *Brit J Nutr* 47:505–520, 1982.
44. Willoughby M, Jewell F: Folate status throughout pregnancy and in postpartum period. *Brit Med J* 4:356, 1968.
45. Qvist I, Abdulla M, Jagerstad M, Svensson S: Iron, zinc and folate status during pregnancy and two months after delivery. *Acta Obstet Gynecol Scand* 65:15–22, 1986.
46. Oppenheim RW: Cell death during the development of the nervous system. *Ann Rev Neurosci* 14:453–501, 1991.
47. Thompson CB: Apoptosis in the pathogenesis and treatment of disease. *Science* 267:1456–1462, 1995.
48. Merry D, Korsmeyer S: Bcl-2 gene family in the nervous system. *Annu Rev Neurosci* 20:245–267, 1997.
49. Schwartzman RA, Cidlowski JA: Apoptosis: The biochemistry and molecular biology of programmed cell death. *Endoc Rev* 14:133–151, 1993.
50. Henderson C: Programmed cell death in the developing nervous system. *Neuron* 17:579–795, 1996.
51. Vekrellis K, McCarthy M, Watson A, Whitfield J, Rubin L, Ham J: Bax promotes neuronal cell death and is downregulated during the development of the nervous system. *Development* 124:1239–1249, 1997.
52. Holmes-McNary M, Baldwin J, Zeisel SH: Opposing regulation of choline deficiency-induced apoptosis by p53 and NF-k B. *J Biol Chem* 276:41197–41204, 2001.
53. Holmes-McNary MQ, Loy R, Mar M-H, Albright CD, Zeisel SH: Apoptosis is induced by choline deficiency in fetal brain and in PC12 cells. *Devel Brain Res* 101:9–16, 1997.
54. Yen CL, Mar MH, Meeker RB, Fernandes A, and Zeisel SH: Choline deficiency induces apoptosis in primary cultures of fetal neurons. *FASEB J* 15:1704–1710, 2001.
55. Yen CL, Mar MH, Zeisel SH: Choline deficiency-induced apoptosis in PC12 cells is associated with diminished membrane phosphatidylcholine and sphingomyelin, accumulation of ceramide and diacylglycerol, and activation of a caspase. *FASEB J* 13:135–142, 1999.
56. Kuan CY, Roth KA, Flavell RA, Rakic P: Mechanisms of programmed cell death in the developing brain. *Trends Neurosci* 23:291–297, 2000.
57. Fisher MC, Zeisel SH, Mar MH, Sadler TW: Inhibitors of choline uptake and metabolism cause developmental abnormalities in neuroulating mouse embryos. *Teratology* 64:114–122, 2001.
58. Fisher MC, Zeisel SH, Mar MH, Sadler TW: Perturbations in choline metabolism cause neural tube defects in mouse embryos in vitro. *Faseb J* 16:619–621, 2002.
59. Paulsen M, Ferguson-Smith AC: DNA methylation in genomic imprinting, development, and disease. *J Pathol* 195:97–110, 2001.
60. Lachner M, Jenuwein T: The many faces of histone lysine methylation. *Curr Opin Cell Biol* 14:286–298, 2002.
61. Reinhart B, Eljanne M, Chaillet JR: Shared role for differentially methylated domains of imprinted genes. *Mol Cell Biol* 22:2089–2098, 2002.
62. Babinger P, Kobl I, Mages W, Schmitt R: A link between DNA methylation and epigenetic silencing in transgenic *Volvox carteri*. *Nucleic Acids Res* 29:1261–1271, 2001.
63. Hendrich B, Bird A: Identification and characterization of a family of mammalian methyl-CpG binding proteins. *Mol Cell Biol* 18:6538–6547, 1998.
64. Jones PA, Takai D: The role of DNA methylation in mammalian epigenetics. *Science* 293:1068–1070, 2001.
65. Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY: Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat Genet* 23:185–188, 1999.
66. Garcia MJ, Martinez-Delgado B, Cebrian A, Martinez A, Benitez J, Rivas C: Different incidence and pattern of p15INK4b and p16INK4a promoter region hypermethylation in Hodgkin’s and CD30-Positive non-Hodgkin’s lymphomas. *Am J Pathol* 161:1007–1013, 2002.
67. Diccianni MB, Omura-Minamisawa M, Batova A, Le T, Bridgeman L, Yu AL: Frequent deregulation of p16 and the p16/G1 cell cycle-regulatory pathway in neuroblastoma. *Int J Cancer* 80:145–154, 1999.
68. Alonso-Aperte E, Varela-Moreiras G: Brain folates and DNA methylation in rats fed a choline deficient diet or treated with low doses of methotrexate. *Int J Vitam Nutr Res* 66:232–236, 1996.
69. Christman JK, Sheikhejad G, Dizik M, Abileah S, Wainfan E: Reversibility of changes in nucleic acid methylation and gene

- expression induced in rat liver by severe dietary methyl deficiency. *Carcinogenesis* 14:551–557, 1993.
70. 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* 90:909–913, 1999.
71. Wolff GL, Kodell RL, Moore SR, Cooney CA: Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. *Faseb J* 12:949–957, 1998.
72. Waterland RA, Jirtle RL: Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol* 23:5293–5300, 2003.
73. Zeisel SH, Mar MH, Howe JC, Holden JM: Concentrations of choline-containing compounds and betaine in common foods. *J Nutr* 133:1302–1307, 2003.