

# FOLIC ACID SUPPLEMENTATION DURING PREGNANCY: FEASIBILITY AND INFANT OUTCOMES

By

GISSELLE MARIE ROSA

(Under the Direction of Lynn B. Bailey)

## ABSTRACT

This investigation is a pilot study assessing the feasibility of implementing a double-blinded randomized control trial of folic acid (FA) supplementation in a prenatal clinic setting. The research question asks if there is a difference in infant birth outcomes in response to 400 µg or 800 µg FA supplementation throughout pregnancy. The hypothesis is being tested in pregnant women receiving prenatal care at Athens Regional Midwifery Clinic. Thus far, 12 participants have completed the study. Mean cord blood serum and RBC folate concentrations are available for 7 infants ( $77.8 \text{ nmol/L} \pm 20.2$  and  $1408.1 \pm 573.8$ , respectively). No differences in gestation age, length, weight, head circumference, or Apgar scores have been observed between both groups. This pilot study provided valuable data regarding the feasibility of conducting a controlled FA intervention study in a prenatal clinic setting and will aid in planning a larger-scale study in the future.

INDEX WORDS: Pregnancy, Folate, Folic acid, Supplementation, Birth outcomes, Cord blood, Midwifery clinic, Pilot study, Feasibility

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GISSELLE MARIE ROSA

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GISSELLE MARIE ROSA

Major Professor: Lynn B. Bailey

Committee: Dorothy B. Hausman  
Richard D. Lewis

Electronic Version Approved:

Suzanne Barbour  
Dean of the Graduate School  
The University of Georgia  
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## **CHAPTER 1**

### **INTRODUCTION**

Folate is a generic term for an essential water-soluble B vitamin, which includes naturally occurring food folate and folic acid, which can be found in fortified cereal grain products and vitamin supplements. The main function of folate in the body is to act as a cofactor in one-carbon metabolism, which includes the de novo synthesis of purines and thymidylate, and the conversion of homocysteine to methionine (Bailey and Gregory 1999, Stover 2010). Adequate folate status is imperative during times of rapid growth and development, such as pregnancy. Due to the role of folate in DNA synthesis and repair as well as epigenetic regulation of gene expression, all cells require folate for proper replication. Definitive evidence has established that during the periconceptional period, low folate status greatly increases fetal risk for a neural tube defect (NTD), which results from the failure of the neural tube to close and/or fully develop during the first month of life (Botto et al, 1999). Adequate folate status is also important throughout pregnancy as it supports adequate fetal growth and maternal tissue growth (IOM 1998). For this reason, the Institute of Medicine (IOM) recommends that pregnant women consume 600 µg Dietary Folate Equivalents (DFEs) per day, equivalent to 400 µg folic acid, throughout pregnancy for the maintenance of maternal serum and RBC folate (IOM 1998, Bailey 2000). The Tolerable Upper Intake Level (UL) for this vitamin is 1,000 µg folic acid per day for all individuals (IOM 1998), though the sole basis for the UL is to prevent “masking” the diagnosis of a vitamin B12 deficiency.

In order to improve blood folate status in women of childbearing age and decrease the risk for NTDs, the Food and Drug Administration issued a final ruling in 1996 that would require all enriched cereal grain products to be fortified with folic acid (Food and Drug Administration 1996). Based on data from an NHANES study, serum and red blood cell (RBC) folate concentrations were higher in the post-fortification cohort compared to the blood folate concentrations from the pre-fortification cohort (serum folate: 13 ng/mL vs. 5.5 ng/mL, respectively; RBC folate: 269 ng/mL vs. 174 ng/mL, respectively; Pfeiffer et al 2007). As a result of folic acid fortification, women of childbearing age in the United States have begun pregnancy with a higher folate status than they would have before fortification efforts and NTD rates have decreased significantly (Honein et al 2001).

While adequate folate status is imperative for the reduced risk for NTDs during the periconceptional period, folate status is also important throughout pregnancy as it supports adequate fetal growth and maternal tissue growth (IOM 1998). The relationship between folate status and pregnancy outcomes have been investigated since 1944, when an association was found between the presence of megaloblastic anemia during pregnancy and infant prematurity (Callender 1944). Conflicting evidence exists for the effect of inadequate folate concentrations on pregnancy and infant birth outcomes. Previous studies have focused on the effects of folate intake and/or folic acid supplementation, and blood folate concentrations on birth outcomes such as birth weight, premature birth, risk for fetal growth retardation, small for gestation age (SGA), and preeclampsia. Some reviews show evidence for decreased risk for low birth weight, SGA, and spontaneous prematurity with higher folate intake or status (Branum et al 2012, van Uiter and Steegers-Theunissen 2013), while the latest Cochrane review did not find any evidence of an effect between folic acid supplementation and a number of birth outcomes (Lassi et al 2013). Nevertheless, many of

these studies have been conducted in European countries, where fortification of grain products is voluntary, and folic acid supplementation protocols are not consistent.

Folic acid supplementation during pregnancy is commonly recommended to assure that women maintain adequate folate status. While the current recommendations indicate that women consume 400 µg folic acid, many over-the-counter supplements contain between 800 µg to 1,000 µg of folic acid, which exceeds the recommendations by at least 2-fold. Due to the success of the mandatory fortification program and prenatal supplementation practices in the United States, women are often exposed to high quantities of folic acid during pregnancy. It was recently estimated that 11% of pregnant women who took supplements exceeded the UL for folic acid (Hoyo et al 2011). While high doses of folic acid during pregnancy have not yet been implicated in adverse birth outcomes, the results from animal studies indicate that offspring from dams fed high folic acid diets during pregnancy and lactation are at risk for metabolic dysfunction in adulthood (Huang et al 2014, Keating et al 2015). To our knowledge, there are no randomized controlled intervention trials to date assessing differences in birth outcomes between infants exposed a dose of folic acid following the current IOM recommendations and those exposed to a dose approximating the amount commonly found in over-the-counter prenatal supplements.

The overall purpose of this study is to generate initial pilot data and determine the feasibility for a larger-scale study in a prenatal clinic setting with sufficient power to address the specific aims of the study. The research question asks if there is a difference in cord blood folate status and infant birth outcomes in infants exposed to either 400 µg or 800 µg folic acid supplementation throughout pregnancy. The hypothesis was tested in pregnant women receiving prenatal care at the Athens Regional Midwifery Clinic (ARMC). The first specific aim of this study is to assess the feasibility of conducting a randomized controlled

intervention trial in a prenatal midwifery clinic setting. It is hypothesized that conducting this trial will be feasible in this type of setting. The second specific aim of this study is to assess the differences in infant birth outcomes and cord blood folate status between infants exposed to 400 µg or 800 µg folic acid throughout pregnancy. It is hypothesized that a difference in cord blood folate parameters and birth parameters will be detected between infants exposed to 400 µg or 800 µg folic acid. Although no differences in cord blood folate status were observed previously in a study in which the effects of folic acid supplements were assessed, the current higher dose (800 µg) is 2-fold higher than that previously compared with a 0 µg dose (Obeid et al. 2010). Additionally, all infants in this study will be exposed to the recommended amount of folic acid to promote adequate maternal folate concentrations (IOM, 1998). This study is significant as doses of folic acid higher than the recommendations could be associated with differences in cord blood folate status and birth outcomes. The results from this study and subsequent larger-scale studies could lead to evidence-based decisions for the reconsideration of folic acid doses in prenatal vitamins by pharmaceutical companies.

Following this chapter, Chapter 2 will include a review of the literature regarding folic acid supplementation during pregnancy and feasibility studies. The first topic includes folate bioavailability and metabolism, folic acid fortification, folate status biomarker assessment, and folic acid supplementation during pregnancy. The second topic includes an overview of findings from pilot studies conducted in pregnant women and issues including retention, compliance, and obtaining dietary records. Chapter 3 includes a manuscript on folic acid supplementation during pregnancy and will contain the methods, results, and discussion of the research. Chapter 4 will contain the concluding remarks pertaining to the current study and areas of future research.

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## **CHAPTER 2**

### **LITERATURE REVIEW**

#### **Part 1: Folic Acid Supplementation During Pregnancy**

##### ***Introduction to folate metabolism and requirements***

Folate is a generic term for an essential water-soluble B vitamin that functions as a cofactor in one-carbon metabolism and includes naturally occurring food folate and synthetic folic acid. Folic acid, found in fortified foods and dietary supplements, exists in the monoglutamate form while folate naturally found in food exists in the polyglutamate form. Folic acid consists of a bicyclic 2-amino-4-hydroxy-pteridine moiety linked to a p-aminobenzoyl-glutamate moiety (Shane 2010). Folate metabolism initially involves the reduction of the pyrazine ring of the pterin moiety to the tetrahydro form.

Tetrahydrofolate (THF) acts as the central folate acceptor molecule during folate metabolism. This molecule can then undergo a variety of interactions in the folate cycle to form THF derivatives, which have the potential to undergo three independent pathways: the de novo synthesis of purines, de novo synthesis of thymidylate, and the conversion of homocysteine to methionine (Bailey and Gregory 1999, Stover 2010). De novo purine formation and purine synthesis provide many of the nucleotides (adenine and guanine) used during human embryonic development and throughout life for the formation of DNA and RNA. Thymidylate biosynthesis occurs through the regeneration of dihydrofolate (DHF) and formation of deoxythymidine 5'-triphosphate



monophosphate (dTMP) required for DNA synthesis and repair. Finally, the remethylation of the amino acid methionine requires the one-carbon transfer from 5-methylTHF, the most bioactive form of folate in the body, to homocysteine through the B<sub>12</sub>-dependent methionine synthase (MS) enzyme. The remethylation of homocysteine to methionine leads to the formation of S-adenosylmethionine (SAM), which functions as a cofactor in methylation reactions, such as methylation of DNA, RNA, phospholipids, and histones. These pathways occur in different cellular compartments (i.e. cytoplasm, mitochondria, and nucleus), though each pathway is interdependent.

A common alteration in folate metabolism occurs through a single nucleotide polymorphism in the methylene tetrahydrofolate reductase (MTHFR) enzyme (Christensen and Rozen 2010). During times of low folate availability, the MTHFR 677 C -> T polymorphism reduces the efficacy of the enzyme, thus impairing the formation of the bioactive form 5-methylTHF. This polymorphism can result in a derangement in homocysteine metabolism negatively, affecting methylation reactions throughout the body.

Due to the role of folate in DNA synthesis and repair, all cells require folate for proper replication. Adequate folate status is imperative during times of rapid growth and development, such as pregnancy. During the periconceptional period folate status affects fetal risk for a neural tube defect (NTD) resulting from the failure of the neural tube to close and/or fully develop (Botto et al 1999). Throughout pregnancy, folate status supports adequate fetal and maternal tissue growth (IOM 1998).

### *Fortification program in the United States*

In order to reduce fetal risk for a NTD in the United States, the Food and Drug Administration (FDA) issued a final rule requiring folic acid fortification of cereal grain products by January 1998 (Food and Administration 1996). All cereal grain products were required to be fortified with 140 µg folic acid per 100 g of the product to increase folic acid intake in women of childbearing age. Neural tube closure occurs 28 days after conception; a time during which many women are unaware they are pregnant. Folic acid fortification improves and maintains an optimal blood folate status in the event that a pregnancy occurs. These new standards mandated by the FDA anticipated an increase in folic acid intake of about 100 µg/d. Surprisingly folic acid consumption increased by >200 µg/d after the initiation of folic acid fortification, excluding intake of folic acid-containing supplements (Quinlivan and Gregory 2003). After fortification of the food supply an increase in serum and RBC folate status was observed in women of childbearing age (Caudill et al 2001) and in the general population (Lawrence et al 1999). Honein et al (2001) observed a 19% decrease in prevalence of NTDs in infants born after mandatory fortification and a 23% decline in spina bifida cases in infants conceived after mandatory fortification. More recently Pfeiffer et al. (2007) observed that serum and red blood cell (RBC) folate concentrations were higher in the 2003-2004 post-fortification NHANES cohort compared to the blood folate concentrations from the 1988-1994 pre-fortification cohort. The significant impact of folic acid fortification in the United States was instrumental in the passage of legislation to mandate folic acid fortification of staple foods by 77 countries as of November, 2012 (Food Fortification Initiative 2014)

### *Bioavailability of folate and dietary needs*

Bioavailability refers to the proportion of an ingested nutrient that is absorbed and is available to be metabolized and used by the body. Bioavailability varies among sources of folate and folic acid (McNulty and Pentieva 2004). Natural folates are in their reduced forms and are prone to oxidation while folic acid, which is found in fortified foods and supplements, is fully oxidized and a more stable form of the vitamin. Natural food folates have an incomplete bioavailability when compared to folic acid due to factors such as intestinal hydrolysis of polyglutamyl forms and influences of food components on digestion and uptake of folate (McNulty and Pentieva 2004). Overall, food folates are approximately 50% less bioavailable than folic acid (IOM 1998). To account for the difference in bioavailability, the Recommended Dietary Allowance (RDA) is stated in Dietary Folate Equivalents (DFEs). One DFE is equal to 1 µg food folate which is equivalent to approximately 0.6 µg folic acid from fortified food or a supplement consumed with a meal or 0.5 µg folic acid taken without food. Alternatively, total food folate in DFEs is calculated as the quantity in µg of food folate plus 1.7 times the µg of folic acid consumed.

The current RDA for all adults is 400 µg DFEs to maintain adequate folate status (IOM 1998). Additional recommendations exist for women of childbearing age to increase folic acid intake to decrease risk for neural tube defects. The Institute of Medicine (1998) recommends that women capable of becoming pregnant should consume 400 µg folic acid from fortified foods or supplements daily. Due to increased nutrient needs to support growth and development, the RDA for women during

pregnancy is 600 µg DFEs, or about 400 µg folic acid, to maintain maternal serum and RBC folate status (IOM, 1998). The Tolerable Upper Intake Level (UL) for this vitamin is 1,000 µg folic acid per day for all individuals (IOM, 1998). The sole basis for the UL is “masking” the diagnosis of a vitamin B12 deficiency when based exclusively on hematological indices (IOM, 1998).

### *Folate biomarkers*

Three biomarkers are currently used to assess folate nutriture: plasma/serum folate concentrations (serum folate), RBC folate concentration (RBC folate), and homocysteine concentration (homocysteine). Serum folate reflects recent folate or folic acid intakes while RBC folate reflects long-term folate intake and tissue stores (Berti et al 2012). The lifespan of a red blood cell is about 120 days; therefore, changes in folate intake can be reflected in the red blood cells in about 2-3 months. Both biomarkers are clinically useful in assessing folate status and are highly correlated (Milman et al 2006). Berti et al (2012) reported that folate status was positively correlated with total folate intake, serum/plasma folate, and RBC folate based on findings from a meta-analysis. Doubling folate intake led to a 47% increase in serum/plasma folate and a 23% increase in RBC folate.

Another biomarker of folate status is homocysteine due to the role of folate as a methyl donor in homocysteine metabolism. A negative correlation was observed between total folate intake and homocysteine, with a 7% decrease in total homocysteine when folate intakes were doubled (Berti et al 2012). Additionally, based on findings from a meta-analysis assessing the effect of folic acid supplementation on homocysteine, it was

concluded that supplementation led to a decrease in homocysteine in a dose-dependent manner with doses of folic acid up to 0.8 mg (Homocysteine Lowering Trialists' Collaboration 2005). Other factors may also influence homocysteine, notably vitamin B12 and B6 nutriture due to their roles as cofactors in enzymatic processes related to homocysteine metabolism. There is evidence to support a negative correlation between B12 status and homocysteine (Selhub et al 2007, Ganji and Kafai 2009). The Homocysteine Lowering Trialists' Collaboration (2005) detected an additional 7% decrease in homocysteine when B12 supplementation was provided in conjunction with folic acid. The evidence is currently mixed regarding the role of B6 status in homocysteine (Dierkes et al 1997, Lee et al 2004, Homocysteine Lowering Trialists' Collaboration 2005). Due to a variety of factors that can influence this marker, homocysteine lacks specificity as a folate status biomarker and, used alone, is not an accepted marker of folate status.

### ***Folate during pregnancy***

Strong evidence suggests that adequate folate status during the periconceptional period greatly decreases the risk for a NTD-affected pregnancy (Czeizel et al 2011). A landmark study by Daly et al (1995) discovered over an 8-fold difference in risk for an NTD-affected pregnancy in women with an RBC folate concentration of  $< 340$  nmol/L when compared to women with a RBC folate concentration of  $\geq 906$  nmol/L. This finding was of particular importance as adequate folate status is defined as RBC folate concentrations  $> 305$  nmol/L due to the negative effects observed in individuals with RBC folate concentrations  $< 305$  nmol/L (such as disordered cell division and mis-

incorporation of uracil in DNA) (IOM, 1998). Studies have also provided evidence indicating that folic acid supplementation during the periconceptional period can prevent first occurrence (Czeizel and Dudás 1992) and recurrence of an NTD-affected pregnancy (Laurence et al 1981).

The physiological changes that occur during pregnancy require sufficient intakes of folate to support rapid growth. Cell replication is increased during this time due to uterine enlargement, placental development, increased maternal RBC formation, and fetal growth (IOM, 1998). Hence, recommendations for adequate folate intake during pregnancy are in place to maintain maternal serum and RBC folate (IOM, 1998). To assure adequate folate status is achieved, folic acid supplementation is commonly prescribed during pregnancy. In a controlled intake study by Caudill et al (1997), the response of 330 µg and 730 µg supplemental folic acid in addition to 120 µg dietary folate was compared against maternal folate status during the second trimester of pregnancy. The higher intake value approximated the previous IOM recommendations for folate intake during pregnancy. The results indicated that 400 µg folic acid intake was adequate to maintain maternal folate status. This study contributed to the evidence on which the current IOM recommendations for folate intake during pregnancy are based. Currently, the IOM recommends that pregnant women consume 600 µg DFEs per day, equivalent to 400 µg folic acid, throughout pregnancy for the maintenance of maternal serum and RBC folate (IOM, 1998, Bailey 2000).

### *Folic acid supplementation during pregnancy*

According to NHANES data collected from 1999-2006, 74% of pregnant women reported taking a multivitamin/mineral supplement containing folic acid (Branum et al 2013). The supplemental folic acid intake for women was reported to be  $817 \mu\text{g/d} \pm 27.6$  (mean  $\pm$  SE). Seventy-two percent of women reported consuming folic acid from supplements, which is equivalent to approximately 2-fold higher intake than that recommended for pregnant women ( $400 \mu\text{g/day}$ ). While the UL for folic acid is  $1,000 \mu\text{g}$ , there is very little evidence to suggest an adverse effect on pregnant women consuming doses exceeding this limit. In a recent study by Hoyo et al (2011) folate and folic acid supplementation intakes were assessed in a sample of pregnant women and women of childbearing age in North Carolina. The results indicate that 11% of pregnant women consumed intakes of doses exceeding the UL for folic acid during pregnancy. Additionally, 5% of pregnant women reported consuming intakes exceeding the UL before pregnancy and during pregnancy.

### *Folate status during pregnancy and birth outcomes*

In the early study by Callender et al. (1944) the relationship between folate status and infant birth outcomes was established in addition to an association between maternal megaloblastic anemia and infant prematurity. Since then, investigations have included other birth outcomes when assessing folate intake or folic acid supplementation response during pregnancy. A study by Baumslag et al (1970) assessed the effect of folic acid supplementation in pregnant Bantu women and White women in Africa. Both groups of women were randomized into three study groups: one receiving an iron supplement,

another receiving 5 mg folic acid in addition to the iron, and the third group receiving 50 µg B<sub>12</sub> in addition to the iron and folic acid. Baumslag et al (1970) concluded that Bantu women receiving folic acid supplementation had fewer incidences of infants who weighed less than 4 lb at birth and of premature birth. In contrast, no effect on infant birth weight or incidence of prematurity was observed in White women taking folic acid supplementation. This discrepancy could be attributable to folate intake from the diet as Bantu individuals have a high intake of maize meal and a low intake of vegetables while White women consume a diet high in uncooked fruits and vegetables.

A study in Alabama assessed the effect of 1 mg folic acid supplementation during pregnancy in women who were at increased risk for fetal growth retardation (FGR) and in women at a low risk for FGR (Goldenberg et al 1992, Tamura et al 1992). The percentage of FGR decreased as maternal folate concentrations increased. Additionally, women whose folate concentrations were above the mean at 30 weeks gestation had infants with higher birth weights while those with folate concentrations below the median had a higher incidence of infants with an Apgar score < 7 at 1 minute.

In pregnant women from a poor, urban city (Camden, NJ), there was a higher incidence of low birth weight (LBW) infants and premature infants born to mothers with a folate intake  $\leq 240$  µg (Scholl et al 1996). In a continuation of the Camden study, the effect of folic acid supplementation (1 mg) on risk for premature birth and LBW was assessed (Scholl et al 1997). The results indicate there was a reduction in risk for preterm delivery and LBW in pregnant women taking prenatal vitamins, especially when vitamin use began



during the first trimester. The aforementioned studies occurred before the start of mandatory fortification of enriched cereal products with folic acid in the United States.

In a more recent study, Bergen et al (2012) reported that women with the lowest concentrations of plasma folate gave birth to infants who weighed 53-125 g less, had over twice the risk of spontaneous prematurity, and had a higher risk for a small for gestational age (SGA) infant. Furthermore, high RBC folate measured at various times during pregnancy was positively associated with birth weight (van Uiter and Steegers-Theunissen 2013). Maternal folic acid supplement use, especially from the second trimester onwards, was positively associated with birth weight. These two studies were conducted in European countries where folic acid fortification of cereal grain products is not mandatory, though many European countries participate in voluntary fortification. These research results are contrary to the findings from the Cochrane review by Lassi et al (2013) regarding folic acid supplementation during pregnancy and infant outcomes. This review did not find evidence of an effect between folic acid supplementation and a number of birth outcomes such as mean birth weight, risk for a LBW infant, low pre-delivery hemoglobin and RBC folate, stillbirth, and preterm birth.

#### *Umbilical cord blood folate assessment*

In addition to assessing maternal folate status, assessing infant folate status at birth may help elucidate the relationship between adequate folate status and adverse birth outcomes. As there is concern that blood tests on infants, especially premature or SGA infants, may lead to detrimental blood and nutrient losses (Carroll et al 2012), umbilical cord blood is often used to assess infant blood parameters at birth. A study comparing

complete blood count tests and hemoglobin concentrations in newborns and umbilical cord blood observed no significant differences between both methods of assessing infant blood values (Carroll et al 2012). This demonstrated that cord blood is an acceptable method of assessing infant blood values at birth, and it has been used to assess infant folate status in several studies (Sweeney et al 2005, Sweeney et al 2009, Obeid et al 2010, Carroll et al 2012).

Early studies observed that high folate activity in the placenta allows it to concentrate folate from the maternal bloodstream into the cord blood (Landon and Oxley 1971). During times of low maternal folate, folate concentration in the cord blood is maintained preferentially over maternal concentration (Giugliani et al 1985). Cord blood folate status has been positively correlated with maternal serum and RBC folate (Giugliani et al 1985). Folate supplementation throughout pregnancy prevents a decrease in RBC and serum folate concentrations in the mother, thereby maintaining adequate levels in the cord blood. This is especially true during the second and third trimesters, which are times of rapid maternal tissue growth and fetal development (McNulty et al 2013).

#### *Oxidized folic acid*

Oxidized, or unmetabolized, folic acid is detected in the blood when the intake of folate and folic acid exceeds the body's capacity to convert it to its reduced form, 5-methylTHF (Kelly et al 1997). It is currently unknown whether oxidized folic acid in the blood poses health risks. Nonetheless, monitoring oxidized folic acid is especially relevant in societies where folic acid fortification of the food supply is mandatory and in

societies with liberal voluntary fortification. As reported in an early study, oxidized folic acid concentrations can be detected in adults in response to consuming a bolus of food fortified with >200 µg folic acid (Kelly et al 1997). The most recent NHANES data indicated that oxidized folic acid was detected in over 95% of individuals aged  $\geq 1$  year regardless of demographic characteristics, fasting status, or supplement use (Pfeiffer et al 2015). Tam et al (2012) reported detectable oxidized folic acid concentrations in women of childbearing age taking two high doses of folic acid supplements (1.1 mg and 5 mg), though there was no significant difference in oxidized folic acid concentrations between the two groups. Another group measured oxidized folic acid in non-fasting adult blood samples and observed oxidized folic acid in the blood of 49 out of 50 participants (Sweeney et al 2009). Further studies from this group reported detectable oxidized folic acid in the majority of maternal and cord blood samples at delivery (Sweeney et al 2005, Sweeney et al 2009). Obeid et al (2010) detected oxidized folic acid in maternal and cord blood, though there was no difference in oxidized folic acid concentration in cord blood between subjects who were exposed to 0 µg or 400 µg folic acid supplements during pregnancy. Studies have yet to assess differences in oxidized folic acid in cord blood in response to a dose approximating the current Institute of Medicine recommendations and higher doses that are found in commercially-available prenatal vitamins. Furthermore, studies have yet to assess the effect of oxidized folic acid concentrations during pregnancy and infant birth outcomes.

### *Folate status during pregnancy and birth outcomes in animal models*

Due to the success of the mandatory fortification program and prenatal supplementation practices in the United States, women are often exposed to high quantities of folic acid during pregnancy. In order to assess the possible effects of high folic acid exposure during pregnancy on the offspring at birth and into adulthood, many animal studies have been performed. When assessing the effect of a high folate diet on embryonic development in mice, Mikael et al (2013) concluded that high folic acid intakes increased incidence of embryonic loss and embryonic delay, and affected heart development by increasing ventricular septal defects and decreasing ventricular wall thickness. Other studies have focused on the effects of high folic acid supplementation on the long-term health outcomes of the offspring. The offspring of dams fed a high folic acid diet during pregnancy and lactation had higher incidences of obesity, glucose intolerance, insulin resistance, and other factors of metabolic dysfunction when exposed to an obesogenic environment (Huang et al 2014, Keating et al 2015). The dose of folic acid provided to the rodents ingesting the high folic acid diet in the aforementioned studies approximate a 10-fold higher dose than the recommended amount, which is equivalent to about 4 mg folic acid for a human adult. Human studies have yet to explore the effects of high folic acid supplementation during pregnancy on long-term outcomes in offspring.

## **Part 2: Feasibility of Conducting a Clinical Trial In a Prenatal Clinic Setting**

### ***Pilot studies***

Assessing the feasibility of implementing a study protocol in a prenatal clinic setting, such as a midwifery clinic, is an important aspect of planning a well-designed clinical intervention study. This can be done by using a pilot study, which is a smaller-scale version of the major study (Teijlingen and Hundley 2002). Pilot studies are key in order to obtain information on three classes of parameters for the design of a study: the administration of the study, the process of a disease, and/or the effect of a treatment (Wittes and Brittain 1990). An advantage of conducting a pilot study is that it can test and uncover any problems in the execution of the study protocol or methodology. It can also help identify ways to improve the research process, whether it is different ways of recruiting participants, distributing questionnaires, or providing additional training to co-investigators.

Previous studies in the area of folate have conducted clinical trials in a prenatal clinic setting, including at least one with pregnant women. The investigators involved in the Generation R Study, which is an ongoing prospective cohort study in The Netherlands, work closely with hospital and community midwives to recruit participants and collect data (Jaddoe et al 2012). The overall purpose of the Generation R study was to explore early environmental and genetic causes that can contribute to normal or abnormal growth and development. One subset of the study focused on folate concentrations during early pregnancy and determined that lower plasma folate and higher homocysteine concentrations were associated with adverse pregnancy outcomes

(Bergen et al 2012). Midwives and obstetricians in the study were involved in disseminating information about the study, assessing eligibility, and recruiting participants. This study demonstrated that a community-based study facilitated by midwives and obstetricians is feasible in this type of setting.

### ***Population of interest***

The population of interest is pregnant women in the United States. In 2008, the estimated pregnancy rate in the United States was 105.5 pregnancies per 1000 women aged 15-44 (Ventura et al 2012). In 2012, the total number of live births was 3,952,841 in the United States and 130,280 in the state of Georgia (Martin et al 2013). The distribution of births in Georgia by race/ethnicity in 2012 was: 46.3% Non-Hispanic White, 34% Non-Hispanic Black, 13.4% Hispanic, 4.5% other, and 1.8% not stated. The sample of women for this research was obtained from the Athens Regional Midwifery Clinic in Athens, Georgia. This clinic is part of the Athens Regional Health System, which serves a 17-county area in northeast Georgia (Athens Regional Health System 2015). According to the most recent estimates, the ARMC delivers about 750 infants per year and the distribution by race/ethnicity of the patients is about 50% Caucasian, 35% Hispanic, and 15% African American (Susan Fisher, personal communication). The sample of births by race/ethnicity that is expected at the ARMC is similar to that of the state of Georgia, with a slight difference in proportion of Hispanic women and African American women.

### ***Retention and compliance***

Retention and compliance are two key aspects of executing successful intervention trials. Recruitment and retention of participants are imperative in assuring that the sample size is sufficient to represent the target population and that the study is powered to test the hypothesis and detect significant differences (Leonard et al 2014). In a recent review of recruitment strategies for young women participating in nutrition research, the authors note that recruitment numbers are dependent on the type of strategies used, the amount of participant burden, and influence of the incentives provided (Leonard et al 2014). Strategies used to recruit young women for nutrition studies included flyers around a university campus, PowerPoint slides in lectures, and email lists. Another successful strategy used to recruit these participants was through advertisements sent through social media such as Facebook and Twitter, as one study recruited more participants in a shorter amount of time using social media than using traditional methods (Leonard et al 2014). Additionally, young women were more likely to participate in studies that did not require high participant burden, though the use of incentives helped increase recruitment numbers.

Once the participants are recruited, however, it is also important to consider methods to maintain or maximize participant retention. In a randomized controlled trial on supplementation, successful retention strategies included text message reminders to take the supplement, providing a list of recommendations to help participants remember to take the supplements, and providing an information sheet about common symptoms related to using the supplement (Leonard et al 2014). Other study designs used email

reminders and face-to-face contact for participant retention. Thus, additional efforts must be made after recruitment to assure as many participants as possible remain in the study.

Measuring compliance in a randomized controlled trial that provides a nutrition supplement as an intervention is an important aspect of accurate reporting and assessment of intervention effects (Kehoe et al 2009). Poor compliance can lead to a study being underpowered to detect significant differences between treatment groups. Therefore, closely monitoring compliance leads to more accurate interpretation of the results, especially in the case of a negative finding. Common methods used to assess compliance include self-reported intake, returned pill counts, and direct observation of the participant ingesting the supplement (Kehoe et al 2009). A factor that can influence compliance of supplement use during pregnancy is motivation for behavior change. One study assessed health behavior change before and during pregnancy, specifically smoking status, fruit and vegetable intake, caffeine intake, and alcohol consumption (Crozier et al 2009). This study concluded that smoking, caffeine intake, and alcohol consumption decreased during pregnancy. The strongest determinants in these behavior changes were education level and age, where women who were younger and less educated tended to make less positive behavior changes during pregnancy. Diligent efforts to increase compliance in participants that meet these criteria should be considered in intervention trials providing nutrition supplements to pregnant women.



### ***Dietary folate intake assessment***

Dietary intake assessment tools can be used to measure exact or estimated nutrient intakes for the analysis of dietary intake patterns. The three most commonly used dietary intake assessment methods are diet records, food frequency questionnaires, and 24 hour recalls (Thompson and Byers 1994). Dietary records require the participant to record the types and amounts of foods and beverages consumed for a certain amount of time, and require some participant training. This allows for accurate data collection as participants measure the exact portion size, preparation method, and brand name of the products consumed. A food frequency questionnaire is a dietary intake method that asks participants to report the frequency in which they consume certain foods or beverages from a list over a certain period of time. Another commonly used dietary assessment method is the 24 hour recall, in which the participant recalls what he/she consumed the previous day to a trained interviewer. This method does not require the participant to be trained or highly literate, and does not place any burden on them.

The Automated Self-Administered 24 hour Recall (ASA24) program developed by the National Cancer Institute is a free online tool that allows participants to self-administer a 24 hour dietary recall (Subar et al 2012). It is based on the United States Department of Agriculture (USDA) Automated Multiple-Pass Method for dietary recalls and uses nutrient level and food group estimates based on the Food and Nutrient Database for Dietary Surveys from the USDA. The ASA24 consists of two applications: the respondent website, in which participants input their own dietary recall data, and the researcher website, in which researchers obtain dietary data from respondents and their

subsequent dietary analyses. The respondent website guides the participant through the process of inputting 24 hour dietary data. It uses probes and questions to obtain specific details about type of food/beverage consumed, serving size, and preparation methods in either English and Spanish. A study by Bjorge-Schohl et al (2014) compared the results of nutrient intakes for food records that were coded by either participants using the ASA24 or researchers using the ESHA Food Processor software program. The results from the self-administered dietary analysis were highly correlated to the results from the diet analysis entered by a trained interviewer, especially averaged dietary records. The findings from this study indicated that using the ASA24 produced similar dietary analysis data when compared to using a trained interviewer to collect dietary information. In a nutrition research study assessing the effects of nutrient supplementation, accurate dietary intake data helps determine the effect of the supplementation itself on the desired outcome while taking dietary intake of the nutrient into account.

## **Summary**

The literature review summarized the current evidence available pertaining to the role of folic acid supplementation during pregnancy and the feasibility of conducting a clinical intervention trial in a prenatal midwifery clinical setting. The first section of this review focused on the metabolism of folate, its bioavailability, the mandatory fortification program, and folate biomarker assessment. Also discussed were the importance of folic acid supplementation during pregnancy, umbilical cord blood assessment, and oxidized folic acid. The second section of the literature review provided an overview of the importance of pilot studies, the population of interest for this study,

participant retention, measuring compliance, and obtaining dietary records. Current gaps in this area include the effects of folic acid supplementation on infant birth outcomes and blood folate concentrations in a setting with mandatory fortification. Additionally, research is lacking in the area of infant birth outcomes and folate concentrations in response to different folic acid supplementation doses, specifically the recommended folic acid level vs. higher doses commonly found in over-the counter prenatal vitamins.

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**CHAPTER 3**

**FOLIC ACID SUPPLEMENTATION DURING PREGNANCY: FEASIBILITY AND  
INFANT BIRTH OUTCOMES<sup>1</sup>**

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<sup>1</sup>Rosa, GR, Park HJ, Andersen VL, Fleming JM, Kauwell GPA, Fischer S, Hausman DB, Bailey LB. To be submitted to the *American Journal of Clinical Nutrition*.

## **Abstract**

Folate is a water-soluble B vitamin that is required for DNA synthesis and repair as well as epigenetic regulation of gene expression. Folate requirements increase during times of rapid growth and development, such as pregnancy. Evidence suggests that women who consume folic acid supplements throughout pregnancy have improved birth outcomes, though the evidence is mixed due to inconsistencies in supplementation doses and dietary folate intakes. The current investigation is a pilot study, designed to assess the feasibility of implementing a double-blinded randomized control trial of folic acid supplementation in a prenatal clinic setting. The research question asks if there is a difference in infant birth outcomes in response to either 400 µg or 800 µg folic acid supplementation throughout pregnancy. It was hypothesized that differences in cord blood folate parameters and birth parameters would be detected between infants exposed to 400 µg or 800 µg folic acid.

The hypothesis is currently being tested in women aged 18 to 40 y with a pre-pregnancy BMI between 18.5 and 35.0 kg/m<sup>2</sup> receiving prenatal care at Athens Regional Midwifery Clinic. Fifty-one participants were enrolled at the beginning of the study and 45% of participants have withdrawn. To date, 12 participants have completed the study. Mean cord blood serum and RBC folate concentrations are available for 7 infants in Group B (77.8 nmol/L  $\pm$  20.2 and 1408.1  $\pm$  573.8, respectively). No differences in gestation age, birth length, birth weight, head circumference, or Apgar scores were observed in the 12 infants born thus far. Following the completion of the study, the study objectives will be addressed with the entire data set.

In conclusion, this pilot study provided valuable data regarding the feasibility of conducting a controlled folic acid intervention study in a prenatal clinic setting and will aid in planning a larger-scale study in this type of setting.

## **Introduction**

Folate is a generic term for an essential water-soluble B vitamin, which includes food folate naturally found in foods, and folic acid, which can be found in fortified cereal grain products and vitamin supplements. The main function of folate in the body is to act as a cofactor in one-carbon metabolism (Bailey and Gregory 1999, Stover 2010). Due to the role of folate in DNA synthesis and repair as well as epigenetic regulation of gene expression, all cells require folate for proper replication. Definitive evidence has established that during the periconceptional period, low folate status greatly increases fetal risk for a neural tube defect (NTD), which results from the failure of the neural tube to close and/or fully develop during the first month of life (Botto et al. 1999). Adequate folate status is also important throughout pregnancy as it supports adequate fetal growth and maternal tissue growth (IOM, 1998). For this reason, the Institute of Medicine (IOM) recommends that pregnant women consume 600 µg DFEs per day, or 400 µg folic acid, throughout pregnancy for the maintenance of maternal serum and RBC folate (IOM, 1998, Bailey 2000).

In order to improve blood folate status in women of childbearing age and decrease the risk for NTDs, the Food and Drug Administration issued a final ruling in 1996 that would require all enriched cereal grain products to be fortified with folic acid (Food and Drug Administration 1996). As a result of folic acid fortification, women of childbearing age in the United States have begun pregnancy with a higher folate status than they

would have before fortification and NTD rates have decreased significantly (Honein et al 2001).

Folate status is also important throughout pregnancy as it supports adequate fetal growth and maternal tissue growth (IOM, 1998). Studies have investigated the relationship between folate status and pregnancy outcomes since 1944, when an association was established between the presence of megaloblastic anemia during pregnancy and infant prematurity (Callender 1944). Conflicting evidence currently exists for the effect of inadequate folate concentrations on pregnancy and infant birth outcomes such as birth weight, premature birth, risk for fetal growth retardation, small for gestation age (SGA), and preeclampsia. Some reviews provide evidence of decreased risk for low birth weight, SGA, and spontaneous prematurity with higher folate intake or status (Branum et al 2012, van Uiter and Steegers-Theunissen 2013) while the latest Cochrane review did not find any evidence of an effect between folic acid supplementation and a number of birth outcomes (Lassi et al 2013). Nevertheless, many of these studies have been conducted in European countries, where fortification of grain products is voluntary, and folic acid supplementation protocols are not consistent.

Folic acid supplementation during pregnancy is commonly recommended to assure that women maintain adequate folate status. While the current recommendations indicate that women consume 400 µg folic acid, many over-the-counter supplements contain between 800 µg to 1,000 µg of folic acid, which exceeds the recommendations by at least 2-fold. Due to the success of the mandatory fortification program and prenatal supplementation practices in the United States, women are often exposed to high quantities of folic acid during

pregnancy. It was recently estimated that 11% of pregnant women who took supplements exceeded the UL for folate (Hoyo et al 2011). While high doses of folic acid during pregnancy have not yet been implicated in adverse birth outcomes, the results from animal studies indicate that offspring from dams fed high folic acid diets during pregnancy and lactation are at risk for metabolic dysfunction in adulthood (Huang et al 2014, Keating et al 2015).

The overall purpose of this study is to generate initial pilot data and determine the feasibility for a larger-scale study with sufficient power to address the specific aims. The research question asks if there is a difference in cord blood folate status and infant birth outcomes in infants exposed to either 400 µg or 800 µg folic acid supplementation throughout pregnancy. The first specific aim of this study is to assess the feasibility of conducting a randomized controlled intervention trial in a prenatal midwifery clinic setting. It is hypothesized that conducting this trial will be feasible in this type of setting. The second specific aim of this study is to assess the differences in infant birth outcomes and cord blood folate status between infants exposed to 400 µg or 800 µg folic acid throughout pregnancy. It is hypothesized that a difference in cord blood folate parameters and birth parameters will be detected between infants exposed to 400 µg or 800 µg folic acid. Although no differences in cord blood folate status were observed previously in a study in which the effect of folic acid supplements were assessed, the current higher dose (800 µg) is 2-fold higher than that previously compared with a 0 dose (Obeid et al. 2010).

## **Methods**

### ***Participants***

This study was a double blind, randomized controlled intervention trial in healthy pregnant women receiving prenatal care at Athens Regional Midwifery Clinic (ARMC). The ARMC is a large regional practice, which delivers approximately 750 babies per year to mothers of varying social-economic class and race/ethnicity (45-50% Caucasian, 15-20% African American, 35% Latina). Women were included in the study if they were aged 18 to 40 y with a pre-pregnancy BMI between 18.5 and 35.0 kg/m<sup>2</sup>. English and Spanish-speaking women of any ethnicity were also included in this study. Women were excluded if they had a pre-existing chronic disease and if they had a multiple-fetus pregnancy. The University of Georgia Institutional Review Board and the Athens Regional Medical Center Institutional Review Board approved all methods and procedures before the study was initiated. This intervention trial was registered in Clinicaltrials.gov (registration identifier NCT02124642).

### ***Recruitment***

Participants were recruited during their first prenatal visit (< 12 wk gestation) by trained midwife research collaborators through the ARMC. The certified nurse midwife collaborators participated in several in-service training sessions regarding all aspects of the study and completed the Collaborative Institutional Training Initiative (CITI) program on human subjects. Recruitment flyers, in English and in Spanish, were placed in the ARMC and the ARMC midwives informed prenatal patients about the study during

their first prenatal visit. The midwives obtained written informed consent from women expressing interest in the study and who were eligible based on inclusion/exclusion criteria.

### ***Intervention and supplement protocol***

Upon consent, participants were randomly assigned to one of two treatment groups: one group receiving the prenatal supplement regimen with 400 µg folic acid and the second group receiving the 800 µg folic acid regimen. The treatment regimens were randomly assigned to “Group A” and “Group B” and, as this an ongoing study, researchers are still blinded to which group is receiving the additional folic acid. The prenatal supplement regimen consisted of 3 pills: a women’s multivitamin/mineral supplement (One-a-Day® Women’s; Bayer Healthcare, USA) containing 400 µg folic acid along with other essential vitamins and minerals, a 200 mg docosahexaenoic acid (DHA) supplement (Life’s DHA™ 200 mg vegetarian capsules; DSM Nutritional Products North America, Parsippany, NJ), and a specially formulated supplement containing 10 mg iron and either 0 or 400 µg folic acid. The formulated capsule was custom made by Westlab Pharmacy and tested by third-party laboratories for accuracy of folic acid (Analytical Research Laboratories, Oklahoma City, OK) and iron (Covance, Madison, WI) content. The prenatal supplement regimen provided each participant with the amounts of vitamins and minerals recommended for a healthy pregnancy by the Institute of Medicine. Additionally, DHA supplements were included in the prenatal supplement regimen as this supports fetal neurodevelopment (Greenberg et al 2008) and prescribing it follows the ARMC’s standards of care. The supplements were provided at

the beginning of enrollment and during each prenatal clinic visit to be taken daily throughout the duration of the pregnancy. Participants were advised to take their prenatal supplement daily, preferably in the evening, and to follow their standard diet while avoiding highly fortified ready-to-eat cereals and fortified snack bars containing >100% of the RDA for folic acid. Both the participants and the researchers were blinded to which treatment the participants received.

### ***Blood sample collection***

Blood samples were collected from participants at baseline (enrollment) and during normally scheduled prenatal visits at the ARMC at 28 wk, and 36 wk visits (Figure 1). Maternal and cord blood samples were collected at delivery. Cord blood was obtained from the umbilical vein upon expulsion of the placenta. Trained personnel at the ARMC practice collected the blood samples following routine draws for clinical and diagnostic testing and no additional needle-pricks were required. This protocol was consistent with the standard of care provided by the clinic and did not disrupt or affect the prenatal services provided to each participant.

### ***Blood sample analysis***

The maternal and cord blood folate concentrations were determined by microbiological assay using *Lactobacillus rhamnosus* (Tamura 1990; Horne and Patterson 1988). The inter- and intra-assay coefficients of variation for serum folate were 8.9 to 10.5% and 6.6 to 6.8%, respectively; whereas those for RBC folate were 3.1 to 3.8% and 3.3 to 4.5%, respectively.



Maternal MTHFR 677C→T genotype was determined after purifying PCR products (QIAquick PCR Purification kit; QIAGEN) and sequencing the DNA templates with an Applied Biosystems Automated 3730 DNA analyzer (Applied Biosystems) at the Georgia Genomics Facility (Athens, GA).

### ***Dietary assessment***

During the first visit, participants were given a three-day recall packet and asked to complete it at approximately 24 and 32 wk of gestation (Figure 1). The packet included dietary information for three 24 h periods on non-consecutive days including one weekend day. The format for the packet followed that of the multi-pass Automated Self-administered 24-hour Recall (ASA24) system by the National Cancer Institute website (Subar et al 2012). When completed, a trained researcher contacted the participants via telephone and used probes and questions from the ASA24 program to obtain specific information about the dietary recalls. The same interviewer entered the intake information obtained through the dietary record and follow-up interview into the ASA24 online program. Participants were also provided the option of entering their dietary records directly into the ASA24 website. While the ASA24 was designed to be self-administered, this procedure allowed participants with limited computer access to continue participating in the study.

### ***Medical records and infant outcomes***

Medical records were accessed to obtain information on maternal baseline characteristics, potential confounders, and birth records. Use of health information,

protected by the Health Insurance Portability & Accountability Act (HIPAA), was authorized through signature of the consent form. Information that was obtained included demographic information, maternal anthropometric data, prescription and non-prescription drug use, laboratory values for clinic-administered testing, and other blood chemistry indices. The Centers for Disease Control's website BMI calculator tool was used to calculate BMI from the height and pre-pregnancy weight listed on the medical record (Centers for Disease Control and Prevention 2014). Infant data obtained through medical records included date and mode of delivery, gestational age, gender, length, weight, head circumference, and Apgar score.

Infant measurements at delivery were obtained by nurses from the Labor and Delivery unit at Athens Regional Medical Center using standard techniques (Koo et al 2004). Infant recumbent length is measured in the supine position to the nearest 0.01 cm using an Infantometer while weight is measured to the nearest 0.01 kg using a digital scale. Head circumference is measured to the nearest 0.1 cm at the largest occipitofrontal circumference using a tape measure. An Apgar test is performed to assess the clinical status of an infant at 1 minute and 5 minutes after birth (American College of Obstetricians and Gynecologists 2006). It is a subjective test in which infants are given a score from 0 to 2 on each of 5 criteria: appearance, heart rate, reflex irritability, muscle tone, and respiratory effort. A score greater than or equal to 7 at each time point is optimal, indicating that the infant will not need additional resuscitation efforts.

### ***Retention and compliance***

In order to maximize retention, participants were contacted every two weeks by telephone, email, or text message. These conversations served to obtain data to complete the Health Behavior questionnaire, provide instructions about completing the 3-day diet recalls, and to answer any questions participants had regarding the study. Additionally, compliance was encouraged through these conversations and the participants were reminded to return their used pillboxes at their subsequent prenatal clinic visit, during which they received a replenished box. Compliance was measured by counting the remaining pills from the returned boxes.

### ***Statistics***

Descriptive statistics were used to describe participant and infant characteristics using SAS (Cary, NC, USA). Differences between participant characteristics, dietary intakes, blood folate concentrations, and infant birth parameters between the two supplementation groups were assessed using two-tailed t tests and chi-squared tests. Pearson's correlation coefficients were calculated to determine the relationship between participant characteristics and blood folate concentrations. The level of statistical significance was defined at  $P < 0.05$ .

## Results

A total of 51 women were recruited into this study (Figure 2) and were randomly assigned to one of the two intervention groups. Table 1 summarizes the clinical and demographic characteristics of all the study participants upon recruitment. In this recruitment cohort, there were significant differences in pre-pregnancy weight ( $P < 0.05$ ), pre-pregnancy BMI ( $P < 0.05$ ), number of previous pregnancies ( $P < 0.05$ ), and parity ( $P < 0.05$ ) between those randomly assigned to Group A and to Group B.

Thus far, 23 participants have discontinued the intervention for reasons listed in Figure 2. There were no significant differences between the characteristics of participants who remain in the study and those who have withdrawn, with the exception of gestation age at recruitment, which was higher in study dropouts ( $P < 0.05$ ; Supplemental Table 2). Among the characteristics of the 28 remaining participants (Supplemental Table 1), pre-pregnancy weight ( $P < 0.05$ ), pre-pregnancy BMI ( $P < 0.05$ ), baseline serum folate concentration ( $P < 0.01$ ), and marital status ( $P < 0.01$ ) are significantly different between participants from the two intervention groups.

The results presented in this report are interim since only a small subset of study participants have delivered their infants to date. Following the birth of infants from all study participants (August, 2015), final data analyses will be conducted.

Twelve participants have given birth and completed the study to date, 4 from Group A and 8 from Group B (Table 2; individual data found in Supplemental Table 3). In this subset, there were no differences in gestation age at recruitment, pre-pregnancy

weight and BMI, weight gain throughout pregnancy, baseline serum folate concentration, baseline RBC folate concentration, or methylenetetrahydrofolate reductase (MTHFR) genotype. Significant differences were observed between the two groups for total folate intake ( $P < 0.05$ ) and total DFEs ( $P < 0.05$ ), with higher intakes reported for Group A. The average compliance rate of the subset of the participants that have completed the study is 90.2%.

Blood folate concentration measurements are available for 7 participants who have delivered their infants to date, all of whom are in Group B. There was no significant difference between maternal serum folate concentrations at baseline and at delivery ( $57.2 \pm 24.9$  nmol/L and  $61.9 \pm 25.4$  nmol/L, respectively;  $P=0.73$ ) or between maternal RBC folate concentrations at baseline and at delivery ( $1306.0 \pm 415.0$  nmol/L and  $1396 \pm 603.2$  nmol/L, respectively;  $P=0.77$ ). The serum and RBC folate concentrations measured throughout pregnancy for the 7 participants are illustrated in Figure 3. Twenty-nine percent of women (2 participants) experienced a decrease in serum folate concentration and 14% of women (1 participant) experienced a decrease in RBC folate concentration from baseline to delivery.

The influence of maternal folic acid supplementation during pregnancy on infant outcomes is presented in Table 3. In the subset of infants delivered to date, there were no significant differences in gestation age, birth length, birth weight, head circumference, or Apgar scores between the two groups (Table 3, individual data found in Supplemental Table 4). Cord blood samples were only available from 6 infants. In this sub-sample, there was a significant correlation between maternal RBC folate concentration at delivery and cord blood serum folate concentration ( $r=0.92$ ,  $P=0.01$ ; Table 4). Similarly, there was

a significant correlation between maternal RBC folate concentration at delivery and infant Apgar score at 1 minute ( $r=-0.97$ ,  $P<0.01$ ).

As previously stated, in the subset of participants who completed the study to date, there were no significant differences in pre-pregnancy BMI and weight gain during pregnancy between the two groups. However, maternal pre-pregnancy BMI was negatively correlated with weight gain throughout pregnancy ( $r=-0.79$ ,  $P<0.01$ ; data not shown). Additionally, maternal RBC folate concentration at delivery was positively correlated with dietary folate intake ( $r=0.94$ ,  $P=0.02$ ), intake of DFEs ( $r=0.91$ ,  $P=0.03$ ), and time taking a folic-acid containing supplement prior to study enrollment ( $r=0.90$ ,  $P=0.04$ ). Partial correlations for these parameters were not conducted as there are insufficient data at this time.

There were no significant correlations between pre-pregnancy BMI, maternal weight gain, or maternal serum folate at delivery and infant birth outcomes. While there were no significant differences in infant birth parameters between both groups, infant weight at birth was significantly correlated with gestation age at birth ( $r=0.66$ ,  $P=0.02$ ; data not shown). Although not statistically significant, infant length at birth was also positively correlated with gestation age at birth ( $r=0.58$ ,  $P=0.08$ ; data not shown).

## **Discussion**

The aims of this study were to assess the feasibility of conducting a randomized controlled intervention trial in a prenatal clinic setting and to assess the response of two different doses of folic acid supplementation during pregnancy on infant birth outcomes. As the study is still ongoing, this thesis includes an interim report inclusive of the infants born to date. A final report will be completed following the birth of all infants from participants enrolled in the study. This trial was designed as a feasibility study to determine the most effective means of conducting a large-scale controlled folic acid supplementation trial in a free-living population of pregnant women. In order to investigate this population, a hospital-based prenatal midwifery clinic was used to obtain a sample that is representative of this population of pregnant women.

Designing and implementing this pilot study in collaboration with the midwife co-investigators elucidated many factors that should be addressed when designing a larger-scale study. One factor that must be addressed is the retention of participants. To date, 45% of participants that were initially recruited have withdrawn from the study. While some were dropped because they no longer met the criteria to be included in the study, many participants voluntarily withdrew. In designing a larger-scale study, stronger efforts must be made to increase participant retention. Some methods that could be implemented include personal contact with the participants throughout the study, stronger incentives to complete the intervention, reducing participant burden and having additional co-investigators working on the study on-site for direct communication with the participants and with the midwives (Leonard et al 2014). In a previous randomized controlled intervention trial, Leonard et al. (2014) incorporated several successful retention

strategies such as text message reminders to take the supplement and providing a list of recommendations to help participants remember to take the supplements. As some participants were dropped from the study due to non-compliance with the supplement protocol, these types of reminders may help the participants take the supplements as directed and thereby remain in the study. The information acquired from this study regarding the attrition rate will be used when performing a power analysis for the large-scale study in the future. This will allow for a more accurate calculation of the number of participants required to provide sufficient power to observe significant results given the attrition rate of 45% observed in this pilot study.

Another factor to consider in planning a future study is selecting subjects based on certain demographic and clinical characteristics that could potentially impact the outcomes of interest. Within the current study, women randomly assigned to Group B had a higher pre-pregnancy weight and a higher BMI than women assigned to Group A. Additionally, participants in Group B had more previous pregnancies and a higher parity than women in Group A. These factors have been known to influence blood folate concentrations (McNulty et al 2013, Tinker et al 2012) and could influence the outcomes of interest. One way in which this could be addressed in the future is through statistically stratifying the participants by BMI category, number of previous pregnancies, and/or parity before randomizing them into an intervention group. This would assure that there is less variability between treatment groups, which could strengthen the results of the study.

Furthermore, controlling for pre-pregnancy and early pregnancy use of a folic-acid containing supplement will be important to assess the impact of the folic acid dose



independent of differences in pre-pregnancy folate status. One way in which this could be addressed in a larger-scale study is through statistical analysis. With a larger number of participants, a separate analysis for those with prior supplementation and those without prior supplementation could be conducted and a differential response to folic acid could be observed between the two subgroups. Another approach to this issue is to recruit women who are planning to become pregnant. The women could then undergo a washout period, begin the prenatal supplement intervention before conception, and continue the intervention throughout pregnancy. This could minimize the influence of pre-pregnancy supplementation, though it would require adjustment of the recruitment strategies used in the study.

Similar to controlling for early pregnancy use of supplementation, accurate measurement of folate and folic acid intake throughout pregnancy can also provide a more accurate measure of supplementation response in future studies. A positive correlation was observed between dietary folate intake, total intake of DFEs, and maternal RBC folate at delivery in the participants who have completed the study to date. The method used in this pilot study was the multiple-pass ASA24 computerized program, which has been validated to produce similar dietary analysis data when compared to using a trained interviewer to collect dietary information (Bjorge-Schohl et al 2014). To remind the participants to complete the records and to provide instructions regarding manually or electronically recording the dietary records, they were contacted via phone call or text message. Despite multiple attempts to contact each participant on a bi-weekly basis, 2 of the 12 participants could not be contacted for instructions on completing the dietary recalls. Therefore, having an on-site trained research interviewer to obtain dietary

information during each participant's regularly-scheduled appointment would yield more complete and accurate folate and folic acid intake information from all participants. This could strengthen the correlation observed between folate intake parameters and blood folate concentrations.

Overall, having a co-investigator or trained researcher located in the midwifery clinic would allow for increased recruitment and retention of participants, a higher response rate for health behavior information and dietary recall data, and easier access to medical records. The bi-weekly telephone conversations with the participants increased the rapport between the researchers and participants, which enhanced trust with the participants and allowed them to ask questions about the study or report any concerns. There were however many individuals who could not be contacted due to inaccurate phone numbers or invalid e-mail addresses which prevented health behavior information and dietary recall data to be obtained. In-person contact with participants during their regularly scheduled visits would allow for better rapport and enhance motivation to remain in the study. It would also increase the likelihood that the participants provide responses to the health behavior questionnaire and dietary recalls. An additional co-investigator in the midwifery clinic would also serve as a resource for the midwife research collaborators in the event that they should have questions about the study protocol or require assistance regarding any aspect of the study.

The results of this preliminary study to date indicate that it is feasible to conduct a randomized controlled intervention trial in a prenatal clinic setting. The strongest aspect of this study is that it allows the subjects to participate in this study with minimum additional burden to their regularly scheduled prenatal visits. This allows for a higher rate

of participation than other types of intervention studies such as controlled feeding studies. While both randomized controlled intervention trials and controlled feeding studies are strong designs, controlled feeding studies are among the best in exploring the metabolic effect of a specific nutrient and its relationship to health outcomes (Most, Ershow et al 2003). Nevertheless, controlled feeding studies require a higher amount of participant burden. This is because all meals and beverages, which are carefully designed and created by the research staff, must be consumed as directed under careful supervision (Most, Ershow, et al 2003). This design also places a higher burden on the researchers as they are required to tailor each meal to the requirements of the feeding protocol as well as ensuring that the energy and nutrient needs of each individual participant are met. Controlled feeding studies have been used in folate research and have played a key role in the development of intake recommendations by the Institute of Medicine. Notably, the controlled feeding study by Caudill et al (1997) determined that 330 µg supplemental folic acid in addition to 120 µg dietary folate was adequate to maintain maternal folate status throughout pregnancy. Randomized controlled intervention trials, especially those that are blinded, can also yield unbiased data that reflects the effects of an intervention in a real-world setting (Kaptchuk 2001). For the purposes of the current and future studies, double-blinded randomized controlled trials are the most feasible for obtaining data from a folic acid intervention on pregnant women in a free-living situation.

The preliminary infant birth outcome data presented in this interim report includes the analysis of blood folate measurements for 7 infants, all of whom were exposed to the supplementation regimen assigned to Group B. As the study is still ongoing, the dose of folic acid provided to these participants remains blinded.

The mean cord blood serum and RBC folate concentrations in the subset of the infants born to date are similar to those reported in a study by McNulty et al (2013) who assessed cord blood serum and RBC folate concentrations in infants of women receiving 400 µg or a placebo during pregnancy. The mean serum folate concentration for the infants in Group B thus far is 77.8 nmol/L, which is lower than the mean observed by McNulty et al in their intervention group (94.9 nmol/L) but higher than their placebo group (65.9 nmol/L). The mean cord blood serum folate concentration in this study was also higher than that observed by Obeid et al (2010), which was 54.4 nmol/L for infants exposed to a 400 µg supplement throughout pregnancy. Differences in mean serum folate concentrations among the 3 studies can be attributed to the environment in which the study was conducted, as each study took place in a different country with varying folic acid fortification regulations.

Interestingly, the mean cord blood RBC folate concentration of the available infant data (1408 nmol/L) was similar to the mean cord blood RBC folate concentration observed in the un-supplemented placebo group reported in the McNulty et al (2013) study (1418 nmol/L). The mean concentration observed in this interim report is lower than what was expected based on the mean cord blood RBC folate value (1993 nmol/L) for the supplemented group in the McNulty et al (2013) study.

Thus far, and contrary to our hypothesis, no differences have been observed in infant birth outcomes between the two groups receiving different doses of folic acid supplementation during pregnancy. No adverse infant birth outcomes have been observed to date according to infant medical records (including prematurity, fetal growth retardation, SGA infant, or LBW infant). Infants within this study were likely protected

from adverse outcomes of low folate status as maternal and cord blood folate concentrations were, for the most part, determined to be adequate. Only one participant thus far had a baseline RBC folate concentration below the cut off of  $< 906 \text{ nmol/L}$  determined to be protective against folate-related NTDs (Daly et al 1995). This participant's RBC folate concentration increased throughout pregnancy in response to supplementation thus decreasing risk for any adverse birth outcomes. Additionally, the mean serum folate concentrations for all maternal participants at baseline ( $65.8 \text{ nmol/L}$ ) and delivery ( $61.9 \text{ nmol/L}$ ) were within the range shown to be at low risk for adverse birth outcomes (Branum et al 2012). Branum and colleagues observed an increased risk for adverse birth outcomes such as birth weight, preeclampsia, and SGA at birth in the lowest quintile of serum folate concentrations ( $\leq 9.6 \text{ nmol/L}$ ) when compared to the highest quintile ( $\geq 25.9 \text{ nmol/L}$ ). The serum folate concentrations of the participants who have completed the study to date are well above the concentration considered by Branum et al. to be at lowest risk. This is to be expected as the population assessed in the current study is exposed to mandatory folic acid fortification while the population studied by Branum et al was not. The effects of high concentrations of serum and RBC folate in cord blood were not determined in the current analysis although they will be further explored upon study completion.

A strength of this feasibility study was its double-blinded, randomized controlled intervention trial design, which will enable the effects of an intervention to be determined in an unbiased manner. Another strength was a strong collaboration with the midwives from the ARMC which allowed us to determine if it was feasible to conduct a nutrition intervention study in a prenatal clinic setting, thereby minimizing participant burden.

Having the midwives as co-investigators involved them in the planning process of this study and their expertise was applied in protocol planning and implementation.

Additionally, implementing the study protocol without disrupting the standard of care provided to each patient at the ARMC was a strength of this study. This aspect of the study reduced the participant and midwife burden as the study participants did not require any extra visits to the ARMC or any extra needle pricks during regularly scheduled blood draws. The findings from this study support the conclusion that a larger-scale intervention study can be conducted within the prenatal clinic coupled with implementation of the proposed changes to enhance compliance and reduce variability.

This study is not without limitations. One limitation of this study is the number of participants that remain in the study which could prevent the study from having sufficient power to address the objectives of this study. Another limitation is the relatively small number of participants that could be contacted by phone on a regular basis. Many participants did not provide the clinic with functioning phone numbers or valid e-mail addresses thus preventing researchers from contacting participants. As previously mentioned, meeting with the participants in-person could strengthen this study as it would allow more data to be collected from all participants and it would help build rapport and improve participant retention.

## **Conclusion**

In conclusion, this interim report addresses the first objective of this research as it demonstrates the feasibility of conducting a nutrition intervention study in a prenatal clinic setting such as the ARMC. This pilot study also offered many ways in which the protocols and procedures could be enhanced in a large-scale study. The secondary

objective of this research is to assess the response of two different doses of folic acid supplementation during pregnancy on infant birth outcomes. While the available preliminary data does not suggest a significant difference in infant birth outcomes in response to two different folic acid supplementation regimens, this study is still ongoing and has not yet been un-blinded. Upon completion of this study, this objective will be addressed with the entire data set and a thorough investigation will be conducted to explore any associations between maternal folate concentrations and infant outcomes. A finalized report will provide a better understanding of the response of two different doses of folic acid supplementation during pregnancy on infant birth outcomes. The results from the finalized report could determine whether doses of folic acid higher than the recommendations could be associated with differences in cord blood folate status and birth outcomes. This preliminary study and subsequent larger-scale studies could lead to evidence-based decisions for the reconsideration of folic acid doses in prenatal vitamins by pharmaceutical companies.

### **Acknowledgements**

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## Interim Results

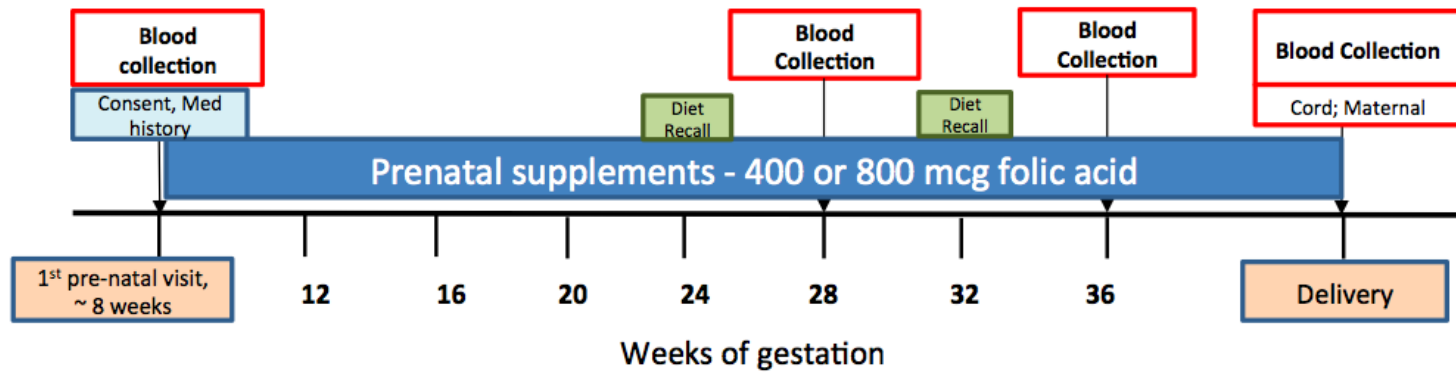
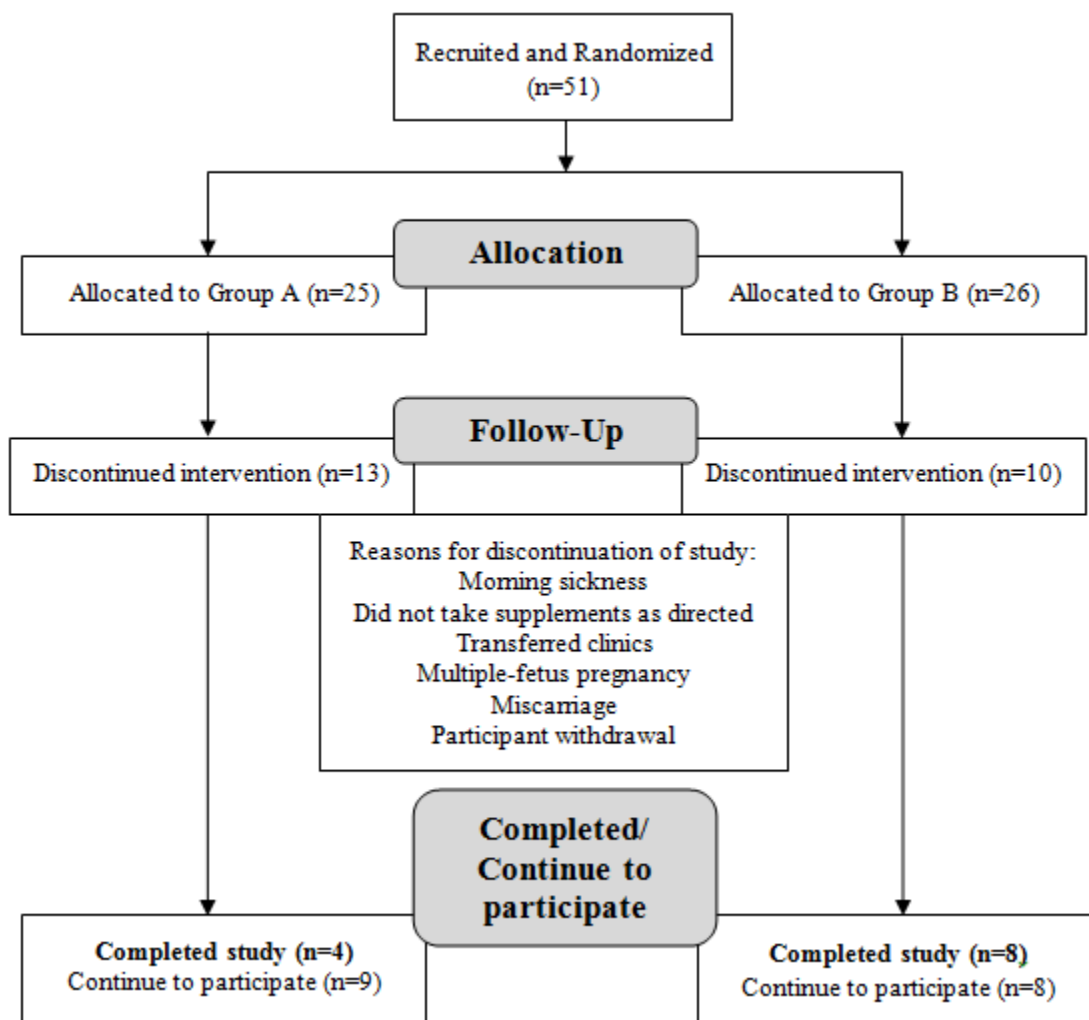


Figure 1: Timeline of study





**Figure 2: Participant flow throughout the study**

**Table 1: Demographic and clinical characteristics of recruited participants randomized to two folic acid supplementation groups<sup>1</sup>**

<b>Group</b>	<b>All</b>	<b>A</b>	<b>B</b>
N (%)	51	25 (49)	26 (51)
Age (y)	27.1 ± 5.4	25.9 ± 4.3	28.3 ± 6.1
Pre-pregnancy weight (kg)	69.7 ± 17.3	63.5 ± 13.9	75.6 ± 18.3*
Pre-pregnancy BMI (kg/m <sup>2</sup> )	26.2 ± 5.5	24.4 ± 5.2	27.9 ± 5.3*
Gestation day upon recruitment (d)	55.6 ± 13.0	56.4 ± 16.0	54.9 ± 9.4
Previous pregnancies	1.2 ± 1.2	0.8 ± 0.7	1.6 ± 1.5*
Parity	0.9 ± 1.0	0.6 ± 0.6	1.2 ± 1.1*
Serum folate at baseline (nmol/L)	72.2 ± 48.2	79.0 ± 41.1	66.0 ± 53.9
RBC folate at baseline (nmol/L)	1234 ± 481.4	1265.2 ± 467.7	1205.7 ± 501.6
<b>Race<sup>1</sup></b>			
White	49.0 (25)	60.0 (15)	38.4 (10)
African American	19.6 (10)	16.0 (4)	23.1 (6)
Hispanic	27.5 (14)	20.0 (5)	34.6 (9)
Other	3.9 (2)	4.0 (1)	3.9 (1)
<b>Insurance Type<sup>2</sup></b>			
Commercial	44.0 (22)	52.0 (13)	36.0 (9)
Medicaid	48.0 (24)	40.0 (10)	56.0 (14)
Other	8.0 (4)	8.0 (2)	8.0 (2)
<b>Marital Status<sup>2</sup></b>			
Married	52.9 (27)	64.0 (16)	42.3 (11)
Single	47.1 (24)	36.0 (9)	57.7 (15)
<b>Genotype<sup>1</sup></b>			
CC	54.9 (28)	56.0 (14)	53.8 (14)
TC	31.4 (16)	40.0 (10)	23.1 (6)
TT	13.7 (7)	4.0 (1)	23.1 (6)
<b>Pre-pregnancy vitamin use<sup>2</sup></b>			
No vitamin use	29.0 (9)	26.7 (4)	31.3 (5)
Multivitamin	12.9 (4)	13.3 (2)	12.5 (2)
Prenatal vitamin	58.1 (18)	60.0 (9)	56.2 (9)
<p>1. Values derived from the 2-sided student t-test and expressed as mean ± SDs unless otherwise indicated.</p> <p>2. Values were analyzed using chi-squared analysis and expressed as % (N)</p> <p>* <math>P &lt; 0.05</math></p>			

**Table 2: Interim demographic and clinical characteristics of participants completing the study to date (May 25, 2015)<sup>1</sup>**

<b>Group</b>	<b>A</b>	<b>B</b>
N (%)	4 (33.3)	8 (66.6)
Age (y)	25.5 ± 5.9	29.4 ± 6.9
Gestation age at recruitment (d)	54.5 ± 11.2	55.4 ± 11.0
Pre-pregnancy weight (kg)	65.5 ± 19.2	66.7 ± 8.9
Pre-pregnancy BMI (kg/m <sup>2</sup> )	24.8 ± 7.4	25.8 ± 5.2
Weight at delivery (kg)	80.5 ± 13.3	77.8 ± 8.2
Weight gain (kg)	15.0 ± 9.3	11.1 ± 3.7
Previous pregnancies	0.75 ± 1.0	2.4 ± 1.8
Parity	0.5 ± 0.6	1.5 ± 1.1
Serum folate at baseline (nmol/L)	65.8 ± 21.7	53.0 ± 25.8
RBC folate at baseline (nmol/L)	1582.7 ± 791.2	1280.1 ± 385
Caloric intake (kcal)	2823.0 ± 1749.0	2037.0 ± 703.6
Total folate intake (µg)	748.5 ± 311.4	389.7 ± 150.9*
Folic acid intake (µg)	360.7 ± 256.9	159.5 ± 62.6
Food folate intake (µg)	388.0 ± 54.5	230.1 ± 114.5
Total dietary folate equivalents (µg)	1001.1 ± 491.7	501.2 ± 330.1*
Race <sup>2</sup>		
White	50.0 (2)	37.5 (3)
African American	0.0 (0)	25.0 (2)
Hispanic	50.0 (2)	37.5 (3)
Insurance type <sup>2</sup>		
Commercial	50.0 (2)	37.5 (3)
Medicaid	50.0 (2)	37.5 (3)
Other	0.0 (0)	25.0 (2)
Marital status <sup>2</sup>		
Married	50.0 (2)	37.5 (3)
Single	50.0 (2)	62.5 (5)
MTHFR Genotype <sup>2</sup>		
CC	50.0 (2)	75.0 (6)
TC	25.0 (1)	12.5 (1)
TT	25.0 (1)	12.5 (1)
Pre-pregnancy vitamin use <sup>2</sup>		
No vitamin use	50.0 (2)	42.9 (3)
Multivitamin	25.0 (1)	0.0 (0)
Prenatal vitamin	25.0 (1)	57.1 (4)
<p>1. Values derived from the 2-sided student t-test and expressed as mean ± SDs unless otherwise indicated.</p> <p>2. Values were analyzed using chi-squared analysis and expressed as % (N)</p> <p>* <math>P &lt; 0.05</math></p>		

**Table 3: Interim infant birth characteristics<sup>1</sup>**

<b>Group</b>	<b>A</b>	<b>B</b>
N (%)	4 (33.3)	8 (66.6)
Gestation age (wk)	40.9 ± 1.8	40.2 ± 0.9
Length (cm)	51.5 ± 1.2	51.3 ± 2.2
Weight (kg)	3.54 ± 0.4	3.59 ± 0.6
Head circumference (cm)	34.2 ± 2.2	35.1 ± 1.3
Apgar score 1 min	7.75 ± 0.5	8.25 ± 0.5
Apgar score 5 min	9.0 ± 0.0	8.9 ± 0.4
Cord serum folate (nmol/L) <sup>2</sup>	--	77.8 ± 20.2
Cord RBC folate (nmol/L) <sup>2</sup>	--	1408.1 ± 573.8
<b>Gender<sup>3</sup></b>		
Male	75.0 (3)	57.1 (4)
Female	25.0 (1)	42.9 (3)
<b>Birth Type <sup>3</sup></b>		
Cesarean Section	75.0 (3)	37.5 (3)
Vaginal Birth	25.0 (1)	62.5 (5)
1. Values derived from the 2-sided student t-test and expressed as mean ± SDs unless otherwise indicated. 2. A total of 7 cord blood samples were obtained and analyzed (Group A N=0, Group B N=7) 3. Values were analyzed using chi-squared analysis and expressed as % (N)		

**Table 4: Interim Pearson's correlations of maternal weight and folate status and infant outcomes**

Variables	Maternal pre-pregnancy BMI*			Maternal weight gain			Maternal serum folate at delivery*			Maternal RBC folate at delivery*		
	N	r	P	N	r	P	N	r	P	N	r	P
Gestation age	12	0.11	0.73	12	0.09	0.78	7	-0.19	0.69	6	0.07	0.89
Infant Length	10	-0.24	0.50	10	0.48	0.16	5	-0.19	0.76	5	0.22	0.73
Infant Weight	12	-0.29	0.36	12	0.45	0.14	7	-0.20	0.66	6	0.18	0.73
Apgar 1 min	12	-0.28	0.38	12	0.24	0.46	7	-0.69	0.08	6	-0.97	<b>&lt;0.01</b>
Apgar 5 min	12	0.02	0.95	12	0.04	0.89	7	-0.18	0.70	6	--	--
Cord blood serum folate*	7	0.34	0.45	7	-0.10	0.84	7	0.72	0.07	6	0.92	<b>0.01</b>
Cord blood RBC folate*	7	0.19	0.69	7	0.01	0.98	7	-0.14	0.76	6	0.62	0.19

\*Log-transformed data

Pearson's correlations of maternal and infant birth outcomes, in which Pearson's correlation coefficient (r) displays strength of correlation. Significance determined at  $P < 0.05$ , as denoted in bold font.

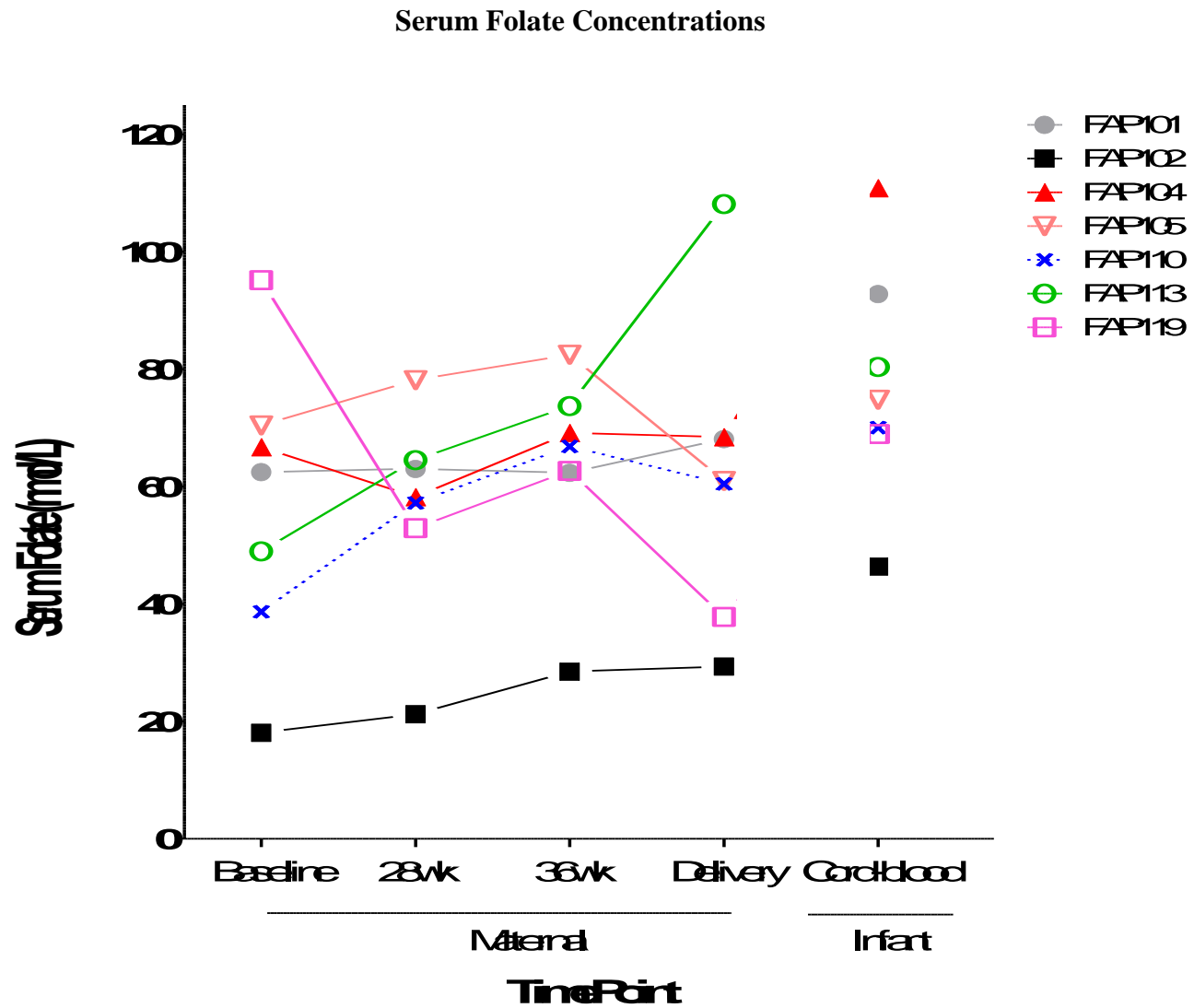
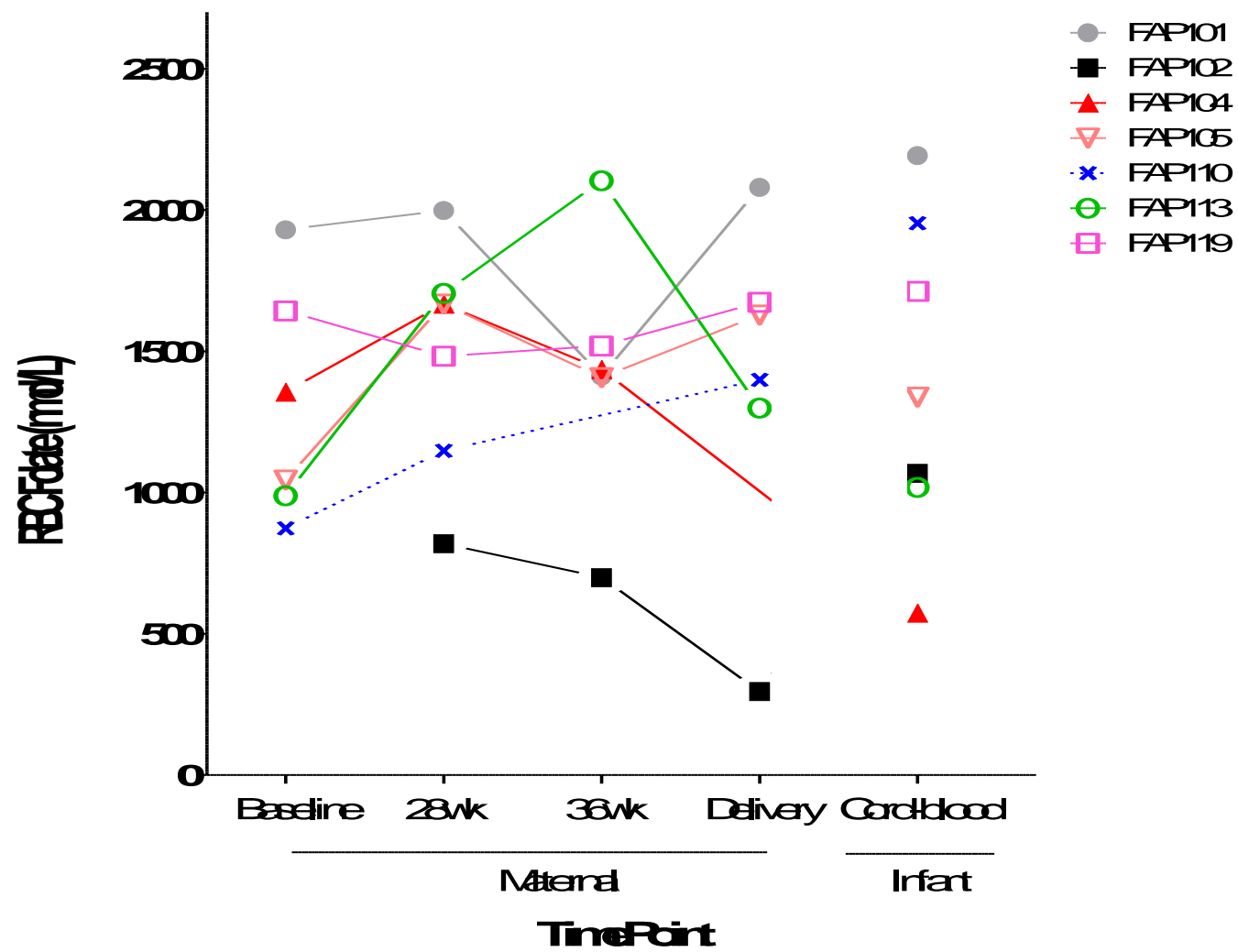


Figure 3: Interim maternal blood folate concentration throughout pregnancy and cord blood

RBC Folate Concentrations



**Supplemental Table 1: Demographic and clinical characteristics of retained participants<sup>1</sup>**

<b>Group</b>	<b>A</b>	<b>B</b>
N (%)	12 (42.9)	16 (57.1)
Age (y)	26.6 ± 4.1	28.3 ± 6.2
Gestation age at recruitment (d)	50.0 ± 14.8	53.6 ± 9.0
Pre-pregnancy weight (kg)	60.7 ± 11.2	72.6 ± 15.6*
Pre-pregnancy BMI (kg/m <sup>2</sup> )	23.3 ± 4.5	27.0 ± 4.8*
Previous pregnancies	0.9 ± 0.8	1.9 ± 1.8
Parity	0.8 ± 0.6	1.3 ± 1.4
Serum folate at baseline (nmol/L)	81.0 ± 28.3	48.2 ± 24.2**
RBC folate at baseline (nmol/L)	1376.0 ± 541.9	1203.3 ± 571.4
Race <sup>2</sup>		
White	58.4 (7)	43.7 (7)
African American	0.0 (0)	18.8 (3)
Hispanic	33.3 (4)	31.2 (5)
Other	8.3 (1)	6.3 (1)
Insurance type <sup>2</sup>		
Commercial	66.7 (8)	33.3 (5)
Medicaid	25.0 (3)	53.3 (8)
Other	8.3 (1)	13.4 (2)
Marital status <sup>2</sup>		
Married	75.0 (9)	31.3 (5)**
Single	25.0 (3)	68.7 (11)**
MTHFR Genotype <sup>2</sup>		
CC	66.7 (8)	56.2 (9)
TC	25.0 (3)	18.8 (3)
TT	8.3 (1)	25.0 (4)
Pre-pregnancy vitamin use <sup>2</sup>		
No vitamin use	22.2 (2)	30.8 (4)
Multivitamin	22.2 (2)	15.4 (2)
Prenatal vitamin	55.6 (5)	53.8 (7)
<p>1. Values derived from the 2-sided student t-test and expressed as mean ± SDs unless otherwise indicated.</p> <p>2. Values were analyzed using chi-squared analysis and expressed as % (N)</p> <p>* P &lt; 0.05</p> <p>** P &lt; 0.01</p>		



**Supplemental Table 2: Comparison between participants remaining in the study and participants who dropped from the study<sup>1</sup>**

<b>Group</b>	<b>Dropped out</b>	<b>Retained</b>
N (%) <sup>2</sup>	23 (45.0)	28 (55.0)
Age (y)	26.6 ± 5.5	27.5 ± 5.4
Gestation age at recruitment (d)	60.0 ± 13.3	52.0 ± 11.8*
Pre-pregnancy weight (kg)	72.3 ± 19.8	67.5 ± 14.9
Pre-pregnancy BMI (kg/m <sup>2</sup> )	27.2 ± 6.0	25.4 ± 4.9
Previous pregnancies	0.9 ± 0.7	1.5 ± 1.5
Parity	0.8 ± 0.7	1.1 ± 1.1
Serum folate at baseline (nmol/L)	85.0 ± 62.6	62.2 ± 30.4
RBC folate at baseline (nmol/L)	1175.2 ± 371.8	1280.0 ± 554.7
<b>Group<sup>2</sup></b>		
A	56.5 (13)	42.9 (12)
B	43.5 (10)	57.1 (16)
<b>Race<sup>2</sup></b>		
White	47.8 (11)	50.0 (14)
African American	30.4 (7)	10.7 (3)
Hispanic	21.8 (5)	32.1 (9)
Other	0.0 (0)	7.2 (2)
<b>Insurance type<sup>2</sup></b>		
Commercial	39.1 (9)	48.2 (13)
Medicaid	56.5 (13)	40.7 (11)
Other	4.4 (1)	11.1 (3)
<b>Marital status<sup>2</sup></b>		
Married	56.5 (13)	50.0 (14)
Single	43.5 (10)	50.0 (14)
<b>MTHFR Genotype<sup>2</sup></b>		
CC	47.8 (11)	60.7 (17)
TC	43.5 (10)	21.4 (6)
TT	8.7 (2)	17.9 (5)
<b>Pre-pregnancy vitamin use<sup>2</sup></b>		
No vitamin use	33.3 (3)	27.3 (6)
Multivitamin	0.0 (0)	18.2 (4)
Prenatal vitamin	66.7 (6)	54.5 (12)
<p>1. Values derived from the 2-sided student t-test and expressed as mean ± SDs unless otherwise indicated.</p> <p>2. Values were analyzed using chi-squared analysis and expressed as % (N)</p> <p>* <math>P &lt; 0.05</math></p>		

**Supplemental Table 3: Individual maternal characteristics for participants completing the study to date**

Subject	Group	Age (y)	Gestation age at recruitment (d)	Pre-preg BMI (kg/m <sup>2</sup> )	Previous Pregnancies	Parity	Race	Insurance Type	Marital Status	Genotype
FAP121	A	20	61	35.7	2	1	H	Medicaid	Single	TC
FAP123	A	32	41	22.3	0	0	W	Commercial	Married	CC
FAP124	A	29	50	19.2	0	0	W	Commercial	Married	CC
FAP127	A	21	66	21.8	1	1	H	Medicaid	Single	TT
FAP101	B	33	63	24.4	2	1	W	Commercial	Married	CC
FAP102	B	21	57	19.8	2	1	AA	Medicaid	Single	CC
FAP104	B	22	47	24.6	2	1	W	Medicaid	Married	CC
FAP105	B	35	71	24.8	3	3	H	Other	Single	TC
FAP110	B	35	57	34.2	3	3	H	Other	Single	CC
FAP113	B	21	38	26.6	0	0	H	Medicaid	Single	TT
FAP119	B	31	46	19.9	1	1	W	Commercial	Married	CC
FAP122	B	37	64	32.1	6	2	AA	Commercial	Single	CC

Subject	Group	Pre-pregnancy Vitamin Use	Final Weight (kg)	Weight Gain (kg)	Baseline Serum Folate (nmol/L)	Baseline RBC Folate (nmol/L)	Total Calories (kcal)	Total Folate Intake (µg)	Folic Acid Intake (µg)	Food Folate Intake (µg)	DEF (µg)
FAP121	A	0	96.0	2.8	59.8	1377.3	--	--	--	--	--
FAP123	A	2	74.0	15.0	84.7	2741.5	1586.2	528.3	179.1	349.5	653.4
FAP124	A	1	86.2	25.4	80.8	1244.3	4059.6	968.7	542.4	426.6	1348.8
FAP127	A	0	65.8	16.8	37.7	967.5	--	--	--	--	--
FAP101	B	2	77.1	14.5	62.5	1929.7	3105.3	636.5	176.4	461.1	759.6
FAP102	B	--	65.0	14.2	18.1	--	--	--	--	--	--
FAP104	B	2	74.6	11.6	66.7	1355.9	1935.8	302.4	154.3	147.9	411.3
FAP105	B	2	68.0	4.6	70.4	1044.2	1440.3	381.4	193.7	187.3	516.8
FAP110	B	0	84.0	7.8	38.7	873.9	1409.9	269.5	79.1	190.4	323.5
FAP113	B	0	87.0	12.2	49.0	988.8	1302.6	247.5	83.8	163.1	306.0
FAP119	B	2	81.0	15.2	95.2	1643.4	2672.6	561.7	257.7	303.7	741.5
FAP122	B	0	86.0	9.0	23.6	1125.0	2392.6	328.8	171.6	157.3	449.3
DFE= Dietary Folate Equivalents											

**Supplemental Table 4: Individual data for infants delivered to date**

Subject	Group	Cord blood serum folate (nmol/L)	Cord blood RBC folate (nmol/L)	Gestation age at birth (mo)	Gender	Length (cm)	Weight (kg)	Head circumference (cm)	Apgar 1 min	Apgar 5 min	Birth Type
FAP121	A	--	--	42.86	F	50.8	3.45	34.5	7	9	C
FAP123	A	--	--	39	F	50.75	3.24	36.2	8	9	C
FAP124	A	--	--	41.86	F	53.3	4.08	--	8	9	V
FAP127	A	--	--	39.86	M	51	3.38	31.8	8	9	C
FAP101	B	92.827	2192.9	40.43	F	51	3.58	34	8	9	V
FAP102	B	46.416	1067.4	40.29	F	50.5	3.46	35.5	9	9	V
FAP104	B	110.839	573.7	39.43	F	--	3.12	--	8	8	V
FAP105	B	74.7995	1338.2	39	--	48.75	2.86	33.5	8	9	C
FAP110	B	70.0815	1953.7	40.43	M	53.34	3.79	36	8	9	V
FAP113	B	80.4095	1018.1	40.86	M	--	3.91	--	8	9	C
FAP119	B	68.9985	1712.3	41.71	F	54.5	4.78	37	8	9	V
FAP122	B	--	--	39.57	M	49.5	3.22	34.5	9	9	C

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## **CHAPTER 4**

### **SUMMARY AND CONCLUSIONS**

Due to its role in DNA synthesis, repair, and epigenetic regulation as a methyl donor, adequate folate status is imperative during pregnancy, which is a time of rapid fetal and maternal tissue growth and development. Definitive evidence has established that during the periconceptional period, low folate status greatly increases fetal risk for a neural tube defect (NTD), which results from the failure of the neural tube to close and/or fully develop during the first month of life (Botto et al. 1999, Daly et al 1995). However, conflicting evidence currently exists for the effect of inadequate folate status on pregnancy and infant birth outcomes. In the United States, folic acid supplementation during pregnancy is commonly recommended to assure that women maintain adequate blood folate concentrations. While the current recommendations indicate that pregnant women consume 600 µg/day DFE, equivalent to 400 µg/day folic acid, many over-the-counter prenatal supplements contain between 800 µg to 1,000 µg of folic acid, which exceeds the recommendations by at least 2-fold.

The current investigation is a pilot study, designed to assess the feasibility of implementing a double-blinded randomized control trial of folic acid supplementation in a prenatal clinic setting. The research question asked if there is a difference in cord blood folate status and infant birth outcomes in infants exposed to either 400 µg or 800 µg/day folic acid supplementation throughout pregnancy. The 400 µg/day dose of folic acid is equivalent to the current RDA for pregnant women (IOM, 1998) and the 800 µg dose



reflects a dose commonly found in over-the-counter prenatal vitamin supplements. The specific aims of the study are 1) to assess the feasibility of conducting a randomized controlled intervention trial in a prenatal midwifery clinic setting; and 2) to assess the differences in infant birth outcomes and cord blood folate status between infants exposed daily to 400 µg or 800 µg folic acid throughout pregnancy.

The hypothesis is currently being tested in pregnant women receiving prenatal care at the Athens Regional Midwifery Clinic (ARMC). Upon consent, participants were randomly assigned to one of the two treatment groups: one group receiving the prenatal supplement regimen with 400 µg folic acid and the second group receiving the 800 µg folic acid regimen. The treatment regimens were randomly assigned to “Group A” and “Group B” and, as this an ongoing study, researchers are still blinded to which group is receiving the additional folic acid. Blood samples were collected from participants at baseline (enrollment) and during normally scheduled prenatal visits at the ARMC at 28 wk, and 36 week visits. Maternal and cord blood samples were collected at delivery. Participants were given a diet recall packet and asked to complete two sets of three-day diet recalls at approximately 24 and 32 weeks of gestation.

As this study is still ongoing, this document is an interim report of findings thus far. In the recruitment cohort, there were significant differences in pre-pregnancy weight ( $P < 0.05$ ), pre-pregnancy BMI ( $P < 0.05$ ), number of previous pregnancies ( $P < 0.05$ ), and parity ( $P < 0.05$ ) between those randomly assigned to Group A or to Group B. Thus far, 23 participants have discontinued the intervention and 12 participants have given birth and completed the study to date (4 from Group A and 8 from Group B).

The mean cord blood serum and RBC folate concentrations in the cord blood samples analyzed to date (n=7) were similar to those reported in previous studies assessing folic acid supplementation response during pregnancy. The mean serum folate concentration for the infants thus far is 77.8 nmol/L, which is lower than the mean observed by McNulty et al (2013) in their 400 µg intervention group (94.9 nmol/L) but higher than their placebo group (65.9 nmol/L). The mean cord blood serum folate concentration in the current study was also higher than that observed by Obeid et al (2010), which was 54.4 nmol/L for infants exposed to a 400 µg supplement throughout pregnancy. The mean cord blood RBC folate concentration observed in this interim report (1408 nmol/L) is lower than what was expected based on the report of a mean cord blood RBC folate value of 1993 nmol/L for the supplemented group in the McNulty et al (2013) study. In the subset of infants delivered to date (12 infants), there were no significant differences in gestation age, birth length, birth weight, head circumference, or Apgar scores between the two groups.

The protocols and methods used in this current study, along with the feasibility data collected thus far, will inform the study design and enhance the quality of a future larger-scale study. Findings from this study will also ensure that variability in participant folate status is reduced and retention of participants is enhanced.

Enhancing the methods for participant recruitment and retention will assure that the sample size is sufficiently powered to test the hypothesis and detect significant differences in future studies (Leonard et al 2014). Implementing methods, such as including personal contact with the participants throughout the study, providing stronger incentives to complete the intervention, reducing participant burden, and having

additional research co-investigators on-site for direct communication may aid in retaining more participants in future studies.

Secondly, placing more stringent inclusion/exclusion criteria to reduce within-group variability or stratifying the participants to control for confounding variables will enhance the quality of the data obtained in the larger scale study in the future. Within the current study, women randomly assigned to Group B had a higher pre-pregnancy weight, BMI, parity, and previous pregnancies than women assigned to Group A. These factors have been known to influence blood folate concentrations (McNulty et al 2013, Tinker et al 2012) and could influence the outcomes of interest. An example of an approach to address differences in pre-pregnancy folate status is to stratify the participants by pre-pregnancy supplement use in future studies. This will decrease the amount of variability in response to folic acid supplementation by decreasing the variability between intervention group characteristics.

Additionally, including more opportunities for investigators to meet with participants in-person will enhance a higher response rate for health behavior information and dietary recall data in future studies. In-person contact with participants during their regularly scheduled visits would allow for better rapport and enhance motivation to remain in the study.

In summary, based on the findings to date included in this interim report, it can be concluded that conducting a double-blinded, randomized controlled folic acid intervention study in a prenatal clinic is feasible. Currently, research is lacking in the area of infant birth outcomes and folate concentrations in response to different folic acid supplementation doses. This study is innovative as it is the first randomized control trial

to assess the response of two different doses of folic acid on infant birth outcomes in an environment with mandatory folic acid fortification. The preliminary data in this interim report is insufficient to base a conclusion regarding hypothesized significant differences in infant birth outcomes in response to two different folic acid supplementation regimens. A finalized report inclusive of the data available for all infants will be completed in the future to fully address the specific aims upon completion of the study.

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## **APPENDICES**

**APPENDIX A**

**RECRUITMENT FLYERS**

## Nutrition Research: Folic Acid Supplementation Study



### Who Can Enroll:

- Healthy pregnant women ages 18-40 years old
- Less than 12 weeks pregnant
- **Enroll at 1<sup>st</sup> prenatal visit – ask midwife for details!**
- Normal weight to moderately obese
- No use of prescription drugs
- No use of alcohol or cigarettes

### Benefits of Participation:

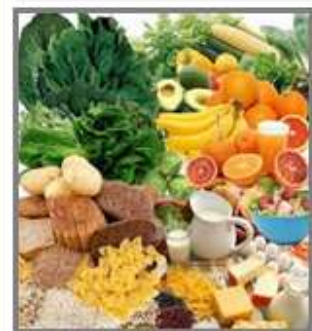
- Free prenatal supplements during entire pregnancy
- Nutrition analysis

### Study Requirements:

- Use provided prenatal vitamins during pregnancy
- Additional blood taken during scheduled blood draws
- Complete 2 dietary records

### Conducted by:

- Folate Research Team at UGA/ ARMC midwifery clinic
- Dr. Lynn Bailey (UGA), principal investigator
- 706-542-4256; folate@uga.edu





# Investigación de Nutrición: Estudio de Suplemento de Ácido Fólico



## Quienes Se Pueden Inscribir:

- Mujeres embarazadas con buena salud de 18 a 40 años de edad
- Embarazada por menos de 12 semanas
- **Inscripción durante primera visita prenatal- Favor preguntara la partera por más detalles!**
- Peso normal o moderadamente obesa
- Que no use medicamento recetado
- Que no fume ni tome alcohol

## Beneficios de Participación:

- Suplementos prenatales gratuitos durante el embarazo
- Análisis nutricional

## Requisitos del Estudio:

- Tomar suplementos prenatales durante el embarazo
- Proveer una toma adicional de sangre en cada consulta
- Completar 2 registros dietéticos

## Conducido Por:

- Grupo de Investigación de Folato UGA/ ARMC clínica de obstetricia
- Dra. Lynn Bailey (UGA), investigadora principal
- 706-542-4256; folate@uga.edu



**APPENDIX B**

**CONSENT FORMS**

**UNIVERSITY OF GEORGIA  
RESEARCH PARTICIPANT INFORMED CONSENT  
AND PRIVACY AUTHORIZATION FORM  
Folic Acid Supplementation in Pregnant Women: Dose Response**

**Researcher's Statement**

We are asking you to take part in a research study. Before you decide to participate in this study, it is important that you understand why the research is being done and what it will involve. This form is designed to give you the information about the study so you can decide whether to be in the study or not. Participation in the study is voluntary. Your decision to participate, or not, will not affect the services or standard of care provided during your prenatal clinical appointments. Please take the time to read the following information carefully. Please ask the researcher if there is anything that is not clear or if you need more information. When all your questions have been answered, you can decide if you want to be in the study or not. This process is called "informed consent." A copy of this form will be given to you.

**Principal Investigator:** Dr. Lynn B. Bailey  
Department of Foods and Nutrition  
Telephone: 706-542-4256  
Email: folate@uga.edu

**Purpose of the Study**

Folate is a general term for a water-soluble vitamin especially important during pregnancy. Folic acid is a form of the vitamin that is used in supplements and fortified foods. Prenatal supplements often contain much different amounts of folic acid and yet scientists and medical practitioners don't know how specific amounts of folic acid affect the blood levels of pregnant women and their babies at birth. The purpose of this study is to determine how levels of folate and related indicators in your blood at different times during pregnancy and in your babies cord blood after birth differ in response to one of two different amounts of folic acid in prenatal supplements. The folic acid doses represent the current Recommended Dietary Allowance (RDA) for pregnant women and a higher dose typically found in over-the-counter prenatal vitamin supplements. Your participation in this study will help provide important new information that will not only inform scientists but will also help guide clinicians who routinely recommend prenatal supplements.

**Eligibility**

You are qualified to volunteer for the study if you are a pregnant patient at Athens Regional Midwifery Clinic and meet other requirements which include the following: (a) 18-40 yrs old; (b) body weight, normal to moderately obese; (c) less than twelve weeks pregnant; (d) carrying only one baby; (e) no history of chronic disease; (f) non-anemic; and (g) not taking prescription drugs. Eligibility for the study will be verified based on meeting the above criteria and perceived willingness to complete study procedures and questionnaires. You may be withdrawn from the study without regard to consent if it is determined that you are carrying more than one baby, you



develop pregnancy-associated complications such as gestational diabetes or hypertension, you fail to take prenatal supplements as directed or to complete other study related procedures, or if you discontinue prenatal care through ARMC midwifery clinic.

### **Study Procedures**

If you agree to participate in the study, you will be asked to take part in the following study related procedures:

**Blood collection** – Blood will be collected during your scheduled pre-natal visits at times that samples are routinely drawn for diagnostics/pregnancy status monitoring. No additional needle-sticks will be required for research purposes. At each collection, a small needle will be inserted into a vein in your arm and an additional 30 mL (about 6 teaspoons) of blood will be taken for research purposes. Blood will be collected during your initial prenatal visit, at clinic visits at 28 and 36 weeks gestation and at delivery and will only take a few minutes. This blood will be used to measure blood folate and related nutritional and genetic indicators. In addition, after your baby is born and the cord has been cut, a blood sample (approximately 5 ml or 1 teaspoon) will be collected from the cord for analysis of folate status indicators. Some of the blood measurements will be done by collaborators at other universities within the United States. The samples will be sent with a participant number code and our collaborators will not be given any information that would allow them to directly identify you. Any information that is discovered from testing of this blood is related to research only and will not be used as therapy or diagnostic testing. For possible analysis in the future of additional folate-related metabolic indicators, a portion of your blood will be saved. Blood samples will be stored with a number code and your personal information will not be associated with your sample. Storage of samples for potential future analysis is not a requirement for participation in this study and you have the right to ask that all of your samples be removed and/or destroyed once the current study is completed. Any unused portions of blood that are collected will be discarded 10 years after completion of the study, per safe handling of hazardous material as defined by The University of Georgia Hazardous Material Safety protocol.

**Vitamin supplement protocol** – As a participant in this study, you will take prenatal supplements that contain one of two doses of folic acid. One of folic acid doses represents the current RDA for pregnant women and the other is a higher dose as typically found in prenatal supplements. There are no known risks related to the consumption of either of the doses of folic acid included with the prenatal supplements. Both supplements contain the same vitamins, minerals, and DHA in amounts that are routinely provided in commercially available prenatal supplements. The only difference in the two supplements is the amount of folic acid.

At your first prenatal visit, you will be provided the first four week supply of prenatal vitamins including one of two doses of folic acid, a prenatal multivitamin/mineral tablet and a DHA (nutrient important for brain development) supplement. The supplements will be packaged as individual daily supplies and you will take all supplements daily throughout your pregnancy from your first prenatal visit until delivery. You will take all the tablets for each day at once and at the same time each day, preferably with your evening meal. To insure that you remember to take your supplements, you will be instructed in the use of a compliance calendar and may receive telephone and/or 'text'

message reminders from project staff. You will be asked to return your pill containers and any unused pills at your next visit. You will receive a new supply of supplements every four weeks through the end of your pregnancy. You will be asked to follow your typical diet, but to refrain from consuming other dietary supplements, multivitamins, and highly fortified cereal products (containing  $\geq 100\%$  the RDA for folate).

Medical records - Information regarding your age, ethnicity, medical history, physical exam findings and blood test results will be obtained from your medical records at ARMC to determine the effect of these factors on the response to the folic acid supplementation. Information will also be obtained from the medical records regarding the date and mode of delivery, gestational age, gender, measurements, Apgar score and blood test results of your baby to determine the effect of the folic acid supplementation on the growth and development of your baby.

The privacy law, Health Insurance Portability & Accountability Act (HIPAA), protects your health information. Researchers may use or disclose protected health information for research purposes only if they have received your authorization for ARMC Midwifery Practice to disclose your information. The researchers will protect this information by using it only as permitted by this Authorization and as directed by state and federal law. If you have any questions and/or wish to revoke this Authorization in writing at any time, you can contact Dr. Lynn B. Bailey (see page 1). This Authorization expires ten years after the completion of the study. If you choose to participate in the research, you must sign this form so that your health information may be used for the research. Your decision to release or not to release this information will not affect the current or future services you receive from the ARMC Midwifery Practice; however, if you do not agree to this, you will not be able to participate in this study. The health information listed above may be disclosed for use in other projects related to pregnancy, nutrition, and infant health. While such disclosure is no longer protected by this authorization, the disclosure of your identifiable health information would only be to researchers who are members of the current research team and who obtain your written consent for involvement in such projects.

Study specific questionnaire - To obtain additional information not available on the medical records you will be contacted by telephone and asked to complete a brief questionnaire. The questions will include information regarding previous and current folic acid supplement use, regular consumption of cereals and other folic acid fortified foods, past and present smoking and alcohol habits, and other lifestyle factors. This questionnaire will be administered by University of Georgia research personnel and the telephone interview should take 15 minutes or less.

Food diaries/diet recalls - Your usual dietary intake at the various stages of pregnancy will be estimated through the use of food diaries and a computer-based diet recall program. You will be provided with Three Day Diet Recall Sheets on which you will record the foods you consume in a food diary format for assigned days at 24 and 36 weeks of gestation. These records will be returned via email or regular mail to UGA project staff. After receipt of the food diaries by study personnel, trained research staff will contact you via telephone to obtain additional and more specific information such as



brands and amounts consumed. The research staff will enter the information obtained through the food diary and follow-up interview into an on-line program for subsequent analysis. It should take about an hour at each of the two collection points to record your information in the food diaries and for the follow-up interview, for approximately two hours total.

#### **Risks and discomforts**

- **Blood draw:** Blood will be drawn for the purposes of this study only at times when samples are already being taken by the clinic for as part of your usual care. There is no additional risk for collecting extra blood for research purposes.
- **Questionnaires/Dietary Recall:** The discomfort or stress that you may face during this research may be associated with the disclosure of information concerning your dietary intake and health history; however it is important to share this information so that your health and nutritional status can be evaluated correctly. All individually-identifiable information will be kept strictly confidential and your name and other identifying information will be kept under lock and key, will not appear on project data files and will not be shared with anyone else.

#### **Benefits**

The information provided by this research study will help the researchers advance their knowledge about how different amounts of prenatal folic acid affect blood folate and other indicators of nutrition status in pregnant women. The study will provide data that will inform clinicians regarding the impact of the current recommended intake of folic acid for pregnant women compared to a higher dose often included in prenatal supplements on both your blood folate levels during pregnancy and the blood level of your baby as determined from your infants' cord blood at delivery. This new knowledge will help guide future decisions regarding the most appropriate dose of folic acid to recommend for prenatal patients. In addition, information regarding how nutrients from dietary sources are associated with nutritional status will provide new evidence for future guidance regarding prenatal dietary intake recommendations.

#### **Incentives for participation**

You will receive your prenatal supplements at no cost as part of the study protocol. Depending on gestational week at enrollment, you may receive prenatal supplements for up to eight months, representing a potential cost savings of up to \$240 (~ \$30 per month). The prenatal supplements will be packaged as four week supplies and will be provided for the duration of participation in the study. If you choose to withdraw from the study at any point or if you are withdrawn from the study without regard to your consent for circumstances as previously indicated, you will not be provided with additional supplements.

We will also provide you with a dietary intake analysis and information on your blood folate levels at various stages of pregnancy.

#### **Privacy/Confidentiality**

Every effort will be taken to protect your identity. No individually-identifiable information about you, or provided by you during the research, will be shared with others without your permission,

except if necessary to protect your rights or welfare (for example, if you are injured and need emergency care), or if required by law. Your participation results, which will include an assigned participant number, and your consent form will not be stored together. A separate list will be the only document linking your name and participant number, and it will be kept along with the consent forms in a locked file drawer, and accessed only by Dr. Bailey and her immediate research team. This list will be destroyed ten years from the end of the study. All other documents, including questionnaires, diet assessment forms and blood sample submission forms will only include your participant number.

This research includes testing for genetic differences that may influence individual response to folate supplementation. Any information obtained from this testing is related to research only, will not be used for diagnostic or therapeutic testing and will not be linked to any individually identifiable information. In the unlikely event that there is a violation in confidentiality, a recent federal law the Genetic Information Nondiscrimination Act (GINA) will help protect you from health insurance or employment discrimination based on genetic information potentially obtained through this research.

This study will be registered at ClinicalTrials.gov, a Web-based publically-available resource that provides patients, healthcare professionals and researchers with information on clinical trials or intervention studies in human volunteers. Study results submitted to this database will be in the form of summary information and will not include any individual data. You will not be identified in this or any other report or publication of this study.

#### **Taking part is voluntary**

Your involvement in the study is voluntary, and you may choose not to participate or to stop at any time without penalty or loss of benefits to which you are otherwise entitled. If you decide to discontinue or withdraw from the study or if the investigator decides to terminate your participation without regard to your consent, the information/data collected from or about you up to the point of withdrawal will be kept as part of the study and may continue to be analyzed, unless you ask to have information that can be identified as yours returned to you, removed from the research records, or destroyed. If you withdraw or are withdrawn from the study, you also have the right to ask for your specimens to be removed from the study and/or destroyed.

#### **If you are injured by this research**

The researchers will exercise all reasonable care to protect you from harm as a result of your participation. If you think that you have suffered a research-related injury, you should seek immediate medical attention and then contact Dr. Bailey right away at (706)-542-4256.

#### **Permission for photograph-taking:**

Please provide initials below if you consent for photography and subsequent use of your image for research-related purposes, such as presentations and publications related to this UGA research study. You may still participate in this study even if you are not willing to have your photograph taken.

\_\_\_\_\_ I am willing to have my photograph taken and used as described above  
\_\_\_\_\_ I do not want to have my photograph taken and/or used as described.

**Permission for contact by UGA research personnel, now and in the future:**

By signing my initials here, \_\_\_\_\_, I agree to allow the investigators of this study to contact me to obtain information required for the Study Specific Questionnaire as previously described.

By signing my initials here, \_\_\_\_\_, I agree to allow the investigators of this study to contact me in the future to request participation in future studies. I understand that at that time, I may refuse any further participation with no negative consequences.

My contact information is:

Telephone Number(s) \_\_\_\_\_ (home) \_\_\_\_\_ (cell)

Address: \_\_\_\_\_

Email: \_\_\_\_\_

**If you have questions**

The main researcher conducting this study is Dr. Lynn B. Bailey, a professor at the University of Georgia. Please ask any questions you have now. If you have questions later, you may contact Dr. Bailey at folate@uga.edu or at (706) 542-4256. If you have any questions or concerns regarding your rights as a research participant in this study, you may contact the Institutional Review Board (IRB) Chairperson at (706)-542-3199 or irb@uga.edu.

**Research Subject's Consent to Participate in Research:**

To voluntarily agree to take part in this study, you must sign on the line below. Your signature below indicates that you have read or had read to you this entire consent form, and have had all of your questions answered.

\_\_\_\_\_  
Name of Researcher

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Date

\_\_\_\_\_  
Name of Participant

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Date

Please sign both copies, keep one and return one to the researcher.



**UNIVERSIDAD DE GEORGIA**  
**CONSENTIMIENTO INFORMADO Y AUTORIZACION PRIVADA**  
**DE PARTICIPANTE DE INVESTIGACION**  
**Suplementación del Ácido Fólico en Mujeres Embarazadas:**  
**Respuesta a la Dosis**

**Declaración de la investigadora:**

Estamos pidiendo que usted participe en una investigación. Es importante que entienda porque se hace la investigación y que involucrará antes de que decide participar. Este formulario está diseñado para darle información sobre la investigación para que pueda decidir si quiere participar o no. La participación en esta investigación es voluntaria. Su decisión participar o no participar no afectará los servicios o el nivel de atención ofrecido en sus citas clínicas prenatales. Por favor, toma el tiempo para leer la información siguiente con cuidado. Por favor, pregunte al investigador si hay algo que no está claro o si necesita más información. Cuando todas sus preguntas han sido contestadas, Ud. puede decidir si quiere estar parte de la investigación o no. Este proceso se llama "consentimiento informado." Una copia de este formulario se le dará a usted.

**Investigadora Principal:** Dra. Lynn B. Bailey  
Departamento de la Nutrición y la Comida  
Teléfono: 706-542-4256  
Correo Electrónico: folate@uga.edu

**Propósito de la Investigación:**

El folato es una palabra general para una vitamina soluble en agua que es importante especialmente durante el embarazo. El ácido fólico es una forma de la vitamina que se usa en suplementos y comidas fortificadas. Suplementos prenatales de menudo contienen cantidades del ácido fólico muy diferentes aún no saben ni los científicos ni los profesionales médicos como ciertas cantidades del ácido fólico afecta a los niveles en la sangre de mujeres embarazadas o sus infantes al nacer. El propósito de esta investigación es determinar como los niveles del folato e indicadores relacionados en su sangre durante varios tiempos del embarazo y en la sangre de cordón de su bebé al nacer sean diferentes en respuesta a una de dos cantidades del ácido fólico en suplementos prenatales. Las dosis representan la Ración Dietética Recomendada (RDR) para mujeres embarazadas y una dosis más alta que se encuentra frecuentemente en los suplementos prenatales sin receta. Su participación en esta investigación ayudará proveer información importante y nueva que no solo informará a los científicos sino también ayudará guiar a los médicos los cuales recomiendan los suplementos prenatales a menudo.

**Elegibilidad**

Usted está calificado ser voluntario para la investigación si eres un paciente embarazado de Athens Regional Midwifery Clinic (Clínica de las Parteras de Athens Regional) y cumple Ud. otros requisitos los cuales incluyen el siguiente: (a) 18-40 años de edad; (b) peso de cuerpo normal a moderadamente obeso; (c) menos que doce semanas de embarazo; (d) llevando a solo un bebé; (e) sin historial médico de enfermedad crónica; (f) sin anemia; (g) no tomando ningún medicamento

recetado. La elegibilidad para la investigación se verificará con los requisitos anteriores y su disposición de completar los procedimientos del estudio y los cuestionarios. Se puede retirarse de la investigación sin tener en cuenta su consentimiento si se determinará que Ud. está llevando más que un bebé, si presenta con complicaciones de embarazo como diabetes gestacional o hipertensión, si no toma los suplementos prenatales como dirigido o completar otros procedimientos del estudio, o si suspende su cuidado prenatal por la Clínica de las Parteras de ARMC.

### **Procedimientos de la Investigación**

Si acepta Ud. participar, se le pedirá hacer los siguientes procedimientos del estudio:

**Colección de sangre**– Se recogerá la sangre durante sus citas prenatales preestablecidas en los mismos tiempos que lo hacen normalmente para los diagnósticos/ el monitoreo del estado del embarazo. No habrá pinchazos de aguja adicionales para fines de la investigación. En cada colección, un aguja pequeña se inserta en su vena y 30 mL (casi 6 cucharaditas) adicionales de sangre se recogerá para fines de la investigación. Se recogerá la sangre durante su visita prenatal inicial, en las visitas de 28 y 36 semanas de gestación y en el nacimiento y solo tomará unos pocos minutos. Esta sangre se usará para medir el folato de sangre y indicadores nutricionales y genéticos relacionados. Adicionalmente, después de nacer su bebé y cortar el cordón umbilical, un poco de sangre (5 mL/ 1 cucharadita) se recogerá del cordón umbilical para análisis de indicadores del estado de folato. Algunas de las medidas se hará por otros colaboradores en otras partes de los Estados Unidos. Los especímenes se enviará con un número de participante, y nuestros colaboradores no recibirán ninguna información que se puede usar para identificarle directamente a usted. Cualquiera información que se descubre de la examinación de esta sangre es solamente para la investigación, y no se usará para tratamiento o exámenes diagnósticos. Se guardará un poco de su sangre para análisis de indicadores metabólicos del folato posible en el futuro. Especímenes de sangre se guardará con un código numérico y su información personal no se asocia con su espécimen. El almacenamiento de su sangre para investigación adicional no es un requisito de esta investigación, y Ud. tiene la derecha pedir que todas sus especímenes serán eliminados/destruidos después de que termina la investigación principal. Cualquiera especímenes de sangre no usadas serán descartadas después de 10 años del final de la investigación, según manejo seguro de materiales peligrosos, definido por el protocolo de Seguridad de Materiales Peligrosos de la Universidad de Georgia.

**Protocolo de suplementación de vitaminas**– Como un participante en esta investigación, Ud. tomará suplementos prenatales que contienen una de dos dosis del ácido fólico. Una de estas dosis representa la RDR corriente para mujeres embarazadas y la otra dosis es más alta y se encuentra típicamente en suplementos prenatales. No hay riesgos conocidos de consumir las dosis del ácido fólico incluido en las vitaminas prenatales. Los dos suplementos contienen las mismas vitaminas, minerales, y DHA en cantidades normalmente encontrado en suplementos prenatales comerciales. La única diferencia en los dos suplementos es la cantidad de ácido fólico.

En su primera visita prenatal, se le proporcionará un suministro de 4 semanas de las vitaminas prenatales incluyendo una de dos dosis del ácido fólico, una pastilla de multi-vitamina/minerales, y un suplemento de DHA (un nutritivo importante para el desarrollo cerebral). Los suplementos serán empaquetados en suministros diarios y usted tomará los suplementos cada día durante su embarazo hasta el parto. Tomará cada tableta para cada día al mismo tiempo cada día, preferiblemente con la cena. Para asegurar que



recuerda tomar los suplementos, se le indicará usar un calendario de conformidad y tal vez recibirá llamadas o mensajes de 'texto' como un recordatorio del personal de la investigación. Se le pedirá devolver sus contenedores de pastillas y cualquieras pastillas no tomadas en su visita próxima. Recibirá un suministro nuevo de suplementos cada cuatro semanas hasta el final de su embarazo. Se le pedirá seguir su dieta normal y abstener de tomar otros suplementos de dieta, multi-vitaminas, o productos de cereal muy fortificados (conteniendo  $\geq 100\%$  la RDR para el folato).

Registros médicos – Información con respecto a su edad, etnicidad, historia clínica, hallazgos del examen físico, y resultados del examen de sangre se obtendrá de sus registros en ARMC para determinar el efecto de estos factores en su reacción a la suplementación del ácido fólico. También se obtendrá información con respecto a la fecha y manera de parto, edad gestacional, género, medidas, la calificación de Apgar, y resultados de examinación de sangre de su niño para determinar el efecto de la suplementación del ácido fólico en el desarrollo y crecimiento de su hijo.

La ley de la vida privada, la Ley de "Portabilidad" y Responsabilidad del Seguro Médico (HIPAA), protege su información de la salud. Investigadores pueden usar o revelar información protegida solamente si han recibido su autorización que La clínica de las Parteras de ARMC puede revelar su información. Los investigadores protegerán esta información por usarla solo como ya está permitida con esta autorización y como dirigida por las leyes del estado y las leyes federales. Si tiene cualquieras preguntas y/o quiere revocar esta autorización al escribir en cualquier momento, puede contactar a Dra. Lynn B. Bailey (ve la página 1). Esta autorización expira diez años después de que termina esta investigación. Si decide participar en esta investigación, hay que firmar este formulario para que se puede usar su información para la investigación. Su decisión dar o no dar a conocer esta información no afectará los servicios que recibes ahora o en el futuro de la clínica de las parteras de ARMC; sin embargo, si no le da permiso, no podrá participar en esta investigación. La información ya descrito puede ser revelado para el uso en otros proyectos sobre el embarazo, nutrición, y la salud infantil. Aún esta revelación no sea protegida por esta autorización, la revelación de su información identificable de la salud solamente sería a los investigadores quienes son miembros de este equipo de investigación y que obtengan su consentimiento escrito para su participación en estos proyectos.

Cuestionario específicamente para el estudio – Para obtener información adicional no contenido en los registros médicos, usted será contactada por teléfono y le pedirá cumplir un cuestionario pequeño. Las preguntas incluirán información con respecto a su uso actual y anterior de suplementos del ácido fólico, su consumo usual de cereales y otras comidas fortificadas con el ácido fólico, hábitos de fumar y tomar alcohol actuales y anteriores, y otros factores del estilo de vida. Este cuestionario se administrará por el personal de investigación de la Universidad de Georgia. La entrevista de teléfono debe tomar 15 minutos o menos.

Los diarios de comida/ Recordatorios de dieta– Su ingesta dietética usual en las varias etapas del embarazo se estimará por el uso de diarios de comida y un programa de recordatorios de dieta basada en la computadora. Se le proporcionará con unos formularios de Recordatorios de Dieta de Tres Días en los cuales recordará las comidas

que consume Ud. en el formato del diario de comida para días asignados en 24 y 36 semanas de gestación. Estos registros se devolverán por correo electrónico o normal al personal del UGA. Después de recibir los diarios de comida, personal capacitado le contactará por teléfono para obtener información adicional y más específica como nombres de marca y las cantidades consumidas. El personal de investigación pondrá la información del diario de comida y la entrevista siguiente en una programa en línea para análisis subsiguiente. Debe tomar una hora en cada de los dos puntos de colección para recordar toda su información en los diarios de comida y para la entrevista, aproximadamente dos horas en total.

#### **Riesgos y molestias**

- **Extracción de sangre:** Se le extraerá sangre para los propósitos de esta investigación solamente cuando ya la están extrayendo en la clínica como parte de su cuidado normal. No hay riesgo adicional de colectar sangre extra para los usos de investigación.
- **Cuestionarios/Recordatorios de la dieta:** La molestia o estrés que puede enfrentar durante esta investigación puede ser asociada con la revelación de información sobre su ingesta dietética e historia clínica; sin embargo es importante compartir esta información para que su estado de salud y nutrición puede ser evaluado apropiadamente. Toda la información que es individualmente identificable será mantenido estrictamente confidencial y su nombre y otra información personal será guardado bajo llave, no aparecerá en archivos de datos del proyecto, y no estarán compartidos con otras personas.

#### **Beneficios**

La información proporcionado por esta investigación ayudará a los investigadores avanzar su conocimiento sobre como cantidades diferentes del ácido fólico prenatal afecta folato de sangre y otros indicadores del estado nutricional de mujeres embarazadas. La investigación proveerá datos que informarán a los médicos según el impacto en su folato de sangre durante el embarazo y el nivel de folato de sangre de su bebé después del parto (según la sangre del cordón umbilical) de la RDR actual del ácido fólico comparado a un dosis mas alto normalmente encontrado en suplementos prenatales comerciales. Este conocimiento nuevo guiará decisiones futuras sobre el dosis más apropiado para pacientes prenatales. Adicionalmente, información sobre como los nutritivos de fuentes dietéticas afectan al estado nutricional proveerá evidencia nueva para dirección futura sobre recomendaciones de ingesta dietética prenatal.

#### **Incentivos de participación**

Recibirá usted sus suplementos prenatales gratis por ser parte del estudio. Tal vez recibirá suplementos prenatales por 8 meses, dependiente en su semana de gestación al inscribirse en la investigación. Esto representa un ahorro potencial de \$240 (~\$30 al mes). Los suplementos prenatales serán empaquetados como suministros de cuatro semanas y se proveerán para la duración entera de su participación en el estudio. Si quiere retirarse de la investigación en cualquier momento o si está retirado sin respecto a su consentimiento para las razones indicadas previamente, no se le proporcionará con suplementos adicionales.

También se le proporcionará con un análisis de ingesta dietética e información de sus niveles del folato de sangre en varias etapas del embarazo.



### **Privacidad/Confidencialidad**

Se hará todo lo posible para proteger su identidad. Ninguna información individualmente identificable sobre usted, o provecho por Ud. durante la investigación, se compartirá sin su permiso, a menos que sea necesario para proteger sus derechos o su bienestar (por ejemplo, si está herida y necesita cuidado de emergencia), o si requerido por la ley. Sus resultados de participación, los cuales incluirán un número de participante asignado, y su formulario de consentimiento no se guardarán juntos. Una lista separada será el único documento que enlace su nombre y número de participación, y se guardará con los documentos de consentimiento en un cajón de archivo bloqueado; a lo cual solamente Dra. Bailey y su equipo de investigación tendrá acceso. Esta lista se destruirá diez años después de que termina la investigación. Todos los otros documentos, incluyendo los cuestionarios, formularios de dieta, y formularios de envío de muestras de sangre solamente incluirán su número de participante.

Esta investigación incluye una examinación de diferencias genéticas que pueden afectar la reacción individual a la suplementación del folato. Cualquiera información que sea obtenida por esta examinación se relaciona solamente con la investigación, y así no será usado para exámenes diagnósticos ni terapéuticos y no serán relacionados con ninguna información individualmente identificable. En el caso improbable de que haya una violación de confidencialidad, una ley federal, el Acto de No Discriminación de Información Genética (GINA) le protegerá de la discriminación en su trabajo o del seguro de salud basada en información posiblemente obtenida por esta investigación.

Este estudio será registrado en ClinicalTrials.gov, un recurso público de internet el cual provee información sobre estudios intervencionistas y ensayos clínicos a los pacientes y profesionales de la salud. Los resultados de este estudio que se presentarán a esta base de datos serán en forma resumida y no incluirán datos individuales. No se le identificará a usted en este o cualquier otro informe o publicación de este estudio.

### **Participar es voluntario**

Su participación en la investigación es voluntaria, y puede decidir no participar o retirar en cualquier momento sin penalización o pérdida de beneficios a los cuales usted tiene derecho. Si decide retirar del estudio o si el investigador decide terminar su participación sin respecto a su consentimiento, la información/los datos recopilados de usted hasta el punto de retiro se mantendrá como parte de la investigación y pueden ser analizados a menos que usted pide que la información suya se devolverá a Ud., quitada de los registros de investigación, o eliminada. Si retira o está retirada del estudio, tiene el derecho de pedir que las especímenes suyas sean eliminadas del estudio o destruidas.

### **Si le causa daño esta investigación**

Los investigadores harán todo lo posible y razonable para protegerle del daño como resultado de su participación. Si piensa Ud. que a sufrido un daño relacionado a la investigación, debe buscar atención médica inmediatamente, y después llama a Dra. Bailey (706)-542-4256.

### **Permiso para sacar fotos:**

Por favor, firme sus iniciales abajo si Ud. da su consentimiento de ser fotografiada y el uso después de su imagen para usos relacionados a la investigación, como presentaciones o publicaciones

relacionadas con esta investigación de UGA. Puede participar en el estudio aún si no quiere ser fotografiada.

\_\_\_\_\_ Doy mi consentimiento ser fotografiada y que mi imagen será usada como ya descrito.  
\_\_\_\_\_ No quiero que saquen mi foto ni que la usen como ya descrito.

**Permiso para el contacto por el personal de investigación de UGA, ahora y en el futuro:**  
Con mi firma de iniciales aquí, \_\_\_\_\_, permito que me pueden contactar los investigadores de este estudio para obtener información necesario para el Cuestionario Específicamente para el Estudio como ya descrito.

Con mi firma de iniciales aquí, \_\_\_\_\_, les permito a los investigadores de este estudio contactarme en el futuro para solicitar mi participación en estudios futuros. Entiendo que en aquel momento, puedo negar participación adicional sin consecuencias negativas.

Mi información de contacto es:

Número(s) de teléfono: \_\_\_\_\_ (Casa) \_\_\_\_\_ (Móvil)

Dirección: \_\_\_\_\_

Correo Electrónico: \_\_\_\_\_

**Si tiene preguntas:**

La investigadora principal es Dra. Lynn B. Bailey, una profesora en la Universidad de Georgia. Por favor, haga cualquieras preguntas ahora. Si tiene preguntas luego, puede contactar a Dra. Bailey por correo electrónico en [folate@uga.edu](mailto:folate@uga.edu) o por teléfono en (706)-542-4256. Si tiene cualquiera pregunta sobre sus derechos como un participante en esta investigación, puede contactar al presidente de la Junta de Revisión Institucional (IRB) por teléfono (706)-542-3199 o correo electrónico: [irb@uga.edu](mailto:irb@uga.edu).

**Consentimiento del Sujeto de Investigación a Participar en la Investigación:**

Para acordar voluntariamente a participar en este estudio, tiene que firmar en la línea abajo. Su firma indica que ha leído este formulario de consentimiento entero, o que ha sido leído para usted, y que todas sus preguntas han sido contestadas.

\_\_\_\_\_  
Nombre del Investigador

\_\_\_\_\_  
Firma

\_\_\_\_\_  
Fecha

\_\_\_\_\_  
Nombre de Participante

\_\_\_\_\_  
Firma

\_\_\_\_\_  
Fecha

Por favor, firme las dos copias, guarde una y devuelva la otra al investigador.

## **APPENDIX C**

### **SIGNED PARTICIPANT WELCOME LETTER**



College of Family and Consumer Sciences  
Department of Food and Nutrition

Welcome to the Folic Acid Supplementation in Pregnancy Study!!

Thank you so much for choosing to participate in this important study which will determine how levels of folate and related indicators in maternal blood at different times during pregnancy and in cord blood at birth differ in response to two different amounts of folic acid in prenatal supplements. Your participation in this study will help provide important new information that will not only inform scientists but will also help guide clinicians who routinely recommend prenatal supplements. Some important points regarding the study are outlined below:

- ❖ You have received a six week supply of supplements - packaged as individual day supply
  - ❖ take all **three tablets every day, at one time** – preferably in the evening
  - ❖ if you take the pills in the morning, **do not take on the morning of return visits to the clinic**
  - ❖ **return the pillbox and any unused pills at each visit.** they will be replaced with new supply
- ❖ Please follow your typical diet, but refrain from other dietary supplements, multivitamins and highly fortified cereal products (containing  $\geq 100\%$  the RDA for folate)
- ❖ Information on your Health Behaviors is an important part of the study. This information will be obtained by means of short questionnaire which should take less than 15 min to complete.  
**Our students will be contacting you soon.** If you cannot complete the questionnaire at the time of the call, please arrange a more convenient time. If you prefer, we can send the questionnaire by email for you to complete at your convenience.
- ❖ You have also been asked to complete two Three Day Dietary Recalls. An instruction sheet, recall forms and a stamped return envelope for the first set of recalls is included in your folder. These will be completed at 24 and 36 weeks of pregnancy. Students will call with a reminder prior to these time points.
- ❖ If you have any questions about the study, please feel free to contact us:

Dr. Hea Jin Park      706-542-5093  
Dr. Dorothy Hausman   706-542-4871  
Dr. Lynn Bailey      706-542-4256



On behalf of the UGA Folate Research Team, thank you again for your participation!

Lynn B. Bailey, PhD  
Principal Investigator

Dawson Hall • Athens, Georgia 30602-3623 • Telephone (706) 542-4869 • Fax (706) 542-5059  
An Equal Opportunity/Affirmative Action Institution



## **APPENDIX D**

### **PARTICIPANT REMINDER CARD**

### Folic Acid Supplementation in Pregnancy Study

#### Important Reminders:

- ❖ The supplements are packaged as individual day supply
- ❖ Take all **three tablets every day, at one time** – preferably in the evening
- ❖ If you take the pills in the morning, **do not take on the morning of clinic visits**
- ❖ **Return all pillboxes and any unused pills at each visit**, they will be replaced with a new supply
  
- ❖ Please follow your typical diet, but refrain from other dietary supplements, multivitamins and highly fortified cereal products (containing  $\geq 100\%$  the RDA for folate)

**APPENDIX E**

**HEALTH BEHAVIOR QUESTIONNAIRES**

## **Folic Acid Supplementation in Pregnancy: Health Behavior Questionnaire**

Date: \_\_\_\_\_ Time: \_\_\_\_\_ Telephone interview completed by: \_\_\_\_\_

Participant number: \_\_\_\_\_

- Have you been taking your prenatal supplements every day as directed? \_\_\_\_Yes  
\_\_\_\_No
- Have you experienced any problems with the supplements? \_\_\_\_Yes \_\_\_\_ No
  - If yes, what? \_\_\_\_\_
- Do you have any questions about the supplements or other aspects of the study?
- Before enrolling in this study, had you heard of folic acid? \_\_\_\_Yes \_\_\_\_ No
  - If yes, how? \_\_\_\_magazine/newspaper/internet \_\_\_\_ radio/TV \_\_\_\_  
schooling \_\_\_\_ doctor/nurse/health professional \_\_\_\_  
family/friends \_\_\_\_ other
- Did you take a multivitamin during the month(s) just before you got pregnant?
  - \_\_\_\_Yes \_\_\_\_ No
  - If yes, what brand? \_\_\_\_\_
  - How often? \_\_\_\_1- 3 times/wk \_\_\_\_4 - 6 times/wk \_\_\_\_every day
  - If no, why didn't you take vitamins? \_\_\_\_didn't think I needed \_\_\_\_too  
expensive \_\_\_\_vitamins gave me side effects \_\_\_\_ did not plan to get  
pregnant \_\_\_\_other
- Did you take a supplement that just contained folic acid during the month(s) just  
before you got pregnant?
  - \_\_\_\_Yes \_\_\_\_ No
  - If yes, why did you take it? \_\_\_\_\_

- How often? \_\_\_\_ 1- 3 times/wk \_\_\_\_ 4 - 6 times/wk \_\_\_\_ every day
- If no, why didn't you folic acid? \_\_\_\_ didn't think I needed \_\_\_\_ too expensive \_\_\_\_ supplements five me side effects \_\_\_\_ did not plan to get pregnant \_\_\_\_ other
- After finding out you were pregnant or may be pregnant and before receiving the supplements from the clinic, did you take any multivitamins or prenatal vitamins?  
\_\_\_\_ Yes \_\_\_\_ No
  - If yes, what brand? \_\_\_\_\_
  - For what period of time? \_\_\_\_\_
  - How often? \_\_\_\_\_
- After finding out you were pregnant or may be pregnant and before receiving the supplements from the clinic, did you take a supplement that just contained folic acid? \_\_\_\_ Yes \_\_\_\_ No
  - If yes, what brand or amount? \_\_\_\_\_
  - For what period of time? \_\_\_\_\_
  - How often? \_\_\_\_\_
- Did you take any dietary or herbal supplement other than multivitamins or folic acid at any time just before or during this pregnancy?
  - \_\_\_\_ Yes \_\_\_\_ No
  - If yes, what kind of supplements, what brand & how often?  
\_\_\_\_\_  
\_\_\_\_\_
- Do you regularly consume any of the following?
  - Ready-to-eat breakfast cereal \_\_\_\_ Yes \_\_\_\_ No \_\_\_\_ Brand \_\_\_\_ times/wk
  - Meal replacement drinks/bars \_\_\_\_ Yes \_\_\_\_ No \_\_\_\_ Brand \_\_\_\_ times/wk
  - Energy drinks \_\_\_\_ Yes \_\_\_\_ No \_\_\_\_ Brand \_\_\_\_ times/wk
  - Protein shakes \_\_\_\_ Yes \_\_\_\_ No \_\_\_\_ Brand \_\_\_\_ times/wk
  - Snack bars \_\_\_\_ Yes \_\_\_\_ No \_\_\_\_ Brand \_\_\_\_ times/wk
  - Spinach, kale or other leafy greens \_\_\_\_ Yes \_\_\_\_ No \_\_\_\_ times/wk
  - Orange juice \_\_\_\_ Yes \_\_\_\_ No \_\_\_\_ Brand \_\_\_\_ times/wk

- In the past, did you ever smoke? \_\_\_\_\_Yes \_\_\_\_\_No  
If yes, for how long? \_\_\_\_\_When did you quit smoking? \_\_\_\_\_
- In the past, did you ever regularly drink more than one serving of alcoholic beverages a day?
  - \_\_\_\_\_Yes \_\_\_\_\_No
  - If yes, how often do you drink 2 or more alcoholic beverages a day? \_\_\_\_\_
- Do you currently drink more than one serving of alcoholic beverages a day?
  - \_\_\_\_\_Yes \_\_\_\_\_No
  - If yes, how often do you drink 2 or more alcoholic beverages a day? \_\_\_\_\_
- Over the past week, about how much time did you spend engaging in physical activities (exercise, walking, gardening, vacuuming, etc)?  
\_\_\_\_\_ (hrs/min) per (day/wk)
- Were your activities spent \_\_\_\_ mainly indoors \_\_\_\_ mainly outdoors \_\_\_\_ half indoors & half outdoors
- Was this similar level of physical activity similar to that before your pregnancy?  
\_\_\_\_\_ Yes \_\_\_\_\_No, more active before pregnancy \_\_\_\_\_No, more active now
- Over the past month, how much time did you usually spend outside each day between sunrise & sunset? \_\_\_\_\_
- Is this amount of time spent outdoors in the sun fairly typical for you at this time of year?  
\_\_\_\_\_yes \_\_\_\_\_ no
- When outside in the sun, did you usually wear a hat, sunscreen or other sun protection? \_\_\_\_\_yes \_\_\_\_\_ no

## Suplementación del Ácido Fólico durante el Embarazo: Cuestionario del Comportamiento de Salud

Fecha: \_\_\_\_\_ Tiempo: \_\_\_\_\_ Entrevista de teléfono hecho por: \_\_\_\_\_

Número de participante: \_\_\_\_\_

- ¿Ha tomado sus suplementos prenatales cada día como dirigida?? \_\_\_\_Sí \_\_\_\_No
- ¿Ha tenido una problema con los suplementos? \_\_\_\_Sí \_\_\_\_ No
  - ¿Cuáles? \_\_\_\_\_
- ¿Tiene unas preguntas sobre los suplementos o otros aspectos del estudio?
- ¿Antes de inscribirse en este estudio, había oído del ácido fólico? \_\_\_\_Sí \_\_\_\_ No
  - ¿Cómo? \_\_\_\_revista/periodico/internet \_\_\_\_ radio/Televisión \_\_\_\_ escuela  
\_\_\_\_ médico/enfermera/profesional de salud \_\_\_\_ familia/amigo  
\_\_\_\_ otro
- ¿Tomaba una vitamina durante el mes antes de la concepción de su hijo?
  - \_\_\_\_Sí \_\_\_\_ No
  - ¿Cuál marca? \_\_\_\_\_
  - ¿Con que frecuencia? \_\_\_\_1- 3 veces a la semana \_\_\_\_4 - 6 veces a la semana \_\_\_\_cada día
  - Si contestó “no”, ¿por qué no tomaba vitaminas? \_\_\_\_no pensaba que era necesario \_\_\_\_demasiado caro \_\_\_\_las vitaminas me causaban efectos negativos \_\_\_\_ no intenté ser embarazada \_\_\_\_otro
- ¿Tomaba un suplemento del ácido fólico durante el mes antes de la concepción de su hijo?
  - \_\_\_\_Sí \_\_\_\_ No
  - ¿Cuál marca? \_\_\_\_\_

- ¿Con que frecuencia? \_\_\_\_ 1- 3 veces a la semana \_\_\_\_ 4 - 6 veces a la semana \_\_\_\_ cada día
- Si contestó “no”, ¿por qué no tomaba el ácido fólico? \_\_\_\_ no pensaba que era necesario \_\_\_\_ demasiado caro \_\_\_\_ me causaba efectos negativos \_\_\_\_ no intenté ser embarazada \_\_\_\_ otro
- Después de realizar que estaba embarazada y antes de recibir los suplementos de la clínica, ¿tomabas unas vitaminas o suplementos prenatales? \_\_\_\_ Sí \_\_\_\_ No
  - ¿Cuál marca? \_\_\_\_\_
  - ¿Qué período de tiempo? \_\_\_\_\_
  - ¿Con qué frecuencia? \_\_\_\_\_
- Después de realizar que estaba embarazada y antes de recibir los suplementos de la clínica, ¿tomabas un suplemento que solo fue ácido fólico? \_\_\_\_ Sí \_\_\_\_ No
  - ¿Cuál marca? \_\_\_\_\_
  - ¿Qué período de tiempo? \_\_\_\_\_
  - ¿Con qué frecuencia? \_\_\_\_\_
- ¿Tomaste un suplemento dietético o herbal que no fue vitaminas o el ácido fólico en cualquier momento durante o antes de este embarazo?
  - \_\_\_\_ Sí \_\_\_\_ No
  - ¿Cuáles tipos, Cuál marca, y con qué frecuencia? \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_
- ¿Consume normalmente unos de los siguientes?
  - Cereal del desayuno \_\_\_\_ Sí \_\_\_\_ No \_\_\_\_ Marca \_\_\_\_ veces/sem
  - Bebidas o barras de reemplazo de comida \_\_\_\_ Sí \_\_\_\_ No \_\_\_\_ Marca \_\_\_\_ veces/sem
  - Bebidas de energía \_\_\_\_ Sí \_\_\_\_ No \_\_\_\_ Marca \_\_\_\_ veces/sem
  - Batido de proteína \_\_\_\_ Sí \_\_\_\_ No \_\_\_\_ Marca \_\_\_\_ veces/sem
  - Bar (de bocadillo) \_\_\_\_ Sí \_\_\_\_ No \_\_\_\_ Marca \_\_\_\_ veces/sem
  - Espinacas, col rizada, otras verduras de hoja verde \_\_\_\_ Sí \_\_\_\_ No \_\_\_\_ veces/sem
  - Jugo de naranja \_\_\_\_ Sí \_\_\_\_ No \_\_\_\_ Marca \_\_\_\_ veces/sem



- ¿En el pasado, jamás ha fumado? \_\_\_\_\_Sí \_\_\_\_\_No  
 ¿Por cuánto tiempo?\_\_\_\_\_¿Cuándo lo dejó?\_\_\_\_\_
  
- ¿En el pasado, jamás tomaba más que una bebida alcohólica al día?
  - \_\_\_\_\_Sí \_\_\_\_\_No
  - ¿Con qué frecuencia tomabas 2 o más bebidas alcohólicas en un día?  
 \_\_\_\_\_
  
- ¿Ahora toma más que una bebida alcohólica al día?
  - \_\_\_\_\_Sí \_\_\_\_\_No
  - ¿Con qué frecuencia tomas 2 o más bebidas alcohólicas al día? \_\_\_\_\_
  
- ¿En la semana pasada, cuánto tiempo pasaba Ud. haciendo actividades físicas (ejercicio, caminando, haciendo quehaceres, etc)? \_\_\_\_\_min/día o  
 \_\_\_\_\_ horas/semana
  
- ¿Fueron estas actividades (elige uno): \_\_\_ adentro \_\_\_ afuera \_\_\_ ½ adentro y  
 ½ afuera?
  
- ¿Esto es un nivel de actividad similar a la suya antes del embarazo? \_\_\_\_\_ Sí  
 \_\_\_\_\_No, fue más activa antes \_\_\_\_\_No, soy más activa ahora
  
- En el mes pasado, cuánto tiempo usualmente pasaba afuera entre la salida y la  
 puesta del sol? \_\_\_\_\_
  
- ¿Es esta cantidad típica para Ud. en este estación del año?  
 \_\_\_\_\_Sí \_\_\_\_\_no
  
- ¿Cuándo pasa Ud. tiempo afuera, usualmente usaba una gorra o protección del  
 sol? \_\_\_\_\_sí \_\_\_\_\_no

## **APPENDIX F**

### **THREE DAY RECALL FORMS**

# THREE DAY DIET RECALL:

## FOLIC ACID SUPPLEMENTATION IN PREGNANCY STUDY

ID# \_\_\_\_\_

### Instructions:

- 1) Please use the attached sheets to record all that you eat for three days during the week of \_\_\_\_\_.
- 2) You will receive a reminder from the research staff when it is time to complete the forms
- 3) Record the information for foods eaten during three 24 hour periods on non-consecutive days, including one weekend day - indicate the date of the recall and day of the week on each sheet
- 4) Use a separate line for each food item. Form can be handwritten – no need to type
- 5) Indicate the time and place (home, work, restaurant, etc.) that the food was eaten and whether it was a snack or part of a meal. (see sheet 1 for a few examples)
- 6) List the food item and approximately how much of it you ate (cups, pieces, etc.).
- 7) Record details, when appropriate, for each food item, including:
  - cooking method (grilled, baked, fried, etc)
  - brand name
  - condiments added (ketchup, salad dressing, butter, etc.)
- 8) Please answer the general questions related to physical and outdoor activities below.
- 9) When the recall sheets for all three days have been completed, please return all four pages (instruction page and three recall pages) to the UGA Folate Team using the self-addressed stamped envelope.
- 10) For questions or assistance please contact: The Folate Research Lab at 706-542-7689

### Health Behavior Questionnaire – follow-up:

- 1) Have you been taking your prenatal supplements every day as directed? \_\_\_\_ Yes  
\_\_\_\_ No
- 2) Have you experienced any problems with the supplements? \_\_\_\_ Yes \_\_\_\_ No  
If yes, what? \_\_\_\_\_

- 3) Over the past week, about how much time did you spend engaging in physical activities (exercise, walking, gardening, vacuuming, etc)? \_\_\_\_\_ min/day or \_\_\_\_\_ hrs/wk
- 4) Were your activities spent (check one): \_\_\_ mainly indoors \_\_\_ mainly outdoors \_\_\_ half indoors & half outdoors
- 5) Over the past month, how much time did you usually spend outside each day between sunrise & sunset? \_\_\_\_\_
- 6) When outside in the sun, did you usually wear a hat, sunscreen or other sun protection? \_\_\_\_\_yes \_\_\_\_\_ no

### THREE DAY DIET RECALL:

## FOLIC ACID SUPPLEMENTATION IN PREGNANCY STUDY

ID# \_\_\_\_\_

Day 1 date: \_\_\_\_\_ Mon    Tue    Wed    Thurs    Fri    Sat    Sun

[illegible]

### THREE DAY DIET RECALL:

## FOLIC ACID SUPPLEMENTATION IN PREGNANCY STUDY

ID# \_\_\_\_\_

Day 2 date: \_\_\_\_\_

Mon

Tue

Wed

Thurs

Fri

Sat

Sun

[illegible]

## FOLIC ACID SUPPLEMENTATION IN PREGNANCY STUDY

ID# \_\_\_\_\_

Day 3 date: \_\_\_\_\_

Mon

Tue

Wed

Thurs

Fri

Sat

Sun

[illegible]

# RECORDATORIO DE DIETA DE 3 DIAS:

## ESTUDIO DE LA SUPLEMENTACIÓN DEL FOLATO DURANTE EL EMBARAZO

ID# \_\_\_\_\_

### Instrucciones:

- 8) Por favor, usa los formularios adjuntos para recordar todo lo que come Ud. para tres días durante la semana de \_\_\_\_\_.
- 9) Recibirá un recuerdo del personal de la investigación cuando el tiempo de llenar el formulario llega.
- 10) Escribe la información para las comidas consumidas durante tres periodos de 24 horas, en días no consecutivos, incluyendo un día de fin de semana- indica la fecha del recordatorio y cual día de la semana en cada hoja.
- 11) Usa una línea diferente para cada ítem de comida. El formulario puede ser escrito con mano- no es necesario escribir a máquina.
- 12) Indica el tiempo y lugar (casa, trabajo, restaurante, etc.) que se comió la comida y si fue parte del desayuno, el almuerzo, la cena, o un bocadillo. (Hay unos ejemplos en página 1)
- 13) Escribe la comida y aproximadamente cuanto comió Ud. (tazas, pedazos, etc.)
- 14) Escribe detalles, cuando son a propósito, para cada comida, incluyendo:
  - método de cocinar (a la parrilla, de horno, frito, etc.)
  - nombre de la marca
  - condimentos usados (salsa de tomate, mantequilla, salsas, etc.)
- 8) Por favor, conteste las preguntas generales abajo sobre actividades físicas y afueras.
- 9) Cuando los formularios para cada de los tres días están cumplidos, por favor devuélvalos (4 páginas, página de instrucciones y tres recordatorios) al Equipo del Folato de UGA usando el sobre con sello que ya tiene dirección.
- 10) Si tiene preguntas o necesita ayuda, por favor llama:  
El laboratorio de Investigación del Folato 706-542-7689

### Cuestionario del Comportamiento de Salud- seguimiento:

- 7) ¿Ud. ha tomado sus suplementos prenatales cada día como dirigido? \_\_\_\_\_Sí  
\_\_\_\_\_No
- 8) ¿Ha tenido una problema con los suplementos? \_\_\_\_\_Sí \_\_\_\_\_ No



¿Cuáles? \_\_\_\_\_

- 9) ¿En la semana pasada, cuánto tiempo pasaba Ud. haciendo actividades físicas (ejercicio, caminando, haciendo quehaceres, etc)? \_\_\_\_\_ min/día o \_\_\_\_\_ horas/semana
- 10) ¿Fueron estas actividades (elige uno): \_\_\_\_ adentro \_\_\_\_ afuera \_\_\_\_ ½ adentro y ½ afuera?
- 11) ¿En el mes pasado, cuánto tiempo usualmente pasaba afuera entre la salida y la puesta del sol? \_\_\_\_\_
- 12) ¿Cuando pasaba Ud. tiempo afuera, usualmente usaba una gorra o protección del sol? \_\_\_\_ sí \_\_\_\_ no

# RECORDATORIO DE DIETA DE 3 DIAS: ESTUDIO DE LA SUPLEMENTACIÓN DEL FOLATO DURANTE EL EMBARAZO

ID# \_\_\_\_\_

Fecha de día 1: \_\_\_\_\_ LVn Mar mie jue vie sab dom

[illegible]

# RECORDATORIO DE DIETA DE 3 DIAS: ESTUDIO DE LA SUPLEMENTACIÓN DEL FOLATO DURANTE EL EMBARAZO

ID# \_\_\_\_\_

Fecha de día 2: \_\_\_\_\_

LVn

Mar

mie

jue

vie

sab

dom

[illegible]

# RECORDATORIO DE DIETA DE 3 DIAS: ESTUDIO DE LA SUPLEMENTACIÓN DEL FOLATO DURANTE EL EMBARAZO

ID# \_\_\_\_\_

Fecha de día 3: \_\_\_\_\_

$$LU_n$$

Mar

mie

jue

vie

sab

dom

[illegible]

## **APPENDIX G**

### **ASA24 INSTRUCTIONS**



Your usual dietary intake and intake of specific nutrients, including folate, will be estimated using the multi-pass Automated Self-administered 24-hour Recall (ASA24™) system hosted through the National Cancer Institute website. This methodology uses multiple probes to capture types and amounts of foods eaten, time and occasion of eating, and additional details related to preparation methods and additions such as condiments. Information from three non-consecutive days including one weekend day is generally required to provide an indication of ‘typical intake’.

Log-in information for the program is provided below. When you log-in you will be asked to supply information about all the food that you ate the previous day (e.g. Monday if log-in is on Tuesday). Once you log-in, you will have until the end of that day to complete the recall – you can come back to it if you are interrupted. You will be asked to complete two sets of dietary recalls – **one at 24 weeks of gestation and one at 32 weeks**. Study personnel will contact you when it is time to complete each set of recalls. For each set of dietary recalls, you will supply information for three days (non-consecutive) including one weekend day. [You will need to log-in separately to complete each days recall.]

**Log-in information:**

**Website:** <https://asa24.nci.nih.gov>

**Username:** FAPREGXX

**Password:** xxxxxxxxx

If you experience problems logging in or using the program please contact:

Folate Research Team: [folate13@uga.edu](mailto:folate13@uga.edu) or 706-542-7689

Dr. Dorothy Hausman: [dhausman@uga.edu](mailto:dhausman@uga.edu) or 706-542-4871

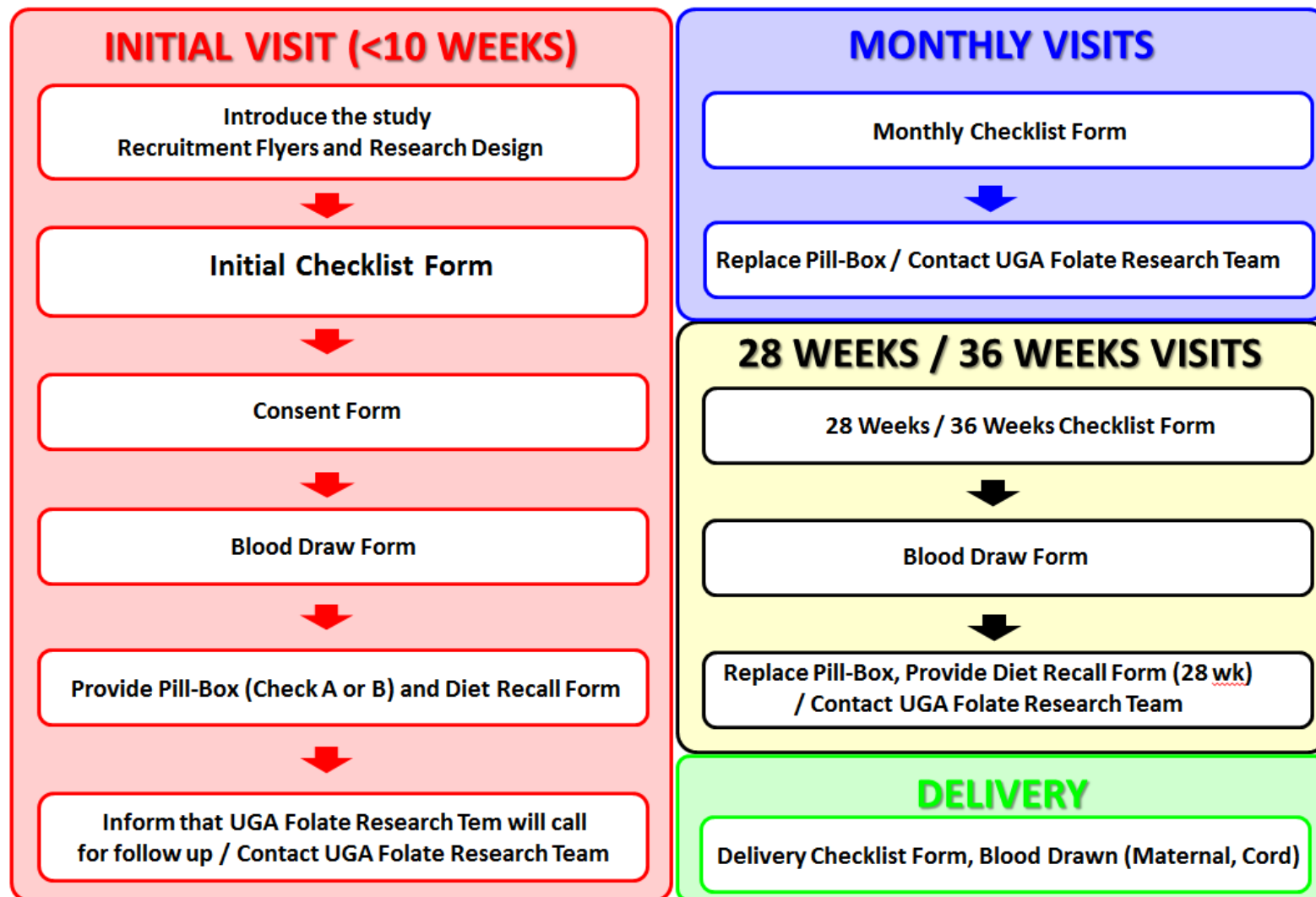
A summary of your dietary intake information will be sent upon completion of the study.

## **APPENDIX H**

### **FLOW CHART FOR CHECKLISTS AND SUPPLEMENTS**

# Folic Acid Supplementation in Pregnancy: Dose Response

Athens Regional Midwifery Clinic & University of Georgia (706-542-7689, folate13@uga.edu)





## **APPENDIX I**

### **BLOOD SAMPLE COLLECTION FORM**

**Folic Acid Supplementation in Pregnancy Study: Blood Sample Collection & Transmittal Form**

Folic Acid Study ID # **FAP #101** 1<sup>st</sup> visit 28 wk 36 wk Delivery

**TIME OF LAST MEAL/SNACK:** \_\_\_\_\_ **Birthdate** \_\_\_\_\_

**Blood Drawn:** Date: \_\_\_\_\_ Time: \_\_\_\_\_

Lavender-top \_\_\_\_ (1 x 10 ml) \_\_\_\_ Mix well; Wrap in foil; Place in refrigerator/cooler

Lavender-top \_\_\_\_ (1 x 10 ml) \_\_\_\_ Mix well; Wrap in foil; Place in refrigerator/cooler

Red-top \_\_\_\_ (1 x 9 ml) \_\_\_\_ Mix well; Wrap in foil; Place in refrigerator/cooler

Comments: \_\_\_\_\_

Midwife / Phlebotomist: \_\_\_\_\_

Delivered to: \_\_\_\_\_ Time: \_\_\_\_\_

Please call **Folate Research Team (706-247-4244 or 706-247-4381)** for sample pick-up.

**Folic Acid Supplementation in Pregnancy Study: Blood Sample Collection & Transmittal Form**

Folic Acid Study ID # **FAP #102** 1<sup>st</sup> visit 28 wk 36 wk Delivery

**TIME OF LAST MEAL/SNACK:** \_\_\_\_\_ **Birthdate** \_\_\_\_\_

**Blood Drawn:** Date: \_\_\_\_\_ Time: \_\_\_\_\_

Lavender-top \_\_\_\_ (1 x 10 ml) \_\_\_\_ Mix well; Wrap in foil; Place in refrigerator/cooler

Lavender-top \_\_\_\_ (1 x 10 ml) \_\_\_\_ Mix well; Wrap in foil; Place in refrigerator/cooler

Red-top \_\_\_\_ (1 x 9 ml) \_\_\_\_ Mix well; Wrap in foil; Place in refrigerator/cooler

Comments: \_\_\_\_\_

Midwife / Phlebotomist: \_\_\_\_\_

Delivered to: \_\_\_\_\_ Time: \_\_\_\_\_

Please call **Folate Research Team (706-247-4244 or 706-247-4381)** for sample pick-up.

## **APPENDIX J**

### **INITIAL CHECKLIST**

## **Initial Checklist**

**TITLE OF STUDY: Folic Acid Supplementation in Pregnant Women: Dose Response**

**PRINCIPAL INVESTIGATOR: Dr. Lynn B. Bailey, University of Georgia**

**IRB PROJECT NUMBER: STUDY00000506 (UGA)**

Date \_\_\_\_\_  
Name \_\_\_\_\_  
Best time to call \_\_\_\_\_  
Participant Number \_\_\_\_\_  
ARMC Midwife \_\_\_\_\_

### **INCLUSION CRITERIA (ALL of below should be checked)**

\_\_\_\_ Age (18-40yrs)  
\_\_\_\_ Week of Gestation (< 12 week)  
\_\_\_\_ BMI \_\_\_\_ (18.5-35.0,)  
    Height: \_\_\_\_ ft. \_\_\_\_ in.  
    Weight: \_\_\_\_ kg.

### **EXCLUSION CRITERIA (NOT ELIGIBLE if checked)**

\_\_\_\_ Chronic disease (diabetes, hypertension, epilepsy, cancer, kidney disease, cardiovascular disease)  
\_\_\_\_ Use of anticonvulsive drugs

### **NEED INFORMATION ON THE FOLLOWING:**

\_\_\_\_ Anemia  
\_\_\_\_ Current illness (pneumonia, urinary tract infection, mononucleosis)  
\_\_\_\_ Smoking  
\_\_\_\_ Alcohol consumption (2 or more drinks per day)  
\_\_\_\_ Vegan dietary regime (excludes all animal products from diet)  
\_\_\_\_ *in vitro* fertilization treatment  
\_\_\_\_ Use of other prescription drugs \_\_\_\_\_  
\_\_\_\_ Use of antibiotics in past 2 weeks

## **CHECK LIST**

- \_\_\_ Blood Drawn (3 tubes)
- \_\_\_ Provide Pill-Box
- \_\_\_ Provide Diet Recall Form/Remind contact from UGA Folate Research Team
- \_\_\_ Notify Dr. Hea Jin Park (706-248-4153) of sample collection
  - Dr. Park (or other Folate Team member) will pick-up sample within 90 min. of collection

## **APPENDIX K**

### **MONTHLY CHECKLIST**

## **Visit Checklist (at every visit)**

**TITLE OF STUDY: Folic Acid Supplementation in Pregnant Women: Dose Response**

**PRINCIPAL INVESTIGATOR: Dr. Lynn B. Bailey, University of Georgia**

**IRB PROJECT NUMBER: STUDY00000506 (UGA)**

Date (Gestational weeks) \_\_\_\_\_

Participant Number \_\_\_\_\_

ARMC Midwife \_\_\_\_\_

### **CHECK POINTS**

\_\_\_\_ Anemia

\_\_\_\_ Pregnancy-associated complications (gestational diabetes, pre-eclampsia)

\_\_\_\_ Acute illness (pneumonia, urinary tract infection, mononucleosis)

\_\_\_\_ Use of prescription drugs Name, duration ( \_\_\_\_\_ )

\_\_\_\_ Use of antibiotics in past 2 weeks Name, duration ( \_\_\_\_\_ )

If any of above is checked, please contact the UGA Folate Research Team

(706-248-4153). Drop decision will be made by the UGA Folate

Research Team and ARMC staff will be notified and to inform the

participant before next visit.

### **CHECK LIST**

\_\_\_\_ Exchange Pill-Box

## **APPENDIX L**

### **28 WEEK CHECKLIST**



## **28 Weeks Checklist**

**TITLE OF STUDY: Folic Acid Supplementation in Pregnant Women: Dose Response**

**PRINCIPAL INVESTIGATOR: Dr. Lynn B. Bailey, University of Georgia**

**IRB PROJECT NUMBER: STUDY00000506 (UGA), ZZZZ (ARMC)**

Date \_\_\_\_\_

Participant number \_\_\_\_\_

ARMC Midwife \_\_\_\_\_

### **CHECK POINTS**

\_\_\_\_ Carrying more than one fetus

\_\_\_\_ Anemia

\_\_\_\_ Pregnancy-associated complications (gestational diabetes, pre-eclampsia)

\_\_\_\_ Acute illness (pneumonia, urinary tract infection, mononucleosis)

\_\_\_\_ Use of prescription drugs: Name, duration ( \_\_\_\_\_ )

\_\_\_\_ Use of antibiotics in past 2 weeks: Name, duration ( \_\_\_\_\_ )

If any of above is checked, please contact the UGA Folate Research Team (706-542-7689, folate13@uga.edu). Drop decision will be made by the UGA Folate Research Team and ARMC staff will be informed to notify the participant.

### **CHECK LIST**

\_\_\_\_ Blood Drawn (Lavender-top 1)

\_\_\_\_ Blood Drawn (Lavender-top 2)

\_\_\_\_ Blood Drawn (Red-top)

\_\_\_\_ Exchange Pill-Box

\_\_\_\_ Provide Diet Recall Form/Remind contact from UGA Folate Research Team

## **APPENDIX M**

### **36 WEEK CHECKLIST**

## **36 Weeks Checklist**

**TITLE OF STUDY: Folic Acid Supplementation in Pregnant Women: Dose Response**

**PRINCIPAL INVESTIGATOR: Dr. Lynn B. Bailey, University of Georgia**

**IRB PROJECT NUMBER: STUDY00000506 (UGA), ZZZZ (ARMC)**

Date \_\_\_\_\_

Participant Number \_\_\_\_\_

ARMC Midwife \_\_\_\_\_

### **CHECK POINTS**

\_\_\_\_\_ Anemia

\_\_\_\_\_ Pregnancy-associated complications (gestational diabetes, pre-eclampsia)

\_\_\_\_\_ Acute illness (pneumonia, urinary tract infection, mononucleosis)

\_\_\_\_\_ Use of prescription drugs: Name, duration ( \_\_\_\_\_ )

\_\_\_\_\_ Use of antibiotics in past 2 weeks: Name, duration ( \_\_\_\_\_ )

If any of above is checked, please contact to UGA Folate Research Team (706-542-7689, folate13@uga.edu). Drop decision will be made by the UGA Folate Research Team and ARMC staff and will be informed and will notify the participants.

### **CHECK LIST**

\_\_\_\_\_ Blood Drawn (Lavender-top 1)

\_\_\_\_\_ Blood Drawn (Lavender-top 2)

\_\_\_\_\_ Blood Drawn (Red-top)

\_\_\_\_\_ Exchange Pill-Box

## **APPENDIX N**

### **DELIVERY CHECKLIST**

## **Delivery Checklist**

**TITLE OF STUDY: Folic Acid Supplementation in Pregnant Women: Dose  
Response**

**PRINCIPAL INVESTIGATOR: Dr. Lynn B. Bailey, University of Georgia**

**IRB PROJECT NUMBER: STUDY00000506 (UGA), ZZZZ (ARMC)**

Date \_\_\_\_\_  
Participant Number \_\_\_\_\_  
ARMC Midwife \_\_\_\_\_

### **Maternal Blood**

\_\_\_\_ Blood Drawn (Red-top) – Top priority  
\_\_\_\_ Blood Drawn (Lavender-top 1) – Second priority  
\_\_\_\_ Blood Drawn (Lavender-top 2)

## **Delivery Checklist**

**TITLE OF STUDY: Folic Acid Supplementation in Pregnant Women: Dose  
Response**

**PRINCIPAL INVESTIGATOR: Dr. Lynn B. Bailey, University of Georgia**

**IRB PROJECT NUMBER: STUDY00000506 (UGA), ZZZZ (ARMC)**

Date \_\_\_\_\_  
Participant Number \_\_\_\_\_  
ARMC Midwife \_\_\_\_\_

### **Cord Blood**

\_\_\_\_ Blood Drawn (Red-top) – Top priority  
\_\_\_\_ Blood Drawn (Lavender-top 1) – Second priority  
\_\_\_\_ Blood Drawn (Lavender-top 2)