Published in final edited form as:
Nutr Rev. 2006 April ; 64(4): 197–203.

Perinatal Choline Influences Brain Structure and Function

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Abstract

Choline is derived not only from the diet, but also from de novo synthesis. It is important for methyl-group metabolism, the formation of membranes, kidney function, and neurotransmission. When deprived of dietary choline, most adult men and postmenopausal women develop signs of organ dysfunction (fatty liver or muscle damage) and have a decreased capacity to convert homocysteine to methionine. Choline is critical during fetal development, when it influences stem cell proliferation and apoptosis, thereby altering brain structure and function (memory is permanently enhanced in rodents exposed to choline during the latter part of gestation).

Keywords

brain development; choline; folate; hippocampus; memory

CHOLINE IS AN ESSENTIAL NUTRIENT

Choline consists of three methyl groups covalently attached to the nitrogen atom of ethanolamine, and is essential for normal functioning of all cells.[1] A major use for choline is as a precursor for the synthesis of membranes. Phosphatidylcholine (sometimes called lecithin) is the predominant phospholipid (>50%) in most mammalian membranes; sphingomyelin is another important choline phospholipid. Only a small fraction of dietary choline is acetylated, catalyzed by the activity of choline acetyltransferase.[2] The product is acetylcholine, an important neurotransmitter. The methyl groups of choline can be made available from one-carbon metabolism upon conversion to betaine,[3] which cannot be reduced back to choline. The interrelationship of this pathway with folate and methionine metabolism is discussed below.

When deprived of dietary choline, most adult men and postmenopausal women develop signs of organ dysfunction (fatty liver or muscle damage)[4,5]; premenopausal women are relatively resistant to choline deficiency (unpublished data). The 1998 Institute of Medicine recommendations on dietary reference intakes included estimates of the Adequate Intake (AI) of choline required by humans (approximately half a gram a day).[6] The USDA recently developed a database of choline content in foods (http://www.nal.usda.gov/fnic/foodcomp/Data/Choline/Choline.html), which shows that excellent sources of dietary choline include liver, eggs, and wheat germ. In foods, choline is found in free and in esterified forms (as phosphocholine, glycerophosphocholine, phosphatidylcholine, and sphingomyelin). There is some evidence that these forms of choline may have different bioavailability,[7] because the lipid-soluble forms bypass the liver when absorbed from the diet, while the water-soluble forms enter the portal circulation and are mostly

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absorbed by the liver. Human milk is rich in choline compounds,[8] and soy-derived infant formulas have lower total choline concentrations than do either human milk or bovine milk-derived formulas.[8] Choline intake by humans on ad libitum diets for males and females averages between 8.4 and. 6.7 mg/kg of choline per day, respectively.[9] However, Shaw et al.[10] studied pregnant women in California and observed intakes that were less than half this amount in 25% of the women studied.

Choline is derived not only from the diet, but also from the de novo synthesis of phosphatidylcholine catalyzed by phosphatidylethanolamine N-methyltransferase (PEMT).[11] This enzyme, which is the most active in the liver, uses S-adenosylmethionine as a methyl donor and forms a new choline moiety.[12] When fed a diet deficient in choline, *Pemt*$^{-/-}$ mice developed fatty liver, severe liver damage, and died; a choline-supplemented diet prevented this[13] and reversed hepatic damage if begun early enough.[14] *Pemt*$^{-/-}$ mice have lower choline pools in liver despite being fed sufficient or supplemental amounts of dietary choline,[15] suggesting that choline production by PEMT is a significant source of choline relative to dietary intake.

CHOLINE, FOLATE, AND METHIONINE METABOLISM ARE INTERRELATED

Choline, methionine and folate metabolism are metabolically interrelated at the point that homocysteine is methylated to form methionine. Thus, any requirement for dietary choline must be considered in relation to these other nutrients. Homocysteine can be methylated to form methionine[16] by two parallel pathways, both of which lower homocysteine concentrations[17] (Figure 1). In the first, vitamins B_{12} and folic acid are involved in a reaction catalyzed by methionine synthase.[18] Deficiency of these nutrients[19,20] or single nucleotide polymorphisms in the genes for the enzymes involved in this pathway[18,20,21] can result in elevated plasma homocysteine concentrations. In addition, tetrahydrofolate is needed to scavenge one-carbon groups when betaine is metabolized.[22] The alternative pathway for the methylation of homocysteine to form methionine is catalyzed by betaine homocysteine methyltransferase.[23] Betaine, derived from dietary choline, is the methyl group donor in this reaction, and supplemental oral betaine can lower plasma homocysteine concentrations.[24,25] We recently reported that humans who are depleted of choline have diminished capacity to methylate homocysteine and develop elevated homocysteine concentrations in plasma after a methionine loading test.[4]

CHOLINE IN PREGNANCY AND LACTATION

Pregnancy and lactation are times when the demand for choline is especially high; transport of choline from mother to fetus[26,27] depletes maternal plasma choline in humans.[28] Thus, the demand for this nutrient is so high that stores are depleted. Pregnant rats have diminished total liver choline compounds compared with non-mated controls and become as sensitive to choline-deficient diets as male rats[29] Because milk contains a great deal of choline, lactation further increases maternal demand for choline, resulting in further depletion of tissue stores.[8,29] Choline nutriture during pregnancy is especially important because it influences brain development in the fetus (see below), and because it is important for maintaining normal plasma homocysteine concentrations during pregnancy.[30] High maternal homocysteine concentrations are associated with increased incidence of birth defects.[31] Molloy et al.[32] reported that, at the time of delivery, plasma choline and betaine concentrations increase in maternal plasma, and that this is highly correlated with an increase in plasma homocysteine concentrations. Molloy speculates that there are two mechanisms that could explain this correlation: 1) pregnancy depletes choline and betaine in the liver (perhaps in order to maintain plasma choline concentrations for delivery to the fetus), resulting in decreased methylation of homocysteine; or 2) pregnancy induces endogenous synthesis of choline via a pathway that

also produces homocysteine. Whatever the mechanism, the supply of choline is critical during pregnancy. Mice that cannot form their own choline (*Pemt*$^{-/-}$) abort pregnancies around 9 to 10 days after gestation unless fed supplemental choline (personal observation).[11]

THE FETUS LIVES IN A HIGH-CHOLINE ENVIRONMENT

Large amounts of choline are delivered to the fetus across the placenta, where choline transport systems pump it against a concentration gradient.[27] Choline concentration in amniotic fluid is 10-fold greater than that present in maternal blood (unpublished observations). Plasma or serum choline concentrations are 6- to 7-fold higher in the fetus and newborn than they are in the adult.[33,34] High levels of choline circulating in the neonate presumably ensure enhanced availability of choline to tissues. Neonatal rat brain efficiently extracts choline from blood,[35] and increased serum choline in the neonatal rat is associated with 2-fold higher choline concentration in neonatal brain than is present later in life. Supplementing choline during the perinatal period further increases choline metabolite concentrations in blood and brain.[36] There is a novel form of phosphatidylethanolamine-N-methyltransferase in neonatal rat brain that is extremely active[12]; this isoform is not present in adult brain. Furthermore, in the brains of newborn rats, S-adenosylmethionine concentrations are 40 to 50 nmol/g of tissue[37]—levels probably sufficient to enable the neonatal form of phosphatidylethanolamine-N-methyltransferase to maintain high rates of activity. As previously mentioned, human and rat milk provide large amounts of choline to the neonate. The existence of these multiple mechanisms, which ensure the availability of choline to the fetus and neonate, suggest that evolutionary pressures favored exposure to high concentrations of choline in utero.

CHOLINE AND BRAIN DEVELOPMENT IN UTERO

Choline is needed for normal neural tube closure in early pregnancy,[38,39] and women in the lowest quartile for dietary choline intake had four times the risk (compared with women in the highest quartile) of having a baby with a neural tube defect.[10] It is widely accepted that folate affects embryogenesis of the brain, and it is recommended that all women be supplemented with folate during the periconceptional period because this reduces the risk for these serious defects in brain development.[6,40] Folic acid administered to women who had previously had a child with a neural tube defect lowered risk of recurrence by 72%.[41] Choline and folate metabolism intersect at pathways for methyl-group donation (see earlier discussion), and it is reasonable to hypothesize that methylation reactions are the mechanism they share in common that influence neural tube closure. As discussed below, folate deficiency and choline deficiency have similar effects on stem cell proliferation and apoptosis in the brain.[42,43]

Choline and folate are also important in later periods of pregnancy, when the memory center of brain (the hippocampus) is developing. Maternal dietary choline supplementation or choline deficiency during late pregnancy are associated with significant and irreversible changes in hippocampal function in the adult rodent, including altered long-term potentiation (LTP)[44-46] and altered memory.[47-52] More choline (about four times dietary levels) during days 11 to 17 of gestation in the rodent increases hippocampal progenitor cell proliferation,[53,54] decreases apoptosis in these cells,[53,54] enhances LTP in the offspring when they become adult animals,[44-46] and enhances visuo-spatial and auditory memory by as much as 30% in the adult animals throughout their lifetimes.[47-49,51,52,55,56] Indeed, adult rodents have a decrement in memory as they age, and offspring exposed to extra choline in utero do not show this "senility."[49,55] Mothers fed choline-deficient diets during late pregnancy have offspring with diminished progenitor cell proliferation and migration and increased apoptosis in fetal hippocampus,[53,54] insensitivity to LTP when they become adult animals,[46] and decremented visuo-spatial and audtory memory.[52]

The effects of perinatal choline supplementation on memory were initially found using radial-arm maze tasks and in the Sprague-Dawley rat strain, but other laboratories have found similar results using other spatial memory tasks, such as the Morris water maze,[57,58] passive avoidance paradigms,[59] and measures of attention[60] in other strains of rats such as Long-Evans[61-63] and in mice.[59] The effects of choline supplementation in utero were also detected in studies on effects of fetal alcohol exposure, where supplementation with choline attenuated behavioral alterations but not motor abnormalities.[64,65] Thus, choline supplementation during a critical period in pregnancy causes lifelong changes in brain structure and function.

The mechanism whereby a choline supplement supplied to the dam results in a permanent change in memory of their offspring has not been fully understood. Though the initial hypothesis was that the effect of neonatal choline supplementation on memory is mediated by increased brain choline, with subsequent increased acetylcholine (a neurotransmitter and neurotrophic factor) release, the amounts of choline that accumulate in fetal brain after treatment of the pregnant dam are not likely of sufficient magnitude to enhance acetylcholine release.[36] Rather, supplementing choline to dams results in significantly greater accumulation of phosphocholine and betaine in fetal brain than in fetuses of controls.[36] Alternatively, the effects of choline on neuronal precursor cell proliferation, differentiation, and apoptosis likely underly the effects on memory. Using mouse neural precursors in vitro, we recently reported that choline deficiency induces significant changes in the expression of genes involved in cell-cycle progression and neuronal differentiation, with the net effect being reduced proliferation and increased neuronal and glial differentiation.[66] The hypothesis of accelerated neuronal differentiation is supported in choline-deficient mouse fetal brain by in vivo studies showing increased expression of several early markers of neuronal and glial differentiation, including calretinin (a calcium-binding protein), MAP-1 (microtubule-associated protein), and vimentin (intermediate filament protein) within the hippocampus area.[67]

EPIGENETIC MECHANISMS FOR CHOLINE EFFECTS ON BRAIN DEVELOPMENT

The effects of choline on neural tube closure and on brain development could be mediated by changes in the expression of genes. Dietary choline deficiency not only depletes choline and choline metabolites in rats, but also decreases S-adenosylmethionine concentrations,[68,69] with resulting hypomethylation of DNA.[70,71] DNA methylation occurs at cytosine bases that are followed by a guanosine (CpG sites)[72] and influences many cellular events, including gene transcription, genomic imprinting, and genomic stability.[73-75] In mammals, about 60% to 90% of 5′CpG-3′ sites are methylated.[76] Many coding and non-coding DNA regions have a higher incidence of CpG repeats than expected (CpG islands), and these islands are the main targets for methylation.[75] Changes in dietary availability of methyl-groups (folate, methionine, and choline intakes) can induce stable changes in gene expression and resulting phenotype.[77,78] When this modification occurs in promoter regions, gene expression is altered[79]; increased methylation is associated with gene silencing or reduced gene expression.[76] In choline-deficient human neuroblastoma cells in culture, methylation of the *CDKN3* gene promoter is decreased, resulting in the overexpression of this gene, which inhibits cell proliferation.[80] 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.[81] Hypomethylation of CpG sites and c-myc gene overexpression occurs in hepatocellular carcinomas induced by a choline-deficient diet in rats.[71] It is also reasonable that maternal diet during pregnancy could alter the methylation status of fetal DNA. For example, 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/mottling of their coats.[77,82] It is clear that the dietary manipulation of methyl donors (either deficiency or supplementation) can have a profound impact upon gene

expression and, by consequence, upon the homeostatic mechanisms that ensure the normal function of physiological processes.

Whether these findings in rodents apply to humans is not known. Of course, human and rat brains mature at different rates, with rat brain comparatively more mature at birth than 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.[83] This area of brain is one of the few areas in which nerve cells continue to multiply slowly throughout life.[84,85] 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 of these are good questions worthy of additional research. The observation by Shaw et al.[10] that women eating low-choline diets have a greatly increased risk for having a baby with a neural tube defect supports the suggestion that the basic research in rodents will be applicable to humans as well.

ACKNOWLEDGMENTS

This work was funded by grants from the National Institutes of Health (AG09525, DK55865, ES012997) and the US Department of Agriculture (2005-3520015247). Support for this work was also provided by grants from the NIH to the University of North Carolina Clinical Nutrition Research Unit (DK56350).

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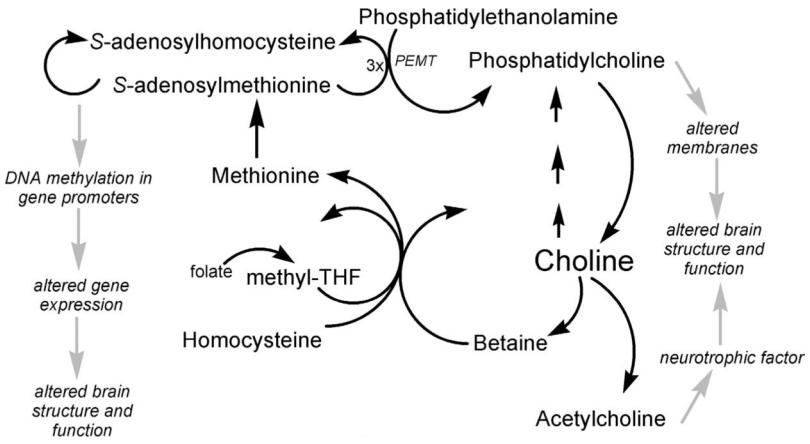

Figure 1.

Metabolism of choline and possible mechanisms for effects on brain structure and function. Choline is acetylated to form acetylcholine, which is a trophic factor for brain. Choline is phosphorylated and then used to form membranes that are required for brain function. Finally, choline is a methyl-group donor that can influence DNA methylation and gene expression, which can, in turn, alter brain structure and function. Methyl-THF = methyltetrahydrofolate.