

Supplemental Choline and Neurodevelopment in Humans

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Background and Rationale:

This research study focuses on how the nutrient choline is needed for normal development of brain. Choline is an essential nutrient that is found in foods such as milk and eggs. In animal models, the stem cells that form the brain's memory center (hippocampus) multiply less and die at a faster rate when pregnant mothers eat less choline, and multiply more when mothers are fed more choline. Thus, the memory area's structure is directly influenced by whether choline was available during a specific critical period in development. Moreover, pups born of mothers who had more choline have permanently improved memory function. Specifically, prenatal choline supplementation in rats facilitates cognitive function and visuospatial memory, whereas choline deficiency impairs divided attention and accelerates the age-related decline in temporal processing.

There are two sensitive periods in rat brain development during which treatment with choline produces enhancement of spatial memory that is lifelong lasting. The first occurs during embryonic days 12-17 (rats give birth on day 21) and the second, during postnatal days 16-30. Choline supplementation during these critical periods elicits a major improvement in memory performance at all stages of training on a 12-arm radial maze. This work has been widely read and is discussed in the US Institute of Medicine's recommendation that an adequate human intake for choline be set.

It is not known if these findings about the development of the memory center in rodents are likely to be true in humans. Human and rat brains mature at different rates; rat brain is comparatively more mature at birth than is the human brain. In our animal models, the critical period for choline availability was during the last third of pregnancy when the memory area of brain is formed. In humans, the development of this area begins in late pregnancy and continues for the first year of life, and in fact there is some continued stem cell proliferation occurring in the memory area in adult humans. However, everything we know about brain development tells us that the processes seen in rodents are the same as those that occur in the developing human brain. For this reason, it is extremely likely that the robust effects we observe for choline in rodent brain have importance in the human. The purpose of this grant proposal is to conduct the first major study in humans to determine whether maternal diet and diet during the baby's first year influences brain development and function.

Goals/Objectives:

- 1) To measure choline and phosphatidylcholine concentrations in plasma of choline-supplemented and non-supplemented pregnant women.
- 2) To analyze choline, phosphatidylcholine, sphingomyelin, betaine, phosphocholine, and glycerophosphocholine concentrations in human breast milk samples from choline-supplemented and non-supplemented mothers.
- 3) To measure the mean change in infant working memory score between control infants and infants whose mothers received an intervention of choline supplements.

Study Design:

The study design is schematized in Figure 1. Specifically, 200 healthy female subjects, pregnant less than 18 weeks, who express the intent to breastfeed their babies, will be enrolled. Subjects will be randomly assigned to one of two treatment arms (n = 100/arm; total of 200 participants) – normal choline (6 placebo corn oil capsules/day), or high choline (6 PhosChol capsules/day delivering approximately 750 mg choline). Subjects will begin to take these supplements at week 18 gestation (as determined by subjects' obstetrician) and will continue through 90 days postpartum. Once enrolled, subjects will come in for 4 follow-up visits at Week 20 gestation, Week 30 gestation, Day 45 postpartum, and Day 90 postpartum. They will also bring their infants in for 4 testing sessions, twice at 10 months of age, and twice at 12 months of age. Details regarding these study procedures are given below.

Diet analysis: Subjects will keep 3-day food records just prior to starting the study, and at 3 additional time points during enrollment. Diet records will be analyzed to determine daily choline and betaine intake levels (based on our chemical analyses of foods). Total choline content of a food is calculated as the sum of amounts of choline, phosphocholine, glycerophosphocholine, lysophosphatidylcholine, phosphatidylcholine and sphingomyelin in the food. Betaine, a metabolite of choline, is calculated independently.

Choline determinations: Plasma choline in mothers will be determined at 20 and 30 weeks pregnancy and at days 45 and 90 postpartum using a mass spectrometric assay. Moreover, breast milk will be collected at 45 and 90 days of lactation and analyzed for choline compounds. In plasma we will only measure choline and phosphatidylcholine concentrations. In milk samples we will analyze choline, phosphatidylcholine, sphingomyelin, betaine, phosphocholine, and glycerophosphocholine, all of which we have previously shown to be present in human milk.

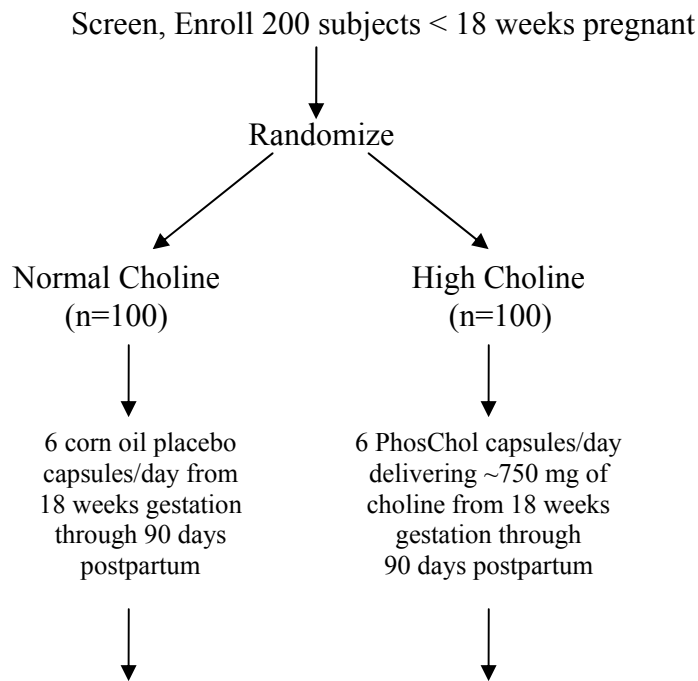
Clinical Laboratory Measures: We will assess iron (iron, iron binding capacity and ferritin) and lead status in the mother at 30 weeks of pregnancy, and in the infant between 6 and 9 months of age, as these can both influence brain development of fetuses.

DNA polymorphisms: We will determine whether women with 11 specific single nucleotide polymorphisms (snps) in genes related to choline and folate metabolism are more sensitive to dietary choline in terms of memory function in their offspring. For example, a number of (snps) have been identified that are common in humans (15% of the population) and that increase requirements for folate as a methyl donor. We and others have also identified snps in the gene that codes for the enzyme that forms choline from S-adenosylmethionine (PEMT); it is this activity that spares some of the dietary requirement for choline.

Maternal Neuropsychology testing: It is likely that some women become deficient in choline while pregnant. Thus, we would like to test whether choline supplementation during pregnancy enhances memory and other aspects of cognitive function, including mood. Measures of cognitive function will be directly compared to maternal blood choline concentrations to assess whether these two factors are potentially related. Cognitive performance in study subjects will be assayed at screening, 30 weeks gestation and 90 days postpartum via a series of standard neuropsychological tests.

Infant memory testing: Each infant will be tested twice at 10 and 12 months of age for short-term visual-spatial memory (working memory) performance and for long-term memory, language and general intelligence. We are focusing on specific aspects of infant memory that are implicated by research on rodents and feasible to measure in infants. Short-term visual-spatial memory, or working memory, which rodents use in maze tasks that are affected by prenatal choline supplementation, emerges in humans by 6 months and shows rapid development thereafter. Our procedures to measure working memory in infants use both gaze and reaching responses in delayed response tasks that challenge working memory capacity for various amounts of information over various delay intervals. Our secondary target, long-term memory, is assessed over a 7-10 day delay interval using a deferred imitation task. Infants will see a novel action performed at an initial testing session and must retain this action and imitate it after a long delay. We will assess early language development using a well-established parent-report instrument. Finally, each infant will be tested on the Mullen scales, which provide a multi-trait index of early intelligence.

Figure 1: STUDY SCHEMA



Mother Visits:

- **Screen <18 weeks gestation:** Informed consent obtained, distribution of 3-day food record, neuropsychology testing, 5-week supply of supplements distributed
- **Week 20 gestation:** Vital signs, weight, blood draw for genetic polymorphisms and choline metabolites, distribution of 3-day food record, 10-week supply of supplements distributed
- **Week 30 gestation:** Vital signs, weight, blood draw for iron/lead status and choline metabolites, neuropsychology testing, distribution of 3-day food record, 17-week supply of supplements distributed
- **Day 45 Postpartum:** Vital signs, weight, blood draw for choline metabolites, subject to bring breast milk sample for choline and metabolites analysis, distribution of 3-day food record, 7-week supply of supplements distributed
- **Day 90 Postpartum:** Vital signs, weight, blood draw for choline metabolites, subject to bring breast milk sample for choline and metabolites analysis, neuropsychology testing

Infant Visits:

- **10-months of age:** Two Cognitive function assessments (separated by 7-10 days)
- **12-months of age:** Two Cognitive function assessments (separated by 7-10 days)

Timeline

Nov 2004-Nov 2005	12 months	Recruitment & Enrollment of 200 subjects
March 2006-May 2007	14 months	Infant testing for 200 babies
June 2007-August 2007	3 months	Data Analysis and Draft Reports
September 1, 2007	-----	Final Report Due