

EXAMINING THE RELATIONSHIP BETWEEN PLASMA  
CHOLINE STATUS AND DIETARY INTAKE OF CHOLINE IN  
PREGNANT WOMEN

BY

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## ABSTRACT

**Background:** Choline is an essential nutrient for maternal health and fetal development of which eggs are the richest source in the typical American diet. A single egg could make a significant difference in choline intake and ultimately plasma choline status.

**Objective:** To determine choline intake from eggs in a population and if this intake predicts choline, phosphatidylcholine and sphingomyelin status in pregnant women.

**Design:** Choline intake from eggs of a subset 357 women from the KUDOS trial at the University of Kansas Medical Center was estimated. Plasma choline, phosphatidylcholine and sphingomyelin status was analyzed using Bligh and Dyer on 201 subjects with available plasma. Simple regression was used to determine presence of significant relationships between choline intake and plasma choline, phosphatidylcholine and sphingomyelin.

**Results:** Women in this study consumed a median of 44.5 mg of choline from eggs daily. No significant correlation was found between choline intake from eggs and plasma choline, phosphatidylcholine, and sphingomyelin.

**Conclusion:** The median choline intake from eggs was less than 10% of the AI for pregnant women. Dietary egg intake was not related to markers of choline status.

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## Table of Contents

<b>Chapter 1: Justification</b> .....	<b>1</b>
Justification for Study.....	1
Statement of Purpose.....	2
Research Questions.....	3
<b>Chapter 2: Literature Review</b> .....	<b>4</b>
Introduction.....	4
Choline in Pregnancy.....	6
Choline in Fetal Development and Postnatal.....	7
Dietary Sources of Choline.....	10
Other Predictors of Choline Status.....	12
Eggs and Heart Disease.....	15
Conclusion.....	18
<b>Chapter 3: Methods</b> .....	<b>20</b>
Study Overview.....	20
Sample.....	20
Inclusion/Exclusion Criteria.....	21
Analysis of Dietary Choline Intake.....	22
Analysis of Demographic Characteristics.....	24
Laboratory Analysis.....	24
Statistical Analysis.....	26
<b>Chapter 4: Results</b> .....	<b>27</b>
<b>Chapter 5: Discussion</b> .....	<b>36</b>
Implications.....	36
Limitations.....	37
Ethics.....	39
<b>References</b> .....	<b>40</b>

## List of Tables and Figures

### In-Text Tables

Table 1: Study Population ethnicity.....	28
Table 2: Study population BMI.....	29
Table 3: Choline Intake from Meats, Eggs and Supplements.....	32

### In-Text Figures

Figure 1: Amount of choline consumed per participant from eggs daily.....	30
Figure 2: Total choline consumed per participant from meats and eggs daily.....	31
Figure 3: Choline intake per participant from supplements.....	32
Figure 4: Choline intake from eggs in relation to plasma choline status.....	34
Figure 5: Choline intake from eggs in relation to plasma phosphatidylcholine status....	34
Figure 6: Choline intake from eggs in relation to plasma sphingomyelin status.....	35
Figure 7: Total choline intake in relation to plasma choline status.....	35
Figure 8: Total choline intake in relation to phosphatidylcholine status.....	36
Figure 9: Total choline intake in relation to sphingomyelin status.....	36

## **Chapter I: Justification**

The need for choline in human development is becoming more apparent in light of growing evidence concerning the role of choline in pregnancy and fetal development (1). In the pregnant woman, choline is necessary for placenta formation, prevention of fatty liver and DNA methylation (1-3). In the fetus, it is necessary for membrane biosynthesis in addition to its other roles in prevention of neural tube defects and possibly augmentation of cognitive function (1).

Choline may come from the diet or from the methylation of ethanolamine in phosphatidylethanolamine (PE) to form phosphatidylcholine (PC) by the action of the enzyme phosphatidylethanolamine-N-methyltransferase (PEMT) (2). The action of this enzyme increases during pregnancy because estrogen increases PEMT activity (3). However, some women have single nucleotide polymorphisms (SNPs) that may prevent or decrease the action of the PEMT enzyme. This leads to less choline being produced endogenously and therefore increases the importance of consuming choline in the diet.

An Adequate Intake for choline in pregnancy was set in the 1998 DRIs. However, according to NHANES data collected in 2003-2004, only 10% of all Americans over 2 years of age consume the Adequate Intake of choline (4). One recent study on choline intake in American pregnant women found that only 5%

of their subjects met the Adequate Intake (3). With this in mind, pregnant women that have these SNPs may not be getting enough choline to ensure optimal fetal development.

Because eggs are the richest source of choline in the American diet (5), a simple analysis of egg intake may be a quick and easy way to estimate choline intake. It has been hypothesized that women who have a low intake of eggs may have a lower choline status than those who have a higher intake of eggs (6). If this turns out to be a good way to approximate the plasma choline status of pregnant women, the woman's health care provider can decide whether or not to recommend choline supplements for the pregnant patient or client in order to facilitate optimal fetal development.

### **Statement of Purpose**

Using data obtained from a cohort of pregnant women enrolled in a study of DHA supplementation during pregnancy in the Kansas City metropolitan area, a retrospective cohort study will be designed. As my primary aim, I will determine how much choline pregnant women actually consume from eggs daily. Secondly, I will examine whether or not the amount of eggs they consume has an effect on their plasma choline, phosphatidylcholine and sphingomyelin levels.



### **Primary Research Question**

What amount of total choline from eggs is consumed by pregnant women?

Hypothesis: Women will on average consume approximately  $\frac{1}{4}$  of the

Adequate Intake of choline daily from eggs.

### **Secondary Research Questions**

Does dietary egg intake predict choline status in pregnant women?

Hypothesis: Dietary egg intake will increase choline status in pregnant women.

## Chapter II: Literature Review

### Introduction

Choline is an essential, water soluble nutrient that has been grouped with the B-complex vitamins by the Institute of Medicine (IOM). This nutrient has only been considered an essential nutrient for a short period of time because of a previous lack of scientific evidence proving its essentiality. The IOM established Adequate Intakes (AI) for infants, children, men, postmenopausal women, premenopausal women, pregnant women and lactating women in 1998. This review will focus on the last three groups of individuals. For premenopausal, pregnant and lactating women, AIs have been set at 425 mg/d, 450 mg/d and 550 mg/d, respectively (7).

Choline was grouped among the non-essential nutrients until 1998, because it can be made endogenously through the phosphatidylethanolamine-N-methyl transferase (PEMT) pathway, largely in the liver (8). The PEMT enzyme catalyzes transfer of three methyl groups from S adenosyl methionine to phosphatidylethanolamine to form phosphatidylcholine and S-adenosylhomocysteine (SAH) (9). This pathway accounts for 30% of the body's supply of choline, while choline-containing foods in the diet account for the other 70% (1).

Choline was also considered “dispensable” because evidence of a true choline deficiency syndrome was lacking (8). However, Zeisel et al. (8) showed that a diet deficient in choline can lead to fatty liver and organ dysfunction. While a true choline deficiency may not be seen due to the endogenous pathway, a diet low in choline can still have consequences.

The consequences of choline deficiency are related to the body’s inability to adequately perform choline’s three major functions. Choline’s first function is its role as a component of phosphatidylcholine (PC) and sphingomyelin (1). Both are major components of the cell membrane (10), and sphingomyelin is an important component of the nerves’ myelin sheath which is necessary for rapid propagation of impulses along the axons of nerves (11). As its second major function, choline is a part of the neurotransmitter acetylcholine, which acts in both the central and peripheral nervous systems (1). In the peripheral nervous system, choline participates in the autonomic nervous system where it has both excitatory and inhibitory functions in skeletal and cardiac muscle (12). In the central nervous system, acetylcholine is mostly involved with memory and learning. Finally, choline serves as a methyl donor for conversion of homocysteine to methionine following its oxidation to betaine (2). Choline interacts with methionine and folate to achieve this one-carbon methylation and reduce the amount of homocysteine in the blood.

While these are the main functions of choline in normal healthy adults, research on the importance of choline during pregnancy has so far been limited to the effect of choline in fetal brain development. With a growing amount of positive evidence during this lifecycle stage, it will be important for dietitians to be able to measure choline status in pregnant women. If blood tests are not available to measure plasma choline, examining the dietary intake of choline-rich foods may be beneficial. However, with other mechanisms playing a role in determining choline status, it is uncertain whether or not a simple analysis of dietary intake of choline-rich foods can be an accurate indicator of plasma choline status in pregnant women.

### **Choline in Pregnancy**

Maternal needs for choline increase during pregnancy due to the changes occurring to support fetal growth. First, there is a rapid rate of cell division and expansion of maternal tissue. Because it is a major component of cell membranes, choline needs increase to support this increased rate of growth (1). Second, phosphatidylcholine (PC) is necessary for the secretion of VLDL from the liver. This action prevents fatty liver due to the maternal hyperlipoproteinemia that can occur during the third trimester of pregnancy (13). Lastly, as methyl donor reactions increase, the need for choline for use in DNA methylation increases as well (3).

In light of the obvious importance of choline during pregnancy, it appears that nature has made it easier for pregnant women to produce enough choline to support maternal growth, as well as the growth of the fetus. Zeisel (14) has written: “It makes sense that evolution would develop mechanisms to assure that women are less susceptible to dietary choline deficiency and have adequate stores of choline prior to becoming pregnant.” No mechanism demonstrates this more than the estrogen induction of the PEMT pathway. Estrogen increases transcription of the PEMT gene (15). During pregnancy, concentrations of estradiol rise almost 60-fold (14), meaning that pregnant women have a much greater capacity to synthesize choline.

However, despite this increased capacity for hepatic production of choline through the PEMT pathway, some women may have single nucleotide polymorphisms (SNPs) in their PEMT gene (3). These polymorphisms can interfere with the endogenous production of choline. With this in mind, women who have these SNPs may be more likely to be choline deficient than those who do not.

### **Choline in Fetal Development and Postnatal**

Evolutionary mechanisms also point to an increased need for choline in fetal development. Choline is actively transported to the fetus against a concentration gradient (16). Plasma choline is 10 times higher in amniotic fluid

than it is in maternal blood, making the nutrient highly available to the fetus (14). This choline concentration remains high in newborns as well. Newborns have a plasma choline concentration approximately 300% higher than a healthy adult ( $33.3 \pm 2.9 \mu\text{mol/L}$  and  $10.9 \pm 0.30 \mu\text{mol/L}$ ), although plasma choline levels vary considerably from infant to infant (17). Whether or not this variability is related to maternal intake is currently unknown.

Maternal and fetal need for choline increases even further during lactation because human milk is choline-rich, at 10-15 times the choline concentration of maternal blood (1, 17). Fischer et al. (3) showed that free choline concentration of breast milk from mothers that did not receive supplemental choline was ~11 fold higher than maternal plasma free choline concentrations. Mothers that were supplemented with choline had a breast milk free choline concentration ~8 fold higher than maternal plasma choline. With this increased choline availability in breast milk, breastfed infants would be expected to have a high intake of choline.

After discussing the mechanisms in place to assure that the mother does not become choline deficient and that the fetus gets adequate amounts, it is important to discuss the functions of choline in fetal development. The four main functions of choline in fetal development include (1): 1) Choline is necessary for membrane biosynthesis just as it is in adults and pregnant women, 2) It is required

for growth of the placenta, 3) It can prevent neural tube defects in the baby, and 4) it can potentially augment cognitive function in the baby.

The last two functions warrant further discussion. Shaw et. al. (18) found that higher intakes of choline and betaine, compared to the lowest intakes, significantly decreased the risk of infants or fetuses with neural tube defects. This is related to the fact that choline and folate are both involved in the methyl-group donation that influences neural tube closure (19). Periconceptional supplementation of folate is recommended because folate deficiency increases the likelihood of neural tube defects (20). No similar recommendation has been made for choline (21).

Choline and folate are also important in the development of the brain's memory center, the hippocampus (6). Studies concerning the effect of choline depletion and supplementation on development of hippocampal neurons and memory have been conducted in rodents (24, 25). Cheng et al. (24) found that rodents undergoing training procedures that had been born to mothers supplemented with additional choline during gestation developed more enduring memories and had fewer responses that are thought to be related to frustration and impulsivity than rodents who received sufficient choline during gestation.

Similarly, Wong-Goodrich et al. (25) found that rodents supplemented with choline performed better than those given usual choline on spatial and

temporal memory tasks. Their research also suggested that choline intake necessary for optimal performance in adulthood depends on choline exposure in the prenatal period. Analogous studies in humans have yet to be performed.

### **Dietary Sources of Choline**

The cytidine diphosphate (CDP)--choline pathway utilizes exogenous (dietary) sources of choline and produces the other ~70% of choline, not produced by the PEMT pathway (24). This pathway is responsible for the transfer of phosphocholine to the sn-3 position of 1,2 diacylglycerol. The phosphatidylcholine produced by these two pathways differs in fatty acid composition. PC produced by the CDP-pathway contains more medium chain saturated fatty acids, while PC produced by PEMT contains more long chain polyunsaturated fatty acids, reflecting the fatty acid composition of phosphatidylethanolamine. However, they are both used to form the basic functions of choline (24).

Choline is found in food as free choline, phosphocholine, glycerophosphocholine, phosphatidylcholine, and sphingomyelin; all of which can be converted to choline or revert to their original form (6). Zeisel et al. (5) performed a nutrient analysis of 145 different foods to determine their choline content when prepared as they normally would be. This experiment contributed to the USDA Database for the Choline Content of Common Foods. Foods with the



highest total choline concentration are: beef liver, eggs, wheat germ, bacon, dried soybeans and pork.

Eggs are among the richest sources of choline in the typical American diet because few Americans regularly consume beef or chicken liver (5). A single egg provides approximately 125 mg of choline. Thus, people who do not eat eggs are potentially at a greater risk for choline deficiency than those who do consume them. Other common sources of choline in the American diet are milk (38 mg in 8 oz) and chicken (56 mg in 3 oz) (25).

Choline's metabolite betaine is also important to take into consideration because betaine intake spares choline (14). Betaine cannot be converted back to choline, but less of the other aforementioned sources of choline in the diet would have to be converted to betaine to be used in methyl donation. Foods with the highest betaine concentration include: wheat bran, wheat germ, spinach, pretzels, shrimp and wheat bread (5).

Some individuals may have an inadequate choline intake because they have been discouraged from consuming choline-rich foods such as eggs and bacon due to their saturated fat and cholesterol content (14). In fact, data from NHANES 2003-2004 show that only 10% of all Americans over 2 years of age consume the Adequate Intake of choline (4). The average choline of adults is approximately 264 mg/d (4) - well below the AI.

According to a study conducted by Fischer and colleagues (3), only 5% of pregnant women met the Adequate Intake for choline without supplementation. The average daily choline consumption was 351 mg, 78% of the AI for pregnant women. Similar findings were reported in a Jamaican study conducted by Gossel-Williams et al. (26), with an average choline intake of 279 mg or 62% of the AI for pregnant women. Interestingly, Fischer's study (3) also found an apparent maximum plasma choline and breast milk concentration. Women supplemented with 750 mg/d of PC achieved a maximal plasma choline concentration after supplementation. The intake-concentration curves did not increase further when dietary sources of choline were added.

### **Other Predictors of Choline Status**

Presence or absence of single nucleotide polymorphisms (SNPs) in the PEMT gene can cause a variation in choline status because they can result in a loss of enzyme function. They are thought to be quite common, with up to half of all Americans possessing some form of a PEMT SNP (15, 27). Adhering to the standard ratio that 30% of the body's PC is produced by the PEMT pathway, these women could be missing some amount of choline from endogenous synthesis.

A study by Zeisel et al. (14) studied men, postmenopausal women and premenopausal women. The participants were placed on a choline deficient diet

and the men and postmenopausal women soon developed signs of organ dysfunction. This was expected because estrogen induces the PEMT gene and allows the body to make it endogenously. Postmenopausal women and men have low levels of estrogen. However, 44% of premenopausal women also developed organ dysfunction. This difference in choline requirement is thought to be related to genetic polymorphisms such as SNPs in the PEMT gene.

To test the effects that these SNPs have on choline status in pregnant women, Fischer et al. (3) conducted an exploratory study on the effect of SNPs on breast milk composition. The authors found that several SNPs were associated with reduced breast milk and plasma choline concentrations. They recommend that the genotype of women be taken into account when recommending choline intakes for the population.

Folate status is also important to choline status in human beings. When folate status is low, choline status tends to be low as well. This is likely due to the fact that when folate is absent, choline is used in greater amounts as a methyl donor to make up for the insufficiency. If the diet and endogenous synthesis does not compensate for this greater need, the person will likely become choline deficient (2).

Folate related choline deficiency was shown in a study by Jacob and colleagues (28). Healthy nonsmoking men and postmenopausal women were fed a

low-folate/low-choline diet for 4-5 weeks, then consumed a low-choline, synthetic folate repletion diet for 2-6 weeks. Folate deficiencies and significant plasma choline depletions were created; however, the depletion was reversed upon folate repletion. It appears from this study that folate 'spares' choline.

In a similar study, Hung et al. (29) studied a small group of African-American, Mexican-American and Caucasian-American women. They all followed a 14 week folate-restricted diet for 7 weeks and a folate-sufficient diet that met the recommended daily allowance for 7 weeks. Choline and betaine were controlled throughout the study. Despite the fact that choline intake remained unchanged throughout the study, during the folate depletion period, PC concentration of all women studied declined. PC depletion was subsequently reversed upon folate repletion in Mexican American and Caucasian American women.

An interesting finding in Hung's study was the lack of response of African-American participants to folate repletion (29). Their PC status remained low despite high amounts of folate. Their folate status also remained lower than Caucasian and Mexican-Americans. This shows that ethnicity may play a role in predicting not only folate status, but choline status as well. Similar low folate and choline status was found in Afro-Caribbean women in Jamaica. Gossell-Williams

et al. (28) found that their participants had low to normal plasma choline during the first trimester.

A similar folate/choline relationship was shown by Abratte et al. (30). Participants were fed a folate restricted diet for 2 weeks and were given 344 mg/d of choline and 122 mg/d of betaine. For the 12 remaining weeks, they were randomized to four groups consuming differing amounts of choline (346 mg/day or 486 mg/day) and betaine (122 mg/day or 349 mg/day). The folate restriction phase was associated with choline depletion, as previous studies have also shown (26, 28, 29). Additionally, the researchers found no significant difference in plasma concentration levels of choline between the groups. This suggests that small changes in choline intake do not have an effect on choline status in women. These participants were not checked for PEMT SNPs and no ethnic breakdown was given.

### **Eggs and Heart Disease**

Eggs have had somewhat of an image problem since the 1970's (31). As the number of Americans with high cholesterol grows, many of them are told by their healthcare providers or by the media to cut down on their cholesterol intake. Although one large egg contains 6g of protein, 1.6g of saturated fat and only 72 calories, eggs are high in cholesterol at 186 mg per large egg (31). This means that an individual instructed to stay below 300 mg of cholesterol per day would

use up over half of their daily requirement with this one item. These people may cut eggs out of their diet because concern for their health or eat only the egg whites. This affects choline intake because all of the choline in eggs is contained in the yolk.

However, despite being high in cholesterol, recent scientific data has been favorable with respect to eggs. Previous studies that have shown dietary cholesterol having an effect on cholesterol have been questioned due to saturated fat in these studies being a confounding factor (31, 32). Saturated fat and *trans* fat have been associated with an increase in LDL oxidation which has been implicated in the development of heart disease. As mentioned above, eggs are actually quite low in saturated fat and contain no *trans* fat. It is the only food that is both high in cholesterol and low in saturated fat (32). This makes it ideal for studying the effects of dietary cholesterol alone on blood cholesterol. A study investigating egg intake and myocardial function found no adverse effects when more than one egg per day was consumed by its participants (33).

Recent studies have found that dietary cholesterol from eggs can possibly raise blood cholesterol (34 - 38). However, it is associated with an increase in both LDL and HDL and does not change the LDL/HDL ratio, which is considered a good indicator for cardiovascular disease risk (39). In addition, the LDL particles that increase after egg consumption are not the small ones associated

with oxidations and heart disease, but instead large buoyant ones that are considered to be much more benign as they are less prone to oxidation (39).

Another reason that dietary cholesterol may not be as bad as previously thought is the fact that much of the cholesterol in the body does not come from the diet. Adults consume between 300 – 450 mg of cholesterol daily, but nearly 800 - 1400 mg is made in the body in order to support membrane and steroid synthesis (40). This suggests that dietary cholesterol may actually have a relatively small effect on blood cholesterol when compared to endogenous cholesterol. In fact, a meta-analysis showed that at intakes between 0 and 400 mg, each 100 mg increase in dietary cholesterol raised plasma cholesterol only a small amount (41).

Despite these findings, some may still need to be careful with their dietary cholesterol. Those with familial hyperlipidemia may be more prone to an increase in blood cholesterol due to (31). In addition, those with type I diabetes may have increased cholesterol absorption (42).

The most recent evidence suggests that eggs can still be a part of a healthy diet (31,38). The high vitamin and low energy content of eggs makes them an ideal part of a healthy diet, therefore most pregnant women should not have a severely restricted whole egg intake. This will allow them to have a good source

of choline in their diets that will benefit not only them, but also their growing baby.

## **Conclusion**

Many Americans do not meet the Adequate Intake of choline, which suggests that most pregnant women are probably not getting sufficient amounts of choline. Eggs are a good way to increase dietary choline intake because they are so rich in the nutrient and low in energy. Recent studies have not shown an increase in endothelial dysfunction related to egg intakes of  $\geq 1$  egg per day. For those that may see an increase in cholesterol, it is unlikely to be the type of LDL particles that are considered atherogenic.

For those pregnant women that don't appear to be meeting the AI for choline through eggs or other sources, it may be wise to consider choline supplements, just as folate supplements are required to assure optimal fetal development. The previously mentioned study by Fischer et al. (3) showed that approximately 750mg/d of choline was needed to achieve a maximal plasma choline concentration and was not associated with any adverse effects. Likewise, Zeisel et al.'s 1991 study (8) used a supplement containing 700mg/d of choline without adverse effects. These studies suggest that choline supplementation in pregnancy is a safe way to increase the concentration of this much needed nutrient if blood plasma choline and dietary recalls point to inadequate intake.



If a correlation is not found between dietary intake and plasma choline status, it may be due to the fact that choline status can be influenced by factors other than dietary intake. Presence or absence of PEMT SNPs can have a significant effect on whether persons are adequate in choline. In addition, folate status and ethnicity have been shown to have an effect as well.

## **Chapter III: Methods**

### **Study Overview**

A database will be built that includes several biological and demographic characteristics of pregnant women that may influence plasma choline status in pregnant women. This database will be used to determine how much dietary choline pregnant women get from eggs. The secondary aim will be to examine the relationship between dietary choline status and plasma choline as well as the status of phosphatidylcholine (PC), sphingomyelin (SM).

### **Sample**

Participants in this study were a subset of women who participated in an ongoing trial known as the Kansas University DHA Outcomes study (KUDOS). Three hundred-fifty seven pregnant women were recruited from the University of Kansas Medical Center in Kansas City, Kansas, St. Luke's Hospital in Kansas City, Missouri and Truman Medical Center in Kansas City, Missouri between October 2005 and January 2010. They were originally enrolled to take part in a clinical trial to determine if a dietary supplement of docosahexanoic acid (DHA) during pregnancy would increase maternal and infant cord blood DHA levels, increase infant visual and cognitive function and reduce toddler distractibility.

## **Inclusion/Exclusion Criteria**

Inclusion criteria for the primary trial were as follows: women must be between 8 and 20 weeks gestation, between ages 16 and 35.99 years, English speaking, available by telephone, expecting a single infant, willing to consume DHA capsules three times per day between enrollment and delivery and willing to return to the study center for follow-up of their infant after delivery. Women were excluded if they had a BMI  $\geq$  40, were under the age of 16 or over the age of 35.99, had serious health risks (e.g cancer, HIV/AIDS, hepatitis, diabetes and lupus, hypertension), non-English speaking, expecting multiple infants, were unwilling or unable to consume DHA capsules three times per day between enrollment and delivery, were unwilling or unable to return to the study center for delivery or were not between the 8<sup>th</sup> and 20<sup>th</sup> week of gestation. The sample size for analysis of dietary intake of choline was 357 women who met these criteria.

Women who were unwilling or unable to return to the infant clinic for follow-up were excluded from the secondary analysis of choline in the diet in relation to plasma choline status. PEMT SNP information, plasma choline, PC and SM were collected for women whose infants participated in follow-up visits up to 18 months of age because PhD candidate Susan Scholtz is currently conducting a project to determine if PEMT SNPs influence infant development. Thus, data was excluded for 149 women who participated in the initial cohort but

did not participate in these follow-up visits. This reduced the sample size to 201 women who are included in the plasma analysis.

### **Analysis of Dietary Choline Intake**

Researchers involved with the DHA study participated in two days of training on how to give a standardized dietary interview. Study participants were asked about the frequency of consuming eggs and animal products that contain DHA at baseline to fill out in order to approximate their DHA intake. The same FFQ will be used to assess their dietary choline intake because DHA and choline are found in many of the same foods. While the aim of this study is to determine the effect of egg intake on plasma choline status, other sources of choline will be included in the database and analyzed. These foods include: liver, fish and meats (including beef, chicken, fish and pork).

Participants were read a list of foods and asked to choose how often they ate each of the listed foods. Choices ranged from “never” to “2 or more times per day”. Written responses were then transferred to a Microsoft Access file and converted into a numerical value which represents how many times each food was eaten per week. For example: “never” became 0 times/1 week = 0, “everyday” became 7 times/1 week = 7, and “2-3 times per month” became 2.5 times/4 weeks = 0.625. Each of these numerical values was multiplied by milligrams of choline in each food to determine dietary intake from choline. Milligrams of choline in

each food were determined using the USDA Database for the Choline Content of Common Foods and Zeisel's article on the choline content of common foods (5).

Total intake of choline from eggs was determined assuming a standard sized large egg was consumed. Frequency of egg intake per day was multiplied by 125 mg to determine daily intake of choline from eggs.

The form and cooking method of the meats in this FFQ are unknown. As a result, the mean amount of choline from the category of meat product was taken to determine an appropriate multiplier. For example, pork could be in several forms such as deli meat, bacon or loin. The foods under the category "pork" in Zeisel's article on the choline content of common foods were averaged to determine the choline content of pork (5).

While not every food containing choline is represented in this FFQ, the biggest sources in the American diet, eggs, meats and liver, are represented. Intake of eggs alone was used to estimate choline intake. In addition, total estimated dietary intake of choline was analyzed.

Supplemental choline was also measured. All women in the study sample gave the researchers the name of the pre-natal supplement they were taking at baseline. The nutritional content of each supplement was entered into a Microsoft Access file. Only a few pre-natal supplements contain choline, so most women in

this study did not have any supplemental choline intake. Women that had no choline in their supplement were given '0 mg' in the 'Supplements' column of the database. Women that did have choline in their supplement had milligrams of choline in the supplement added to the column.

### **Analysis of Demographic Characteristics**

The women in this study were asked to fill out a general information form at baseline that included their self-reported pre-pregnancy weight, self-reported height and ethnicity. Height of the mothers was also officially taken at the infant clinic at the infants' four-month follow-up visit. Official height was used to calculate BMI. In the few cases where official height was not available, self-reported height was used. A t-test for significance was used to determine the difference in official height and self-reported height of all women in the database. It was found that there was no significant statistical difference between the two values ( $p = 0.49$ ). Therefore, self-reported height is considered here to be a valid measurement of the height of the mothers.

### **Laboratory Analysis**

On the morning following childbirth, maternal blood samples were collected by venipuncture. After collection, the samples were immediately placed on ice and centrifuged (3000g for ~10 minutes) at 4°C. The resulting plasma was

aliquoted and stored at – 80°C until transport by FedEx on dry ice overnight. The samples were stored between 2 and 5 years depending on when the samples were taken. The analysis took place in March 2012 at the University of North Carolina.

Maternal choline status, as well as status of phosphatidylcholine and sphingomyelin, were quantified directly on samples extracted using the extraction procedure Bligh and Dyer (23). Aqueous and organic compounds were separated, analyzed and quantified directly using liquid chromatography/electrospray ionization-isotope dilution mass spectrometry (LC/ESI-IDMS) after the addition of internal standards labeled with stable isotopes to correct for recovery (44).

### **Database Categories**

The final list of categories to be included in the database is as follows: Study DHA assignment, primary participant number, maternal age, maternal ethnicity, maternal pre-pregnancy BMI, choline intake from eggs, choline intake from meat sources, choline intake from supplements, estimated total dietary choline intake, maternal plasma choline status, maternal plasma phosphatidylcholine status and maternal plasma sphingomyelin status.

## **Statistical Analysis**

Regarding the primary research question, the amount of choline from eggs and meats was determined as described in the methods above. The range, mean, median and mode of dietary choline intake from eggs and meats were then calculated.

For the second research question, a simple regression analysis was performed to determine relationship between dietary egg intake and several markers of choline status including plasma phosphatidylcholine, plasma sphingomyelin and plasma choline.



## Chapter IV: Results

An analysis of demographic characteristics determined that most women in this study were Black /African American or White/Caucasian. Forty-one percent of women identified as Black/African American and 44% identified as White/Caucasian. The third most common race represented was White/Hispanic; 7.84% of participants identified with this group. American Indian, Asian, Hawaiian or other Pacific Islander represented less than 2% of the total sample.

**Table 1**  
Maternal race

	<b>Number of Participants</b>	<b>Percentage</b>
<b>Black or African American</b>	147	41.18
<b>White</b>	176	49.30
<b>Hispanic</b>	28	7.84
<b>American Indian</b>	2	0.56
<b>Hawaiian or Pacific Islander</b>	2	0.56
<b>Asian</b>	1	0.28
<b>Other</b>	1	0.28
<b>Total</b>	357	100

**Table 2**  
Maternal BMI of sample

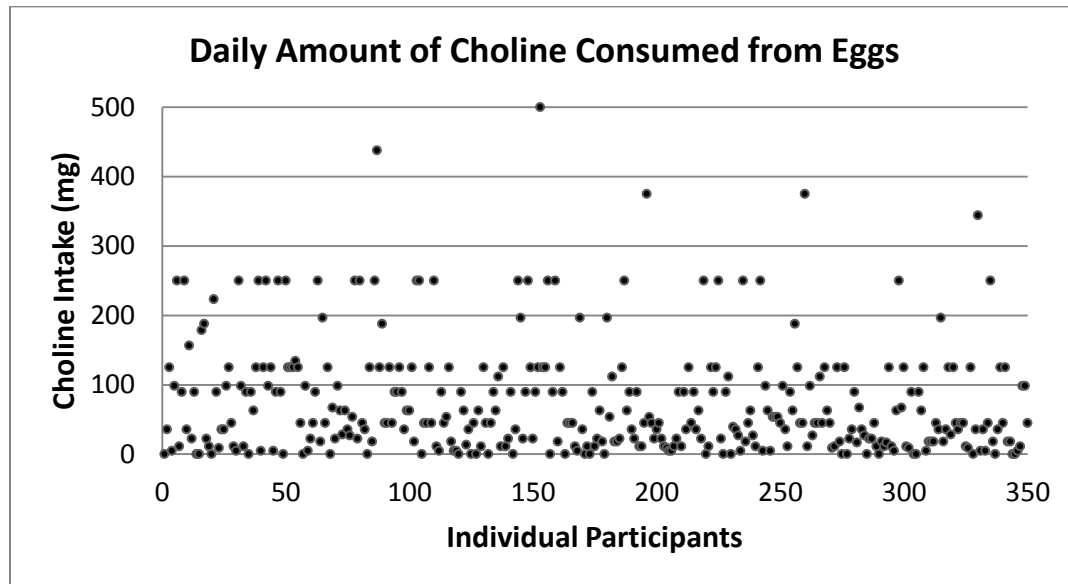
	<b>BMI</b>
<b>Average</b>	25.10
<b>Minimum</b>	14.25
<b>Median</b>	24.21
<b>Maximum</b>	39.96

Upon analysis of dietary choline intake from eggs, a wide range of intakes was found. Choline intake from eggs ranged from 0 mg a day to 500 mg a day. On average, it was found that pregnant women in this study consumed a 73 mg of choline daily. The median amount was 44.5 mg. The large difference between the median and average suggests that the average would not be an appropriate characterization of choline content from eggs and the median would be a more suitable number.

The most frequent amount of choline consumed was 125 mg – the equivalent of the choline content in a single egg. Forty-four (12%) of the participants consumed one egg per day. Only one participant met the adequate intake for choline through eggs alone by consuming four eggs per day - 500 mg of choline.

**Figure 1**

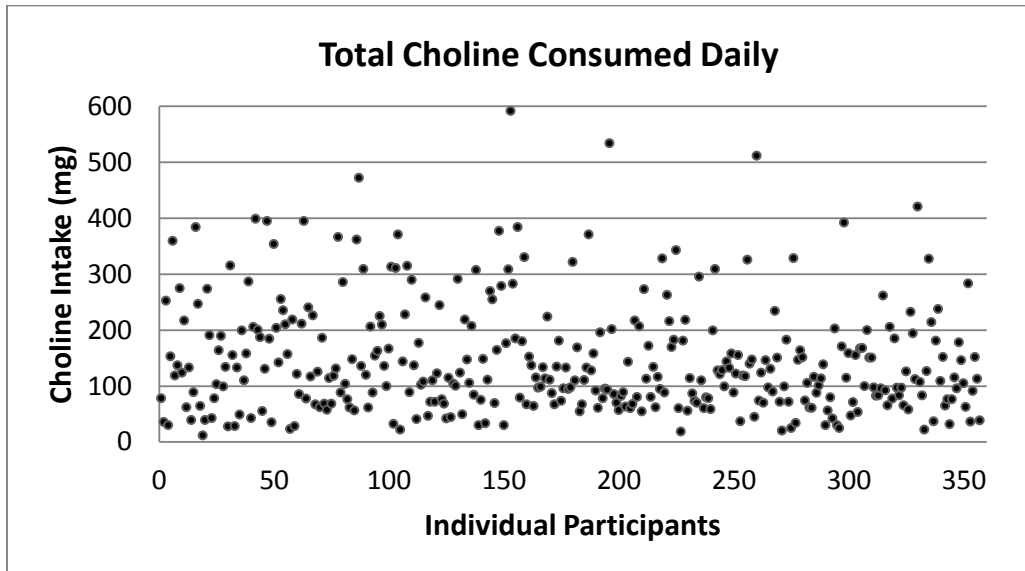
Amount of choline consumed per participant from eggs daily



This population consumed an average of 72.32 mg of choline from the meat categories available on the FFQ. A t-test for significance showed no difference between amount of choline consumed from eggs and amount of choline consumed from meats ( $p = 0.95$ ). However, the median intake from meats was 17.94 mg higher than the median intake of choline from eggs – 62.58 mg.

**Figure 2**

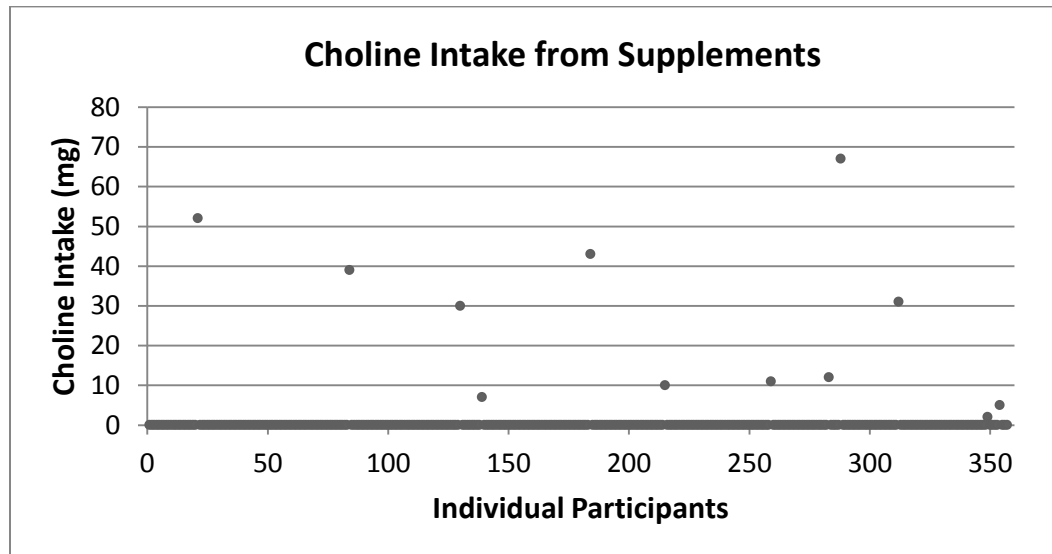
Total choline consumed per participant from meats and eggs daily



In an analysis of supplement content by fellow Masters Student Mallory Bratton, it was found that only twelve (3%) of the participants in this study consumed a supplement containing choline. Supplemental choline intake ranged from 0 – 67 mg per day. The median intake of choline in this population was 0 mg.

**Figure 3**

Choline intake per participant from supplements



**Table 3**

Choline Intake from Meats, Eggs and Supplements

	<b>Choline Intake from Eggs</b>	<b>Choline Intake from Meats</b>	<b>Choline Intake from Supplements</b>	<b>Choline Intake from All Represented Dietary Sources</b>
<b>Average</b>	72.019	72.32	0.87	144.34
<b>Mode</b>	125	100.97	0	67.07
<b>Minimum</b>	0	0	0	11.16
<b>1<sup>st</sup> Quartile</b>	16.74	41.60	0	74.60
<b>Median</b>	44.64	62.58	0	116.38
<b>3<sup>rd</sup> Quartile</b>	98.21	92.44	0	186.08
<b>Maximum</b>	500	282.71	67	591.1

## Plasma Analysis

Analysis by Bligh and Dyer yielded an average plasma choline of 6.59  $\pm$  2.20, an average plasma phosphatidylcholine of 2225.05  $\pm$  493.65 and an average plasma sphingomyelin of 529.99  $\pm$  123.86.

**Table 3**

Choline, phosphatidylcholine and sphingomyelin status of participants

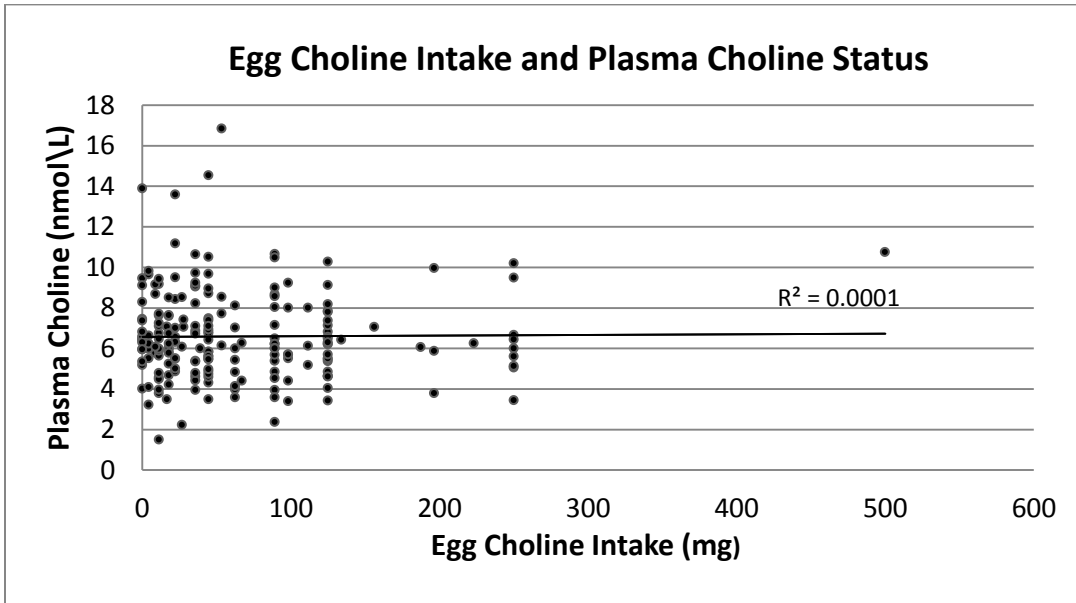
	<b>Choline</b>	<b>Phosphatidylcholine</b>	<b>Sphingomyelin</b>
<b>Minimum</b>	1.5	595.00	126.48
<b>Mean</b>	6.59	2225.05	529.99
<b>Median</b>	6.22	2160.29	527.85
<b>Maximum</b>	16.84	4170.02	865.81

No correlation was found between race and status of choline ( $r = 0.17$ ,  $p = 0.01$ ), phosphatidylcholine ( $r = 0.003$ ,  $p = 0.44$ ) or sphingomyelin ( $r = 0.004$ ,  $p = 0.95$ ).

No significant correlation was found between choline intake from eggs and plasma choline status ( $r = 0.01$ ,  $p = 0.88$ ), plasma phosphatidylcholine status ( $r = -0.033$ ,  $p = 0.62$ ) or plasma sphingomyelin status ( $r = -0.035$ ,  $p = 0.64$ ). Total choline intake showed no correlation when analyzed with plasma choline status ( $r = -0.005$ ,  $p = 0.94$ ), plasma phosphatidylcholine status ( $r = -0.04$ ,  $p = 0.53$ ) or plasma sphingomyelin status ( $r = -0.03$ ,  $p = 0.66$ ).

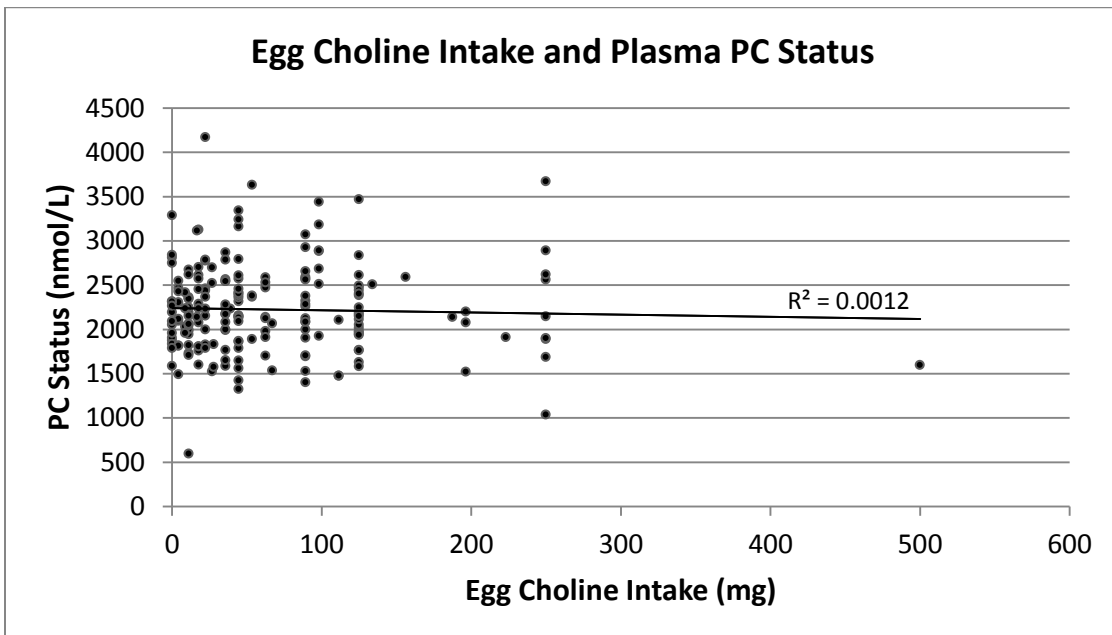
**Figure 4**

Choline intake from eggs in relation to plasma choline status



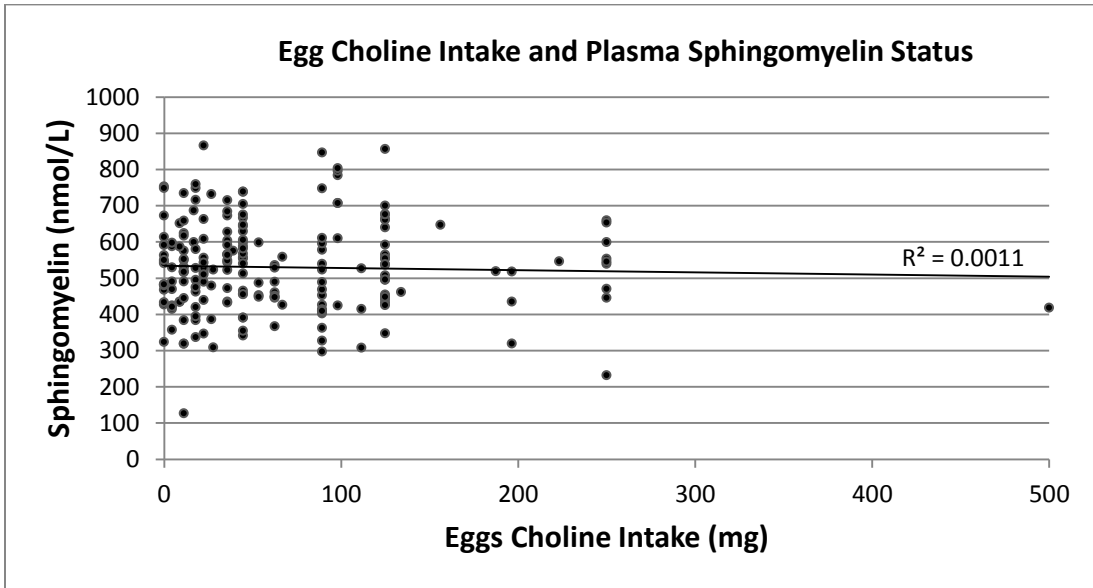
**Figure 5**

Choline intake from eggs in relation to plasma phosphatidylcholine status



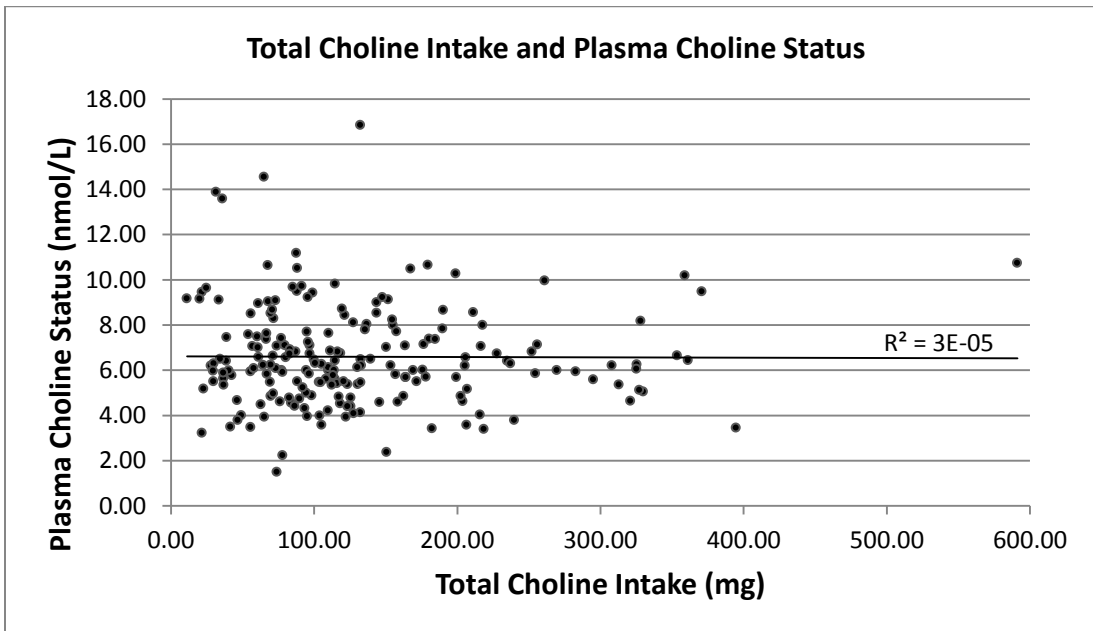
**Figure 6**

Choline intake from eggs in relation to plasma sphingomyelin status



**Figure 7**

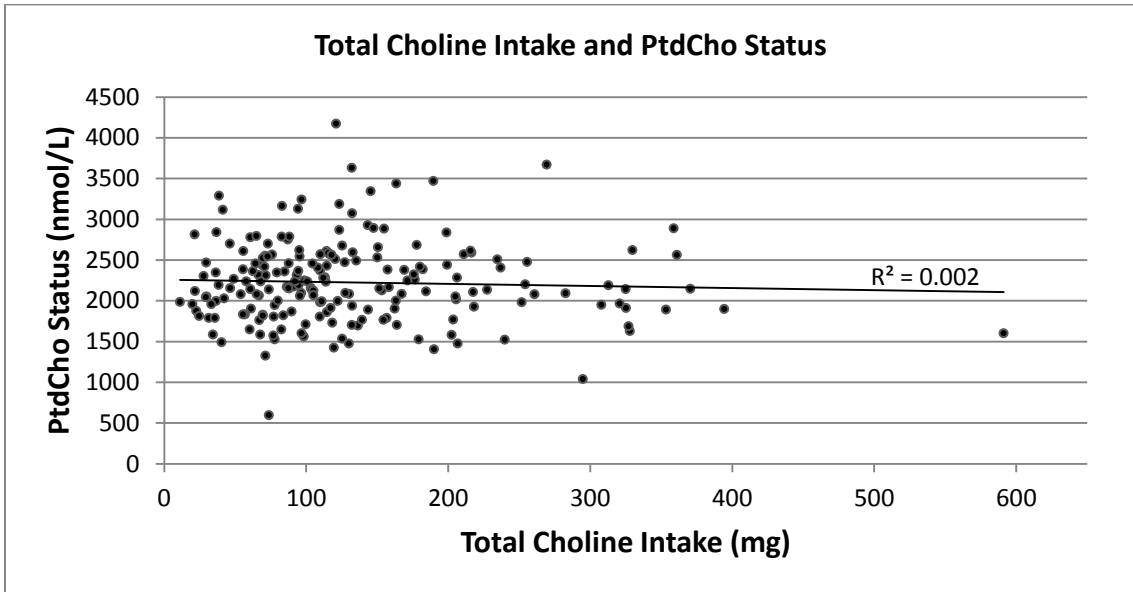
Total choline intake in relation to plasma choline status





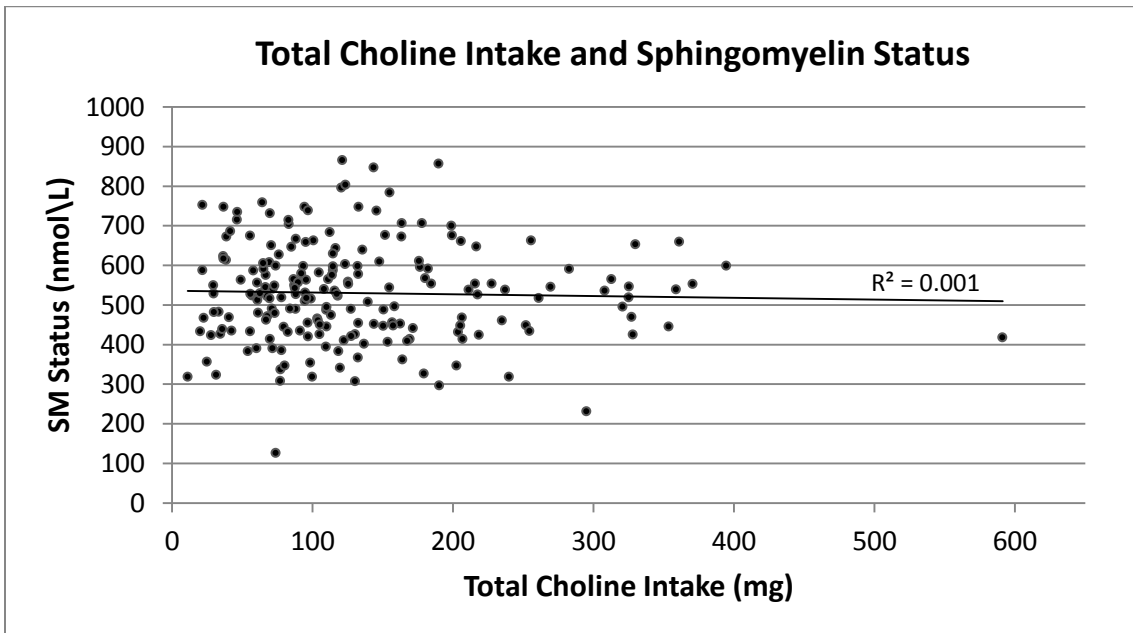
**Figure 8**

Total choline intake in relation to phosphatidylcholine status



**Figure 9**

Total choline intake in relation to sphingomyelin status



## Chapter V: Discussion

### Implications

Choline can come from the diet and from endogenous synthesis; however, many persons have functional SNPs in their PEMT gene that reduce their ability to synthesize choline. This study attempted to determine whether choline intake could be a predictor of choline status regardless of SNP status. If this study had found a correlation between dietary egg intake and plasma choline status, this may have been considered a cheap and simple way to analyze whether or not a pregnant woman had enough choline in her diet to support her growing fetus.

This study found that on average, women aren't receiving a large amount of choline from eggs, nor are they receiving much from supplements. This means that unless choline intake from other sources is high, most women will not be meeting the adequate intake recommended by the IOM for pregnant women.

However, the analysis of dietary intake also found that choline intake from meats isn't very high either. When all common sources of meat are considered – pork, chicken, beef and fish – it was determined that average and median intake were not all that different from egg intake. This does lend weight to the theory that eggs are a very important source of choline in the diet; however, intake of

eggs alone will not usually allow a person to meet the adequate intake unless 4 eggs per day are eaten on a daily basis.

No correlation was found between choline intake and any of the three measure of choline status used in this study. This lack of correlation suggests that dietary choline intake cannot be considered an accurate predictor of choline status. This may be due to the presence of PEMT SNPs that decrease choline status in many women. In a future study, this samples' dietary intake of choline will be co-varied with PEMT SNP status.

Most women in this study had very low plasma choline compared to what is considered normal -  $> 10$  nmol/L for non-pregnant women and  $> 14.5$  nmol/L for pregnant women (26). This low value may be due to the time that the plasma samples were taken. One day postpartum, and in a fasting state, women will be unlikely to have optimal choline stores. However, phosphatidylcholine and sphingomyelin should be more consistent as they are concentrated in the plasma membrane and less likely to fluctuate with feeding and fasting.

### **Limitations**

This secondary study has a few unavoidable limitations. First of all, these participants were chosen from an ongoing study that focused on DHA supplementation in pregnant women. Therefore the dietary questions asked were

not created with the specific aim of measuring choline intake. Foods that contained choline were missing from this food frequency questionnaire. In particular, dairy products were not recorded even though they are a good source of choline.

Another potential limitation is that responses may not accurately represent a typical person's diet. It is difficult to pinpoint intake of choline to the exact milligram if people are unclear on exactly how often they eat a particular food. In addition, the portion size and cooking method of each food was not asked. A 24-hour diet recall would not give an accurate reflection of the diet either. The best instrument that could have been used in this study, short of direct observation in a feeding clinic, would be a food record. This would require the mothers be dedicated to writing down everything they ate or drank during a period of at least a week. This may not have been plausible for some mothers or may have required greater compensation.

A third limitation is that the study sample size was not based on a statistical determination of power and effect size. Because no equivalent studies have been conducted so far, I was unable to determine the effect size of this particular study. The study sample may or may be sufficiently powered to detect a significant relationship between the variables being studied.

Lastly, because most of the lab work for this study was completed at the University of North Carolina, I was unable to measure folate status of the women involved in this study. Because several studies have found a relationship between plasma choline status and folate status, the study would be more complete if this variable was able to be added as a dependent variable in the regression analysis. However, most women in this study consumed pre-natal supplements with folate, so it may be safe to assume that most women were in good folate status. I was also unable to determine the lipid panel of these women so I was also unable to determine whether or not the amount of egg intake had any effect on their lipid panel. However, because total cholesterol often increases in pregnancy, this would not be an ideal population to measure whether or not eggs had any impact on lipid levels.

### **Ethics**

The University of Kansas Medical Center's Human Subjects Committee reviewed the procedures for the original KUDOS Trial. Participants in this study read and signed informed consent paperwork. Upon beginning this secondary study I underwent online Human Subjects training in order to be added as study personnel. The procedures in this secondary study were covered under the original KUDOS protocol.

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