A Research Proposal: Prenatal Choline Supplementation and Infant Cognitive Development During the First Year of Life: A Randomized Controlled Trial

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Abstract

Choline is a nutrient of interest during pregnancy due to its role in processes that contribute to fetal neurodevelopment. Currently, human studies utilizing supplemental choline during pregnancy are limited. The purpose of this proposal is to explore the impact of prenatal choline supplementation in the second and third trimesters on infant cognitive development. During the duration of this 40-month randomized controlled trial, we will recruit 300 expectant mothers and follow their infants through the first year of life. Mothers will be randomly assigned to receive 150 mg or 600 mg daily choline supplementation during pregnancy. This study will utilize the Bayley Scales of Infant and Toddler Development-4th edition (Bayley-4) to assess infant development at 1 month and 12 months of age. Anticipated results indicate that infants born to mothers in the 600 mg/day supplementation group will score significantly higher in the Cognitive domain of the Bayley-4 at both the 1-month (p<.01) and 12-month (p<.001) evaluations. No significant differences in scores will be seen within the Language and Motor domains. The proposed study will contribute to the early human research on prenatal choline intake and child cognitive development. Continued research on this topic could potentially provide rationale for increasing the Adequate Intake (AI) of choline during pregnancy and for the use of choline supplementation as a means of supporting fetal neurodevelopment.

Keywords: pregnancy, prenatal, choline, supplementation, infant, cognitive development

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Table of Contents

Chapter 1: Introduction 8

Background 9

Problem Statement 11

Purpose of the study 11

Research Question 11

Hypotheses 12

Nature of the Study 12

Definitions 13

Assumptions 13

Limitations 14

Delimitations 14

Significance 16

Summary 16

Chapter 2: Literature Review 18

Literature Research Strategy 19

Background 21

Impact of Prenatal Nutrition on Fetal Growth 21

The Chinese Famine: A Natural Case Study 23

Maternal Dietary Patterns During Pregnancy, Child Cognition, and Behavior 25

Vegetarian Diet and Child Cognitive Development. 29

Mediating Factors Between Prenatal Diet and Child Development. 30
Prenatal Micronutrient Intake and Child Cognitive Function 33
Vitamin D 33
Vitamin B12. 36
Docosahexaenoic acid (DHA). 38
Choline. 40
Research Methodology 45
Summary 47
Chapter 3: Methodology 48
Research Protocol 48
Research Question 48
Hypotheses 49
Study Design 50
Setting and Sample 50
Population 51
Recruitment 51
Study Protocol and Intervention 53
Data Collection Process 56
Instrumentation 57
Data Analysis Plan 58

Threats to Validity 59

Ethical Procedures 59

Summary 60

Chapter 4: Anticipated Results 61

Baseline Characteristics 61

Bayley-4 Scores at 1 and 12 Months 63

Summary 66

Chapter 5: Discussion 67

Interpretation of Results 67

Characterization of the Study Population 68

Bayley-4 Scores 68

Comparison to Other Studies 69

Strengths and Limitations 70

Suggestions for Future Studies 72

Conclusion 73

References 74

Appendix A 84

Appendix B 100

Appendix C 103

Appendix D 108

Appendix E 109

List of Tables 110

List of Figures 111

Chapter 1: Introduction

Maternal diet and nutritional status play integral roles in fetal growth and development (Li et al., 2019). An embryo's brain begins to develop during week five of pregnancy, and continues to develop throughout infancy, childhood, and early adulthood (*Fetal Development*, 2022). Since the mother's nutrient intake and stores are the only source of nutrition for the fetus, maternal diet directly impacts fetal development and birth outcomes. Some of these impacts are well established. As a result, the Recommended Daily Allowance (RDA) of many micronutrients has been adjusted to meet the needs of pregnant individuals (Kominiarek & Rajan, 2016). Decades of research have confirmed the importance of sufficient folic acid intake for the prevention of neural tube defects, as well as the role of calcium and vitamin D in the development of fetal bones and teeth (*Nutrition During Pregnancy*, 2022). Adequate vitamin A intake is necessary for cell proliferation and differentiation, and the omega-3 fatty acid docosahexaenoic acid (DHA) is essential for fetal brain development (Kominiarek & Rajan, 2016). The RDAs of iron and iodine are also increased in pregnancy due to their contributions to fetal neurodevelopment (Li et al., 2019). Human research studies on choline supplementation are limited as there are ethical concerns regarding the choline status of individuals who would be assigned to the control group (Cheatham, 2019). A primary ethical concern is that individuals may not be meeting the AI for pregnancy of 450 mg/day of choline through dietary sources alone and those in the control group would be unable to supplement with additional choline. Although human studies are limited, there is a large body of animal studies that support maternal supplementation of choline during fetal development.

While research on prenatal nutrition and fetal neurodevelopment exists, there is still much that remains unknown, including with regard to choline, about the effects of maternal dietary intake on the cognitive function of offspring in infancy and further into adulthood. In 1990, British epidemiologist David Barker hypothesized that a stimulus, such as a nutrient deficiency, can influence programmed changes during critical periods of fetal development and, as a result, make a fetus susceptible to certain disease states such as diabetes, hypertension, and cardiovascular disease later in life (Kwon & Kim, 2017). This idea, now recognized as fetal programming, can also offer insight into how the physiological, metabolic, and structural changes occurring during fetal development may contribute to cognitive development and function.

Background

Choline's contributions to biochemical and physiological processes are extensive. Maternal choline intake influences lipid metabolism, neurotransmission, and methylation (Irvine et al., 2022). Choline serves as a methyl donor, so it plays a role in DNA methylation and has been linked to cognitive outcomes in children. Large amounts of choline-derived phospholipids are needed during fetal development to sustain functions such as rapid cell division, growth, and myelination (Korsmo et al., 2019). The neurotransmitter acetylcholine is also derived from choline and affects many of the processes in the developing brain. Adequate amounts of choline are needed to prevent neural tube defects and are important for hippocampal development which impacts functions such as learning and memory. Through its effect on neurodevelopment, maternal choline intake may impact cognitive outcomes such as processing speed, visuospatial memory, attention, self-regulation, and visual acuity.

Choline is synthesized by the body, but additional choline intake is needed through diet or supplementation for optimal health (Korsmo et al., 2019). During pregnancy, large amounts of choline are transferred from the mother to support placental function and fetal development. The current recommendation for choline intake is 425 mg/day in non-pregnant women and 450 mg/day in pregnant women. Common dietary sources are meat, poultry, fish, eggs, and dairy products. Plant-based foods such as cruciferous vegetables, nuts, seeds, whole grains, and certain beans also provide choline (*Office of Dietary Supplements - Choline*, n.d.-a). Although the American Academy of Pediatrics recognizes choline as an important nutrient for neurodevelopment, it is estimated that less than 10% of pregnant women achieve target intake levels. The tolerable upper intake level (UL) of choline is 3.5 g/day for adults, but according to a study by Bailey et al. (2019) which used the National Health and Nutrition Examination Surveys (NHANES) from 2005-2014 to examine usual nutrient intake distributions of pregnant women aged 20-40 years, the mean choline intake (through foods and supplements) for pregnant women was only 322 mg choline/day.

As previously mentioned, human-based randomized controlled trials on prenatal choline supplementation and offspring cognitive development are limited. Studies by Caudill et al. (2018) and Bahnfleth et al. (2019, 2022) demonstrated improvements in offspring cognitive functioning in the highest choline supplementation groups (930 mg choline/day) versus the 480 mg choline/day group. The use of choline supplementation as a neuroprotective agent against prenatal alcohol exposure and mental illness are also current research topics of interest (Hunter et al., 2021; Jacobson et al., 2021, Ross et al., 2016; Warton et al., 2021). This study will add to

the current body of research and specifically explore the differences in dose-response between two levels of maternal choline supplementation and infant cognitive function.

Problem Statement

Choline is recognized as an important micronutrient for neurodevelopment, yet very few human studies have investigated the impact of prenatal choline supplementation on infant (defined as a post-natal age of 12 months or less) cognitive outcomes.

Purpose of the study

The purpose of this double-blind randomized controlled trial is to analyze the impact of different levels of choline supplementation during the second and third trimesters of pregnancy on infant cognitive outcomes in the first year of life. Infant cognitive outcomes will be evaluated by the Bayley Scales of Infant and Toddler Development- 4th edition. This screening tool assesses infant neurodevelopment in five domains including Cognitive, Language, Motor, Social-Emotional and Adaptive domains. The outcome of the study will contribute to the limited amount of human research on choline supplementation during pregnancy and offspring cognitive function.

Research Question

Does prenatal choline supplementation during the second and third trimesters in amounts exceeding the Adequate Intake, compared with lower amounts of supplementation, result in significantly higher infant cognitive outcomes [evaluated by the Bayley Scales of Infant and Toddler Development 4th edition (Bayley-4)] at 1 month and 12 months of age?

Hypotheses

H_0: The amount of prenatal choline supplementation has no effect on infant cognitive outcomes as measured by the Bayley Scales of Infant and Toddler Development- 4th edition (Bayley-4) at both 1 and 12 months of age.

H_1: Infants of mothers receiving higher amounts of prenatal choline supplementation, compared with lower amounts, will have significantly higher cognitive outcomes as measured by the Bayley Scales of Infant and Toddler Development- 4th edition (Bayley-4) at both 1 and 12 months.

Nature of the Study

The proposed double-blind randomized controlled trial will occur over a 40-month period. The study will examine the impact of different levels of maternal choline supplementation during pregnancy on infant cognitive development as assessed by the Bayley-4 at 1 and 12 months of age. Pregnant women will be recruited through an open enrollment process managed by Obstetrics and Gynecology (OB-GYN) clinics within the Froedtert & Medical College of Wisconsin (MCW) Health Network in southeastern Wisconsin. Participants will be randomized to receive either 150 mg choline/day supplementation or 600 mg choline/day supplementation. Research staff will also be recruited from Froedtert & MCW Health Network and student volunteers will be recruited from local academic institutions.

The primary instrumentation used in this study will be the Automated Self-Administered 24-hour Dietary Assessment tool (ASA24) and the Bayley-4. Maternal sociodemographic data will be collected through an online questionnaire. A Chi-Square Goodness-of-Fit test will be used for maternal demographic comparisons among the treatment groups. For ratio data,

including maternal age, pre-pregnancy BMI, average monthly household income, dietary choline intake, and infant birth weight, an independent t-test will be used to test for any differences among the treatment groups. Independent t-tests will also be used to evaluate the results of each sub domain of the Bayley-4 at 1 month and 12 months. This will determine if significant differences exist between treatment groups with regard to infant Bayley-4 scores.

Definitions

Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tool: a dietary assessment tool based on the United States Department of Agriculture's (USDA) Automated Multiple-Pass Method (*ASA24® Evaluation & Validation*, 2022)

Bayley scales of Infant and Toddler Development- 4th edition (Bayley-4): a screening tool used to assess infant development in five domains including Cognitive, Language, Motor, Social-Emotional and Adaptive domains (Balasundaram & Avulakunta, 2021)

Choline chloride: a water-soluble and synthetic form of choline that has been shown in rodent studies to provide superior bioavailability to the brain as compared to phosphatidylcholine (Caudill et. al, 2018)

Non-compliance: refers to compliance with the provided supplement regimen; defined as intake of less than 70% of provided prenatal or choline supplements for 2 or more months of study duration

Preterm birth: babies born before 37 weeks gestation

Assumptions

A key assumption in this project is that participants will provide honest answers on 24-hour food recalls. A percentage of the study participants will likely not be meeting the AI for

choline through their dietary intake. Participants will adhere to study protocol, including travelling to the study location for infant testing at 1 and 12 months and only taking the provided prenatal supplements. Participants will return all unused supplement packets during the monthly collection period. Participants will only be patients of the Froedtert & MCW Health Network, so it is assumed that the patient demographics will be similar to those of other hospital systems in the Milwaukee area. It is also assumed that there will be enough members of the targeted population willing to participate in the study and minimum sample size will be met.

Limitations

This study is expected to have multiple limitations. First, the study population will only be recruited from one hospital system in southeastern Wisconsin, so the results cannot necessarily be generalized to the broader population. Average monthly dietary choline intake will be tracked using the ASA24. The ASA24, while a detailed tool, is self-reported and the results are susceptible to reporting inaccuracies. Lastly, attrition is expected between the 1-month and 12-month Bayley-4 evaluations. This could impact the results of the study, as the sample sizes will likely differ at these two stages of analysis.

Delimitations

This study will require participants to be at least 18 years of age, so pregnant individuals less than 18 years will be excluded. Participants will be excluded if they are at more than 14 weeks of gestation during the time of recruitment. Individuals of all races and ethnicities will be eligible for inclusion, but all participants must be assigned female at birth. Additional maternal exclusions include a diagnosis of anemia, kidney, or liver blood markers outside of the normal

range, cardiovascular or pulmonary diseases, cancer, diabetes, self-reported tobacco, drug or alcohol use or presence of complications such as preeclampsia or gestational diabetes. Individuals experiencing hyperemesis gravidarum (as this could have a significant impact on dietary intake), an allergy to fish or soy (the prenatal vitamin will likely contain one or both of these allergens) and/or non-compliance with the supplement regimen (defined as intake of less than 70% of provided prenatal or choline supplements for 2 or more months of study duration) will also be excluded. Infant exclusions will include premature birth (<37 weeks) and diagnosis of conditions that impact cognitive function, such as Down Syndrome. This study will only assess supplementation in the second and third trimesters of pregnancy, so the impact of choline intake during the first gestational trimester cannot be accounted for. Additionally, data on pre-conception dietary choline intake, postnatal dietary choline intake during lactation, and infant dietary choline intake will not be collected, although these factors could potentially influence outcomes. Indicators of parental cognitive functioning and development, such as parental intelligence quotient (IQ), will not be included in this study. Controlling for such variables would require a greater time commitment from parents, as well as additional resources. Post-natal supplementation and diet will not be controlled for; these factors can impact levels of infant choline intake through formula and breastmilk. The Centers for Disease Control and Prevention (CDC) recommend children be introduced to solid foods around the age of 6 months, and the results of the study will not account for this dietary intake (*When, What, and How to Introduce Solid Foods*, 2021). Another delimitation is that the study will only assess offspring cognitive development at 1 and 12 months and will not address long-term impacts of choline supplementation during pregnancy.

Significance

Results from this study will contribute to the limited body of evidence from human studies that evaluate prenatal choline intake and offspring cognitive development. Since this study will include a treatment group receiving 600 mg choline supplementation, it could support evidence that suggests that the current choline AI of 450 mg/day in pregnant women needs to be increased for improved cognitive functioning in offspring. Study outcomes could provide a basis for continued research on the use of choline supplementation to combat risk factors for impaired neurocognitive development, such as prenatal alcohol exposure.

Summary

Maternal nutritional status during pregnancy plays a significant role in fetal development. While the importance of adequate intake of nutrients such as folate, DHA, iron, iodine, and choline with regards to fetal neurodevelopment is well-recognized, there is still much to learn about the relationship between micronutrient intake and offspring cognitive functioning. The proposed study aims to explore the differences in dose-response between two levels of maternal choline supplementation and infant cognitive function using a larger sample population compared to recent human RCTs with similar interventions.

The results of this study can contribute to the limited body of evidence suggesting that higher-dose choline supplementation during pregnancy is associated with improvements in offspring cognitive abilities. Results could also potentially support evidence that the AI of maternal choline intake during pregnancy should be increased to enhance cognitive functioning in children. Upcoming chapters will provide a review of the current literature on maternal

dietary intake and offspring cognitive functioning, as well as study methodology, anticipated results, and a discussion of the presented material.

Chapter 2: Literature Review

Maternal diet and nutritional status play integral roles in fetal growth and development (Li et al., 2019). An embryo's brain begins to develop during week five of pregnancy, and continues to develop throughout infancy, childhood, and early adulthood (*Fetal Development*, 2022). Since the mother's nutrient intake and stores are the only source of nutrition for the fetus, maternal diet directly impacts fetal development and birth outcomes. Some of these impacts are well established. As a result, the Recommended Daily Allowance (RDA) of many micronutrients has been adjusted to meet the needs of pregnant individuals and their developing offspring (Kominiarek & Rajan, 2016). Decades of research have confirmed the importance of sufficient folic acid intake to reduce the risk of neural tube defects (*Nutrition During Pregnancy*, 2022). Calcium and vitamin D are required for the development of fetal bones and teeth. Adequate vitamin A intake is necessary for cell proliferation and differentiation, and omega-3 fatty acids are essential for fetal brain development (Kominiarek & Rajan, 2016). The RDAs of other micronutrients such as iron and iodine are also increased in pregnancy due to their contributions to fetal neurodevelopment (Li et al., 2019).

While research on prenatal nutrition and fetal neurodevelopment exists, there is still much that remains unknown about the lasting impacts of maternal dietary intake on cognitive development and behavior into childhood and adulthood. In 1990, British epidemiologist David Barker hypothesized that a stimulus, such as nutrient deficiencies, can influence programmed changes during critical periods of fetal development and, as a result, make a fetus susceptible to certain disease states such as diabetes, hypertension, and cardiovascular disease later in life (Kwon & Kim, 2017). This concept, now recognized as fetal programming, can also offer insight

into how a nutrient deficiency can potentially impact the physiological, metabolic, and structural changes occurring during fetal development and subsequently may impact cognitive development and functioning.

Research involving pregnant women, fetuses and neonates is essential to further our understanding of fetal programming, but the risks of this research present ethical concerns ("Mothers Matter: Ethics and Research During Pregnancy," 2013). To address these concerns, the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Science Research established guidelines for research involving these populations. These guidelines were closely considered during the development of the proposed study. The complete list of regulations is extensive and, while necessary to protect the wellbeing of mothers and offspring, it contributes to the challenges of conducting research among the prenatal and neonate populations.

Literature Research Strategy

This literature review explored a variety of topics related to prenatal nutrition and cognitive development. Online databases including PubMed, Google Scholar, and Primo (at Mount Mary University's Haggerty Library) were used to conduct this literature search. To be used in the literature review, studies needed to be original research and had to be published within the last 10 years. Initial search terms included "prenatal nutrition AND fetal development," "maternal diet AND fetal development," and "prenatal diet AND fetal growth." This approach yielded articles that suggested a wide range of potential research topics such as micronutrient supplementation, overall diet quality, impact on fetal growth indicators and biometrics, and implications in later childhood such as cognitive function and disease states. To

narrow the review, I searched the terms “prenatal nutrition AND asthma in children,” and “maternal diet AND asthma in children.” The interventions and independent variables present in the studies were still too broad, so search terms were further specified to “maternal vitamin D intake AND asthma in children,” and “prenatal vitamin D intake AND asthma in children.” Studies were available, but original studies published within the last 10 years were limited.

Upon re-examining the initial search terms, the topic of prenatal nutrition and cognitive development stood out. Searching “prenatal nutrition AND cognitive development,” and “maternal diet AND cognitive development” yielded articles with similar research approaches, including studies that explored behavioral impacts in childhood, in addition to cognitive development and neurodevelopment. After considering the complexity of a research study extending into adolescence, the search was narrowed down even further using the terms “prenatal nutrition AND infant cognitive development,” “prenatal diet AND infant cognitive outcomes.” These terms yielded studies exploring the relationship between micronutrients such as vitamin B12, DHA, and choline and infant cognitive function. The final search terms utilized included “prenatal choline intake AND infant cognitive outcomes” and “prenatal choline intake AND infant cognitive development.”

Abstracts were reviewed to determine if articles were applicable to this literature review. Once an article was selected, the reference list was reviewed to see if there were any other studies on the topic that could be considered for inclusion. If a study had a relevant title and was published in the last 5 years, the Google search engine was used to look up the journal article and identify if the full text was accessible for subsequent review.

Background

Impact of Prenatal Nutrition on Fetal Growth

Pregnant individuals have increased nutritional needs including micronutrients, protein, and water to support the growth and development of a healthy fetus (*Pregnancy and Nutrition*, n.d.). Intake of these nutrients, among others, is used to determine maternal diet quality and diversity. Karimi et al. (2022) studied the relationship between maternal dietary diversity and newborn anthropometric measures using participants from their prospective cohort. Trained nutritionists collected pre-pregnancy dietary data at the first meeting using Food Frequency Questionnaires (FFQ), and intake during pregnancy was measured twice by 24-hour food recalls. Each participant received scores for their dietary diversity status (DDS) and nutrient adequacy ratios based on the RDA for each individual nutrient. Neonatal anthropometric measurements and indices were collected at birth. Karimi et al. found significant inverse relationships between maternal DDS during pregnancy and neonatal birth weight, weight for age Z-score and BMI for age Z-score, as well as maternal nutritional adequacy both before and during pregnancy and neonatal birth weight and weight for age Z-score. They concluded that dietary diversity during pregnancy and maternal nutritional adequacy before and during pregnancy are related to neonatal anthropometric indices at birth. Ancira-Moreno et al. (2020) came to similar conclusions in their PRINCESA cohort study. Maternal diet was assessed in the second and third trimesters using a multiple pass method 24-hour dietary recall. Researchers used the Mexican Dietary Guidelines to categorize foods eaten into 12 major food groups and subsequently composed a Maternal Diet Quality Score (MDQS). Factor analysis was used to determine dietary patterns. Women were determined to have either a “healthier dietary

pattern" or a "mixed dietary pattern" during both the second and third trimesters of pregnancy. Ancira-Moreno et al. identified that higher MDQS during the second and third trimesters was associated with a reduced risk of neonatal low body weight (as classified by the World Health Organization) compared to lower scores.

Additional studies by Chia et al. (2018) and Emond et al. (2020) assessed the impact of diet quality on measures of fetal growth as determined by neonatal growth indices. Increased adherence to the Healthy Eating Index for Pregnant women in Singapore (HEI-SCP), a measure of diet quality, was associated with longer birth length and lower measures of neonatal adiposity (Chia et al., 2018). However, adherence to HEI-SCP was not associated with occurrence of preterm birth or birth weight. Emond et al. (2018) utilized the Alternative Healthy Eating Index-2010 to determine diet quality. Higher quality diets were considered to be composed of more nutrient dense foods such as those with higher levels of fiber and unsaturated fats, and lower levels of saturated fats, trans fats, sodium, and added sugar. Adherence to higher quality diets was associated with reduced risk of having a baby born small for gestational age (SGA). This is an important measure because SGA likely correlates with restricted intrauterine growth. Emond et al. point out that restricted uterine growth can potentially lead to "catch-up" growth and an increased risk of chronic diseases such as metabolic disorders later in life.

Many of the concerns regarding fetal development have been linked to undernutrition or malnutrition, but there is a growing interest in exploring the impact of maternal overnutrition on fetal growth and development. Recent studies have found that maternal diet also affects factors such as neonatal body composition. A study by Shapiro et al. (2016) had

participants record their diets using the Automated Self-Administered 24-hour Diet Recall (ASA24). Researchers then scored those assessments using the Healthy Eating Index. The results revealed that poorer maternal diet quality during pregnancy was associated with higher neonatal fat mass and percentage of fat mass. This is concerning because neonatal adiposity is associated with higher childhood BMI levels and an increased incidence of overweight and obesity in early childhood (Moore et al., 2020).

Ancira-Moreno et al. (2020), Chia et al. (2018) and Emond et al. (2020) acknowledged that women with higher measures of dietary quality tended to be older, while Chia et al. and Emond et al. also noted that these women were less likely to smoke and had higher education levels. Socioeconomic status had an important role in the significance of the results determined by Karimi et al. (2022). These factors require consideration because many pregnant women, especially those living in developing countries, do not receive optimal nutrition due to factors like low socio-economic status, poor dietary adequacy, and food insecurity. Nearly 30% of low-income-households in the United States were food insecure in 2020 (*Food Insecurity - Healthy People 2030 | health.gov*, n.d.). Food-secure individuals of higher socio-economic status can struggle with adequate dietary diversity, so women facing the previously mentioned barriers are at an even greater disadvantage when it comes to maintaining optimal nutrition during pregnancy to support fetal development (Karimi et al. 2022).

The Chinese Famine: A Natural Case Study

Longitudinal studies, particularly those spanning from birth (or earlier) to adulthood, come with a myriad of challenges such as ethical concerns, the impact of changing dietary

status over time, and attrition (Twisk & de Vente, 2002; Weaver & Miller, 2017). However, investigating the long-term impact of maternal prenatal nutrition on offspring is essential to establishing the connections between nutrient intake at the earliest stages of development and future health outcome measures (Weaver & Miller, 2017). As He et al. (2018) points out, famines can be used as natural case studies. The drastic shift in food availability during such times provides an opportunity to assess the impact of prenatal malnutrition on offspring, compared to offspring born in the years directly before and after the famine. Several studies on the 1944-1945 Dutch Famine did not show any association between prenatal famine exposure and adult cognitive function; however, a study on the Chinese Famine by He et al. found correlations between prenatal famine and adult cognitive impairment.

The Chinese Famine was one of the most severe famines in recent human history, causing the death of 15-30 million people (He et al., 2018). It began during a push to industrialize China, which led to a decrease in grain production of 70% over the course of two years. During this time, death rates climbed, and fertility rates dropped steeply. The study by He et al. used data from the Second National Sample Survey on Disability, which included information on cognitive impairment in China. For the study, “prenatal famine” was defined as maternal exposure to famine during the 300 days before delivery. Cognitive impairment was identified through a questionnaire screening and medical diagnosis by a psychiatrist (defined as an IQ < 70 with age of onset less than 18 years). He et al. used difference-in-difference (DID) models to look at regional variates of famine exposures across birth cohorts. After comparing the results of adults exposed to prenatal famine from 1959-1961 to those born in the two years before and after the Chinese Famine, He et al. determined that maternal prenatal famine

exposure was associated with impaired cognitive function in offspring (both men and women) living in rural areas, but no significant association was found among men and women living in urban areas. The results of this study provide insight into the influence of prenatal malnutrition on cognitive function in adulthood, specifically among those who may have less access to appropriate quantity and diversity of food sources.

Maternal Dietary Patterns During Pregnancy, Child Cognition, and Behavior

Indicators of behavioral problems in childhood are typically categorized into internalizing and externalizing spectrums (Willner et al., 2016). Externalizing symptoms include "aggression, conduct problems, delinquent behavior, oppositionality, hyperactivity, and attention problems." Internalizing symptoms include "anxiety, fear, sadness/depression, social withdrawal, and somatic complaints." Research has demonstrated that co-occurrence of these two spectrums is not uncommon among children. The following studies will explore the connection between maternal dietary intake during pregnancy, child cognition, and behavioral concerns.

Mahmassani et al. (2021), in their study evaluating relationships between prenatal diet quality, child cognition, and behavior, followed 1580 mother-child pairs from the Project Viva prospective birth cohort. Maternal diet was evaluated in the first and second trimesters of pregnancy using a modified version of the Mediterranean Diet Scores for pregnancy (MDS-P), and the Alternate Healthy Eating Index (AHEI-P). Points were awarded based on the intake of certain foods and food groups with higher scores representing better adherence to dietary standards.

Child cognitive and behavioral outcomes were measured at infancy, early, and mid-childhood using standardized tests and questionnaires including the Visual Recognition Memory (VRM) paradigm, the Peabody Picture Vocabulary test- 3rd edition, (PPVT-III), the Wide Range Assessment of Visual Motor Abilities (WRAVMA) and the Kaufman Brief Intelligence test- 2nd edition (KBIT-II) (Mahmassani et al., 2021). These tools evaluate skills such as an infant's ability to memorize, recognize, and show preference to a newly introduced stimulus, fine motor, visual spatial, and visual motor abilities, and verbal and nonverbal intelligence.

After the children completed the tests at the mid-childhood visit, parents and teachers completed the Behavioral Rating Inventory of Executive Function (BRIEF) which assessed executive function behaviors at home and school (Mahmassani et al., 2021). Parents also filled out the Strengths and Difficulties Questionnaire (SDQ) which assessed the child's social, emotional, and behavioral functioning. As a comparative measure, maternal cognition was evaluated using PPVT-III, KBIT-II, and the Home Observation Measurement of the Environment-Short Form (HOME-SF). HOME-ST measured the mother's cognitive stimulation and emotional support for the child at home.

After evaluating all variables, the authors determined that there was no relationship between maternal diet and child VRM scores in infancy, but higher adherence to the MDS-P was associated with better verbal and nonverbal intelligence, as well as fewer metacognition problems in mid-childhood (Mahmassani et al., 2021). Additionally, mothers with higher adherence to dietary standards during pregnancy had children with better visual spatial skills in early childhood and with better verbal intelligence and executive function in mid-childhood. Looking at the study population, a high proportion of white, college-educated mothers in a

higher household income bracket, it is reasonable to assume that many of the participants were not at risk for malnutrition due to environmental factors. Through this study, Mahmassani et al. were able to draw the conclusion that overall maternal dietary quality is associated with certain aspects of child cognitive function and behavior, but it would be valuable to see how this relationship changes in populations more severely impacted by malnutrition.

Mortaji et al. (2021) also explored the connections between prenatal diet and executive function in children, with the added variable of postnatal home environment. Similar to many of the studies discussed in this review, they used participants from a large longitudinal cohort. Eight-hundred and eight children ages 3-4 were selected from the Canadian Maternal infant Research on Environment Chemicals (MIREC) cohort to complete the behavioral assessments. This study specifically addressed adaptability outcomes and externalizing and internalizing problems.

Information on maternal diet was collected using FFQs and Healthy Eating Index (HEI) scores were subsequently determined to provide a measure of overall dietary quality (Mortaji et al., 2021). Quality of the home environment was assessed using Home Observation for Measurement of the Environment (HOME) scale. This tool is commonly used in research to assess the home environment and evaluate the quality of stimulation and support provided to the child. During statistical analysis, Mortaji et al. adjusted for maternal education, as they note that mothers who are more highly educated tend to provide more stimulation and interaction with their child at home. They also controlled for maternal depressive symptoms because depression can influence parenting behaviors, as well as dietary intake. Mortaji et al. (2021) identified that prenatal maternal diet quality was associated with better child working

memory, planning skills and adaptability in less stimulating environments. Environments with higher levels of stimulation, however, decrease the association between maternal diet and child behavior. The conclusion suggests the finding that prenatal diet may have a bigger impact in those children living in less optimal home environments. Mortaji et al. also noted that the lack of observed associations between maternal diet quality and internalizing and externalizing problems may be related to the age of the children, and reassessment at a later age may reveal different results.

While Mahmassani et al. (2021) and Mortaji et al. (2021) looked at prenatal nutrition and executive function behavior, including internalizing and externalizing problems, the study by Galera et al. (2018) specifically explored the relationship between prenatal diet and hyperactivity-inattention and conduct problems in childhood. As previously mentioned, hyperactivity-inattention and conduct problems fall under the construct of externalizing behavior (Liu, 2004). The presence of externalizing behavior in childhood can be concerning because it is recognized as a risk factor for “juvenile delinquency, adult crime, and violence”.

Galera et al. (2018) used data from the EDEN mother-child cohort study in France. Data was collected during pregnancy, and multiple times between birth and 8 years of age. The data was compiled from medical records, interviews, and questionnaires completed by the parents during all phases of the study. Mothers completed FFQs shortly after birth, and results were grouped into either “Healthy” or “Western” dietary practices. Parents then completed Strength and Difficulties Questionnaires (SDQs) at three points during early childhood and these results were used to identify each child’s patterns of externalizing behavior.

Through their analysis, Galera et al. (2018) determined that maternal low "Healthy" and high "Western" dietary patterns during pregnancy were significantly associated with increased levels of hyperactivity-inattention in children, even after adjusting for confounding variables such as the children's dietary intake, socioeconomic factors, and maternal depression. No significant relationship was identified between prenatal dietary patterns and conduct problems in children. As Galera et al. mentioned in their discussion, many prior studies failed to separate the issues of hyperactivity-inattention and conduct problems in their assessments. By looking at these variables separately, the authors were able to determine the association between maternal diet and hyperactivity-inattention- a primary symptom of attention deficit hyperactivity disorder. This study demonstrates the importance of recognizing the unique components of child cognition and behavior, and assessing these variables independently to gather an accurate understanding of how they are related to maternal dietary intake.

Vegetarian Diet and Child Cognitive Development. The studies by Galera et al. (2018), Mahmassani et al. (2021) and Mortaji et al. (2021) considered general dietary patterns but did not consider the unique impacts of a vegetarian diet on child cognition. As of 2018, 5% of U.S. adults consider themselves to be vegetarian (Hrynowski, 2023). If not followed appropriately, individuals who adhere to a vegetarian diet are at risk of consuming inadequate amounts of nutrients such as vitamin B_{12}, vitamin D, omega-3 fatty acids, calcium, iron, and zinc which all play unique and important roles in fetal development (Craig, 2010). Crozier et al. (2019) collected dietary data and fasting blood samples for both vegetarian and non-vegetarian mothers in either early pregnancy (11 weeks), late pregnancy (34 weeks), or at both points. Children of these mothers completed testing to assess cognitive function 6-7 years of age.

Testing included the Wechsler Abbreviated Scale of Intelligence to evaluate intelligence quotient (IQ) and the Cambridge Neuropsychological Test Automated Battery (CANTAB) to evaluate executive function. Researchers found that following a vegetarian diet in either stage of pregnancy (early, late, or both) was not associated with any negative effects on cognitive outcomes as determined by CANTAB. Vegetarian mothers tended to breastfeed longer, have lower incidence of tobacco use, higher IQ and higher educational level. It is worth acknowledging that in their studies, Chia et al. (2018) and Emond et al. (2020) also indicated that mothers with a lower incidence of tobacco-use and higher education levels consumed higher quality diets.

Mediating Factors Between Prenatal Diet and Child Development. Dawson et al. (2021) and Rijlaarsdam et al. (2016) took unique approaches to study the topic of prenatal nutrition and child development and behavior by evaluating the roles of maternal gut microbiota (Dawson et al., 2021) and insulin-like growth factor 2 gene methylation (Rijlaarsdam et al., 2016) as mediators between these variables. Dawson et al. (2021) undertook the first human study to look at maternal gut microbiota and child behavior. Researchers used fecal samples collected during pregnancy to complete 16S rRNA gene amplicon sequencing and measure both alpha and beta diversity of the gut microbiota. Alpha diversity metrics provide information on the richness and evenness of the microbial community within an individual sample, whereas beta diversity metrics are used to identify how samples differ from one another (Kers & Saccenti, 2022).

Maternal dietary intake was collected from the Dietary Questionnaire for Epidemiological Studies (DQES) and each mother received scores representing their adherence

to the dietary patterns (Dawson et al., 2021). The dietary patterns identified from the DQES data included a "modern healthy dietary pattern" and a typical Western diet. Parents also completed the Child Behavior Checklist at 2 years of age. The results of the analysis revealed that higher scores on the healthy dietary pattern were associated with a greater degree of alpha diversity in the gut microbiota (Dawson et al., 2021). Beta diversity analysis showed that maternal prenatal gut microbiota was significantly different in mothers of children in the elevated behavioral group compared to those with children in the normative behavioral group, with specific regard to internalizing symptoms. Although there was no evidence of an association between prenatal alpha diversity and externalizing behaviors, there was evidence of an indirect pathway between healthy maternal diet and lower child internalizing behaviors. Higher scores on the Western diet (poorer diet quality) were significantly associated with higher internalizing scores in children, but Dawson et. al did not find evidence of a direct correlation between healthy dietary patterns and child behavior dysregulation. Overall, the results illustrate an indirect link between prenatal diet, maternal gut microbiota composition, and behavioral outcomes in children, and therefore, additional studies are needed to further explore these findings.

Prenatal diet is associated with DNA methylation of insulin-like growth factor 2 (IGF2), which is involved in fetal neural development (Rijlaarsdam et al., 2016). Rijlaarsdam et al. investigated the role that IGF2 methylation has in the relationship between an unhealthy prenatal diet and ADHD, using data from the Avon Longitudinal Study of Parents and Children, specifically, a sample of participants involved in a study of DNA methylation. The sample included 164 youth, 83 with early-onset persistent (EOP) conduct problems and 31 with low

conduct problems. Cord blood collected at birth and blood samples collected from children at age 7 were used to determine levels of IGF2 methylation. Food Frequency Questionnaires were collected from the mother at 32 weeks gestation, as well as for the child at 3, 4.5, and 7 years of age. Children's ADHD symptoms were assessed at ages 7, 10, and 13 using the Development and Well-being Assessment. In this assessment, parents answered questions relevant to ADHD, oppositional defiant disorder, conduct disorder, generalized anxiety disorder, and major depressive disorder. Rijlaarsdam et al. showed that prenatal "unhealthy diet," specifically a high-fat and high-sugar diet, was associated with IGF2 methylation at birth among youth with both EOP conduct problems and low conduct problems. In EOP youth, higher IGF2 methylation at birth predicted ADHD symptoms, but the same prediction was not seen in youth with low conduct problems. These two results demonstrate that a prenatal "unhealthy" diet is associated with higher ADHD symptoms indirectly via higher IGF2 methylation at birth.

Several large-cohort studies have established that better adherence to a "healthy" prenatal diet with higher intake of fruits, vegetables, whole grains and healthy fats, and lower intake of red meat, processed foods, and discretionary foods, has beneficial associations with child functional and behavioral measures (Galera et al.,2018; Mahmassani et al.,2021). Mortaji et al. (2021) drew a similar conclusion but specified that the impact of maternal diet during pregnancy only positively impacted child executive function in less stimulating environments. Some studies evaluated additional variables such as home environment (Mortaji et al., 2021), vegetarian diet (Crozier et al., 2019), maternal gut microbiota (Dawson et al., 2021), and levels of IGF2 DNA methylation (Rijlaarsdam et al., 2016). Galera et al. (2018) found that a less healthy maternal diet was associated with a higher risk of externalizing problems in children,

but Mortaji et al. (2021) reported no association between maternal diet and externalizing or internalizing problems. The challenge, and opportunity, with this topic is the extensive range of variables that can be studied. Childhood is a time of significant cognitive and behavioral development, and subsets of child cognitive function and behavior are often interconnected, so this may contribute to the mixed results seen among current research.

Prenatal Micronutrient Intake and Child Cognitive Function

Vitamin D. In the discussion thus far, the studies that explored prenatal nutrition and child cognitive function and development have included rather general dietary patterns. While it is important to explore the impact of overall diet, research involving micronutrient status and supplementation is essential to continue to identify the roles specific micronutrients serve in our biological systems, as this data is used to set dietary guidelines (Harvard Health, 2021). The following discussion will address current research on the effects of vitamin D, vitamin $B_{12,}$ docosahexaenoic acid (DHA) and choline intake (or status) during pregnancy on child cognitive function.

Chawla et al. (2017) looked at the connection between prenatal vitamin D concentration and children's social and emotional development. Vitamin D receptors are found extensively in human neurons and glial cells, so it is logical to examine the effect of prenatal vitamin D intake on neurocognitive development. Chawla et al. included 218 mother-infant pairs from the Newborn Epigenetic Study displaying a racial/ethnic breakdown of 36% White, 30% Black, and 34% Hispanic. All participants had prenatal 25-hydroxyvitamin D (25(OH)D) concentrations available and previously completed the Infant Toddler Social Emotional Assessment (ITSEA) when the child was one year old.

The ITSEA scores calculated for externalizing, internalizing, dysregulation, and social-emotional competence behaviors (Chawla et al., 2017). The assessment also included domains that may reflect early signs and symptoms of Autism Spectrum Disorder (ASD). Plasma 25(OH)D concentrations were measured from blood samples in the first or second trimesters, and values were divided into quartiles representing serum vitamin D levels. Black women had a significantly lower 25(OH)D distribution compared to White and Hispanic women. For this reason, Chawla et. al conducted analyses stratified by race/ethnicity. They also controlled for season of blood draw, as plasma 25(OH)D measurement includes both food and UVB light derived sources of Vitamin D. Race and ethnicity strongly influenced the association between vitamin D concentrations and ITSEA scores. Among White and Hispanic infants, lower quartiles of 25(OH)D were associated with less favorable dysregulation scores (dysregulated sleep or eating) and ASD social competence scores (imitative play, eye contact). Lower quartiles of 25(OH)D were associated with more favorable scores for internalizing behaviors, AND social competence, and ASD problem behavior (repetitive behavior, difficulty adjusting to change) in Black infants. Chawla et al. discussed that the results pertaining to Black infants opposed their original hypothesis and speculated that this could be related to the smaller sample size.

Darling et al. (2017) also used the data of over 5000 mother-child pairs from a large cohort study, the Avon Longitudinal Study of Parents and Children (ALSPAC), to explore serum vitamin D status during pregnancy and neurodevelopmental outcomes in childhood. In order to be included in the study, mother-child pairs needed to have one recorded maternal measure of vitamin D status during pregnancy and at least one child neurodevelopmental outcome on record between 6 months and 9 years of age. Potential neurodevelopmental tests included the

ALSPAC pre-school development test, assessment of IQ at 8 years and assessment of reading ability at 9 years. The primary notable outcome was that children of vitamin D deficient mothers had an increased likelihood of scoring lower on the gross-motor and fine-motor domains of the ALSPAC pre-school development tests when tested at 30 months and lower social development scores at 42 months compared to the children of vitamin D sufficient mothers.

Instead of utilizing cohort data, Voltas et al. (2020) conducted a randomized controlled trial of 422 mother-infant pairs to study the effect of vitamin D status during pregnancy on infant neurodevelopment. At weeks 12 and 36 of pregnancy, researchers evaluated maternal serum vitamin D levels, dietary data using a validated FFQ, and administered the State-Trait Anxiety Inventory to determine the mothers' emotional status during pregnancy. At the 40-day postpartum visit, qualified staff conducted the Bayley Scales of Infant and Toddler Development-3rd edition (Bayley-III) to assess infant cognitive development and the Prenatal Stress Index Form 4th Edition to evaluate attachment between the mother and child. Only the cognition, motor skills and language subscales were considered during the Bayley-III assessment. After controlling for mothers' attachment to the infant and their mental state during pregnancy, Voltas et al. found that inadequate vitamin D levels (<30 nmol/L) during the first trimester were associated with poorer cognitive and language domain outcomes, and inadequate levels in the third trimester were associated with poorer outcomes in the motor skills domain. Highly deficient vitamin D levels (<20 nmol/L) were associated with poorer language performance. About 80% of the mothers had either deficient or insufficient levels of vitamin D in the first trimester, although these levels improved slightly by the third trimester.

The National Institutes of Health currently recommend pregnant and breastfeeding individuals consume 15 mcg (600 IU) vitamin D per day (*Office of Dietary Supplements - Vitamin D*, n.d.). Current data shows that prevalence of vitamin D deficiency is significantly higher among individuals of Asian, Black African, and mixed ancestry compared to individuals of White European Ancestry (Sutherland et al., 2021). Considering the large variations in effects of 25(OH)D based on maternal race/ethnicity as seen in the study by Chawla et al. (2017), it is essential to further understand the role of prenatal vitamin D status on fetal neurodevelopment and how this might impact child behavior.

Vitamin B12. Srinivasan et al. (2017) used data collected in a parent study by Duggan et al. (2014) to assess the impact of vitamin B12 supplementation during pregnancy and early lactation on infant neurocognitive function. The original randomized controlled trial consisted predominantly of women of lower socioeconomic status in Bangalore, India. Healthy mothers were provided either oral B12 supplements or a placebo at or before 14 weeks of gestation, and the supplementation continued through 6 weeks postpartum. Blood samples were tested for B12, total homocysteine (tHcy), methylmalonic acid (MMA) and erythrocyte folate concentrations throughout pregnancy and after birth. Duggan et al. found that infants of the mothers supplemented with B12 had significantly higher B12 levels.

Using these results, Srinivasan et al. (2017) examined the correlation between the B12 levels of the infants and their neurocognitive development. The authors used the Bayley Scales of Infant and Toddler Development 3rd edition (Bayley-III) to assess neurocognitive development at 9 months of age because developmental functions such as language, increased motor coordination, and increased engagement tend to appear at this time. The Bayley -III

scales assess multiple developmental domains, but for the purpose of this study, only cognitive, language and motor functions were targeted. A questionnaire was also provided to parents to assess aspects of the home environment and parenting behavior. The biochemical measures collected by Duggan et al. (2014) were used in the analysis by Srinvasan et al. (2017). Although no relationship was identified between maternal B12 supplementation and infant neurocognitive outcomes at 9 months, elevated tHcy levels throughout pregnancy were associated with poorer performance in the language expression domain, and higher levels in the first trimester were associated with poorer fine motor function. Elevated tHcy levels are significant in this context because they are recognized as a biomarker of deficiency for several B vitamins, especially folate deficiency. Srinvasan et al. concluded that the data presents an opportunity to further study the impact of longer-term supplementation in pregnant women with elevated tHcy levels or B12 deficiency.

Thomas et al. (2018) conducted an additional follow up study, using Bayley -III to assess child cognitive function at 30 months of age. Unlike Srinvasan et. al (2017), the authors found that children of mothers who received oral B12 supplementation scored significantly higher on the expressive language domain at 30 months. Thomas et al. (2018) also reported that children of mothers with elevated tHcy in the second and third trimesters scored significantly lower on the expressive language and gross motor domains compared with children of mothers with normal tHcy levels. These results align with the relationships identified by Srinvasan et. al (2017) between maternal tHcy levels during pregnancy and child cognitive function at 9 months and provide a foundation for continued research on the association between maternal B12 supplementation and offspring cognitive functioning.

While Srinvasan et al. (2017) and Thomas et al. (2018) analyzed the effects of maternal B12 supplementation on child cognitive outcomes, Lai et al. (2019) used an observational approach to study the relationship between plasma vitamin B12 v concentrations during pregnancy and cognitive outcomes at age two. Lai et al. used mother-infant pairs from the Growing Up in Singapore Towards Health Outcomes cohort. Maternal plasma B12 and tHcy levels were measured at 26-28 weeks gestation and child cognitive development was evaluated using Bayley-III at 24 months. Maternal diet was also assessed using 24-hour recall questionnaires at 26-28 weeks gestation. The authors found that 57.5% of the cohort had deficient or insufficient levels of vitamin B12. Infants of mothers with B12 deficiency, as well infants of mothers with a co-occurrence of B12 and B6 insufficiency had lower scores in the Cognitive domain. Deficiency or insufficiency was not associated with other Bayley-III subscales. Although the study by Lai et al. (2019) did not implement B12 supplementation, the results indicate a possible relationship between maternal B12 intake and infant cognitive outcomes and the large percentage of the cohort experiencing B12 deficiency and insufficiency indicates this as an important topic for continued prenatal education and research.

Docosahexaenoic acid (DHA). DHA is an essential omega-3 fatty acid, recognized for its role in brain development and function (Horrocks & Yeo, 1999). Using data from a randomized trial of maternal DHA supplementation in pregnancy, Mulder et al. (2018) conducted a follow-up study on fetal DHA status and the impact on child neurodevelopment. In the original study, women were randomized to receive 400 mg/d DHA supplementation or a placebo from 16 weeks gestation to delivery. Dietary intake was assessed using FFQs and maternal DHA status was evaluated using measurement of erythrocytes at 16- and 36-weeks gestation. Neural

development was assessed using standardized tests when the child was 5.75 years old. These tests assessed measures of memory, visual-motor integration, language development, attention, and impulsivity. Results from the primary study showed that insufficient DHA intake during pregnancy was correlated with a greater risk of receiving lower scores on infant neurodevelopment tests at 18 months of age, but Mulder et al. did not find the same association in the children reassessed at 5.75 years. The authors found that maternal DHA status was positively associated with child performance on tests evaluating language and short-term memory. It is important to clarify that maternal DHA supplementation and DHA status are related, but they are not identical measures, as dietary intake of DHA-containing foods can also contribute to DHA status.

In another randomized control trial conducted by Colombo et al. (2019), pregnant mothers received supplementation of 600 mg/d of DHA or a placebo from 14.5 weeks through delivery. Children of these mothers underwent cognitive and behavioral testing from the ages of 10 months to 6 years. Assessments included a combination of standardized tests, laboratory-based tasks and parent-reported measures that evaluated a variety of cognitive functions such as problem solving, spatial memory, literacy, rule-learning and self-regulation of mood and attention. Maternal serum DHA levels at study enrollment were associated with productive vocabulary at 18 months and DHA supplementation was associated with a significant reduction in preterm birth and improved development of attention in infancy. Researchers noted that consumption of breastmilk and formulas may have provided infants with adequate amounts of DHA to support cognitive development, thus impacting the ability to discern the benefits of supplementation during pregnancy.

The studies by Srinvasan et al. (2017) and Mulder et al. (2018) exemplify the nuance of nutritional research, especially when looking at intergenerational effects. Both studies had to control for variables such as parenting behaviors and involvement, parental IQ, and number of parents and children in the home, which could've influenced child neurodevelopment test scores. Colombo et al. (2019) highlighted another commonly experienced barrier. This being, as a child gets older, it can be more difficult to isolate the effect of prenatal diet intake on child development because data could be masked by the dietary pattern of children.

Choline. Human research studies on choline supplementation are limited (Cheatham, 2019). Recently, the investigation into choline's role in neurodevelopment and neuroprotection has been of increasing interest. Jacobson et. al (2018) and Warton et al. (2021) explored the possibility of mitigating the effects of prenatal alcohol exposure (PAE) with high levels of choline supplementation during pregnancy. Both studies utilized the same sample population consisting of 69 heavy drinkers in Cape Town, South Africa. Individuals were considered heavy drinkers during pregnancy if they consumed an average of 2 or more standard drinks a day or had 2 or more incidents of binge drinking (4 or more drinks per occasion). Participants received either 2 grams choline supplementation or a placebo daily from mid-pregnancy through delivery. Jacobson et al. measured eyeblink conditioning at 6.5 months and assessed recognition memory and processing speed using the Fagan Test of Infant Intelligence at 6.5 and 12 months. The children of choline-treated mothers were significantly more likely to meet the criterion for eyeblink conditioning than the placebo group, after the infants whose mothers had very poor (<20%) adherence to the choline-supplementation regimen were excluded from analysis. The authors also reported that infants in the choline treatment group had higher

novelty preference scores at 12 months. Novelty preference scores are an indicator of visual recognition memory. This is determined by evaluating the ability of an infant to recall a familiar stimulus, such as a photograph, and differentiate it from a new stimulus. The recognition memory scores can be used as a predictor of intellectual function in adolescence.

In the supplemental study by Warton et al. (2021), infants underwent Magnetic Resonance Imaging to assess regional brain volume between 1-7 weeks. The authors found that normalized volumes (regional volume divided by total intracranial volume) were larger in 6 of the 12 brain regions in the treatment group when compared to the placebo group. The larger volumes also correlated with the level of maternal adherence to the supplementation regimen. Jacobson et al. (2018) and Warton et al. (2021) demonstrated choline's potential neuroprotective properties against PAE.

Caudill et al. (2018) studied the effect of maternal choline supplementation during pregnancy on infant processing speed, which is recognized as a measure of cognition. Women entering their third trimester of pregnancy were recruited to participate in a double-blind randomized controlled trial. A group of 29 participants were randomly assigned to receive 480 or 930 mg choline/day through a strictly controlled dietary and supplement regimen. Women had to consume at least one meal and supplement onsite Monday through Friday, and all other meals were provided for the mothers to take home. To ensure compliance, participants filled out a daily food checklist. Researchers then gathered maternal and newborn medical information from charts at the time of delivery. In phase two of the experiment, 24 of the infants from the original 29 mothers were brought to laboratory visits at 4, 7, 10, and 13 months of age to participate in visual attention tasks designed to measure saccade (rapid eye

movement) reaction time. Both mean saccade reaction time and predictive saccades were analyzed. Caudill et al. estimated mean saccade reaction time for infants in the 930 mg choline/day group was significantly faster than the reaction time for infants in the 480 mg choline/day group when averaged across all ages, as well as both unpredictable and predictable sequence types. There was no significant effect of maternal choline intake on the number of predictive saccades during infant testing.

According to the National Institutes of Health, the current recommended choline intake during pregnancy is 450 mg/day (*Office of Dietary Supplements - Choline*, 2021). Caudill et al. (2018) concluded that choline supplementation during the third semester of pregnancy, at levels exceeding the current Adequate Intake (AI), was shown to improve infant processing speed relative to maternal consumption levels. The authors only evaluated infant saccade reaction times as a measure of cognitive function, so the results can only be applied to cognitive benefits.

In two separate 7-year follow up studies, Bahnfleth et al. (2019, 2022) examined the longevity of the cognitive outcomes identified in the controlled choline feeding study by Caudill et al. (2018). In one study, children were tested using the Sustained Attention Task (SAT) (Bahnfleth et al., 2022). The SAT is a computer-administered evaluation that requires the individual to indicate whether or not a visual signal appeared on the screen for the duration of 216 trials. It is designed to assess cognitive control of voluntary attention. Essentially, this test evaluates the child's ability to remain engaged and correctly identify the presence or absence of a visual stimulus during a prolonged decision-making process.

In the second study, children repeatedly performed a color-location memory task which required them to recall the location of dots on a cartoon figure after either a 1 or 8 second retention interval (Bahnfleth et al., 2019). The task increased in difficulty as the levels of the evaluation progressed. Bahnfleth et al. (2019, 2022) found that the 7-year-old children born to women in the 930 mg choline/day group achieved both higher SAT scores and passed more levels in the color-location memory task than the children of women in the 480 mg choline/day group, indicating improved attentional control and memory span among the children whose mothers received higher levels of prenatal choline supplementation. Twenty of the initial 26 children involved in the study by Caudill et al. (2018) were included in the final analyses of both studies. The results of these studies reinforce the findings by Caudill et al. and show that the benefits of choline supplementation at 930 mg/day extend from infancy into early childhood. Although Caudill et al. and Bahnfleth et al. (2019, 2022) utilized strong study designs, the sample size was small, so this limits the ability to generalize their findings.

Ross et al. (2016) looked at the interaction between high-dose oral choline supplementation and the CHRNA7 (α7- nicotinic acetylcholine receptor) gene which has been associated with schizophrenia, autism, and ADHD (Allen-Brady et al., 2009; “Rare Chromosomal Deletions and Duplications Increase Risk of Schizophrenia,” 2008; Williams et al., 2012). Mothers were either given a 6300 mg phosphatidylcholine supplement (providing about 900 mg choline) or a placebo each day, beginning at 16 weeks gestation. Infants continued to receive choline supplementation at lower doses until 3 months of age. As a means to control for dietary intake, all mothers were provided nutrition education to encourage similar dietary choline intake during pregnancy. Parents completed the Child Behavior Checklist to evaluate

internalizing and externalizing factors when their child reached 40 months of age. Children of participants in the choline treatment group reported fewer attention problems and less social withdrawal than children in the placebo group.

Expanding on the study by Ross et al. (2016), Hunter et al. (2020) looked specifically at the data associated with Black mothers and children. This only included a sample size of 25 participants. As a comparative measure to the Black American mothers, the authors enrolled an additional 166 women from rural Uganda. During initial data collection, Black women had higher instances of self-reported depression symptoms and negative experiences in childhood. Interestingly, plasma choline levels were significantly lower in Black American women compared to white American women and the sample population of Ugandan women, so there seem to be factors other than ethnic background that are contributing to the choline levels in Black American women. It is worth noting that factors such as household income and obesity were not related to plasma choline concentration, as these factors are often associated with poorer dietary intake. Results from the Child Behavior Checklist indicated that male children of choline supplemented Black American mothers scored significantly lower in the Withdrawn and Attention problems domains, compared with the placebo treated mothers and offspring. No significant differences were noted among the child female population. Symptoms of social withdrawal and attention problems are some of the earliest signs of schizophrenia, so the results from both Ross et al. (2016) and Hunter et al. (2021) provide a foundation for continued research into the use of choline supplementation as a preventative measure for mental illness (Reichenberg et al., 2010).

Choline supplementation during pregnancy was shown to have potentially positive effects with regard to enhancing cognitive benefits and providing protection against prenatal alcohol exposure and mental illness in childhood (Bahnfleth et all, 2019, 2022; Caudill et al., 2018; Hunter et al., 2021; Jacobson et al., 2021, Ross et al., 2016; Warton et al., 2021). While all of these studies are randomized controlled trials, they also include small sample sizes. Future studies need to be conducted on larger populations to reinforce these results and provide stronger evidence for the use of prenatal choline supplementation as a means of supporting fetal neurodevelopment.

Research Methodology

Considering the limited research on prenatal choline supplementation and infant cognitive development, the proposed study is loosely based off of the design by Caudill et al. (2018) which included treatment groups of 480 mg choline/day or 930 mg choline/day through a controlled study diet and supplementation. Due to the size of the study, individual diets will not be controlled. Members of two treatment groups will receive either 150 mg/day or 600 mg/day of choline chloride supplements. Supplement quantities are based off of a study conducted by Bailey et al. (2019) that examined usual nutrient intake distributions of pregnant women aged 20-40 years based on National Health and Nutrition Examination Surveys (NHANES) from 2005-2014. This study reported that the mean choline intake (through foods and supplements) for pregnant women was 322 mg choline/day. Based on this average choline intake of 322 mg choline/day, supplementation of 150 mg choline/day will provide an estimated 472 mg choline/day which is enough to meet the recommendation of 450 mg/day during pregnancy. Supplementation of 600 mg choline/day will provide an estimated total of

922 mg choline/day. Supplemental choline will be provided in the form of choline chloride. Rodent research has shown that choline chloride provides greater bioavailability of choline to the brain compared with phosphatidylcholine, which is commonly used in studies providing choline supplementation (Cheng et al., 1996).

Supplements will be provided on a 30 day-cycle, and participants will be required to return the supplement package every month as a way to track compliance. The process to track compliance is based off of the study by Thomas et al. (2018). Participants with a compliance rate of less than 70% during 2 or more months of the study duration will be excluded for lack of compliance. The compliance rate requirement of 70% is comparable to the criteria used by Ross et al. (2019).

The ASA24 will be used to collect prenatal maternal dietary information, as seen in the study by Shapiro et al. (2016). This tool, created by the National Cancer Institute, is based on the United States Department of Agriculture's (USDA) Automated Multiple-Pass Method, and validation and evaluation studies indicate that this system is closely aligned with standardized interviewer-administered 24-hour recalls (*ASA24® Evaluation & Validation*, 2022). Using the ASA24 will allow participants to complete their food recalls autonomously and reduce the amount of labor required from research staff.

Instead of assessing infant cognitive function through the completion of visual attention tasks, the proposed study will use the Bayley Scales of Infant and Toddler Development as was done in studies by Lai et al. (2019), Srinivasan et al. (2017), Thomas et al. (2018), and Voltas et al. (2020). The Bayley Scales of Infant and Toddler Development- 4th edition (Bayley-4) will be used in place of Bayley Scales of Infant and Toddler Development- 3rd edition (Bayley-III), as

Bayley-4 takes less time to administer and has a greater sensitivity and accuracy than Bayley-4 (Balasundaram & Avulakunta, 2021).

Summary

Much of the research conducted in the past focused on the importance of prenatal nutrition with regard to basic fetal development and birth outcomes. The impact of prenatal nutrition on cognitive function and behavior of offspring, however, is of growing interest. The study by He et al. (2018) revealed that prenatal nutrition, especially in severe circumstances such as prenatal famine, has a lasting effect on the cognitive functioning of offspring. This literature review has shown that general dietary patterns, as well as individual micronutrient intake, can influence cognitive development and function in infancy and into childhood. The aim of this study is to expand on the limited body of evidence from human research demonstrating that choline supplementation during pregnancy has potential neurodevelopmental and neuroprotective benefits for infants (Bahnfleth et al, 2019, 2022; Caudill et al., 2018; Hunter et al., 2021; Jacobson et al., 2021, Ross et al., 2016; Warton et al., 2021). Future studies should include larger sample sizes and explore various levels of choline intake and supplementation, as further evidence could provide a rationale for increasing choline recommendations during pregnancy. The methodology of the proposed study will be further detailed in the upcoming chapter.

Chapter 3: Methodology

Maternal nutritional status during pregnancy impacts the health of both the mother and the fetus. Many micronutrients, such as choline, are essential for fetal brain development and have been associated with cognitive development in children (Irvine et al., 2022). The American Academy of Pediatrics recognizes choline as an important nutrient for neurodevelopment, yet it is estimated that less than 10% of pregnant women achieve the target intake level of 450 mg/day (Korsmo et al., 2019). Although choline is synthesized by the body in small amounts, additional intake is needed through diet or supplementation for optimal health. As a methyl-group donor for DNA methylation, choline plays a complex role in brain development and function (Irvine et al., 2022). Adequate amounts are needed to prevent neural tube defects and for hippocampal development since this impacts functions such as learning and memory. Very few human studies have investigated the impact of prenatal choline intake on infant cognitive outcomes. This chapter will address the methodology of the proposed study including research protocol, study design, data collection and analysis plan, threats to validity, ethical procedures, and a summary of the provided information.

Research Protocol

Research Question

Is prenatal choline supplementation during the second and third trimesters of pregnancy associated with improved infant cognitive outcomes [evaluated by the Bayley Scales of Infant and Toddler Development 4th edition (Bayley-4)] at 1 month and 12 months of age?

Hypotheses

H_0: The amount of prenatal choline supplementation has no effect on infant cognitive outcomes as measured by the Bayley Scales of Infant and Toddler Development- 4th edition (Bayley-4) at both 1 and 12 months of age.

H_1: Infants of mothers receiving higher amounts of prenatal choline supplementation, compared with lower amounts, will have significantly higher cognitive outcomes as measured by the Bayley Scales of Infant and Toddler Development- 4th edition (Bayley-4) at both 1 and 12 months.

Table 1

Research Questions and Variables

Research Question	**Independent**	**Dependent**	**Confounding**
Does prenatal choline supplementation during the second and third trimesters in amounts exceeding the Adequate Intake, compared with lower amounts of supplementation, result in significantly higher infant cognitive outcomes [evaluated by the Bayley Scales of Infant and Toddler Development 4th edition (Bayley-4)] at 1 month and 12 months of age?	Prenatal choline supplementation (150 mg/day or 600 mg/day)	Infant cognitive outcomes	Prenatal dietary choline intake (other than supplementation), dietary choline intake that is higher or lower than expected, parental cognition (IQ, etc.), home environment

Study Design

The study will be a 40-month, double-blind randomized controlled trial, with two treatment arms. Randomization will help to minimize potential confounders. The two treatment arms will include a 150 mg choline/day supplementation group and a 600 mg choline/day supplementation group. Infants will complete the Bayley-4 assessment at 1 month and 12 months of age to provide data on developmental progress.

Setting and Sample

A sample size of at least 200 participants will be needed when accounting for attrition and exclusions (e.g., non-compliance, gestational diabetes or pre-eclampsia diagnosis) that occur during the study. This sample size was based off of similar studies that assessed prenatal micronutrient supplementation and child cognitive function and neurodevelopment, such as those by Mulder et al. (2018) and Thomas et al. (2018).

Live births for Milwaukee, Waukesha, and Washington counties totaled to 18,995 in 2016 (*2016 Births to Wisconsin Residents by County,* 2020). While there are other maternity clinics, hospitals, and birthing centers available to the surrounding population, the Froedtert & MCW Health System is one of the primary health care systems in the area.

Supplement preparation and infant testing and evaluation will be conducted at Town Hall Health Center in Menomonee Falls, WI. Town Hall Health Center is part of the Froedtert & Medical College of Wisconsin (MCW) Health System and has an Obstetrics and Gynecology (OB-GYN) clinic onsite. Froedtert & MCW Health System services Milwaukee, Waukesha, and Washington counties and this clinic location is fairly centralized among those counties. Froedtert & MCW Health System was selected as the source of the sample population due to

potential funding through grants provided by the Medical College of Wisconsin's Children's Research Institute.

Population

Eligible study subjects will be pregnant women aged 18 years or older who are at 14 or less weeks of gestation at the time of recruitment. The participants must be patients of the Froedtert & Medical College of Wisconsin (MCW) Health Network Bayley-4 and, as a result, will likely reside in Milwaukee, Waukesha, or Washington counties. Participants (or legal guardians of the infants) will need to be able to provide their own transportation to Town Hall Health Center in Menomonee Falls, WI for infant testing at 1 and 12 months.

Recruitment

Pregnant women will be recruited through an open enrollment process (January 2024 to July 2025) from the 17 OB-GYN clinics managed by the Froedtert & MCW Health Network located in Milwaukee, Waukesha, and Washington counties in southeastern Wisconsin. Research staff will request that all OB-GYN clinics that are part of the Froedtert & MCW Health Network send out study information via electronic messaging to their active patient list on a monthly basis, as well as have fliers with a brief overview of the nature of the study and contact information available in each clinic. Any patient that attends an appointment at one of these clinics and is in their first trimester of pregnancy will also be given an informational handout with details on the study and who to contact to enroll. Participants will be asked to complete an initial screening survey to gather demographic data and information on the criteria listed in Table 2. Maternal exclusion criteria were based upon the randomized controlled trial by Caudill et al. which investigated the effects of prenatal choline supplementation on infant processing

speed (2019). Potential study participants will receive this screening form via encrypted email, and identification of any exclusion criteria will inform the patient that they are not eligible to participate in the study. Study volunteers will review all completed surveys within a 72-hour period. Patients who do not identify any exclusion criteria will have their survey information sent to the research team for review, and eligibility will be confirmed by cross-checking patient information through Epic Electronic Medical Records. Once a patient is confirmed to be an appropriate study participant, they will receive a consent form and additional details on the study process and expectations.

To incentivize participation, marketing material will emphasize that participants will be provided with free prenatal vitamins during the second and third trimesters of pregnancy, and parents will receive feedback on their child's cognitive development after completing Bayley-4. Families will also be provided with a packet of resources highlighting nutritional recommendations and strategies to support the continued cognitive development of their child after completion of the study.

Table 2

Inclusion and Exclusion Criteria

Inclusions	**Exclusions**
≥ 18 years, any race and ethnicity, assigned female at birth, less than 14 weeks gestation at the time of recruitment, singleton pregnancy, patient in the Froedtert & MCW Health Network, ability to provide personal transportation to research clinic for infant cognitive screenings, current email address and regular computer access	Maternal: Multiple pregnancy, anemia, kidney/liver blood markers outside of the normal range, cardiovascular or pulmonary diseases, cancer, diabetes, self-reported tobacco, drug or alcohol use or presence of complications such as preeclampsia or gestational diabetes, non-compliance with supplement regimen, hyperemesis gravidarum, fish or soy allergy Infant: premature birth (<37 weeks), genetic conditions that are known to impact cognitive development such as Down syndrome

Study Protocol and Intervention

Primary research staff will be recruited through Froedtert & MCW Health Network. Research staff will include dietitians, psychiatrists, and other medical professionals qualified to conduct the Bayley-4 assessment. Students will be recruited for volunteer-based positions from undergraduate and graduate programs (such as dietetics, nursing, and psychology) at Mount Mary University, Marquette University, Milwaukee Area Technical College and University of Wisconsin-Milwaukee. Individuals in volunteer-based positions will require CITI training. Volunteers will be responsible for duties such as reviewing initial screening forms, assembling monthly supplement packages, and reaching out to participants to schedule infant cognitive testing.

After completing the enrollment process, participants will be randomized into 2 groups using an online clinical trial randomization tool. Members of these groups will receive either 150 mg/day or 600 mg/day of choline chloride supplements. Supplemental choline will be provided in the form of choline chloride. Rodent research has shown that choline chloride provides greater bioavailability of choline to the brain compared with phosphatidylcholine, which is commonly used in studies providing choline supplementation (Cheng et al., 1996). Choline chloride supplements will be purchased from Balchem Human Nutrition & Health in the form of a water-soluble powder.

As previously mentioned, the tolerable upper intake level (UL) of 3.5 g choline/day for adults was established to prevent hypotension and fishy body odor (Korsmo et al., 2019). To address ethical concerns, any ASA24 that reports choline intake of 2500 mg or more will be flagged. The participant associated with the ASA24 will be contacted by a registered dietitian from the research staff and provided basic education on the risks of excessive choline intake. The value of 2500 mg was chosen to account for the possibility that the individual is part of the 600 mg choline supplementation treatment group and to consider potential reporting error when completing the ASA24.

Each participant will also receive a Nature Made Multi + DHA prenatal vitamin throughout the duration of the study, or a prenatal vitamin of similar micronutrient composition with USP Verified Mark and will be discouraged from utilizing additional supplements. The USP Verified Mark reflects that a specific dietary supplement passed the verification process and meets the quality standards set by U.S. Pharmacopeia (*Dietary Supplements & Herbal Medicines | USP*, n.d.). Women with fish or soy allergies will be excluded

from the study due to the use of fish oil as the primary source of DHA in majority of the prenatal vitamins reviewed for this study. Vegan prenatal vitamins utilize marine algae as their source of DHA, but these varieties tend to be significantly more expensive. The incidence of finned fish and soy allergies in the United States are very low (1% and 0.4%), so excluding these individuals should not have a significant impact on sample size (*Common Allergens - Peanut, Egg, and Sesame Allergies | FARE*, n.d.). Mothers will be instructed to follow their health care provider's guidance with regard to supplementation postpartum. The research team will seek funding for the prenatal and choline chloride supplements through the FDA Office of Women's Health.

The choline chloride supplements will be provided in the form of a lemon-lime powder that can be mixed into water. Participants will receive a new supply of either the 150 mg or 600 mg choline chloride supplement, as well as the prenatal multivitamin, every 30 days. The supplement packets will be prepared onsite at the dedicated research space at Town Hall Health Clinic by research staff and the choline supplements will not be labeled with any information identifying the dosage. The supplements will be sent in an unmarked package (aside from participant name and address) with return postage, and participants will need to send back the package to the research clinic at the end of the 30-day cycle. To maintain blinding, none of the staff responsible for packaging and sending supplements will be involved with infant cognitive testing. Members of the research team who conduct data analysis will not take part in any aspects of the intervention, including supplement preparation or administration of the Bayley-4.

After giving birth, the mother will no longer be provided with prenatal vitamin or choline supplementation and will be expected to report the use of any postnatal vitamin intake. Research staff will contact participants at 1 week and 10 months postpartum to schedule a time for infant developmental testing. A parent or guardian will fulfill study guidelines by bringing the infant to the research center for testing at 1 ±0.25 months and 12 ±0.5 months of age. A qualified member of the research team will conduct the Bayley-4 testing at each appointment.

Data Collection Process

Maternal sociodemographic information will be assessed through a secure online questionnaire. Maternal dietary intake during pregnancy will be collected through the Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tool. Participants will be required to complete the ASA24 within 2 weeks of their enrollment date and on a monthly basis until they give birth. The research team will provide each participant with a window of time in which they can complete their ASA24 each month. At least 2 of these monthly recalls must be completed on a weekend. Registered dietitians from the research team will be responsible for managing the data from each ASA24 and reporting the estimated dietary choline intake for each participant. Information on dietary choline intake will be used during the data analysis process to determine average dietary choline intake for each treatment arm.

Infant anthropometric data, measured and recorded by nursing staff, will be gathered through the Epic Electronic Health Record (EHR). Anthropometric data, including standard growth measures of weight, will be collected at birth as well as at each infant cognitive testing session (at age 1 month and 12 months). The Bayley-4 will be administered to infants at 1 ±0.5 months and 12 ±0.5 months by trained clinical staff. Trained clinical staff could include a

psychologist, pediatrician, occupational therapist, speech and language pathologist, or pediatric nurse practitioner (Balasundaram & Avulakunta, 2021).

Instrumentation

The ASA24 will be used to collect prenatal maternal dietary information. This tool, created by the National Cancer Institute, is based on the United States Department of Agriculture's (USDA) Automated Multiple-Pass Method, and validation and evaluation studies indicate that this system is closely aligned with standardized interviewer-administered 24-hour recalls (*ASA24® Evaluation & Validation*, 2022). The ASA24 collects and analyzes dietary data, providing estimated nutrient intake. Using the ASA24 will allow participants to complete their food recalls autonomously and reduce the amount of labor required from research staff.

The Bayley Scales of Infant and Toddler Development 4th edition (Bayley-4) will be used to evaluate infant cognitive development. For decades, researchers and clinicians have used various versions of the Bayley Scales of Infant and Toddler Development to monitor children's developmental progress and identify developmental delays (Alfonso et al., 2022) . The 4th edition of the formal assessment is published by Pearson Clinical and can be used with children aged 16 days to 42 months. It assesses Cognitive, Language, Motor, Social-Emotional, and Adaptive Behavior domains. For the purposes of this study, children will only be evaluated on the Cognitive, Language, and Motor domains, as they were standardized together and are known for having exceptional validity and reliability. Additionally, the items in these domains were designed utilizing an integrated neuro-development model. A summary of the underlying skills assessed in these domains can be found in Appendix C. Each domain has a set number of items and is scored using a polytomous system (one that allows for more than two scoring

options) with each item receiving either a 2, 1, or 0 signifying that a skill has been mastered, is emerging, or is not yet present. The individual item scores are totaled to produce an overall score for each domain. The Bayley-4 takes less time to administer and has a greater sensitivity and accuracy than the Bayley-III (Balasundaram & Avulakunta, 2021).

Data Analysis Plan

This study will include two treatment arms. Participants will be randomized to receive either 150 mg choline supplementation/day or 600 mg choline supplementation/day. Mean and standard deviation will be calculated for ratio data including maternal age, pre-pregnancy BMI, average monthly household income, dietary choline intake, infant birth weight, and Bayley-4 domain scores. Graphical representations of means, medians and distributions of the Bayley-4 domain scores can be found in Figures 2 and 3, and the specific values for means will be included in the explanation of results.

Inferential statistics will be reported as *p*-values. A Chi-Square Goodness-of-Fit test will be used for maternal demographic comparisons among the treatment groups. For ratio data, including maternal age, pre-pregnancy BMI, average monthly household income, dietary choline intake, and infant birth weight, an independent t-test will be used to test for any differences among the treatment groups. Descriptive statistics (means, standard deviations, number in subsamples, percentages) and inferential statistics for the participant baseline characteristics can be found in Table 4. Independent t-tests will be used to examine any significant differences between the scores of the two treatment groups within each domain of Bayley-4 at 1 month and 12 months. The independent and dependent variables can be found in Table 3.

Table 3

Description of Variables

Variable Type	Variable Name	Potential responses	Variable Source	Level of Measurement
Independent	Level of choline supplementation	150 mg choline/day or 600 mg choline/day	Assigned group (based on randomization)	Nominal
Dependent	Bayley-4: Cognitive domain	40-155	Bayley-4 assessment	Ratio
Dependent	Bayley-4: Language domain	40-155	Bayley-4 assessment	Ratio
Dependent	Bayley-4: Motor domain	40-155	Bayley-4 assessment	Ratio

Threats to Validity

Since the sample population will only reside in southeastern Wisconsin (likely Milwaukee, Waukesha, and Washington counties), results cannot be generalized to the entire population. The accuracy of nutritional data will also be a threat to validity, as participants may under or over report their food and beverage consumption when completing the ASA24. Attrition could also be a threat to validity. The expected time commitment for the mother-infant pairs to complete the study will be approximately 18-20 months. It is possible that the drop-out rate may be higher than accounted for when determining required sample size.

Ethical Procedures

Approval from the Mount Mary University Institutional Review Board (IRB) will be attained prior to beginning the study. To ensure privacy and protection of patient medical information, all members of the research staff and volunteers will be required to have up to date Health Insurance Portability and Accountability Act (HIPPA) training. Staff and volunteers

will receive training and must maintain HIPPA compliance throughout the entire research process. All study participants will be required to complete the Informed Consent process. Participant names will not appear on any study materials. Each participant will be assigned an identification number for the duration of the study to help maintain privacy and to ensure proper blinding. The study will have dedicated computers that are password-protected; only the necessary research staff will have access to the password.

Summary

The proposed randomized controlled trial will examine the differences in dose-response between two levels of maternal choline supplementation and infant cognitive function as assessed by Bayely-4 at 1 and 12 months of age in two treatment groups (150 mg choline supplementation/day and 600 mg choline supplementation/day. Due to the timing of infant cognitive testing, the maximum duration of the study would be approximately 40 months (recruitment occurring from January 2024 to July 2025 and infant cognitive testing for the last recruitment group occurring in April 2027). It is anticipated that prenatal choline supplementation of 600 mg/day, vs 150 mg/day, during the second and third trimesters will result in infants scoring significantly higher in the Cognitive domain of the Bayley-4 at both the 1-month and 12-month assessments. Further research will be needed to expand on these results and build on the existing body of evidence demonstrating the potential neurodevelopmental benefits of choline supplementation. Additional research could also either reinforce the adequacy of the existing AI for choline during pregnancy, or provide enough evidence to support increasing these recommendations.

Chapter 4: Anticipated Results

This chapter will present the expected results from the proposed study. In total, 300 expectant mothers will be recruited from Froedtert & MCW Health Network between January 2024 and July 2025. A mother-infant pair will be identified as one participant. Participants will be randomly assigned to treatment groups with 150 assigned to the 150 mg choline/day group and 150 assigned to the 600 mg choline/day group. It is expected that 266 participants will complete treatment, with attrition due primarily to miscarriage and non-compliance. An additional 26 participants will be excluded due to preterm births. Miscarriage and preterm birth rates are estimated based on the most recent data from the March of Dimes (*Distribution of Gestational Age Categories: Wisconsin, 2021*, n.d.; *Miscarriage*, n.d.). Figure 1 reports the total number of infants anticipated to complete Bayley-4 testing at 1 month of age. Additional attrition is anticipated between the 1-month and 12-month testing.

Baseline Characteristics

It is anticipated that there will be no significant differences in baseline characteristics between the two treatment groups. A Chi-square Goodness of Fit test will be used for maternal demographic comparisons. Further data on baseline characteristics can be found in Table 4.

Figure 1

Flowchart Depiction of Participants From Study Enrollment Through Intervention and Infant Behavioral Assessment at 12 Months of Age

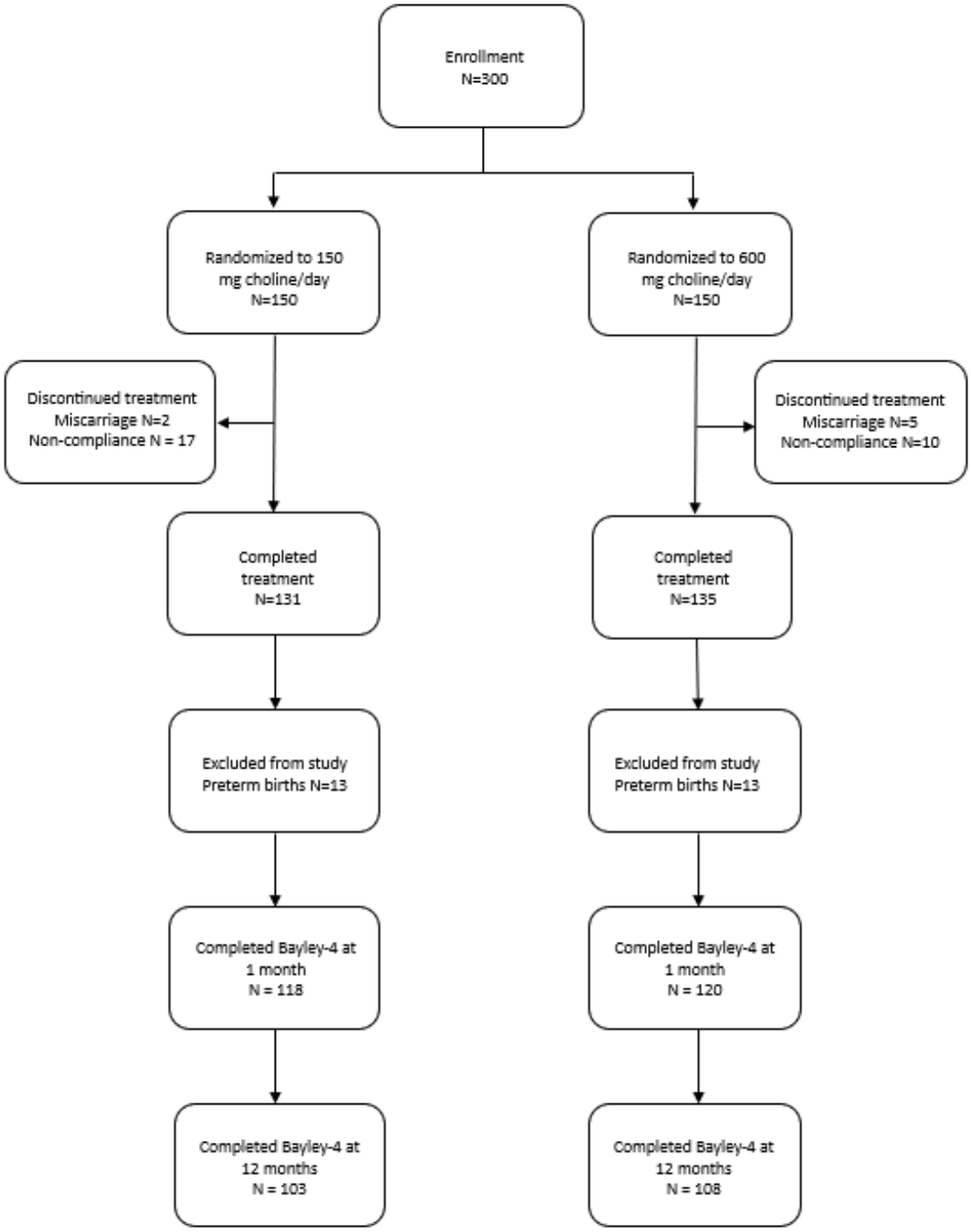


Table 4

Baseline Characteristics of 150 mg/day and 600 mg/day Choline Supplementation Groups

Note. BMI = body mass index.

Baseline Characteristic	150 mg choline/day group n=118	600 mg choline/day group n=120	*p*
Maternal race, No. (%)			0.94
White	86 (72.9)	92 (76.7)	
Black	23 (19.5)	21 (17.5)	
Asian	3 (2.5)	3 (2.5)	
Indigenous or Alaska Native	1 (0.8)	1 (0.8)	
Multiracial	5 (4.2)	3 (2.5)	
Maternal ethnicity, No. (%)			0.24
Non-Hispanic	116 (83.1)	106 (88.3)	
Hispanic	20 (16.9)	14 (11.7)	
Maternal education, No. (%)			0.82
Did not graduate high school	6 (5.1)	6 (5.0)	
High school/high school equivalent	61 (51.7)	62 (51.7)	
Undergraduate/Advanced Degree	19 (16.1)	24 (20.3)	
Maternal age, M (SD), *y*	30.1 (6.1)	28.8 (5.2)	0.10
Maternal pre-pregnancy BMI, M (SD)	27.7 (4.2)	28. (4.1)	0.06
Average monthly household income, M (SD), *USD*	5746 (1487)	5786 (1300)	0.99
Maternal dietary choline intake, M (SD), *mg*	313 (73)	316 (61)	0.71
Birth outcomes			
Infant female sex, No. (%)	59 (0.5)	67 (55.8)	0.37
Infant birth weight, M(SD), *kg*	3.19 (0.49)	3.20 (0.50)	0.91

*$p < 0.05$ indicates statistical significance

Bayley-4 Scores at 1 and 12 Months

Figures 2 and 3 depict expected results for the Bayley Scores for Infant and Toddler Development- 4th edition. Standard, or composite, domain scores will be used in the analysis since they have the greatest internal consistency reliability among the different scoring methods available for the Bayley-4 (Alfonso et al., 2022).

The initial Bayley-4 evaluations will take place when each infant reaches approximately 1 month of age. It is anticipated that a total of 238 infants will complete the first round of

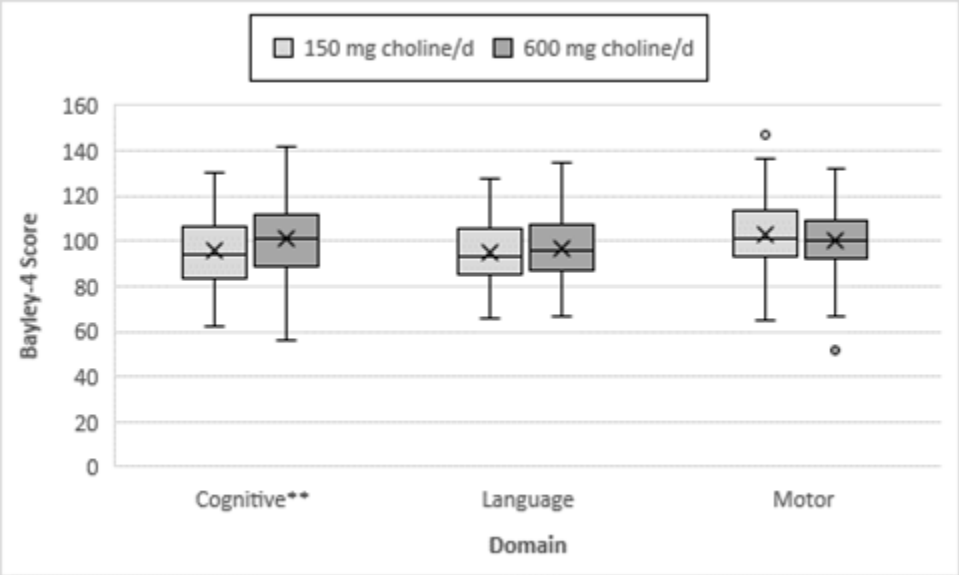
testing, including 118 from the 150 mg supplementation group and 120 from the 600 mg supplementation group. Infants in the 150 mg supplementation group averaged 98 points in the Cognitive domain, compared with an average of 103 points from infants in the 600 mg supplementation group. Results of an independent t-test indicate that infants from the 600 mg supplementation group scored significantly higher in the Cognitive domain than infants from the 150 mg supplementation group at the 1-month evaluation ($p<.01$).

A total of 27 mother-child pairs were unavailable for the Bayley-4 evaluation at 12 months, resulting in final treatment group populations of 103 infants in the 150 mg supplementation group and 108 infants in the 600 mg supplementation group. Similar to the results at 1 month, it is anticipated that infants in the 600 mg supplementation group will achieve higher scores in the Cognitive domain compared to the 150 mg supplementation group. Infants from the 150 mg supplementation group scored an average of 100 points in this domain, while infants from the 600 mg supplementation group scored an average of 106 points. The results of the second Bayley-4 assessment were consistent at the first, as infants from the 600 mg supplementation group scored significantly higher in the Cognitive domain than infants from the 150 mg supplementation group at the 12-month evaluation ($p<.001$).

Independent t-tests were also conducted on the Language and Motor domain scores. The resulting *p*-values indicate no significant differences in scores between the 150 mg and 600 mg supplementation groups at the 1-month and 12-month assessments.

Figure 2

Bayley-4 Domain Scores at 1 Month of Age

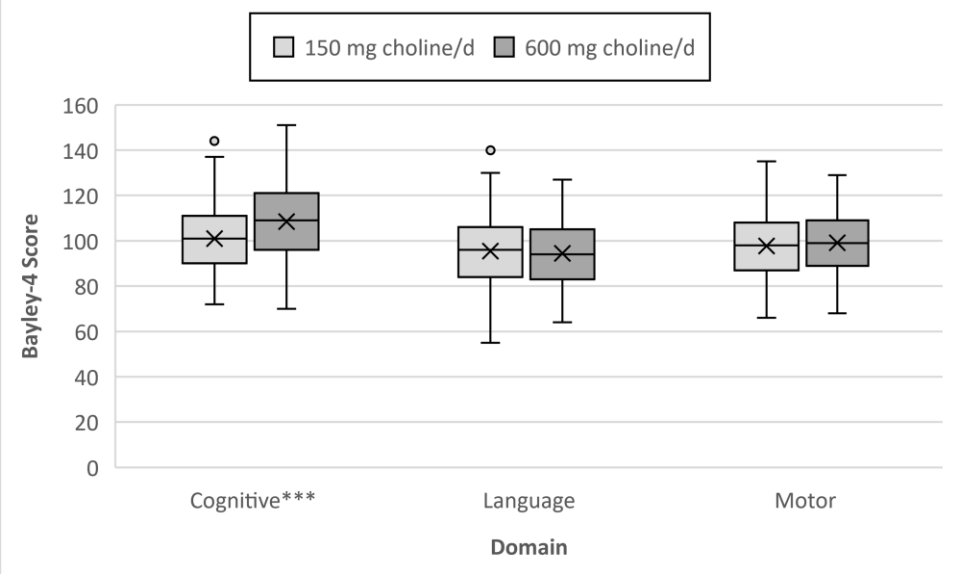


Note. The box and whisker plot displays the distribution and variation of the standard scores of the Cognitive, Language, and Motor domains for the two treatment groups (n=238). The box displays the interquartile range (central 50% of data points), and the whiskers indicate variability outside of the upper and lower quartiles. Open circles represent outliers, the line in the middle of the box represents the median value and "X" represents the mean value. Bayley-4 = Bayley Scores of Infant and Toddler Development- 4th edition.

**$p < 0.01$; $p < 0.05$ indicates statistical significance.

Figure 3

Bayley-4 Domain Scores at 12 Months of Age



Note. The box and whisker plot displays the distribution and variation of the standard scores of the Cognitive, Language, and Motor domains for the two treatment groups (n=211). The box displays the interquartile range (central 50% of data points), and the whiskers indicate variability outside of the upper and lower quartiles. Open circles represent outliers, the line in the middle of the box represents the median value and "X" represents the mean value. Bayley-4 = Bayley Scores of Infant and Toddler Development- 4th edition.
***$p < 0.001$; $p < 0.05$ indicates statistical significance.

Summary

Anticipated results of the Bayley-4 domain scores are shown in Figures 2-3, with significant differences anticipated between treatment groups in the Cognitive domain. It is expected that the differences in Cognitive domain scores will be seen at both the 1-month and 12-month evaluations. Chapter 5 will further discuss these results, in addition to their relevance to existing studies, strengths and limitations of the proposed study, and suggestions for expanding on this research in the future.

Chapter 5: Discussion

Choline is a nutrient of interest during pregnancy due to its involvement in fetal neurodevelopment (Korsmo et al., 2019). Adequate intake of prenatal choline can help prevent neural tube defects. It also contributes to the development of the hippocampus which impacts processes such as learning and memory. The majority of people in the United States consume inadequate levels of choline (*Office of Dietary Supplements - Choline*, n.d.-a). Although choline is produced endogenously, additional choline must be obtained through dietary sources for optimal health. Current research suggests that prenatal choline supplementation may provide positive effects with regard to enhancing cognitive benefits and providing protection against prenatal alcohol exposure and mental illness in childhood (Bahnfleth et al., 2019, 2022; Caudill et al., 2018; Hunter et al., 2021; Jacobson et al., 2021, Ross et al., 2016; Warton et al., 2021). However, due to the limited number of human studies utilizing prenatal supplemental choline, there is a lack of generalizable evidence supporting the ties between higher levels of choline intake and child cognitive development. This chapter will provide an interpretation of the anticipated results of the proposed study, compare these results to the studies conducted by Ross et al. (2016), Jacobson et al. (2018), Cahill et al. (2018), and Bahnfleth et al. (2019, 2022), and discuss strengths, limitations, and suggestions for continued research on this topic.

Interpretation of Results

In this proposed 40-month randomized controlled trial, expectant mothers will receive either 150 mg or 600 mg daily choline supplement. This study will utilize the Bayley Scales of Infant and Toddler Development- 4th edition to evaluate the impact of these two different levels of choline supplementation during the second and third trimesters of pregnancy on child

cognitive development in the first year of life. It is anticipated that the null hypothesis will be rejected, and the alternative hypothesis will be accepted. It is expected that the children of mothers in the 600 mg choline/day supplementation arm will score higher in the Cognitive domain of the Bayley-4 than children of mothers in the 150 mg choline/day supplementation arm, and that this trend will be seen during testing at both 1 month and 12 months.

Characterization of the Study Population

The study population will include pregnant women of at least 18 years of age who are patients of the Froedtert & MCW Health Network. Most of the data for expected maternal demographics were based on information from the U.S. Census Bureau for Milwaukee, Waukesha, and Washington counties. Froedtert & MCW obstetrics and birth services are only located in these three counties, so it is assumed the study population will also reside in these areas. Anticipated birth outcomes including occurrence of preterm birth, low birth weight, and miscarriage were based on the most recently published data from the March of Dimes. Results from a study by Bailey et al. (2019) were used to determine expected average maternal dietary choline intake during pregnancy. It is anticipated that the population characteristics of the mothers and infants in both treatment groups will be statistically similar.

Bayley-4 Scores

The findings of this research revealed that children born to mothers who received 600 mg choline supplementation/day performed better in the Cognitive domain of the Bayley-4 assessment than children born to mothers who received 150 mg choline supplementation/day. According to the Descriptive Classification System for the Bayley-4, standard scores of 90-109 fall within the 25^{th}-74^{th} percentiles and are considered “average” (Alfonso et al., 2022). This

indicates that the mean scores from infants in both treatment groups are classified as "average", although infants in the 600 mg supplementation group scored significantly higher overall in this domain.

The Language and Motor domains are standard components of the Bayley-4 assessment. They evaluate skills such as verbal working memory, complex concepts, and visual tracking and focus (Alfonso et al., 2022). Due to the lack of current evidence connecting prenatal choline supplementation and intake to the skills included in the Language and Motor domains, they are not addressed in the hypotheses for this study. However, since the Bayley-4 has not been used before in a prenatal choline supplementation study, the resulting Language and Motor domain scores may provide additional context for any potential relationship between choline and the developmental skills evaluated in these domains.

Comparison to Other Studies

The proposed study will evaluate infant cognitive development using the Bayley-4, while the studies by Cahill et al. (2018), Bahnfleth et al. (2019, 2022), Jacobson et al. (2018), and Ross et al. (2016) utilized other methods to assess cognitive development. Cahill et. al (2018) used saccade reaction time (rapid eye movement) to measure infant processing speed, while Bahnfleth et al. (2019, 2022) used Sustained Attention Task and color-location memory task to evaluate attention and memory span during a 7-year follow up of the same participant cohort. Jacobson et al. (2018) measured eyeblink conditioning and used the Fagan Infant Test of Intelligence to assess visual recognition memory and processing speed. In the study by Ross et al. (2016), the Child Behavior Checklist was used to assess internalizing and externalizing factors, including attention problems. These tools specifically evaluate the cognitive functions

of processing speed, attention, and memory tasks, which are comparable to several of the skills evaluated in the Cognitive domain of the Bayley-4. The complete summary of skills assessed by the Bayley-4 can be found in Appendix D.

Cahill et al. (2018) and Jacobson et al. (2018) found that infants of mothers who received at least 960 mg choline/day (through diet and/or supplementation) during treatment showed improved visual attention and recognition memory compared to the other treatment groups (430 mg choline/day and placebo). Bahnfleth et al. (2019, 2022) found these same benefits to be present at 7 years of age, and Ross et al. (2016) reported that children of participants in the choline treatment group (900 mg/day) had fewer attention problems than children in the placebo group at 40 months of age. Based on the results of these studies and the specific skills assessed in the Cognitive domain of the Bayley-4, infants of the 600 mg choline supplementation group are expected to perform better than infants of the 150 mg supplementation group in the Cognitive domain at both 1 and 12 months.

Strengths and Limitations

One strength of this proposed study is the sample size. After accounting for attrition, the choline supplementation studies discussed in the literature review ranged in sample size from 24-62 participants, whereas the proposed study will recruit 300 participants. The larger sample size will provide greater statistical power and should reduce the margin of error. Another strength is the use of the Bayley-4 to measure infant cognitive development. This tool is widely used in research to formally assess cognitive development in children 16 days-42 months of age (Balasundaram & Avulakunta, 2021). The 4th edition of Bayley-4 has a greater level of clinical sensitivity and accuracy compared to previous editions. Another strength, in

addition to the use of choline supplements, is that all participants will be provided with a daily prenatal vitamin for the duration of their pregnancies. This will help ensure adequate overall maternal micronutrient intake during pregnancy. Additionally, this study will minimize the effects of non-compliance by requiring all participants to return their empty or unused packets of prenatal vitamin and choline supplements on a monthly basis and excluding any participant who is less than 70% compliant with the treatment regimen for 2 or more months of the trial period.

The proposed study includes several limitations. Since this is not a controlled feeding study, there will be no strict control for maternal dietary choline intake during pregnancy. The Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tool will provide data on average monthly dietary choline intake which will be used to determine if intake is similar between treatment groups. However, the ASA24 relies on self-reporting, so the results are susceptible to reporting accuracies and personal bias. Study participants will only include patients of the Froedtert & MCW Health System, which has birth centers that serve Milwaukee, Waukesha, and Washington counties of southeastern Wisconsin. Therefore, the results cannot be generalized outside of this region. Another limitation is that parental intelligence and other indicators of parental cognitive functioning are not measured in this study. Measures of cognitive ability, such as intelligence quotient (IQ) and attention-related processes such as information-processing speed, have strong genetic components (De Geus et al., 2001). Controlling for such variables would require a greater time commitment from parents, as well as additional resources.

Suggestions for Future Studies

One of the greatest challenges of conducting nutritional research with pregnant women is the ethical concern that comes along with manipulating nutrient intake during pregnancy. While it is typically seen as beneficial to have a control group, it would be unethical to prohibit choline supplementation during pregnancy knowing that a majority of the population is not meeting the current Adequate Intake level. It will be important for future studies to continue to explore the benefits of choline intake at or above the AI during pregnancy. This can be accomplished by studying different levels of maternal choline intake (provided through a combination of diet and supplementation) to identify the minimum level at which significant benefits to cognitive development are seen. The current AI for pregnancy was based on evidence related to the choline intake needed to prevent liver dysfunction in the male population (Institute of Medicine [US] Standing Committee [IMSC] and its Panel on Folate, Other B Vitamins, and Choline, 1998). Therefore, strong evidence demonstrating a positive influence of higher levels of choline intake on child cognitive development may provide rationale for increasing the AI of choline during pregnancy.

It would also be interesting to see the effects of continuing choline supplementation during lactation. This could provide an additional measure of control but would also introduce another variable. Not every mother will breastfeed during the first year of life, and it would become even more complicated to account for infant choline intake once they begin eating solid foods.

Another step for future studies would be to increase the study duration. This may be challenging, as study of this duration would require additional funding, staffing, and a larger

sample size to account for the attrition expected during a potentially decades-long research process. A study of this nature would also require the use of additional instruments validated to assess cognitive development during different life stages. Evaluating infant cognitive development after prenatal choline supplementation is an essential step, but a long-term goal should be to assess the efficacy of choline supplementation on cognitive development into adulthood.

Conclusion

Research on the connection between prenatal diet and child development is important, yet challenging. Human studies on the impact of prenatal choline intake are limited in part due to ethical concerns. Recent research has shown that higher levels of prenatal choline intake via supplementation are associated with improved child cognitive development, particularly processing speed and attention and memory-related tasks. The proposed study will evaluate the impact of two different amounts of prenatal choline supplementation on child cognitive development in the first year of life. Cognitive development will be assessed by the widely used and validated Baley Scales of Infant Development- 4th edition. The results of this study will contribute to early human research demonstrating the benefits of prenatal choline intake on child cognitive development. Continued research on this topic could potentially provide rationale for increasing the AI of choline during pregnancy and the use of choline supplementation as a means of supporting fetal neurodevelopment.

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Appendix A

IRB Application

Mount Mary UNIVERSITY

Mount Mary University
Institutional Review Board (IRB) for
the Protection of Human Subjects

Application for IRB Review

DATA COLLECTION CANNOT BEGIN
UNTIL THE IRB HAS APPROVED THIS PROJECT

Directions:

- Faculty and student researchers, as well as student research advisors, should **read all relevant information on the University IRB page in My Mount Mary before initiating an application**. This includes full knowledge of the US Department of Health and Human Services Code of Federal Regulations Title 45 (Public Welfare), Part 46 (Protection of Human Subjects). http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html.
- All applicants must verify completion of Human Subjects Training. See http://www.citiprogram.org.
- The IRB application must be filed and approved by the IRB prior to any Mount Mary University faculty, staff, or student (undergraduate or graduate), initiating a research project/study.
- If there is a cooperating institution, attach a copy of their IRB approval.
- In the case of a student research project, the student may complete the IRB application but the student's research advisor must sign and submit the application to the IRB for approval. It is the responsibility of the faculty research advisor to ensure that student applications and all attachments (e.g., informed consent forms and survey instruments) are in their final edited form. Even though a student research project may qualify as exempt from full IRB review, the research advisor may request the student to complete and submit a full IRB application.
- Complete this application using your word processing program (e.g. Word), then send it on or print it out and obtain signatures from all investigators and advisors. (Handwritten applications will not be accepted.) For your benefit, save the completed application on your computer in case it needs to be revised and resubmitted.
- This is a professional document; please check spelling, grammar and punctuation.
- Submit an electronic copy, via email, of the completed application with required signatures and attachments, in a single pdf, to Tammy Scheidegger, IRB Chair, scheidet@mtmary.edu. You will receive an email verifying receipt of the application.
- Allow a minimum of 30 working days to process your application. Make sure this time-frame is accounted for when considering initiation of data collection and due dates for student projects. Please be aware that if, upon completion of the application, you find that no exemptions apply to your research, your application will need to go through a full IRB Committee review which can take as many as 60 days to be completed.
- For class projects you must submit IRB applications to the IRB Chair by October 31st of the fall semester and March 31st for the spring semester. For summer classes, please consult with the IRB Chair.
- Upon receipt of the IRB letter of approval, data collection may begin.

I. **Required Documentation** - ***No action will be taken without these attachments.***

Are the following attached to the IRB application?

Informed Consent Document	Yes	Informed Consent Documents should include an explanation of procedures, risk, safeguards, freedom to withdraw, confidentiality, offer to answer inquiries, third party referral for concerns, signature and date. See Appendix A and use the **MMU Informed Consent Template to avoid delays in the process.**
Survey/Interview Instrument(s)	Yes	If a survey is being administered in any written format (e.g., Google Forms, Survey Monkey, Qualtrics), a copy of that survey must accompany this application. If a survey/interview is being conducted verbally, a copy of the introductory protocol/comments and survey questions being asked must be attached to this application. If survey/interview includes focus group questions, a complete list of the question must be attached. For research using a published/purchased instrument, a photocopy of the instrument will suffice.
Verification of Human Subjects Training	Yes	Copy of transcript, certificate or other evidence that ALL members of the research team have completed the required training.
Copy of cooperating institution's IRB approval.	Yes	Not required if there is no cooperating institution.

II. **Investigator(s):**

Name: *Lauren B. Potvin* Phone: *763-250-0912*

Affiliation with Mount Mary University (e.g. faculty, student, etc.): *Graduate Student* Email: *lbondy@mtmary.edu*

Signature: Date:

Name: Phone:

Affiliation with Mount Mary University: Email:

Signature: Date:

If student, list Research Advisor and complete the application. Research Advisor must provide requested information and verify.

Research Advisor's Name: *Janine Bamberger* Department: *Dietetics*
Email: *bambergj@mtmary.edu* Phone: *414-930-3264*

Research Advisor: Have you completed Human Subject's Training? Yes No

Research advisor's signature indicates responsibility for student compliance with all IRB requirements.

Signature: Date:
Research Advisor

Individuals who participate in research play an important and active role in the advancement of knowledge. In recognition of their important contributions to research, humans will be referred to as "participants" rather than "subjects."

III. **Project Description – Required by all applicants**
Instructions: Briefly describe the proposed project including the sample and methodology (e.g. human subjects, data collection, data analysis and instruments).

1) Objectives (purpose of project):

This double-blind randomized controlled trial will analyze the relationship between different levels of choline supplementation during the 2nd and 3rd trimesters of pregnancy and infant cognitive outcomes in the first year of life.

2) Relevance to practice/body of knowledge:

Choline is essential for fetal brain development, yet it is estimated that less than 10% of pregnant women achieve the target intake levels of choline. Studies utilizing supplemental choline during pregnancy are limited. The proposed study will contribute to the early human research on prenatal choline intake and its impact on child cognitive development. Continued research on this topic could potentially provide rationale for increasing the AI of choline during pregnancy and the use of choline supplementation as a means of supporting fetal neurodevelopment.

3) Describe the research design (e.g. subject/participant selection and assignment, design, intervention, data analysis):

The study will be a double-blind randomized controlled trial, with two treatment arms. The two treatment arms will include a 150 mg choline/day supplementation group and a 600 mg choline/day supplementation group. A minimum of 300 study subjects will be recruited through an open enrollment process from January 2024-July 2025. Eligible study subjects will be pregnant women aged 18 years or older who are at 14 or less weeks of gestation at the time of recruitment. The participants must be patients of the Froedtert & Medical College of Wisconsin (MCW) Health Network. Research staff will also be recruited from Froedtert & MCW Health Network and student volunteers will be recruited from local academic institutions.

Participants will be randomized into one of the two treatment groups and provided with a new supply of either the 150 mg or 600 mg choline chloride supplement, as well as a prenatal multivitamin, every 30 days. The supplement packets will be prepared onsite at the dedicated research space at Town Hall Health Clinic by research staff and the choline supplements will not be labeled with any information identifying the dosage. After giving birth, the mother will no longer be provided with prenatal vitamin or choline supplementation and will be expected to report the use of any postnatal vitamin intake. To track compliance, the supplements will be sent in a package with return postage, and participants will need to send back the package to the research clinic at the end of the 30-day cycle. Participants will be required to complete an Automated Self-Administered 24-hour Dietary Assessment within 2 weeks of their enrollment date and on a monthly basis until they give birth. Registered dietitians from the research team will be responsible for managing the data from each ASA24 and reporting the estimated dietary choline intake for each participant.

Research staff will contact participants at 1 week and 10 months postpartum to schedule a time for infant cognitive testing. A parent or guardian will fulfill study guidelines by bringing the infant to the research center for cognitive testing at 1 ±0.25 months and 12 ±0.5 months of age. A qualified member of the research team will conduct the Bayley-4 testing at each appointment.

A Chi-Square Goodness-of-Fit test will be used for maternal demographic comparisons among the treatment groups. An independent t-test will be used to evaluate for any differences among the treatment groups with regard to maternal age, pre-pregnancy BMI, average monthly household income, dietary choline intake, and infant birth weight. Additionally, independent t-tests will be used to examine the relationships between the two treatment groups and each domain of the Bayley-4 at 1 month and 12 months.

4) What measurement/data collection tools are being used?

Automated Self-Administered 24-hour Dietary Assessment tool (ASA24), Bayley Scales of Infant and Toddler Development- 4th edition (Bayley-4)

IV. **Additional Project Information – Required by all applicants**

1) What human subjects training has the researcher completed (e.g. course work, online certification)?

The researcher has completed CITI training.

2) What process is used for obtaining informed consent? See Appendix A for consent content requirements and use the template, available on the MMU IRB webpage, when constructing your informed consent form.

Informed consent application is attached.

3) Does the research include special populations?

• Minors under 18 years of age?	Yes	No
• Persons legally incompetent?	Yes	No
• Prisoners?	Yes	No
• Pregnant women, if affected by research?	Yes	No
• Persons institutionalized?	Yes	No
• Persons mentally incapacitated?	Yes	No

If YES, describe additional precautions included in the research procedures.

The guidelines established by the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Science Research were closely considered during the development of the proposed study. The study will not include a placebo group. The National Health And Nutrition Examination Surveys from 2005-2014 reported a mean choline intake of 322 mg/day for pregnant women. This value guided the decision to establish the lower-level choline supplementation for this study at 150 mg/day, as average dietary intake and supplementation would exceed the adequate intake of 450 mg choline during pregnancy. Participants are instructed to follow their health care provider's guidance on postnatal supplementation.

4) Does the research involve any of the following procedures?

• False or misleading information to subjects?	Yes	No
• Withholds information such that their informed consent might be questioned?	Yes	No
• Uses procedures designed to modify the thinking, attitudes, feelings, or other aspects of the behavior of the subjects?	Yes	No

If YES, describe the rationale for using procedures, how the human subjects will be protected and what debriefing procedures are used.

5) Does the research involve measurement in any of the following areas?

- Sexual behaviors? Yes No
- Drug use? Yes No
- Illegal conduct? Yes No
- Use of alcohol? Yes No

If YES, describe additional precautions included in the research procedures...

6) Are any portions of the research being conducted online?

- Survey posted on a website? Yes No
- URL for survey includes information that could identify participants? Yes No
- Invitation to participate sent by email? Yes No
- Items use drop-down box? Yes No
  If yes, assure that items allow choice of "no response"
- Will you be recording virtual interviews? Yes No

Audio only Video only Audio & Video

If video recording is being used, assure anonymity by only recording audio unless the research necessitates visual recording.

If YES, to any of the above items, describe additional procedures.

All online communication, such as provision of screening surveys, will be conducted via encrypted email.

7) Describe the methods used to ensure confidentiality of data obtained.

Each participant will be assigned an identification number for the duration of the study to help maintain privacy and to ensure proper blinding. The study will have dedicated computers that are password-protected; only the necessary research staff will have access to the password. Any physical materials will be kept in a locked filing cabinet. Participant names will not appear on any study materials other than the supplement-containing packages. The supplement-containing packages will include participant name and address but will otherwise be unmarked. All online communication will be conducted via encrypted email. The research staff and volunteers will be required to have up to date Health Insurance Portability and Accountability Act (HIPPA) training.

Risks and Benefits

1) Describe risks to the subjects and the precautions that will be taken to minimize them. (Risk includes any potential or actual physical risk of discomfort, harassment, invasion of privacy, risk of physical activity, risk to dignity and self-respect, and psychological, emotional or behavioral risk.)

It is possible that participants could have adverse effects to the choline supplement or prenatal vitamin provided, such as gastrointestinal issues like constipation, nausea, and bloating. Study subjects can choose to discontinue use and remove themselves from the study at any point in time. The prenatal vitamins contain soy and fish products, but participants will have the opportunity to identify if they have one of these allergies during the initial participation survey. Any participant with a fish or soy allergy will not be eligible to participate in the study.

2) Describe the benefits to subjects and/or society. (These will be balanced against risk.)

The proposed study will provide subjects with free prenatal vitamins during the second and third trimesters of pregnancy. Some of these participants may not otherwise have adequate knowledge or finances to adhere to a prenatal vitamin regimen. The parents will also gain information on their child's cognitive development during the Bayley-4 evaluations and have the opportunity to discuss the results with the trained clinical staff member who administers the assessment. Finally, the results of this study could continue to the growing body of research on the topic and provide evidence to support the inclusion of choline as part of a standard prenatal vitamin regimen.

V. **Is the proposed project "research" as defined by Institutional Review Board requirements? - Required by all applicants**

Per 45 CRF 46.102: "Research is defined as a systematic investigation, including research development, testing, and evaluation, designed to develop or contribute to generalizable knowledge. Activities that meet this definition constitute research for purposes of this policy, whether or not they are conducted or supported under a program that is considered research for other purposes."

Per HHS.gov and the Office for Human Subjects Research (https://www.hhs.gov/ohrp/regulations-andpolicy/requests-for-comments/draft-guidance-activities-deemed-not-be-research-public-healthsurveillance/index.html#:~:text=For%20purposes%20of%20the%202018,by%20a%20public%20health%20aut hority), the following activities are deemed **not** to be research:

- Scholarly and journalistic activities (e.g., oral history, journalism, biography, literary criticism, legal research, and historical scholarship), including the collection and use of information, that focus directly on the specific individuals about whom the information is collected.
- Public health surveillance activities, including the collection and testing of information or biospecimens, conducted, supported, requested, ordered, required, or authorized by a public health authority. Such activities are limited to those necessary to allow a public health authority to identify, monitor, assess, or investigate potential public health signals, onsets of disease outbreaks, or conditions of public health importance (including trends, signals, risk factors, patterns in diseases, or increases in injuries from using consumer products). Such activities include those associated with providing timely situational awareness and priority setting during the course of an event or crisis that threatens public health (including natural or man-made disasters).
- Collection and analysis of information, biospecimens, or records by or for a criminal justice agency for activities authorized by law or court order solely for criminal justice or criminal investigative purposes.
- Authorized operational activities (as determined by each agency) in support of intelligence, homeland security, defense, or other national security missions.

A human subject is defined as a living individual about whom an investigator obtains either 1) data through intervention or interaction with the individual; or 2) identifiable private information. In social science research, human subjects may be referred to as research subjects or research participants.

Does the research involve human subjects/participants or official records about human subjects/participants?

Yes No

If "no", STOP here, and submit application.

If the results will be available in the library, presented at a professional conference (includes any presentation to group(s) outside of the classroom), or published, please check the Yes box:

Yes No

If "yes", proceed to SECTION VI.

If "no, STOP here, and submit application.

VI. **Exemptions - Required by all applicants**

Are you requesting exemption from IRB review in one of the federally approved categories?

Yes No

If yes, please reference OHRP website http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html and continue with application.

1) Does the research meet the criteria for exempt category 1 (education)? [45 CFR 46.104(d)(1)]? Is the research conducted in established or commonly accepted educational settings (e.g. schools, Universities, or other sites where educational activities regularly occur)?

Yes No

Does the research study involve only normal education practices that are not likely to adversely impact students' opportunity to learn required educational content or the assessment of educators who provide instruction? This includes most research on regular and special education instructional strategies, and research on the effectiveness of or the comparison among instructional techniques, curricula, or classroom management methods.

Yes No

If both questions are answered "yes" stop here, and submit application.

2) Does the research meet the criteria for exempt category 2 (specific procedures) [45 CFR 46.104(d)(2)]? Does the research involve only the use of educational tests, survey procedures, interview procedures or observation of public behavior (including visual or auditory recording)?

Yes No

Does this research meet at least one of the following criteria:

- The information obtained is recorded by the investigator in such a manner that the identity of the human subjects cannot readily be ascertained, directly or through identifiers linked to the subjects

  Yes No
- Any disclosure of the human subjects' responses outside the research would not reasonably place the subjects at risk of criminal or civil liability or be damaging to the subjects' financial standing, employability, educational advancement, or reputation. Yes No

If the primary question and either of the two sub-questions are answered "yes", stop here, and submit the application.

3) Does the research meet the criteria for exempt category 3 [45 CFR 46.104(d)(3)]? Does the research involve benign behavioral interventions in conjunction with the collection of information from an adult subject through verbal or written responses (including data entry) or audiovisual recording and prospectively agrees to the intervention and information collection and at least one of the following criteria is met:

- The information obtained is recorded by the investigator in such a manner that the identity of the human subjects cannot readily be ascertained, directly or through identifiers linked to the subjects

  Yes No
- Any disclosure of the human subjects' responses outside the research would not reasonably place the subjects at risk of criminal or civil liability or be damaging to the subjects' financial standing, employability, educational advancement, or reputation

  Yes No
- The information obtained is recorded by the investigator in such a manner that the identity of the human subjects can readily be ascertained, directly or through identifiers linked to the subjects

  Yes No

For the purpose of this provision, benign behavioral interventions are brief in duration, harmless, painless, not physically invasive, not likely to have a significant adverse lasting impact on the subjects, and the investigator has no reason to think the subjects will find the interventions offensive or embarrassing. Provided all such criteria are met, examples of such benign behavioral interventions would include having the subjects play an online game,

having them solve puzzles under various noise conditions, or having them decide how to allocate a nominal amount of received cash between themselves and someone else.

If the research involves deceiving the subjects regarding the nature or purposes of the research, this exemption is not applicable unless the subject authorizes the deception through a prospective agreement to participate in research in circumstances in which the subject is informed that he or she will be unaware of or misled regarding the nature or purposes of the research.

If the answer to this question is "yes" stop here, and submit application.

4) Does the research meet the criteria for exempt category 4 (existing data/specimens) [45 CFR 46.104(d)(4)]? Does this research use secondary data (*i.e., secondary research/data uses consists of identifiable private information or identifiable biospecimens, if at least one of the following criteria is met: (i) The identifiable private information or identifiable biospecimens are publicly available; (ii) Information, which may include information about biospecimens, is recorded by the investigator in such a manner that the identity of the human subjects cannot readily be ascertained directly or through identifiers linked to the subjects, the investigator does not contact the subjects, and the investigator will not re-identify subjects; (iii) The research involves only information collection and analysis involving the investigator's use of identifiable health information when that use is regulated under 45 CFR parts 160 and 164, subparts A and E, for the purposes of "health care operations" or "research" as those terms are defined at 45 CFR 164.501 or for "public health activities and purposes" as described under 45 CFR 164.512(b); or (iv) The research is conducted by, or on behalf of, a Federal department or agency using government-generated or government-collected information obtained for non-research activities, if the research generates identifiable private information that is or will be maintained on information technology that is subject to and in compliance with section 208(b) of the E-Government Act of 2002, 44 U.S.C. 3501 note, if all of the identifiable private information collected, used, or generated as part of the activity will be maintained in systems of records subject to the Privacy Act of 1974, 5 U.S.C. 552a, and, if applicable, the information used in the research was collected subject to the Paperwork Reduction Act of 1995, 44 U.S.C. 3501 et seq.*) for which consent is not required?

Yes No

Does the research involve only the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens?

Yes No

Will the information be recorded by the investigator in such a manner that the subjects cannot be identified directly or through identifiers linked to the subjects? (See Appendix B)

Yes No

If all answers are "yes," stop here, and submit application.

5) Does the research meet the criteria for exempt category 5 (federal program research) [45 CFR 46.104(d)(5)]? Is this research or a demonstration project that is conducted or supported by a Federal department or agency, or otherwise subject to the approval of department or agency heads (or the approval of the heads of bureaus or other subordinate agencies that have been delegated authority to conduct the research and demonstration projects), and that are designed to study, evaluate, improve, or otherwise examine public benefit or service programs, including procedures for obtaining benefits or services under those programs, possible changes in or alternatives to those programs or procedures, or possible changes in methods or levels of payment for benefits or services under those programs (i.e., such projects include, but are not limited to, internal studies by Federal employees, and studies under contracts or consulting arrangements, cooperative agreements, or grants. Exempt projects also include waivers of otherwise mandatory requirements using authorities such as sections 1115 and 1115A of the Social Security Act, as amended)?

Yes No

Does the research involve studying, evaluating or examining federal public benefit or service programs?

Yes No

Is the research conducted through a federal agency?

Yes No

If all of the answers are "yes" stop here, and submit application.

6) Does the research meet the criteria for exempt category 6 (taste and food quality) [45 CFR 46.104(d)(6)]?

Does the research involve a taste and food quality evaluation or consumer acceptance study?

Yes No

Does the wholesome food consumed contain no additives, or a food ingredient at or below the level and for a use found to be safe, or agricultural chemical or environmental contaminant at or below the level found to be safe, by the Food and Drug Administration or approved by the Environmental Protection Agency or the Food Safety and Inspection Service of the U.S. Department of Agriculture?

Yes No

If all of the answered are "yes" stop here, submit application.

7) Does the research meet the criteria for exempt category 7 (Storage or maintenance for secondary research for which broad consent is required) [45 CFR 46.104(d)(7)]? Does the research involve the storage of secondary research data for which broad consent is required (contains identifiable private information or identifiable biospecimens for potential secondary research)?

Yes No

8) Does the research meet the criteria for exempt category 8 (Secondary research for which broad consent is required) [45 CFR 46.104 (d) (8)]? Does the research involve the use of identifiable private information or identifiable biospecimens for secondary research use?

Yes No

Are **all** of the following criteria met: (i) Broad consent for the storage, maintenance, and secondary research use of the identifiable private information or identifiable biospecimens will be obtained; (ii) Documentation of informed consent or waiver of documentation of consent will be obtained; (iii) the research to be conducted is within the scope of the broad consent referenced in paragraph (i) of this section; and (iv) the investigator will not include returning individual research results to subjects as part of the study plan. This provision does not prevent an investigator from abiding by any legal requirements to return individual research results.

Yes No

If no exemptions apply, your application will need to go through a full IRB Committee review. Be advised that this process can take as many as 60 days to be completed.

Appendix A: Required Elements of Informed Consent

Please use the template provided on the MMU IRB website for constructing your Informed Consent Document

Informed consent is the process of communicating to a prospective participant, in easy-to-understand language (usually sixth- to eighth-grade level), all that he or she needs to know about participating in a research project, and then obtaining the prospective participant's agreement to participate. The following ten elements of consent are widely recognized and, except under certain specific conditions, must be included in all consent processes and forms:

1. An explanation of the study, including goals, procedure, and a statement that the study is research.
2. A description of what participants are expected to do and expected length of participation.
3. A description of any likely risks or discomforts for the participants. Potential harm should be explained in language that participants can understand and that relate to everyday life.
4. A description of any likely benefits to the participant or to others.
5. A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the participant.
6. A statement describing the level of privacy assured for collected information (anonymous, confidential) and how private information and information security will be managed.

7. An explanation of whom to contact for answers to questions about the research. When a Mount Mary student is the principal investigator, the name and phone number of a supervising faculty member is required.
8. An explanation of whom to contact for concerns about the participant's privacy and rights, which for Mount Mary University is its IRB Chair.
9. For research involving more than minimal risk, a statement describing any compensation for injuries and contact information. (Minimal risk is a risk of harm to the participant that is no greater than the risk encountered in normal, day-to-day activities or during routine physical or psychological examinations.)
10. A statement that research participation is voluntary and the participant may withdraw from participation at any time, without penalty or loss of benefits to which the participant is otherwise entitled. If the participant is a patient or client receiving medical, psychological, counseling, or other treatment services, there should be a statement that withdrawal from the study will not jeopardize or otherwise affect any treatment or services the participant is currently receiving or may receive in the future. Participants also should be told whether their data will be destroyed should they withdraw from the study. If a survey instrument or interview questions are used and some questions deal with sensitive issues, the participants should be told they may refuse to answer individual questions.

Appendix B: IRB De-Identification Standard for Information

Protecting the privacy of research participants is a general concern in the vast majority of research projects. The degree to which privacy needs to be ensured or maintained depends on the nature of the particular research, its setting, and the research participants. Researchers share a general obligation to design their research to reduce the risks of disclosure of collected information about individual research participants. Thus, the present standard for de-identification of information is useful as a guide to protecting privacy even when it is not required or fully required. In this regard, the researcher should consider the following question when collecting and handling data.

Does the information I am accessing, recording, and/or disclosing contain identifiers? Simple access to information may be without concern, for example when the researcher is an employee who routinely handles the records in carrying out his or her position. But, the presence of identifiers in any recorded or disclosed information in the research means the information is not anonymous and so does not meet the IRB de-identification standard, which in some cases may also disqualify the research from exemption from IRB review. The IRB de-identification standard includes all 18 direct identifiers specified in the HIPAA Privacy Rule de-identification standard - 45 CFR 164.514(b). Below are listed specific direct and indirect identifiers that lead to information not being anonymous.

Identifiers: Direct; Indirect

One way to distinguish between information that is truly anonymous and information that is simply being kept confidential is to determine whether the data set contains direct or indirect identifiers. Information in a data set with either direct or indirect identifiers is not anonymous.

Direct Identifiers include:

- Names
- Addresses
- Telephone and fax numbers
- Email addresses, IP addresses, and URLs
- Social Security numbers
- Medical record numbers
- Account numbers, such as those associated with bank accounts or health plans
- License or certificate numbers, including driver's license numbers
- License plate numbers and other vehicle identifiers
- Fingerprints, voiceprints, or full-face photographic images
- Other unique characteristics or identification numbers (example student ID numbers)

Indirect Identifiers can be combined with publicly available information to identify individuals. The determination of indirect identifiers depends on the nature of the research participants. For example, in a study of residents of the state of Wisconsin, the information that someone graduated from one of the UW system schools probably would not be a unique identifier. However, in a study of small business leaders in Racine, WI, the same information might well apply to only one individual. In general, if any single variable in a data set applies to fewer than five participants, it is considered a potential indirect identifier.

Examples of indirect identifiers include:

- Detailed geographical information, such as state, county, or census tract of residence
- Organizations to which participants belong
- Educational institutions from which participants graduated
- Exact occupations
- Places where participants grew up
- Many dates, e.g. birth dates, hospital admission dates, high school or University graduation dates, etc.
- Detailed income information • Offices or posts held by participants.

Appendix B

Research Participant Information and Consent Form

Mount Mary UNIVERSITY

Research Participant Information and Consent Form

Mount Mary University

Title of Study: Prenatal Choline Supplementation and Infant Cognitive Development During the First Year of Life: a Randomized Controlled Trial

Invitation to Participate and Purpose of the Research

You are invited to participate in a research study that seeks to evaluate the differences in dose-response between two levels of prenatal choline supplementation and infant cognitive outcomes in the first year of life. The study will aim to determine if higher levels of choline supplementation are associated with indicators of improved infant cognitive development. There will be two treatment groups including a 150 mg per day choline supplementation group and a 600 mg per day choline supplementation group. These treatment levels were carefully established so that the recommended Adequate Intake of choline during pregnancy can be realistically met through supplement and dietary intake. Participants will be asked to comply with the daily choline supplement and prenatal vitamin regimen, complete monthly 24-hour food recalls, and bring their child to Town Hall Health Center in Menomonee Falls, WI for a 1-hour cognitive and behavioral assessment when they reach 1 month and 12 months of age. Data will be de-identified and analyzed by researchers. Participants must be 18 years of age or older and less than 14 weeks gestation during initial enrollment in the study. Expected length of participation is 18-20 months.

Benefits and Risks

This research is designed to benefit the dietetics profession by evaluating the benefits of choline supplementation on infant cognitive development and potentially contributing evidence to support the inclusion of choline as part of a standard prenatal vitamin regimen. Although participants may not benefit personally from being in this research study, findings generated by this research may add new knowledge to the field of nutrition and dietetics, as well as prenatal health, in general. There will be no monetary compensation. However, study participants will be provided with monthly prenatal vitamins for no cost. Potential risks include standard adverse side effects from prenatal vitamin intake such as constipation and bloating. Study subjects are free to discontinue supplement use and end their participation in the study at any time. Please address any questions or issues of concern to the researchers using the contact information provided below.

Confidentiality

All information obtained will be kept confidential by the researchers who will be the only people with access to the data. Information obtained will be stored electronically and will be password protected. Per the U.S. Office of Human Research Protections (code §46.115), all data will be destroyed 3 years after the end of data collection. Paper files will be shredded, and electronic files will be deleted. Individual participants will not be identified in any report or publication about this study.

Contact Information

If you have questions about this research study, your rights as a research subject, or would like to know the outcome of the research, please contact Janine Bamberger, (414) 930-3264, bambergj@mtmary.edu and Lauren Potvin, (763) 250-0912, bondyl@mtmary.edu. If you have any questions regarding your rights

or privacy as a participant in this study, please contact Dr. Tammy Scheidegger, Mount Mary University Institutional Review Board Chair, 2900 North Menomonee River Parkway, Milwaukee, Wisconsin, 53222-4597, telephone (414) 930-3434 or email scheidet@mtmary.edu.

Consent

By signing below, you are indicating that you have read this consent form, have been given the opportunity to ask questions, and have agreed to voluntarily participate. You may withdraw from participation at any time, or refuse to answer any question herein, without penalty or loss of benefits to which other participants are entitled.

You may request a copy of this page for your records. Thank you for your participation.

Signature of participant_____ Date _____

Other Possible Elements Needed

A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the participant. For research involving more than minimal risk, a statement describing any compensation for injuries and contact information. (Minimal risk is a risk of harm to the participant that is no greater than the risk encountered in normal, day-to-day activities or during routine physical or psychological examinations.) If the participant is a patient or client receiving medical, psychological, counseling, or other treatment services, there should be a statement that withdrawal from the study will not jeopardize or otherwise affect any treatment or services the participant is currently receiving or may receive in the future. Participants also should be told whether their data will be destroyed should they withdraw from the study. If a survey instrument or interview questions are used and some questions deal with sensitive issues, the participants should be told they may refuse to answer individual questions.

Appendix C

Participant Screening Survey

Personal and Demographic Information

The information gathered through this survey will help us determine if you are eligible to participate in the research study.

Name:

First Name Last Name

Date of Birth:

Month Day Year

Mailing Address:

Street Address

Street Address Line 2

City State / Province

Postal / Zip Code

Primary Contact number:

Area Code Phone Number

Email:

example@example.com

Language(s) spoken:

☐ English ☐ Spanish ☐ Other

What category best describes your race:

- ☐ Indigenous or Alaska Native
- ☐ Asian
- ☐ Black or African American
- ☐ White
- ☐ I choose not to answer
- ☐ Multiracial
- ☐ Other

Please specify your ethnicity:

- ○ Hispanic or Latino
- ○ Not Hispanic or Latino

Highest level of education completed:

- ○ Did not graduate high school
- ○ High school/high school equivalent
- ○ Undergraduate or advanced degree
- ○ Other

Average monthly household income:

Medical and Substance Use History

The information gathered through this survey will help us determine if you are eligible to participate in the research study.

Select your current pregnancy status:

- ○ Singelton (one baby)
- ○ Twin or multiple (two or more babies)

Have you ever been or are you currently being treated for any of the following medical conditions?

- ☐ Cancer
- ☐ COPD/Emphysema
- ☐ Diabetes mellitus
- ☐ Heart disease/heart attack
- ☐ Gestational diabetes
- ☐ Preeclampsia
- ☐ Hyperemesis gravidarum
- ☐ Iron deficiency/anemia
- ☐ Kidney disease
- ☐ None
- ☐ Liver disease
- ☐ Other

Please list any other medical conditions not listed above:

Please list any complications with previous pregnancies/birts/deliveries:

Primary Care Provider Name:

First Name | Last Name | MD. DO I Suffix

Primary Care Provider Phone Number:

Area Code | Phone Number

Your other healthcare providers:

Has there been any alcohol, tobacco, or recreational drug use since you've been aware of your current pregnancy?

- ○ Yes
- ○ No

If yes, please list the substance and current frequency of use:

Any other information regarding substance use that you feel your provider needs to know?

Thank you for completing the survey!

If you are deemed eligible to participate in the research study, one of our staff members will contact you with further details.

Submit

Appendix D

Summary of Underlying Skills Assessed by the Bayley-4

Table 4.1 Summary of Underlying Skills Assessed by the Bayley–4

Cognitive Scale	Language Scale	Motor Scale	Social–Emotional Scale	Adaptive Behavior Scale
• Attention • Imitation • Habituation • Basic concepts • Acquired knowledge • Working memory • Problem solving • Learning • Numerical concepts • Classification • Planning • Cause and effect • Processing speed • Imagination	• Concrete terms • Abstract terms • Social interaction • Complex concepts • Verbal working memory • Non-specific vocalizations • Sophisticated sounds • Articulation • Gesturing • Vocabulary knowledge	• Visual tracking and focus • Sensorimotor behaviors and reflexes • Grasping • Visual-motor integration • Writing skills such as imitating, tracing, and copying • Fine motor speed • Head and trunk control • Motor planning • Locomotion such as walking, running, hopping, etc. • Throwing (e.g., a ball)	• Sensory processing • Self-regulation • Engaging in relationships • Using emotions purposely • Communicating via emotional signals or gestures • Using emotional signals or gestures to solve problems • Using symbols or ideas to convey intentions/feelings • Using symbols or ideas to express complex needs • Creating bridges between emotions and ideas	• Listening • Understanding • Talking • Self-care • Relating to others • Playing

Sources: Aylward (2020), Bayley and Aylward (2019b, 2019c), and Chapters 2 and 3

Note. From "Essentials of Bayley-4 Assessment," by A. D. Turner, J. R. Engler, and V. C. Alfonso, 2022, John Wiley and Sons. Reprinted with permission.

Appendix E

Nature Made Prenatal Vitamin Label

Supplement Facts
Serving Size 1 Softgel

Amount Per Softgel	% Daily Value for Pregnant Women and Lactating Women
Calories 5	
Vitamin A (as Beta Carotene) 770 mcg	59%
Vitamin C (as Ascorbic Acid) 85 mg	71%
Vitamin D_3 (as Cholecalciferol) 25 mcg (1000 IU)	167%
Vitamin E (as d-Alpha Tocopherol) 15 mg	79%
Vitamin K (as Phytonadione) 90 mcg	100%
Thiamin (as Thiamine Mononitrate) 1.4 mg	100%
Riboflavin 1.4 mg	88%
Niacin (as Niacinamide) 18 mg	100%
Vitamin B_6 (as Pyridoxine Hydrochloride) 1.9 mg	95%
Folate 1330 mcg DFE (800 mcg Folic Acid)	222%
Vitamin B_{12} (as Cyanocobalamin) 5.2 mcg	186%
Biotin 30 mcg	86%
Pantothenic Acid (as d-Calcium Pantothenate) 6 mg	86%
Calcium (as Calcium Carbonate) 150 mg	12%
Iron (as Ferrous Fumarate) 27 mg	100%
Iodine (as Potassium Iodide) 150 mcg	52%
Magnesium (as Magnesium Oxide) 45 mg	11%
Zinc (as Zinc Oxide) 11 mg	85%
Omega-3 Fatty Acids (from Fish Oil Concentrate)[11] 260 mg	*
Omega-3 Docosahexaenoic Acid (DHA)[11] 200 mg	*
Omega-3 Eicosapentaenoic Acid (EPA)[11] 60 mg	*

*Daily Value not established.

SUGGESTED USE:
Adults, take 1 softgel daily with water and a meal for optimal absorption.
Store tightly closed, in a cool, dry place, out of reach of children.
Do not use if imprinted seal under cap is broken or missing.

✓ No Artificial Flavors
✓ Gluten Free

CAUTION:
If you are taking medication or have blood clotting issues, consult your physician before use.

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List of Tables

Table 1: Research Questions and Variables……47

Table 2: Inclusion and Exclusion Criteria……51

Table 3: Description of Variables……57

Table 4: Baseline Characteristics of 150 mg/day and 600 mg/day Choline Supplementation Groups……61

List of Figures

Figure 1: Flowchart Depiction of Participants From Study Enrollment Through Intervention and Infant Behavioral Assessment at 12 Months of Age……60

Figure 2: Bayley-4 Scores at 1 Month of Age……63

Figure 3: Bayley-4 Scores at 12 Months of Age……64