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## THE FETAL ORIGINS OF MEMORY: THE ROLE OF DIETARY CHOLINE IN OPTIMAL BRAIN DEVELOPMENT

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### Abstract

Fetal nutrition sets the stage for organ function in later life. In this review we discuss the fetal and neonatal origins of brain function. Numerous research observations point to the importance of choline for the developing fetus and neonate. This essential nutrient is involved in 1-carbon metabolism and is the precursor for many important compounds, including phospholipids, acetylcholine, and the methyl donor betaine. Dietary intake of choline by the pregnant mother and later by the infant directly affects brain development and results in permanent changes in brain function. In rodents, perinatal supplementation of choline enhances memory and learning functions, changes that endure across the lifespan. Conversely, choline deficiency during these sensitive periods results in memory and cognitive deficits that also persist. Furthermore, recent studies suggest that perinatal choline supplementation can reduce the behavioral effects of prenatal stress and the cognitive effects of prenatal alcohol exposure in offspring. The likely mechanism for these effects of choline involves DNA methylation, altered gene expression, and associated changes in stem cell proliferation and differentiation. The currently available animal data on choline and hippocampal development are compelling, but studies are needed to determine whether the same is true in humans.

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There is a growing body of evidence indicating that fetal and perinatal nutrition and growth influence organ function in adult life (eg, blood pressure,<sup>1</sup> heart disease,<sup>2</sup> diabetes<sup>3</sup>). Evidence also indicates that fetal and perinatal nutrition influence brain function in later life. Iron,<sup>4</sup> zinc,<sup>5</sup> and folate<sup>6</sup> nutrients in the fetus have been shown to alter brain development, and there is compelling data showing that another important nutrient, choline, is essential for brain formation. The human requirement for choline was officially recognized with the establishment of adequate intake recommendations by the Institute of Medicine in 1998<sup>7</sup> (adequate intake for infants age 0 to 6 months, 18 mg/kg/day). Choline is required for the structural integrity and signaling functions of cell membranes, methyl group metabolism, and neurotransmitter synthesis.<sup>8</sup> Some of the choline needed to sustain normal organ function is synthesized de novo, mainly in the liver,<sup>9</sup> when phosphatidylethanolamine is methylated by phosphatidylethanolamine *N*-methyltransferase (PEMT) to form phosphatidylcholine. However, this mechanism does not always meet the demands for choline, and humans eating diets deficient in choline develop fatty liver, liver damage, and muscle damage.<sup>10,11</sup> In this review, the main focus is on choline's role in brain development and function during the perinatal period.

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## CHOLINE, FOLIC ACID, AND METHIONINE METABOLISM ARE RELATED

The close interrelationship of choline, folic acid, vitamin B<sub>12</sub>, and methionine metabolism intersects at the formation of methionine from homocysteine. Methionine can be formed through 2 pathways: from homocysteine, using methyl groups donated by methyltetrahydrofolate, or from methyl groups derived from betaine (which is derived from choline).<sup>7</sup> A disturbance in 1 of these metabolic pathways results in compensatory changes in the other. For example, if 1 nutrient is in short supply, the other nutrient may be able to meet some of the demand for it. Rats ingesting a low-choline diet have shown diminished tissue concentrations of methionine and *S*-adenosylmethionine,<sup>12</sup> as well as total folate.<sup>13</sup> Humans deprived of dietary choline have difficulty removing homocysteine after a methionine load and develop elevated plasma homocysteine concentrations.<sup>14</sup> Methotrexate, which is widely used in the treatment of cancer, psoriasis and rheumatoid arthritis, limits the availability of methyl groups by competitively inhibiting dihydrofolate reductase, a key enzyme in intracellular folate metabolism. Rats treated with methotrexate have diminished pools of all choline metabolites in the liver.<sup>15</sup> Choline supplementation reverses the fatty liver caused by methotrexate administration.<sup>16-18</sup> Thus, methionine, methyltetrahydrofolate, and choline are fungible sources of methyl groups. The fact that several parallel pathways exist to help ensure an adequate supply of methyl donors demonstrates the physiological importance of these compounds.

## DIETARY SOURCES OF CHOLINE

The first database of choline content in foods is now available for scientists and clinicians to use in assessing choline intake in humans<sup>19,20</sup> (see also <http://www.nal.usda.gov/fnic/foodcomp/Data/Choline/Choline.html>). Daily human choline intake on an ad libitum diet averages 8.4 mg/kg for males and 6.7 mg/kg for females.<sup>21</sup> However, Shaw et al,<sup>22</sup> studying pregnant women in California, observed intakes of less than half this amount in 25% of the women studied. Choline is found in a wide variety of foods; excellent sources include liver, eggs, and wheat germ. In foods, choline exists in free and esterified forms (as phosphocholine, glycerophosphocholine, phosphatidylcholine, and sphingomyelin). Although these forms are likely fungible, there is some evidence that they may have different bioavailability in neonates<sup>23</sup> because the lipid-soluble forms bypass the liver when absorbed from the diet, whereas the water-soluble forms enter the portal circulation and are mostly absorbed by the liver. Human milk is rich in choline compounds, and soy-derived infant formulas have lower total choline concentrations than either human milk or bovine milk-derived formulas.<sup>24</sup>

## CHOLINE AND THE FETUS

Choline is important during the perinatal period, especially for spinal cord and brain development. There is a high rate of transfer of choline across the placenta,<sup>25,26</sup> which actually depletes maternal stores of choline.<sup>27</sup> After birth, the baby gets choline from breast milk. (Note that infant formulas do not always emulate human milk in terms of choline content.<sup>28</sup>) This choline comes from the transfer of choline from maternal blood into milk against a concentration gradient by the mammary epithelial cells.<sup>29,30</sup> Blood and tissue concentrations of choline (and esterified forms of choline) are extremely high in the fetus and neonate.<sup>31,32</sup> In the brain, a specific carrier mechanism transports free choline across the blood-brain barrier at a rate proportional to the serum choline concentration.<sup>33</sup> This choline transporter has an especially high capacity in the neonate.<sup>34</sup> High choline concentrations in the brain and spinal cord are important for neural tube closure and brain development.

### Choline Is Needed for Normal Neural Tube Closure

One of the great successes of nutritional science has been the identification of folate's role in normal neural tube closure. Adequate dietary folate intake by the mother during pregnancy can prevent 50% or more of neural tube defects (NTDs) in infants.<sup>6</sup> As discussed earlier, choline and folate metabolism are highly interrelated. Inhibition of choline uptake and metabolism is associated with the development of NTDs in mice.<sup>35,36</sup> Recent evidence suggests this may also be the case in humans; a retrospective case-control study (400 cases and 400 controls) of periconceptional dietary choline intake in women in California found that the women in the lowest quartile for daily choline intake had a 4-fold greater risk of having a baby with an NTD than the women in the highest quartile for intake.<sup>22</sup>

### Choline Availability Alters Brain Hippocampal Development

In rodent models, maternal dietary choline intake influences brain development (specifically development of the hippocampus, the brain's memory center). In rats and mice, embryonic days 11 to 18 (corresponding to day 56 of pregnancy through several months after birth in humans) is the critical period for development of the hippocampus and septum.<sup>37</sup> More choline (about 3 times the dietary levels) during days 11 to 18 of gestation results in increased cell proliferation and decreased apoptosis in rodent fetal hippocampal progenitor cells.<sup>38,39</sup> Morphological alterations occur in the brain after choline supplementation during fetal life, including larger soma and increased numbers of primary and secondary basal dendritic branches.<sup>40,41</sup>

The brief exposure to extra choline in utero and subsequent changes in hippocampal structure result in enhanced long-term potentiation, (an electrophysiological property of the hippocampus),<sup>42-44</sup> and enhanced visuospatial and auditory memory (by as much as 30%) throughout the lifespan.<sup>41,45-50</sup> Indeed, adult rodents decrement in memory as they age, but offspring exposed to extra choline in utero do not show this "senility."<sup>47,50</sup> We discuss effects on memory in more detail later in this review.

There is a dose-response relationship for exposure to choline in utero. Mothers fed choline-deficient diets during late pregnancy had offspring with diminished progenitor cell proliferation and increased apoptosis in the fetal hippocampus,<sup>38,39</sup> insensitivity to long-term potentiation in adulthood,<sup>42</sup> and decremented visuospatial and auditory memory.<sup>49</sup>

It is interesting to note that the effects on hippocampal development of supplemental choline in rodents are not seen when pups are supplemented during the first 2 weeks after birth, but again become apparent with treatment during postnatal days 16 to 30.<sup>50</sup> It is likely that the high choline content of rat milk<sup>29</sup> naturally supplements the pups during early postnatal life and thus obscures the effect of added choline. The 2 periods of enhanced sensitivity to choline correspond to the periods for neurogenesis (prenatal; the formation of cholinergic neurons) and synaptogenesis (prenatal and postnatal; the formation of nerve-to-nerve connections) in the hippocampus and basal forebrain. In humans, the architecture of the hippocampus continues to develop after birth, and it closely resembles the adult structure by age 4 years.<sup>51</sup> The hippocampus is 1 of the few areas of the brain in which nerve cells continue to multiply slowly in adults.<sup>52,53</sup> Extrapolating from the rodent data, human sensitivity to the developmental effects of choline would occur in utero through perhaps up to age 4 years.

### Choline Effects in Models of Memory and Learning

As discussed earlier, choline supplementation or deficiency in utero and/or during the early neonatal period results in permanent alteration of the structure of the memory center—the hippocampus—of rodents. These structural changes have effects on memory function. Tonjes et al<sup>54</sup> showed that depriving neonatal rats (embryonic day 3 to 14) of maternal contact resulted

in altered memory, and that these effects of deprivation could be reversed by choline. Animals treated with supplemental choline during this period exhibited significantly higher memory capacity in adulthood than animals who received choline later (embryonic day 15 to 28).<sup>54</sup> Subsequently, Meck et al<sup>48</sup> fed pregnant rat dams 3 times the normal dietary choline levels and observed that their pups, studied at age 60 days, performed more accurately on both working and reference memory components of memory tests on a radial maze than did controls. The performance differences were evident from the initial testing and continued throughout training.

Perinatal choline supplementation also enhanced timing and temporal memory.<sup>46</sup> Investigators found significant differences related to proactive interference between choline-supplemented rats and controls.<sup>49</sup> Proactive interference refers to the interference of memories from previous experiences with current memory; an example would be when a person thinks she remembers where she parked her car but is confusing the actual location with the location from a previous trip. When trials were massed in this later study, choline-supplemented rats showed little proactive interference, whereas controls exhibited moderate levels and choline-deficient rats displayed high levels of proactive interference. Prenatal supplementation enhanced simultaneous temporal processing (ie, the animal's ability to divide attention between multiple stimuli presented in parallel), increased attention to both the preferred and lesser preferred signal, and delayed age-related decline in simultaneous temporal processing (evaluated in animals age 24 to 36 months). On the other hand, whereas prenatal choline deficiency also increased attention to the preferred signal, it decreased attention to the lesser preferred signal and accelerated age-related decline.<sup>47</sup> These studies in elderly rats confirm that prenatal exposure to choline supplementation enhances memory function across the lifespan. The converse is also upheld—prenatal choline deficiency impairs memory.<sup>50</sup>

Interestingly, neuroprotective effects of choline administration also have been observed. When an N-methyl-D-aspartate receptor antagonist was administered to pregnant dams over a 6-day period, choline supplementation protected against neurotoxicity from this chemical and subsequent changes in brain function in both adolescent and adult off-spring.<sup>55,56</sup> Another protective effect of prenatal choline supplementation has been observed: Offspring of dams fed either control or choline-deficient diets had highly impaired performance after seizures, whereas offspring of choline-supplemented dams showed no impairment.<sup>57</sup>

Fetal alcohol syndrome is an important concern of pediatricians.<sup>58</sup> Researchers have evaluated whether postnatal choline treatment could reduce the cognitive deficits associated with prenatal ethanol. Animals that had been exposed to ethanol in utero and not treated with choline performed poorest on all memory tasks, whereas the ethanol-exposed, choline-treated animals performed significantly better.<sup>59</sup> In addition, the performance of the choline-treated did not differ significantly from that of any of the control groups. Furthermore, postnatal choline exposure improved performance in all groups, but the effect was greater in ethanol-treated groups. Follow-up studies by this group examined perinatal choline supplementation in rats that had been exposed to alcohol neonatally. These animals demonstrated hyperactivity compared with controls and performed poorly on reversal learning tasks; perinatal choline supplementation ameliorated this hyperactivity and improved reversal learning task performance.<sup>60,61</sup> These findings suggest that perinatal choline supplementation may alter some of the structural and functional changes brought on by early alcohol exposure, and that these effects last beyond the period of supplementation.

Currently, there are no published studies in humans confirming whether choline supplementation during pregnancy enhances memory performance in offspring. A pilot study is ongoing at the University of North Carolina at Chapel Hill.

## Possible Mechanisms for the Effects of Choline on Neural Tube and Brain Development

The mechanism whereby choline supplementation (or choline deficiency) in pregnant dams results in permanent changes in memory of their offspring has not been fully elucidated. Although the initial hypothesis was that the effect of neonatal choline supplementation on memory is mediated by increased brain choline levels with subsequent increased acetylcholine release, the amounts of choline that accumulate in fetal brains after treatment of pregnant dams are not likely of sufficient magnitude to enhance acetylcholine release.<sup>62</sup> Rather, supplementing choline to dams results in significantly greater accumulation of phosphocholine and betaine in fetal brains than is seen in controls.<sup>62</sup>

The effects of choline on neural tube closure and 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,<sup>12,63</sup> with resulting hypomethylation of DNA.<sup>64,65</sup> DNA methylation occurs at cytosine bases that are followed by guanosine (CpG sites)<sup>66</sup> and influences many cellular events, including gene transcription, genomic imprinting, and genomic stability.<sup>67-69</sup> In mammals, between 60% and 90% of 5'-CpG-3' islands are methylated.<sup>70</sup> When this modification occurs in promoter regions, gene expression is altered;<sup>71</sup> increased methylation is associated with gene silencing or reduced gene expression.<sup>70</sup> In choline-deficient cells in culture, methylation of the cyclin-dependent kinase inhibitor 3 gene promoter is decreased, resulting in overexpression of this gene, which inhibits cell proliferation.<sup>72</sup> We replicated this observation in brains of fetuses from choline-deficient mothers and found that cyclin-dependent kinase inhibitor 3 was hypomethylated and overexpressed in the neuroepithelium of the fetal hippocampus (submitted for publication); we suggest that this is the likely molecular mechanism for decreased stem cell proliferation in brains of these fetuses. This is not an outlandish hypothesis, because we already know that dietary intake of methyl donors in pregnant mice can permanently alter the expression of genes that control coat color in their pups.<sup>73,74</sup> It is clear that the dietary manipulation of methyl donors (either deficiency or supplementation) can have a profound impact on gene expression and consequently on the homeostatic mechanisms that ensure the normal function of physiological processes.

## CHOLINE REQUIREMENTS MAY VARY WITH SEX

Premenopausal women, relative to males and postmenopausal women, have enhanced capacity for *de novo* biosynthesis of choline moiety through PEMT in the liver. This likely reflects some evolutionary pressure to optimize choline status in females capable of becoming pregnant. Female rats are less sensitive to choline deficiency than are male rats,<sup>75</sup> and female mice produce more phosphatidylcholine via the PEMT pathway than do male mice.<sup>76</sup> Estrogen status may be important for this increased PEMT activity;<sup>77</sup> compared with controls, estradiol-treated castrated rats have greater hepatic PEMT activity.<sup>78</sup> Thus, estrogen could be the mediator of increased PEMT activity in women. During pregnancy, estradiol concentration rises from approximately 1 nM to 60 nM at term,<sup>79,80</sup> suggesting that the capacity for endogenous synthesis of choline should be greatest during fetal development. As noted earlier, demand for choline is especially high during pregnancy and lactation; transport of choline from mother to fetus<sup>25,81</sup> depletes maternal plasma choline in humans.<sup>82</sup> Thus, despite an enhanced capacity to synthesize choline, the demand for this nutrient is so high that it depletes the stores. If endogenous choline biosynthesis were defective, then dietary intake requirements during pregnancy likely would be much greater.

We are in the process of identifying genetic polymorphisms in genes that greatly increase the likelihood that women require increased amounts of choline in the diet. We have examined genes of choline metabolism and identified a polymorphism in the promoter region of the *PEMT* gene (-939G→C; rs12325817), which is associated with greatly increased

susceptibility to choline deficiency in women (submitted for publication). The frequency of this variant allele was 0.74. We need to determine whether women with this genetic polymorphism are especially likely to need more choline during pregnancy.

## QUESTIONS FOR FUTURE RESEARCH

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? Does choline supplementation of pregnant women result in babies with enhanced memory? Are the women who are eating low-choline diets and have an increased risk of having babies with a neural defect<sup>22</sup> also at risk of having babies with diminished memory function? Do women with genetic polymorphisms in genes of choline metabolism need more dietary choline? All of these are good questions that merit additional research.

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### Glossary

CpG, Cytosine base followed by a guanosine; NTD, Neural tube defect; PEMT, Phosphatidylethanolamine N-methyltransferase.

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