THE RELATION OF MATERNAL IRON AND ZINC STATUS TO COGNITIVE DEVELOPMENT IN TODDLERS

By

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THE RELATION OF MATERNAL IRON AND ZINC STATUS ON COGNITIVE DEVELOPMENT IN TODDLERS

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Abstract:

The objective of the study was to determine relations between toddler cognition and maternal perinatal iron and zinc biomarker levels; toddler cognition and infant Visual Information Processing (VIP); and toddler cognition and maternal dietary iron and zinc intake. The current project is a follow up to a study, which analyzed the relation between maternal micronutrient levels and infant cognition for breastfed infants (n = 132). The current study included mother/toddler pairs (n = 36) when toddlers were 2.5 to 4 years old (mean = 3.16).

At three months postpartum multiple maternal iron indicators and zinc levels were measured, and mothers completed a Dietary History Questionnaire to measure dietary iron and zinc intake. Infants completed the VIP procedure at three and nine months old. Toddler cognition was determined by multiple measures, including the Pearson Picture Vocabulary Test, a Distractibility activity, and an Imitation task. At the toddler visit, mothers completed the MacArthur Bates Communicative Development Inventory (CDI)

Mothers' mean total dietary iron and zinc intake met the Estimated Average Requirement. Descriptive statistics showed mothers were well nourished with serum zinc levels and multiple measures of iron within the recommended range except for increased soluble transferrin receptors (sTfR). Results for the first hypothesis indicated positive relations for maternal sTfR and toddler Distractibility (Total Looking Time) (p=0.042) and for maternal hemoglobin (Hgb) and CDI Vocabulary (p=0.036) and Mean Length of Utterance (MLU) (p=0.014).

The other two hypotheses, toddler cognition and infant VIP as well as toddler cognition and maternal dietary iron and zinc were not supported.

In conclusion, different pools of maternal iron, but not zinc, impacted specific domains of toddler cognitive development. Maternal Hgb, which reflects oxygen carrying capacity, was positively related to toddler language development. Increased maternal sTfR, which indicates decreased iron status, was related to increased toddler distractibility. The research indicated generally adequate maternal iron levels in healthy mothers impacted long-term development in healthy toddlers. Since maternal iron levels were positively significantly related to toddler cognitive development, the results support the Fetal Origins Hypothesis.

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CHAPTER I

INTRODUCTION

This research project is based on the Fetal Origins Hypothesis, also known as the Barker Hypothesis or Fetal Programming Hypothesis; this theory considers the effect of maternal nutritional status during pregnancy on the health of the infant not only during infancy but also into childhood and throughout the lifespan (Ellison, 2005). Barker formulated the Fetal Origins Hypothesis in 1994, which is why it is also known as the Barker Hypothesis.

Research in relation to birth weight and chronic diseases later in life, in particular type two diabetes, coronary heart disease, hypertension and stroke, was based on observational studies conducted in the early 1900s (Barker, 2012). Restriction of calories during pregnancy has been shown to be related to obesity, dyslipidemia, insulin resistance, and coronary heart disease in the offspring. These morbidities are dependent on when in pregnancy the calorie restriction occurs. Other chronic diseases related to fetal development include asthma and obstructive lung disease (Barker, 2001). Evidence suggests osteoporosis, polycystic ovarian syndrome, mental disorders including depression and schizophrenia, as well as cancers including breast, ovary, and prostate, are related to fetal development.

The Fetal Origins Hypothesis suggests a trade off for energy in relation to growth (Ellison, 2005). The fetal response to an energy deficit is to maintain blood glucose levels for the developing brain (Barker, 2003). However, glucose maintenance for the brain is at the expense of the muscles and other tissues. Therefore, energy to the fetus is either utilized for brain growth or for somatic body growth (Ellison, 2005). Though this trade off begins during development *in utero*, the Fetal Origins Hypothesis believes the tradeoff may last long term. For instance, if the fetal brain is spared while *in utero*, later in life when nourishment may be less than adequate, the brain will again be spared. On the other hand, if fetal body growth is spared while *in utero*, later in life when nourishment may be less than adequate, the body will again be spared. The long-term trade off may continue during the offspring's pregnancy (Barker, 2004). When an offspring becomes pregnant, how she nourishes her fetus may be determined by how her mother nourished her when she was *in utero*.

Significance

The purpose of the current project is to better understand the relation between maternal nutrition and the cognition of toddlers aged 2.5 to 4 years old. More specifically, iron and zinc will be considered due to their function in neurological development (Fretham, Carlson, & Georgieff, 2011). Iron needs are increased in pregnancy due to the increase in blood volume and to meet the needs of the growing fetus as well as the placenta (Institute of Medicine (IOM), 2001). Fetal growth also requires increased zinc for cell differentiation and cell development.

Anemia is generally diagnosed by hemoglobin (Hgb) levels; iron deficiency anemia (IDA) in pregnancy is defined as hemoglobin (Hgb) less than 11 g/dL (Centers for Disease Control and Prevention (CDC), 2014b; World Health Organization (WHO), 2014). Decreased Hgb, anemia, is seen globally in 38% of pregnant women and 43% of children under five years old (CDC, 2014b). Hgb is found in erythrocytes, also known as red blood cells (RBC), which is where two-thirds of bodily iron is located (IOM, 2001). However, Hgb levels are also affected by Vitamin B_{12} and folate.

Iron status can be assessed by multiple variables, including but not limited to serum iron, ferritin, transferrin (Tf), and soluble transferrin receptors (sTfR). Ferritin is considered the most sensitive measure for iron stores, and decreased serum ferritin is indicative of decreased or depleted iron stores (CDC, 2014a). When iron in the tissues decreases, sTfR increases to increase the uptake of iron (Mei et al., 2011).

Due to difficulty in assessing zinc status, it is challenging to detect marginal zinc deficiency (Vale et al., 2014). However, insufficient zinc consumption during preconception and/or during pregnancy may play a role in cognitive development (Black, 2003). Women who have a marginal zinc status prior to pregnancy may develop zinc insufficiency or deficiency due to the increased needs for zinc during pregnancy for the mother as well as for the fetus (National Institute of Health (NIH), 2014b). Also, marginal zinc status may be exacerbated as women are encouraged to take iron supplements during pregnancy, and iron supplementation may inhibit zinc absorption.

The current study aims to contribute data to assist in filling the gap in the literature related to healthy toddlers and healthy mothers since research in this area tends to focus on infants, school aged children, and/or adolescents (Chang, Zeng, Brouwer, Kok, & Yan, 2013; Tran et al., 2013). In relation to iron, studies focus on maternal and/or child iron deficiency (ID) and/or iron deficiency anemia (IDA) instead of focusing on a non-anemic status in healthy individuals (Black, 2012; Chang et al., 2013; Habte et al, 2013; Tran et al., 2013). In general, studies have been conducted on animals or on individuals in non-developed countries that may have other nutritional concerns rather than on healthy humans (Chang et al., 2013; Habte et al., 2013; Mihaila et al., 2011). Additionally, research in cognitive development in relation to iron and zinc tends to be related to the status of the child rather than the status of the mother (Osendarp, Murray-Kolb, & Black, 2010). Therefore, the current project may assist to further advance information on the Fetal Origins Hypothesis.

This theory is becoming more known to the lay public; for instance, there was an article in Time magazine (Paul, 2010). Therefore, the findings may advance the theory as well as emphasize the importance of prenatal nutrition to the lay public. Moreover, research using the Fetal Origins Hypothesis tends to explore chronic disease, such as coronary heart disease and type two diabetes. However, cognitive development will be considered in the scope of this project.

Research Questions

The purpose of this research project is to determine if toddler cognition is related to maternal iron and/or zinc status during pregnancy and early infancy. Maternal iron and zinc statuses were determined when infants were approximately three months old using multiple measures of iron and serum zinc levels. Infants involved in the project were born full-term and as a singleton birth.

At three and nine months of age, Visual Information Processing (VIP) was used as an assessment for infant memory and processing speed. Cognitive assessments in toddlers from 2.5 to 4 years old included the Peabody Picture Vocabulary Test (PPVT), an Imitation task, and a Distractibility activity. A maternal assessment of toddler language development, the MacArthur Bates Communicative Development Inventory (CDI), was also used.

Hypotheses of this study were assessed when the children were from 2.5 to 4 years old.

I. Toddler cognition will be positively correlated with increased maternal iron and zinc biochemical levels when assessed at three months postpartum.

Additional hypotheses will be considered:

II. Toddlers who showed shorter looking times during the Visual Information Processing test at nine months old, which is indicative of faster processing speed, will have better performance on cognitive tests when measured at 2.5 to 4 years old.

III. Toddlers' increased cognition will be positively related to higher levels of maternal iron and zinc dietary intake during pregnancy and three months postpartum.

Study Design

Participants were recruited through researchers visiting the local hospital during breastfeeding courses and events where mothers would be generally found. Signs were also posted around the local area in Stillwater, OK. Finally, word of mouth also brought in participants. Inclusion criteria in the initial infant study was the infants were required to be predominantly breastfed for the first three months of life; this indicates they were fed less than 28 fluid ounces of formula per week. In addition, infants were to have been born full-term and at a healthy weight, 6.5 to 9.5 pounds. Lastly, both the mother and the infant were to have no serious health conditions. For the toddler study, since it was a follow-up study, the participants in the infant study were called back to ask if they were willing to participate. Monetary incentive ($120 total) was paid for both parts of the study.

A total of 132 mother/infant pairs were included in the original infant study. The follow up toddler study included a total of 47 mother/toddler pairs. Approximately 36 participants completed all the necessary assessments for inclusion in the current project. The study was funded primarily by the United States Department of Agriculture (USDA) and was approved by the Oklahoma State University Review Board.

CHAPTER II

REVIEW OF LITERATURE

The literature review in relation to iron and zinc's effects on cognitive development has been divided into six sections. First, an overview of the Fetal Origins Hypothesis will be considered. Secondly, understanding toddler iron and zinc requirements as well as toddler intake levels of iron and zinc is important. Because the median population of toddlers meets or exceeds dietary recommendations for iron and zinc intake (Butte et al., 2010), potential cognitive differences may be related to pre-natal maternal intake. Thirdly, toddler cognitive development will be discussed. Fourthly, looking at the literature in relation to iron supplementation randomized control trials (RCT) as well as review articles is important to understand the relation of iron to cognition. Fifthly, looking at the literature in relation to zinc RCT as well as review articles is important to understand the relation of zinc to cognition. Lastly, animal studies will be considered in relation to brain development. Animal studies consisted of rat studies as well as rhesus monkeys studies. The animal studies also consider iron and zinc in relation to cognitive development.

Fetal Origins Hypothesis

Within the Fetal Origins Hypothesis, there are three concepts (Ellison, 2005). The position of the first concept is cause and effect. The cause is non-normative development during the fetal period, and the effect is chronic disease later in life. Low birth weight infants may have fewer cells in their key organs, such as the kidney (Barker, 2003). For instance, the decreased number of cells in the kidney was related to a decreased number of glomeruli. Blood pressure was related to the number of glomeruli, while reduced glomeruli levels related to hypertension.

The position of the second concept of the Fetal Origins Hypothesis is related to constraint (Ellison, 2005). Due to inadequate nutrition and/or maternal stores for normative development, the fetus cannot physiologically develop to its full potential. Disease later in life may be related to restricted fetal growth (Barker, 2003). This may be due to hormones and metabolism while *in utero*. For instance, if a fetus does not receive enough calories *in utero*, it may alter how it handles food and nutrients. The altered hormones and/or metabolism may continue after birth, despite adequate nutrient conditions. Low birth weight followed by accelerated weight gain has been related to insulin resistance. The position of the third concept of the Fetal Origins Hypothesis is related to adaption (Ellison, 2005). In this concept, the fetus is able to adapt to inadequate nutrition through physiologically normal development.

Furthermore, there are three domains (Ellison, 2005). The domains include growth, maintenance, and reproduction. Within this thesis project, the domain considered is growth. Despite the name Fetal Programming, the programming continues past the fetal period and throughout development (Barker, 2001). Fetal adaptations to nutrient imbalances may permanently affect development.

The fetus receives nutrients from the placenta through the turnover of protein and fat in maternal tissues. Pre-pregnancy maternal size and body composition affects fetal growth and development (Barker, 2003). The composition of maternal diet also affects fetal growth (Barker, 2012). For instance, increased protein intake in late pregnancy was related to increased birth weight (Robinson, Moore, Owens, & McMillen, 2000). On the other hand, increased carbohydrate intake in early pregnancy was related to decreased birth weight. This may be due to decreased micronutrients from the diet, as an increase in calories is not necessarily indicative of an increase in nutrients (Barker, 2001).

Research suggests low birth weight was linked to an energy deficit for the infant while *in utero* (Ellison, 2005). The lack of nourishment *in utero* may lead to limitations of fetal brain development. On the other hand, the fetus may adapt to the lack of nourishment and fetal brain development may be spared. During fetal development, the fetus exhibits developmental plasticity (Barker, 2003). Plasticity is the ability of the fetus to adapt in response to its environment; thus, brain development may be spared from an energy deficit. Plasticity is a defense mechanism for adaptation of the fetus for survival (Barker, 2004). During sensitive periods of development, the fetus, infant, or child exhibits more developmental plasticity than less sensitive periods of development. The timing of sensitive periods is not well defined, but it has been suggested to include stages from as early as late gestation to as late as three years old (Thomas, Grant, & Aubuchon-Endsley, 2009). Aside from the brain, only the liver and the immune system continue to have developmental plasticity postnatally (Barker, 2012). The brain is considered only half developed at birth, and the liver continues to have plasticity until approximately five years old (Barker, 2001).

Despite the focus on birth weight within Fetal Origins Hypothesis, birth weight alone is an inadequate description of fetal growth and development (Barker, 2004). Low birth weight may be related to a small mother and smaller accommodations for the fetus rather than inadequate nutrition. Maternal birth weight was indicative of her offspring's birth weight (Barker, 2001). There was a positive correlation with maternal body mass index (BMI) and birth weight (Robinson et al., 2000). Birth weight was affected until maternal weight reaches more than 120% of ideal body weight. Low birth weight may also be due to early birth (Barker, 2001). Additionally, gene-nutrient interactions as well as gene-environment interactions may affect birth weight (Barker, 2004). However, birth weight is a convenient marker and offers an estimation for fetal development. Other factors affecting birth weight may include multiple births rather than a singleton birth and maternal disease (Robinson et al., 2000). Research has shown childhood weight gain modifies the relation of birth weight to adult cardiovascular disease (Barker, Eriksson, Forsen, & Osmond, 2002).

Toddler Intake Levels

The importance of toddler recommendations for iron and zinc as well as intake levels will be considered. Recommendations for iron and zinc from birth to four years old from the Institute of Medicine (IOM) will be discussed.

Recommendations for iron intake from the Institute of Medicine (IOM) for the first six months of life are dependent on the infant receiving adequate iron from maternal iron stores *in utero* (NIH, 2014a). The Adequate Intake (AI) for zero to six-month-old infants for iron is 0.27 mg per day (IOM, 2001). This was based on the average amount of iron concentration in human milk. In infants seven to 12 months old, iron recommendations increase (IOM, 2001). For this age group the Estimated Average Requirement (EAR) for iron is 6.9 mg/day and the RDA is 11 mg/day. For toddlers, recommendations decrease since infants grow more rapidly than toddlers. For one to three year olds, the EAR for iron is 3.0 mg/day and the RDA is 7 mg/day. The changes are due to growth, tissue storage, and increase in blood volume. The difference between the EAR and the RDA is due to the calculation in relation to the population. The EAR recommendations are based on meeting the requirements for 50 percent of the population, and the RDA recommendations are based on meeting the requirements for 97.5 percent of the population.

Also from the IOM, the AI for zinc is 2 mg/day in infants zero to six months old, which was determined by the mean intake of zinc in healthy, breastfed infants (IOM, 2001). For infants seven to 12 months old, the EAR for zinc is 2.5 mg/day and the RDA is 3 mg/day. For one to three year olds, the EAR for zinc is 2.5 mg/day and the RDA is 3 mg/day.

A recent study found that the mean iron and zinc in two groups of toddlers aged 12 to 23 months and 24 to 47 months met the EAR (Butte et al., 2010). The toddlers' mean intake for both iron and zinc were not only met, but also exceeded the EAR recommendations. Furthermore, the lowest dietary intake group, the tenth percentile, also exceeded the EAR recommendations. The upper limit (UL) for zinc intake is 7.0 mg/day, and the median for zinc intake exceeded the UL in 24 to 47 months at 8.7 mg/day. It is known that phytates and fiber may inhibit zinc absorption; however, mean dietary fiber was below the AI for toddlers.

Previously Skinner et al. (1999) found mean iron intake to be less than the RDA for 27-month-old and 34-month-old boys and girls and for 48-month-old girls. Furthermore, results showed zinc intake to be less than the RDA for all age groups. However, the RDA for iron and zinc at the time of the study was 10 mg/day; the RDA for zinc has since decreased. Therefore, the discrepancy in the findings between Skinner (1999) and Butte (2010) was due to the differences within dietary recommendations between the EAR and the RDA as well as a decrease in the RDA for zinc. When considering the iron and zinc values in relation to the current EAR and RDA, all values from toddlers from two to five years old meet current requirements (Skinner et al., 1999; IOM, 2001).

In summary, changes in dietary recommendations have been updated during the time between the Skinner article (1999) and the Butte article (2010). Toddlers in the Butte article met and exceeded the EAR for both iron and zinc, and the UL was exceeded for zinc. This may be because the main source of zinc is in fortified cereals (NIH, 2014b). Fiber may inhibit absorption of iron and zinc; however, the AI for fiber in the Butte article (2010) was not met in toddlers, thus it is likely not a concern for iron and zinc absorption. Toddler intake rather than blood levels are generally used since biochemical levels rapidly change due to growth (Hermoso et al., 2011). Also, because of rapid physiological changes there is not a consensus for blood values in children. Toddler recommendations as well as infant and child recommendations are based upon adult recommendations (IOM, 2001). Therefore, the recommendations for individuals who are not adults are less than perfect.

Toddler Cognitive Development

Toddler cognition is composed of multiple components. Different components of cognitive development include language, long-term recall, and attention (Bauer, 2002; Kannass & Colombo, 2011; Vohr, 2007). Language can be measured as both receptive language and expressive language (Vohr, 2007). It is more comprehensive to assess both receptive and expressive language to better understand language development since they are both important for the fundamentals of communication. Moreover, vocabulary can be measured both verbally as well as non-verbally (Hoffman, Templin, & Rice, 2012). Toddler vocabulary may also be measured with maternal questionnaires (Fenson, Pethick, Renda, & Cox, 2000). Long-term recall considers remembering a past experience without an ongoing stimulation of the experience (Bauer, 2002). Long-term recall is a portion of the complex cognitive domain of memory. Lastly, attention is comprised of multiple factors, such as attention span, persistence, perseverance, and distractibility (Kannass & Colombo, 2011). Assessing multiple areas of toddler cognition gives a better overall picture of development. Assessments consist of standard expectations for toddlers; however, specific scores to measure toddler cognitive development for certain age ranges are not always well defined.

A maternal report measure for toddler vocabulary is the MacArthur Bates Communicative Development Inventory (CDI) III (Fenson et al., 2000). This questionnaire assesses toddler expressive language. More specifically, only words that the toddler says, also known as produced vocabulary, are assessed. Children may understand words that they hear, also known as receptive vocabulary, but this maternal report measure does not assess what the toddlers hear.

Scoring for the MacArthur Bates CDI is broken up into multiple sections (Fenson et al., 2007). The sections are broken up into vocabulary, sentences, usage, and mean length of utterance (MLU). The vocabulary section assesses words children say and receives a score from zero to one hundred. The sentences section assesses the combination of words children say, and it is scored from one to twelve. Furthermore, the usage section assesses the toddler's language use, and it is also scored from zero to twelve. Lastly, MLU is assessed by averaging the number of words from three of the longest sentences the toddler has said recently.

The scores that are expected in toddlers vary dependent on toddler age and gender (Fenson et al., 2007). For instance, the MacArthur Bates CDI III is intended for 30 to 37 month olds. The expected scores are divided into four age groups for 30 to 31 months old, 32 to 33 months old, 34 to 35 months old, and 36 to 37 months old. The scores are then broken down into percentiles. For example, a score of 67 for vocabulary is in the 50th percentile for 30 to 31 month old toddler girls. On the other hand, a score of 51 for vocabulary is in the 50th percentile for 30 to 31 month old toddler boys. In general, females score higher than males for all four sections, vocabulary, sentences, usage, and MLU.

Fenson (2007) assessed validity and reliability for the MacArthur Bates CDI by using internal consistency and test-retest reliability. Internal consistency was computed by using Cronbach's alpha on multiple scales for vocabulary, which included words and gestures understood (0.95), words and gestures produced (0.96), and words and sentence words produced (0.96). However, internal consistency was lower for words about time (0.65) and question words (0.68). Test-retest reliability was measured by sending mothers a second MacArthur Bates CDI approximately two months following the first one. When reliability was measured by Pearson correlations, the two surveys, the test and the retest, indicated *r* values in the upper 0.80s, and *p* values of less than 0.01. Moreover, reliability for the short form was compared to the longer forms (Pan et al., 2004). Reliability was also assessed by the Cronbach's alpha for children's raw vocabulary production scores (0.99). Similarly, an additional study indicated a comparison of the short form to the long form was highly reliable (*r*=0.99) (Fenson, et al., 2000). Both articles indicated the short forms were highly reliable when compared to the longer forms. Overall, when maternal designation of toddler vocabulary was used, it was more advantageous than clinical observation. Caregivers interact with toddlers more than clinicians interact with them, which is why a maternal measure may be more reliable (Fenson et al., 2007). Recent PubMed searches using the key words McArthur Bates, vocabulary, nutrition, iron, toddlers, and children did not include articles for toddler or child outcomes for cognition.

Toddler language can also be assessed through toddler observations (Hoffman et al., 2012). The Peabody Picture Vocabulary Test (PPVT) is widely used to assess vocabulary. Unlike the MacArthur Bates CDI, toddler receptive language is assessed rather than toddler expressive language. This means words toddlers know are considered, rather than if the toddler says the words. More specifically, vocabulary is assessed for verbs, nouns, and adjectives. Four pictures are shown to the toddler, and he/she is asked to point at the correct one. Therefore, this is a non-verbal measure of vocabulary. There are twelve sets of four pictures in each sequence, and if the toddler misses enough pictures in a sequence, that sequence stops. The next sequence will start until the toddler misses too many words for the test to continue or when the test has gone through all of the sequences. Scoring for the PPVT is standardized for age and gender (Hoffman et al., 2012). The average score is 100 with a standard deviation of fifteen.

Previous research was conducted between toddler nutritional status and PPVT scores (Hubbs-Tait et al., 2009; Nyaradi et al., 2013). Nyaradi (2013) measured toddler diets at one, two, and three years old, and a diet score was given based off of the Eating Assessment in Toddlers. The PPVT was measured at ten years old. Higher toddler diet scores at two and three years old was associated with increased vocabulary at ten years old, yet the association was non-significant. Likewise, Hubbs-Tait (2009) measured iron levels in children with a mean age of four years old. The PPVT was also measured at four years old. There was a positive significant relation between toddler transferrin receptors and toddler PPVT scores. Therefore, nutritional status in toddlers had a long-term impact for child vocabulary, and iron levels in toddlers were predictive of toddler vocabulary (Nyaradi et al., 2013; Hubbs-Tait et al., 2009).

Another assessment of toddler cognition is imitation (Bauer, 2002). Elicited imitation includes giving a child tasks to imitate with a certain object. This assessment measures memory, in particular memory involved in recalling specific information, as well as long-term recall processes. The measure can assess cognition in infants as young as nine months old through adulthood, and memory is more reliable in individuals two years old and older. However, it is still not well understood why some infants and toddlers are better able to encode, store, and recall more information than other infants and toddlers who are the same age. In general, older children are able to recall more information than younger children. In addition, older children are better able to recall information after delays. This means after the imitation task is demonstrated, older children are able to recall the task after a time lapse and use their long-term memory.

The ability to use long-term memory is indicative of brain development since long-term memory requires using multiple regions of the brain (Bauer, 2002). For instance, when the imitation task is shown, the memory is formed in the temporal lobe and stored in the hippocampus. After the time lapse, the memory is retrieved through the prefrontal cortex. Therefore, both storage and retrieval are assessed through imitation. Imitation scores are based on the number of steps for the task (which varies dependent on the age of the infant or child being assessed) as well as the correct sequence for the task.

Research has been conducted in relation to maternal iron status and toddler memory (Riggins, Miller, Bauer, Georgieff, & Nelson, 2009). Maternal ferritin from cord serum was measured at birth, and toddler memory was assessed via imitation at three and four years old.

The results suggested maternal ferritin levels were significantly associated with imitation task performance in toddlers. Therefore, maternal iron levels impacted long-term recall in toddlers.

An additional assessment for toddler cognition is distractibility (Kannass & Colombo, 2011). Distractibility is a construct for attention. Other constructs of attention include attention span, persistence, and perseverance. Distractibility is a competition for attentional focus, and it can be assessed by the latency to orient to the distraction. Moreover, distractibility is variable dependent on attention to the stimulus, the age of the participant, and the nature of the distracting stimulus. Therefore, the child is less distractible if he/she is focused on the stimuli. As age increases, resistance to the distracting stimulus also increases. Lastly, characteristics of the distracting stimulus, such as volume and complexity, also affect distractibility.

Distractibility can exist in multiple forms and be scored in multiple ways (Kannass & Colombo, 2011). A distracting stimulus may be intermittent or continuous. Intermittent distractors occur periodically and randomly throughout the task, and continuous distractors occur for the entire duration of the task. The distractibility task is recorded then coded. Scoring is conducted as the time in seconds for the latency to look at the distracting stimulus, the number of times the toddler looked at the distracting stimulus, and the total time in seconds spent looking at the distracting stimulus. A look is generally defined as looking at the distracting stimulus for at least one second.

Nutrition and distractibility has been considered in previous research (Jensen et al., 2010). Breastfeeding mothers from delivery until four months postpartum were given 200 mg/day of decosahexaenoic acid (DHA) supplements compared to a non-DHA vegetable oil supplement. Attention in five year olds was assessed by the Leiter International Performance Scale-Revised using the Sustained Attention portion of the measure. Children from the DHA supplement group had significantly improved performance in comparison to the control vegetable oil group. Therefore, maternal nutritional status during lactation impacted attention in children. Moreover, another article also considered maternal DHA and infant/toddler attention (Columbo et al., 2004). Mothers were given high DHA (135 mg/each) eggs in the supplement group or ordinary (35 mg/each) eggs in the control group during the third trimester. Infant attention was assessed at four, six, and eight months old for by visual habituation. Toddler attention was assessed at 12 and 18 months old via a distractibility task. Infants at four and six months old in the high maternal DHA group showed significantly increased visual habituation compared to the control. Toddlers in the high maternal DHA group were significantly less distractible as assessed by turns to the distractor and latency to look at the distractor. Therefore, maternal nutritional status during the third trimester impacted visual habituation in infants and distractibility in toddlers. Overall, the research articles indicate a long-term impact on distractibility related to perinatal nutrition (Jensen et al., 2010; Columbo et al., 2004).

In summary, toddler cognition can be assessed by multiple measures (Bauer, 2002; Fenson et al., 2000; Fenson et al., 2007; Hoffman et al., 2012; Kannass & Colombo, 2011). Different assessments consider different types of cognition, such as vocabulary, imitation, or distractibility. The assessments to measure toddler cognition may be completed through maternal questionnaires or toddler tasks. Measuring toddler cognition is complex since toddlers may not cooperate in the tasks, which makes maternal measures beneficial. Maternal nutrition has been linked to long-term cognitive development (Columbo et al., 2004; Hubbs-Tait et al., 2009; Jensen et al., 2010; Nyaradi et al., 2013; Riggins et al., 2009). Furthermore, the scores generally vary dependent on toddler age and gender. Toddler expectations are not well defined due to the challenges for assessing toddler cognition, such as age, gender, and cooperation.

Iron

IDA in individuals is the most prevalent mineral deficiency worldwide (WHO, 2014). According to the National Health and Nutrition Examination Survey (NHANES) from 1999-2006, ID was found in 18% percent of pregnant women in the United States (Mei et al., 2011). The prevalence of ID has been shown to increase throughout pregnancy. For instance, women in their second or third trimester were more likely to have ID than women in their first trimester. Additionally, in the United States, ID was found in 14% of one to two year olds, 4% of three to five year olds, and 9% of women of childbearing age (CDC, 2014a). Even if ID or IDA in pregnant women was not prevalent, iron inadequacy may exist. This may be due to the increasing numbers of RBC needed by the developing fetus as well as the placenta. It is suggested that iron requirements for the fetus are met before the iron requirements for the mother (IOM, 2001).

Iron status can be assessed by multiple measures of iron, such as hemoglobin (Hgb), soluble transferrin receptor (sTfR), ferritin, and serum iron. Hgb is an oxygen carrying protein found in red blood cells (RBC) (NIH, 2014a). Hgb is believed to contain approximately 70 percent of the body's total iron. The body regulates Hgb, and it may not decrease until iron deficiency (ID) or iron deficiency anemia (IDA) is severe. Moreover, Hgb is a typical clinical measurement used to assess iron. A more sensitive marker for iron status is sTfR. As iron in the tissue decreases, the concentration of sTfR increases (Skikne, 2008). Ferritin is an intracellular protein that stores iron and releases it under conditions of insufficiency or deficiency (NIH, 2014a). Decreased ferritin levels may indicate early ID or storage depletion (National Library of Medicine (NLM), 2014). However, perinatal ferritin may be decreased due to increased iron needs in pregnancy, and the mother and the fetus may have depleted iron stores. Therefore, sTfR is a useful indicator for iron status perinatally. Serum iron measures the amount of iron in the blood that is bound to transferrin, an iron transport protein. Overall, there are multiple measures of iron, and each iron measure is related to specific iron pools in the body.

Iron plays specific roles in growth and development; for instance, iron was related to tissue differentiation (Fretham, Carlson, & Georgieff, 2011). Tissue differentiation is important for fetal brain development. Unfortunately, the mechanisms on a cellular level concerning how iron is related to these cognitive behaviors are still unclear. Despite the lack of clarity for the mechanisms, research showed iron is important for cognitive development. For example, children with ID exhibited developmental delays as well as cognitive impairment when cognition was measured by mental and motor tests (IOM, 2001). Therefore, ID has functional consequences in relation to cognitive development.

Pregnant women as well as infants and toddlers who are rapidly growing require a higher amount of iron (Osendarp et al., 2010). Iron concentration in the infants' brain is at the highest at birth. Iron levels in infants' brain decreases through weaning then begin to increase as the diet changes with age. The increase in iron levels in infants is related to an increase in receptors within the brain, particularly transferrin (Tf) receptors. Also in relation to brain development, infancy and late toddlerhood are when hippocampal development peaks (Lozoff et al., 2006). ID affects the structural maturation of the hippocampus from late gestation through approximately two to three years old (Fretham et al., 2011). Iron is used for myelination during development. IDA negatively affects myelination, which may decrease the efficiency of learning related to reduced processing speed. An additional iron function is related to neurotransmitters, specifically dopamine. Iron concentrations and distribution in brain are dependent on the stage of brain development (Beard & Connor, 2003). Yet, the data in relation to specific milestones for development during toddlerhood is limited (Rosales, Reznick, & Zeisel, 2009).

Research for perinatal iron status in infants is limited due to the wide belief that infants are protected from maternal iron deficiency (ID) unless the mother has iron deficiency anemia (IDA) (Lozoff et al., 2006). IDA in infants was related to decreased developmental scores as well as decreased scores in relation to cognitive function tests and educational achievement in children older than two years old (Grantham-McGregor & Ani, 2001). Moreover, children who were previously IDA as infants had decreased performance scores in relation to mental, motor, and social/emotional functioning tests as well as decreased test scores for neurocognitive tests in preschool, school age, and adolescence (Lozoff et al., 2006).

Some studies indicated children benefit from iron treatment in fortified infant formulas, foods, or supplementation in relation to cognitive development (Aukett, Parks, Scott, & Wharton, 1986). One group of toddlers was supplemented 24 mg/day of ferrous sulfate for two months with vitamin C in comparison to another group of toddlers who took a placebo (Aukett et al., 1986). Other researchers compared a group of toddlers who were supplemented 50 mg/day for two months in comparison to a group of toddlers who were supplemented with a multivitamin without iron (Metallinos-Katsaras et al., 2004). Overall these two research articles concluded supplementation was shown to have more benefit in IDA individuals.

On the other hand, other studies have indicated no effect from iron treatment (Metallinos-Katsaras et al., 2004). A review article concluded iron supplementation had a positive non-significant association with mental development (Sachdev, Gera, & Nestel, 2004). Typically infants who had lower iron status before supplementation showed the most improvement in relation to iron status and in the Bayley Scales of Infant Development (BSID) (Beard & Connor, 2003). Supplementation benefits are dependent on an appropriate duration of use, which is not well defined (Rosales et al., 2009). In relation to supplementation studies, it is important to consider the characteristics of the control group, such as diet, nutritional concerns and other factors, and how these factors impact the results (Thomas et al., 2009).

Results from a randomized control trial (RCT) in rural China showed a correlation between maternal prenatal IDA with mental development in infants at 12, 18, and 24 months old (Chang et al., 2013). Infants in rural China showed lower mental development at 12, 18, and 24 months of age when their mothers had prenatal IDA. However, the marker used to determine IDA was Hgb, which may also be impacted by other nutritional deficiencies, such as folate and Vitamin B_{12} (Habte et al., 2013). Another iron study correlated antenatal Hgb levels to cognitive development (Tran et al., 2013). The research considered common mental disorders, such as depression and/or anxiety, in relation to infant cognitive development. Depression and/or anxiety are prevalent in pregnancy and postpartum. The article concluded there was a positive correlation between mothers with prenatal IDA who exhibited common mental disorders and their six-month-old infants who had lower cognitive development.

Children who were previously ID at birth received poor scores during an elicited imitation task (Fretham et al., 2011). This showed an impairment in learning as well as in memory. This may be due to the rapid development of the hippocampus portion of the brain during this time period. If plasticity is decreased due to a lack of iron, this may explain the decreased ability for learning as well as for memory.

A review article concluded that perinatal ID exposure increases the risk of children failing to reach educational milestones (Radlowski & Johnson, 2013). This may be due to developmental delays in infancy and/or toddlerhood including motor development, IQ, learning, and memory. When IDA infants are compared to non-IDA infants, the average mental development scores were decreased in the IDA infants (Lozoff et al., 2006). It is believed prenatal iron protects infants from IDA until six months old, but prenatal IDA may be related to infants with IDA (IOM, 2001).

The origins of cognition as well as chronic disease may be linked to generational roots. Offspring eggs are produced by her mother since a girl is born with all of the eggs she will ever have (Barker, 2012). It has been suggested that maternal fetal growth may affect their offspring's fetal growth (Barker, 2003). A determinant of birth weight is the mother's birth weight (Barker, 2001). Furthermore, applying the Fetal Origins Hypothesis to toddler cognition suggests perinatal ID may affect generations of individuals with ID rather than just individuals.

In summary, iron needs are increased in pregnancy as well as infancy (Osepdarp, Murray-Klob, & Black, 2010). Infants and toddlers up to two years old with IDA have decreased developmental scores, cognitive function test scores, and educational achievement (Grantham-McGregor & Api, 2001). Children who were IDA at birth exhibited reduced scores for elicited imitation (Fretham et al., 2011). In addition, children with IDA have an increased benefit from iron supplementation compared to children without IDA (Black, 2012). However, there is not a consensus for the benefit of iron supplementation in children (Hermoso et al., 2011). There is a complication for how IDA is identified since IDA is commonly diagnosed by decreased Hgb levels, which may be impacted by folate and Vitamin B_{12} (Habte et al., 2013). Maternal IDA was related to lower infant mental development, and children exposed to maternal perinatal ID had an increased likelihood of not reaching educational markers (Radlowski & Johnson, 2013). In conclusion, maternal ID and IDA affected child outcomes. The benefit from iron supplementation was dependent on multiple variables, such as the iron status of the individual, the type of iron supplement received, and the dose of the iron supplement.

Zinc

Zinc is generally measured as serum zinc. However, serum zinc is a poor indicator of zinc status (Vale et al., 2014). The amount of zinc in the serum is minimal in comparison to total body zinc. The majority of zinc in the body is found in skeletal muscle and bone (IOM, 2001). Moreover, serum zinc levels are affected temporarily by multiple factors including muscle and bone catabolism as well as recent food intake (Vale et al., 2014). Serum zinc is well maintained by the body without noticeable changes despite dietary zinc restrictions or increases, which makes it a poor indicator for supplementation benefits (IOM, 2001). The zinc in the RBC may contaminate serum zinc; this makes plasma zinc concentration a more sensitive indicator of zinc status.

Zinc needs are increased in pregnancy due to its role in cell growth and differentiation as well as gene expression (IOM, 2001). Zinc accumulates in the mother and the fetus due to the increase in tissue from the fetus and the placenta. In lactation, zinc needs are also increased, yet research does not provide evidence of a relation between increased dietary zinc intake and increased zinc concentration in breastmilk.

Zinc is also important for development since it plays a role in cell growth and differentiation. The importance of zinc for growth is widely accepted (Salgueiro et al., 2002). Zinc deficiency was related to stunted growth (IOM, 2001). It has been hypothesized that zinc affects growth due to the concentration of zinc in skeletal muscle, bone, and adipose tissue. Additionally, zinc supplementation was related to an increase in mean weight-for-age as well as increased linear weight gain in comparison to a placebo. Zinc is related to bone growth through stimulating osteoblasts, cells that synthesize bone, and inhibiting osteoclasts, cells that reabsorb bone tissue (Solomons, 2013). Specific roles of zinc are related to memory and learning, which are crucial during early development (Darton-Hill, 2013). However, zinc bioavailability may be affected due to plant-based diets as well as iron and folate supplementation competing for absorption (Salguiero et al., 2002).

An observational study showed serum zinc declined as pregnancy progressed (Tamura, Goldenberg, Johnston, & DuBard, 2000). However, maternal zinc supplementation indicated null results in relation to the mental development index (MDI) in infants, behavior or growth in infants, and neurologic development in children five years old (Hamadani, Fruchs, Osendarp, Khatun, & Grantham-McGregor, 2001; Hamadani, Fuchs, Osendarp, Huda, & Grantham-McGregor, 2002; Tsenenobu, Goldenberg, Ramey, Nelson, & Chapman, 2003).

Zinc supplementation during pregnancy showed interesting results. Prenatal zinc supplementation (25 mg/day of zinc in an oral dose) during the second half of pregnancy showed no correlation with mental, psychomotor, or neurological development when the child was five years old (Tsenenobu et al., 2003). This may be because supplementation occurred in the second half of the pregnancy and central nervous system (CNS) development begins early in the pregnancy. The participants were from low socio economic status (SES) homes, which may have contributed to similar cognitive development in children if they did not receive educational stimulation.

When zinc supplements were given to women in their second half of pregnancy with a dose of 30 mg/day in the slums of Dhaka, Bangladesh, there was no significant effect on behavior or growth when the infants were 13 months old (Hamadani et al., 2002). Behaviors measured included nonverbal ability, verbal ability, conceptual ability/IQ, visual sequential memory score, auditory sequential memory score, and gross motor scale score. However, also in the slums of Dhaka, supplemented zinc was related to lower MDI scores at seven and 13 months old (Hamadani et al., 2001). Results are confounded by the fact that zinc may not be the only micronutrient in need of supplementation in the slums. Despite null or non-significant results between prenatal zinc supplementation and toddler cognition, zinc is important for growth and development (Osendarp et al., 2001). For instance, when zinc supplements were given to pregnant women in their first trimester until birth with a dose of 30 mg per day, there was a relation between the zinc supplements and decreased low birth weight as well as decreased instances of diarrhea in six-month-old infants.

In infants and children, supplementation trials indicated interesting results (Hamadani et al., 2001). Zinc was supplemented in infants for the first year of life with a dose of 5 mg/day in comparison to a placebo. The results indicated similar MDI means, yet significantly more non-supplemented infants scored lower on the MDI than the supplemented infants (Castillo-Duran et al., 2001). On the other hand, zinc supplementation in children from one to 36 months old did not result in the same positive correlations as zinc supplementation prenatally (Sazawal et al., 2007). Zinc was supplemented for over a year with a dose of 5 mg/day in infants one to 12 months and 10 mg/d in children 12 to 36 months old in comparison to a placebo. A non-significant relation to decreased mortality was found. The study was conducted in Pemba, Zanzibar. Again, research conducted in non-developed areas may have other nutrition concerns outside of zinc.

Zinc is important for multiple areas of cognitive development. Zinc deficiency was correlated with cognitive development in toddlers and pre-school aged children through decreased attention, activity, and motor development (Bhatnagar & Taneja, 2001). Furthermore, zinc in school-aged children suggested an effect from zinc deficiency on academic performance, specifically in relation to reading. In particular, reasoning and attention were the primary differences between supplemented and non-supplemented groups. Additionally, another review article concluded poor prenatal zinc status had a negative effect on fetal brain development (Hambridge, 2000). Yet, this is not well understood, but it is suggested it may be related to a growth limiting zinc deficiency. However, data do not suggest a relation between maternal zinc status and infant neurobehavioral development (Shah & Sachdev, 2006).

Similar to iron, there is not an agreement for the relation of zinc and cognition. Furthermore, zinc supplementation during pregnancy indicated null results in multiple studies (Hamadani et al., 2001; Hamadani et al., 2002; Tsuenobu et al., 2003). Zinc supplementation in children did not show statistically significant results in comparison to a placebo (Castillo-Duran et al., 2001). However, zinc deficiency was shown to have a relation to toddler and child cognitive performance (Bhatnagar & Taneja, 2001). In conclusion, the benefit of supplementation in pregnancy, infancy, and childhood is unknown.

Animal Studies

Animal studies suggest that infant and child ID is related to hippocampal development, which is rapid in infants and toddlers (Fretham et al., 2011). Another possible pathway is in relation to the neurotransmitter dopamine. There may be a defining developmental age where iron repletion may not reverse behavioral consequences of ID; however, this developmental age is not yet determined.

An animal study has also considered iron in relation to fetal development (Mihaila et al., 2011). In a rat model, Mihaila et al. (2011) manipulated iron intake as well as monitored development at various time periods during pregnancy. Maternal iron restriction in rats during preconception as well as during the first week of gestation was associated with developmental changes. There were increased neuronal defects when iron was restricted during the first week of gestation in comparison to iron restriction during the second or third week of gestation. The ID rats in comparison to the control rats showed a significant decrease in spinal cord tissue as well as cerebellum tissue in the fetal rats. The decrease was greatest in the cerebellum tissue. The cerebellum controls motor function as well as attention and language in humans. Therefore, the research suggests a lack of cerebellum development may correlate with impaired motor learning, and/or inability to focus, which may be related to distractibility.

Rodent models have linked early prenatal ID to multiple alterations in neurotransmission (Lozoff & Georgieff, 2006). ID in rats was induced in gestation for two groups, prenatal iron repletion therapy and postnatal iron repletion therapy (Lozoff, 2007). Prenatal iron repletion therapy in rats in the third week of gestation increased fetal iron levels. Neurological changes were seen in the areas of the basal ganglia and the hippocampus as well as in the process of myelination in both groups. Also, developmental test scores were decreased for both groups. Therefore, repletion therapy did not reverse the consequences of perinatal ID.

Rhesus monkey models consider perinatal iron with growth and development (Golub, Hogrefe, Germann, Capitano, & Lozoff, 2006a; Golub et al., 2006b). Rhesus monkeys who had diet induced IDA during gestation delivered infant monkeys with low Hgb at birth (Golub et al., 2006b). Iron deprivation decreased multiple iron measures, such as Hgb and ferritin. However, there was not a significant difference in growth between IDA infant monkeys and the control infant monkeys. Furthermore, another rhesus monkey study included three groups, prenatal iron-deprived rhesus monkey infants, postnatal iron-deprived rhesus monkey infants, and the control rhesus monkey infants (Golub et al., 2006a). The rhesus monkey infants' weights were not affected among the three groups at one, two, three, and four weeks old nor at two and four months old. Gross motor function and reach/fine motor function tests also showed no difference among the three groups. Cognition in infant monkeys was assessed with novelty preference as well as object permanence. Novelty preference was not significantly different amongst all three groups. Infant rhesus monkeys who were prenatally iron deprived did not have significant cognitive differences compared to the control group. Yet, infant rhesus monkeys who were postnatally iron deprived showed a significant difference in object permanence in relation to the control.

Another study with rhesus monkeys considered cerebral spinal fluid (CSF) (Gloub, 2010). CSF was used to measure neurotransmitters, such as dopamine, epinephrine, norepinephrine, and serotonin. CSF was obtained from three infant monkey groups including, maternal monkey IDA, infant monkey IDA, and the control. IDA groups were recovered from IDA at 12 months old. The previously maternal and infant IDA monkeys CSF exhibited decreased dopamine and increased norepinephrine in comparison to the control.

In a long-term study with the rhesus monkeys, consequences of ID in relation to cognition were considered (Gloub, 2010). Cognition was measured by three variables, discrimination reversal, delayed non-match to sample, and concurrent object discrimination.

When the ID and IDA induced rhesus monkeys were juveniles, they were less distractible and more object oriented in comparison to the control. Though there was a difference it was non-significant. Gloub (2010) suggested the timing and the duration of the ID are important factors for long-term consequences for cognitive functioning.

Animal studies also examine zinc in relation to cognitive development (Sandstead, Frederickson, & Penland, 2000). Zinc deprivation in rats was related to fetal brain malformations. Zinc is important for specific brain regions (Gower-Winter & Levenson, 2012). Similar to iron, zinc is important for neurodevelopment of the cerebellum. Again, the cerebellum is essential to motor function, attention, and language. Furthermore, zinc deprivation in rats during the third week of gestation was related to lower DNA in the brain as well as decreased brain growth (Sandstead et al., 2000). Spatial learning and memory in infant rats was decreased in prenatal zinc deficiency when compared to prenatal zinc supplementation and a prenatal control group (Boroujenji et al., 2009). Infant rats from the prenatal zinc supplementation group had impaired spatial learning and memory. Therefore, prenatal zinc is important for brain development in fetal rats.

Despite conflicting evidence for zinc and cognition, zinc is known to be essential for normal brain development (Adamo & Oteiza, 2010). Severe prenatal zinc deficiency was related to fetal brain malformations in rats. Marginal zinc status during gestation and early postnatal development was related to decreased neurotransmission and cell signaling in rats. The mechanisms are unknown, yet there are speculations to explain these results. It is suspected brain development may be related to the increase of oxidative stress as well as zinc's role on the immune system since zinc is a known antioxidant.

Animal restriction models contribute to iron and zinc research (Mihaila et al., 2011; Sandstead et al., 2000). Iron deprivation in rats during pregnancy was related to decreased spinal cord tissue and cerebellum tissue in comparison to the control (Mihaila et al., 2011). Rhesus monkeys who were prenatally IDA from iron deprivation were gave birth to infant monkeys who were also IDA (Golub et al., 2006). Yet, object permanence was decreased in prenatally iron deprived infant monkeys when compared to postnatally iron deprived infant monkeys and the control infant monkeys but was not significantly different in relation to novelty preference. Lastly, zinc deprivation in rats was related to brain malformations as well as decreased brain growth (Sandstead et al., 2000). In conclusion, iron and zinc deprivation in rats was related to CNS tissue development, and iron deprivation in perinatal rhesus monkeys was related to decreased infant monkey iron levels at birth and decreased developmental scores in infancy and later in childhood.

Summary

The current project considers the Fetal Origins Hypothesis, which theorizes maternal nutritional status during fetal development is related to the long-term health of the fetus (Barker, 2012). More importantly, iron and zinc were considered since they are nutritionally relevant during growth. In particular, maternal iron and zinc levels are important for fetal brain development. In addition, the fetal brain is believed to be only half developed at birth (Barker, 2001). The research in relation to brain development and the Fetal Origins Hypothesis contains considerably less information when compared to somatic development (Barker, 2012). Consequently, a focus on the Fetal Origins Hypothesis for maternal iron and zinc in relation to toddler cognition is unique and may further emphasize the importance of maternal nutrition for long-term development of the offspring.

Considering maternal iron and zinc levels in relation to toddler cognition rather than toddler iron and zinc is useful because toddlers' dietary intake of iron and zinc meets the EAR (Butte et al., 2010). Also, using toddler iron and zinc blood levels is a poor indicator of status since toddler levels change frequently because of growth, and standards for blood values in children are not consistent (Hermoso et al., 2011). Furthermore, the recommendation for dietary iron and zinc in toddlers is based on adult values that are scaled down (IOM, 2001). Iron levels are maintained for several months, and zinc levels are highly controlled by the body. The maternal blood values at three months postpartum reflect iron and zinc availability to the fetus. Therefore, using maternal iron and zinc levels rather than toddler iron and zinc levels may be a more useful indicator of long-term development and thus follows the model of the Fetal Origins Hypothesis.

Toddler cognitive development can be assessed in various ways (Bauer, 2002; Fenson et al., 2000; Fenson et al., 2007; Hoffman et al., 2012; Kannass & Colombo, 2011). There are both maternal questionnaires as well as toddler tasks to assess toddler cognition. Moreover, there are multiple areas within toddler cognition. For instance, vocabulary, distractibility, and imitation measure different cognitive domains. Measuring toddler cognition can be challenging since toddlers may not cooperate in the given tasks, and results can be affected by toddler age and gender. Therefore, maternal questionnaires may be more helpful than toddler tasks. Overall, measuring toddler cognitive development is tricky and flawed since there is no standard method for interpreting the assessment results. Yet, despite the difficulty there has been an association between nutrition and cognitive development (Columbo et al., 2004; Hubbs-Tait et al., 2009; Jensen et al., 2010; Nyaradi et al., 2013; Riggins et al., 2009).

Researching iron is important due to the increased need for iron during pregnancy as well as during infancy and toddlerhood (IOM, 2001). The need for iron increases as pregnancy progresses; therefore, the prevalence of ID and IDA increases as pregnancy progresses (Mei et al., 2011). Iron is important for neurodevelopment, in particular for hippocampal development and myelination (Lozoff et al., 2006). Additionally, iron is the most concentrated in an infant's brain at birth and continues to decrease until the diet changes and includes more iron (Osendarp, Murray-Klob, & Black, 2010).

Children and adolescents with ID or IDA exhibited decreased scores for cognitive function tests, neurocognitive tests, mental, motor, and social/emotional functioning tests (Grantham-McGregor & Ani, 2001; Lozoff et al., 2006). The literature for iron supplementation and cognition contains a gap in relation to toddlers, especially compared to children and adolescents. Iron supplementation in toddlers has shown both null results as well as a positive correlation to mental development (Aukett et al., 1986; Metallinos-Katsaras et al., 2004). This may be because of the difference in iron status prior to supplementation since benefits are more prominent in individuals with ID or IDA (Sachdev et al., 2004). For instance, if children or adolescents were ID or IDA before iron supplementation, cognitive benefits were increased compared to individuals who were non-ID or non-IDA receiving supplementation. Moreover, the appropriate dose and duration for supplementation is not well defined (Rosales et al., 2009). The research reviewed considered supplemented toddlers for two months (Aukett et al., 1986; Metallinos-Katsaras et al., 2004).

Zinc needs are also increased during growth and development (IOM, 2001). It is accepted that zinc is essential for normative brain development (Adamo & Oteiza, 2010). The difficulty in researching zinc is the lack of a sensitive marker for zinc status (Vale et al., 2014). However, serum maternal zinc levels decreased as pregnancy advanced (Tamura, et al., 2000), yet maternal zinc supplementation was not related to the mental development index in infants, behavior or grown in infants, or neurological development in five year olds (Hamadani et al., 2001; Hamadani et al., 2002; Goldenberg, et al., 2003). Therefore, the research regarding the relation of maternal zinc and cognition in children indicated null results (Hamadani et al., 2001; Hamadani et al., 2002; Tamura et al., 2000; Tsuenobu et al., 2003). However, zinc supplementation prenatally produced positive results not related to cognition, such as increased birthweight, increased growth, and decreased the prevalence of diarrhea at six months old.

However, these effects were dependent on when supplementation occurred in pregnancy, the dose of zinc, and when the outcomes were measured (Hamadani et al., 2001; Hamadani et al., 2002; Tsuenobu et al., 2003). Furthermore, research tended to be in developing nations. Individuals in developing nations may be deficient in other micronutrients outside of zinc. Therefore, the literature lacks a conclusion in relation to maternal zinc and child outcomes.

Animal studies indicate the importance of prenatal iron and zinc for brain development. In restriction models in rodents, rodents who were iron deprived gave birth to pups with poor brain development (Lozoff & Georgieff, 2006; Mihaila et al., 2011), and pups who were zinc deprived prenatally had similar outcomes of poor brain development (Boroujenji et al., 2009; Gower-Winter & Levenson, 2012; Sandstead et al., 2000). Rodent models provide interesting information in regards to iron and zinc and brain development. However, rodent brains are significantly different compared to human brains.

Research in rhesus monkeys is more related to human brain development due to the increased similarities in rhesus monkey brains and human brains (Gloub et al., 2006a). Despite the increased similarities between humans and rhesus monkeys compared to humans and rodents, rhesus monkeys lack the sophisticated communication and processing abilities of humans. When cognition was measured via object permanence, infant rhesus monkeys prenatally exposed to IDA and corrected for IDA at birth did not show significant differences in relation to the control (Golub et al., 2006a). Yet, infant rhesus monkeys postnatally exposed to IDA who were iron sufficient at birth did show statistically significant negative differences in relation to the control. The results indicated iron delivery to the fetus is highly controlled by the placenta independent of maternal iron status. In the infant rhesus monkeys born from IDA mothers and born with IDA, the measured cerebral spinal fluid (CSF) indicated decreased dopamine and increased norepinephrine in comparison to the control (Golub, 2010).

In conclusion, the current research project is based on the Fetal Origins Hypothesis with a focus on the relation between maternal iron and zinc and toddler cognition. Moreover, iron and zinc have been shown to be important in brain development as well as in cognitive performance in both humans and animals. Since toddlers meet dietary recommendations, differences in cognition may be related to prenatal maternal status rather than toddler status (Butte et al., 2010). This focus is important due to the gap in the literature for Fetal Origins Hypothesis in relation to cognitive development. Overall, a focus on Fetal Origins Hypothesis may further emphasize the importance of maternal nutrition (Barker, 2012).

CHAPTER III

METHODOLOGY

Participants

The participants included 36 mother/toddler pairs when the toddlers were 2.5 to 4 years old (mean = 3.16). The toddler participants were initially studied as mother/infant pairs from 2008 to 2010 to consider the relation between maternal micronutrient levels and infant cognitive development. There were 132 mother/infant pairs in the original sample. The infants were predominantly breastfed until three months old. Predominantly breastfed was defined as consuming less than 28 ounces of formula per week. Other inclusion criteria included full-term singleton birth infants. On the other hand, exclusion criteria included maternal blood transfusion during delivery, maternal illness, and infant illness during recruitment. Therefore, all toddlers were born healthy from healthy mothers. The mother/infant pairs participated in assessments when the infants were three, six, and nine months old. Both the original study and the current study were approved by the Oklahoma State University Review Board.

Between May 2012 and March 2013, 47 mother/toddler pairs returned to be reevaluated. The original sample of the 132 mother/infant pairs included ten toddlers who were older than four years old when the study began and 12 who were less than 2.5 years old when the funding expired. Of the remaining eligible mother/toddler pairs, 43% returned. The MacArthur Bates Communicative Development Inventory (CDI), a maternal measure for toddler vocabulary, was to be completed by mothers and returned through the mail. Out of the 47 mother/toddler participant pairs, only 36 mothers (77%) returned the MacArthur Bates CDI. Therefore, the final sample of mother/toddler pairs (n = 36) represented 27% of the original mother/infant pairs (n = 132). Incentives were given for both infant and toddler data collection sessions, equaling $120. The project was supported by National Research Initiative Grant 2008-35200-18779 from the USDA National Institute for Food and Agriculture and an OSU Provost's Multidisciplinary Grant.

Procedures

When the infants were three months old, the mothers completed a dietary history questionnaire (DHQ) that covered the last 12 months (NIH, 2007). Maternal iron and zinc status were determined at three months postpartum by hematological assessment. Furthermore, when the infants were three and nine months old, they participated in visits to examine Visual Information Processing (VIP), an assessment of infant cognitive development. At the follow-up visit when the participants were 2.5 to four years old, toddler cognition and language assessments were conducted. Mothers provided demographic information at this follow-up visit. Demographic characteristics considered included ethnicity, maternal education, and household income. Two of the participants preferred not to disclose information concerning their household income. After the follow-up visit, mothers filled out the MacArthur Bates CDI and returned it through the mail.

Maternal Measures

Maternal Diet

A diet history questionnaire (DHQ) was filled out by the mothers at the three-month postpartum visit (NIH, 2007). The food frequency questionnaire was completed online and assessed maternal diets for a year, from the start of pregnancy until three months postpartum. A total of 124 questions were asked in relation to food intake, portion sizes, and dietary supplements. The online DHQ measure was scored electronically using Diet*Calc Software (version 1.4.3, 2005, National Cancer Institute, Bethesda, MD). Validity of the DHQ was assessed by the Eating at America's Table Study (EATS) (Subar et al., 2001). Participants in the EATS validation study completed a DHQ in addition to a Block food frequency questionnaire (FFQ) or a Willett FFQ. Coefficients for correlations and attenuation with 24-hour recall results were increased in the DHQ compared to the Block FFQ and the Willett FFQ.

For the current project, maternal dietary iron intake was separated into dietary iron, supplemental iron, and total iron intake. Equally, maternal zinc intake was separated into dietary zinc, supplemental zinc, and total zinc intake. Total intake was the sum of both the dietary intake and the supplemental variables. Total maternal intake was compared to the Estimated Average Requirement (EAR). For iron, the EAR is 22 mg/day during pregnancy and 6.5 mg/day during lactation (IOM, 2001). For zinc, the EAR is 9.5 mg/day during pregnancy and 10.4 mg/day during lactation.

Maternal Biochemical Recommendations

Individuals with ID or IDA can be identified by blood values below the normal range (NLM, 2014). The recommended range for Hgb for adult women is 12.3 to 15.3 g/dL. Levels of sTfR less than 4.4 mg/L are indicative of adequate iron status, levels of sTfR 4.4 to eight mg/L are indicative of iron insufficiency, and levels of sTfR greater than eight mg/L are indicative of poor iron status and ID (Mei et al., 2011; Skinke, 2008). However, ranges for pregnancy and lactation are not well established (Mei et al., 2011). The recommended range for ferritin is 12 to 150 ng/mL (NLM, 2014). The recommended range for serum iron is 60 to 170 μg/dL.

Maternal Blood Sampling and Analysis

Maternal blood was drawn at the three-month postpartum visit. Two blood samples were taken at the University clinic using Sarstedt sterile safety-multifly needles and Sarstedt monovette 7.5 mL trace-mineral free syringes (Sarstedt Inc., Newton, NC) without ethylenediamine tetraacetic acid (EDTA) and analyzed by the Nutritional Sciences Department's laboratory at Oklahoma State University. Serum zinc (reference range 0.6 to 1.0 mg/L) and multiple measures of iron were assessed (IOM, 2001). Hemoglobin (Hgb) was analyzed from the whole blood as part of a complete blood count (CBC) at the local hospital's clinic. In the Nutritional Sciences Department's laboratory, the whole blood was centrifuged to separate the blood from the serum and transferred to mineral free vials and frozen. If the serum sample was hemolyzed it was unable to be analyzed because the RBC's intracellular minerals were mixed with serum iron and serum zinc. In the current study, one serum sample could not be included in the analysis, probably due to hemolysis. Serum iron and serum zinc were analyzed in batches via inductively coupled plasma mass spectrometry (ICP-MS). The ICP-MS was calibrated with human serum standards (Utak Laboratories Inc., Valencia, CA). For analysis each individual serum sample was diluted in a 0.1% high purity nitric acid. A serum sample of 200 µL was diluted to 5 mL. The internal standard for the ICP-MS was Gallium. After every ten samples a standard serum was analyzed to ensure consistency of the instrument. Lastly, ferritin and sTfR were measured from serum by specific enzyme-linked immunosorbent assay (ELISA) kits obtained from Ramco Laboratories, Inc. (Stafford, TX). All samples were run in duplicate to increase reliability.

Toddler Cognitive Measures

Infant Visual Information Processing

In the infant portion of the study, Visual Information Processing (VIP) was used to assess infant cognition at three and nine months old (Colaizzi, Grant, Thomas, Aubuchon-Endsley, & Kennedy, 2014). During the VIP test, the infants were in a low lighting room placed in front of a 22-inch computer screen in a car seat, and his/her mother sat behind him/her. At the start of the procedure, familiarization was conducted. The familiarization face was chosen randomly for each infant and was not used as a stimulus at any other age. During the familiarization, infants were shown the stimulus on the computer screen until the infant looked away for one second. The screen would be blank for two seconds, and then the same familiarization face was shown again. This procedure continued until the habituation criteria were met, namely, after the infants looked at the stimulus two consecutive times with a mean look time less than half the time of the two previous longest looks. The screen would again be blank for two seconds, and then the familiar face and a novel face were shown on the computer screen next to each other. Both faces would remain on the screen until the infant looked at either face or both faces for ten seconds at three months old or five seconds at nine months old. The screen would be blank for one second, and then the faces were shown again but in reversed positions.

The computer program was able to calculate the looking times in seconds and determine the visual habituation rate. During the procedure, an experimenter watched the infants on a television screen and coded the infants' looking times, both towards the stimulus and away from the stimulus. The procedure was also video recorded to ensure reliability. In this study the variable for the VIP considered was the longest look at the nine month old visit. In addition, the longest look change measured the decrease in longest look duration from three months old to nine months old. The underlying cognitive domain considered was processing speed, with shorter looking times indicating faster processing (Colaizzi et al., 2014).

Toddler Peabody Picture Vocabulary Test

Four different measures were used to assess toddler cognition. The first measure was the standard Peabody Picture Vocabulary Test (PPVT). This test measured the child's receptive vocabulary (Hoffman, Templin & Rice, 2012). For instance, if there were four pictures, a duck, a dog, a cat, and an elephant, the child would be asked to point at the duck. If the child pointed at the duck, it was scored as a correct response. If the child pointed at one of the other animals, it was scored as an incorrect response. There were multiple sections of the test with ten words in each section. With the progression of the sections, the words became increasingly more challenging. After the child missed the word, the next set of words was tried. If the child missed eight words out of the ten in one section, the test would cease. The raw score was converted into a standard score with the mean as 100 and a standard deviation (SD) of 15. The PPVT standard score is age-adjusted. Therefore, this standard score was used in the analysis.

MacArthur Bates Communicative Development Inventory

The McArthur Bates Communicative Development Inventory (CDI) asked the mother to indicate which words, phrases, and gestures her son/daughter were familiar with (Skarakis-Doyle, Campbell & Dempsey, 2009). The form used was for the current project was the MacArthur Bates CDI Vocabulary Checklist III (2007), which was revised from the 2000 version (Fenson et al., 20007). Research has shown the test to be valid and reliable in estimating expressive vocabulary in toddlers (Fenson, Pethick, Renda, & Cox, 2000; Fenson et al., 2007; Pan, Rowe, Spier, & Tamis-Lemonda, 2004).

The vocabulary checklist contained 100 words, and the mother specified only words that the child used (Fenson et al., 2007). Vocabulary usage was indicated by morphology and syntax. Morphology was determined by four questions indicating the use of plural, possessive, progressive, and past tense words. Language and syntax use were determined by 14 questions. Lastly, longest length of utterance was determined through word combinations. If such word combinations are indicated, mothers are then asked to provide three examples of complex utterances that the child has recently said.

The MacArthur Bates CDI Vocabulary Checklist III (2007) used is intended for 30 to 37 month olds and measures multiple aspects of vocabulary (Fenson et al., 2007). Therefore, the raw scores were used rather than the standardized scores, as our participants were aged 30 to 48 months old. The variables for this measure included vocabulary, sentences, usage, and mean length of utterance (MLU). Vocabulary indicated words known while usage indicated words used. Sentences indicated the sentence complexity while MLU indicated the length of the toddler's sentences. The MacArthur Bates CDI was distributed at the toddler visit and was to be returned in the mail. This increased the number of toddlers who were excluded due to missing data because 11 out of 47 forms (23%) were not returned.

Toddler Imitation Task and Toddler Distractibility Activity

Next, the third test and fourth toddler cognitive tests were administered in sequence. The third test was an Imitation task, which was adapted for this project from Bauer (2002). In part one of the Imitation task, the child was given a toy and shown a nine-step procedure involving a toy truck. A researcher demonstrated the procedure twice. The child was then given a different toy to be used for the Distractibility activity.

Lastly, the fourth test, Distractibility, was measured within the Imitation task. The Distractibility activity was adapted for this project from Kannass and Oakes, (2008) and Kannass, Oakes, and Shaddy (2006). While the child played with a different toy that was received after part one of the Imitation task, a video clip was played with sound at an appropriate audio level. The video would continue to play for approximately seven seconds and then was turned off. The researcher observed the child's focus in relation to the toy or the distraction in relation to the video. It was determined how long the toy was focused on, how long resistance to the video occurs, and how long the video was watched. The child was asked if he/she would like to play with the same toy or if he/she would like a new toy. The procedure was repeated three times. Throughout this measure, the child was videotaped to determine the length of time in seconds the child played compared to the length of time the child was distracted. The variables assessed for the Distractibility activity were the latency to the first look, the total number of looks, and the total looking time. The latency to the first look measured how long in seconds it took for the child to be distracted by the video. The total number of looks indicated how many times the child looked at the video. Lastly, the total looking time was a sum of all of the seconds the child spent looking at the video.

After 15 minutes, the approximate time it takes to complete the Distractibility activity, the second part of the Imitation task was administered. The child was asked to demonstrate the nine steps shown prior to the Distractibility activity. Imitation score included two measures; the first was the number of steps the toddler repeated the steps and the second was if the toddler repeated the steps in the correct sequence (Bauer, Wenner, Dropik, & Wewerka, 2000). The Imitation task score was a sum of the total number of the nine individual steps completed and the total number of the eight pairs of steps, such as 1-2, 2-3, and etc., completed in the correct order. Therefore, a maximum of seventeen points was possible from the sum of the nine individual steps and the eight pairs of steps. Elicited imitation can be defined as memory in relation to specific facts, which is what was being measured during this test. The Imitation task score is reported in the results as *imitation*.

Statistical Analysis

Descriptive statistics were used to determine the mean and the standard deviation (SD) for continuous variables, such as serum zinc, serum iron, ferritin, Hgb, sTfR, infant VIP variables, the Peabody Picture Vocabulary Test (PPVT) Standard Score, Imitation, Distractibility for first look, total number of looks, and total looking time, the MacArthur Bates Communicative Development Inventory (CDI) Raw Scores for vocabulary, sentences, and usage, and the mean for the longest utterance (MLU), and toddler age. Next, descriptive statistics analyses were performed as frequency distributions for categorical variables, including maternal employment, maternal marital status, maternal education, family income, toddler race, and toddler gender.

Since the current study was a follow up study and did not contain all of the participants from the previous study, an independent sample t-test was performed to determine the relation of maternal blood values between the participants in the previous study who did not return and the participants in the current study. Additionally, an independent sample t-test was conducted to determine the relation of infant VIP values between the participants in the previous study who did not return and the participants in the current study. The independent t-test was performed due to the loss of participants from the original sample to the current sample. Non-significant results indicate the two groups are not different from each other on these measures.

The primary hypothesis considered the relation between multiple measures of toddler cognition and maternal perinatal iron and zinc levels. All relations were measured by partial correlations except for the PPVT as the standardized score already controls for age and gender. In this case, a bivariate correlation analysis was conducted. Within the partial correlation, toddler age and gender were controlled for due to the change in cognitive development within 2.5 to four year olds and the difference in developmental rate between genders. Iron measures included serum iron, ferritin, soluble Transferrin Receptor (sTfR), and hemoglobin (Hgb). Also, zinc status was measured as serum zinc. Toddler cognition was measured by four methods, which included the PPVT, Imitation task, Distractibility activity, and the MacArthur Bates CDI. The PPVT used the standardized score. Distractibility was assessed by multiple variables, which included first look, longest look, and total looking time. The MacArthur Bates CDI also provided multiple variables, which included, vocabulary, sentences, usage, and mean length of longest utterance (MLU).

The secondary hypothesis, considered the relation between the longest look duration at nine months old and the longest look change from three months old to nine months old and multiple measures of toddler cognition. The relations were measured by partial correlations. The VIP was log transformed for the longest look because the results were skewed. The log transformation made the VIP numbers have a more normal distribution. The natural log was multiplied by the raw value to equal the log-transformed value. In addition, the relation between the VIP change in time from three months old to nine months old and multiple measures of toddler cognition was also analyzed. The toddler cognitive development variables used are consistent with the previous hypothesis. Again, toddler age and gender were used as a control with the exception of PPVT as noted previously.

The third and final hypothesis considered the relation between maternal dietary iron and zinc intake during gestation and the first three months of lactation and multiple measures of toddler cognition. The relations were measured using partial correlations. Toddler cognitive development was measured as with the previous hypotheses. Again, toddler age and gender were used as control variables with the exception of PPVT. Dietary maternal iron and zinc intake was determined through the DHQ (NIH, 2007). Maternal dietary iron intake was separated into dietary iron, dietary iron supplement, and total dietary iron intake. Similarly, maternal dietary zinc intake was separated into dietary zinc, dietary zinc supplement, and total dietary zinc intake. Maternal dietary intake indicated the amount from food while the supplement indicated the amount from prenatal vitamins or an additional supplement. Total dietary intake was the sum of the intake from food and from the supplement.

Data were analyzed using IBM® SPSS® Statistics version 20.0.0. Significance for the project was set at *p* less than 0.05.

CHAPTER IV

FINDINGS

After the descriptive data are discussed, the information regarding the three different research questions is presented. The first research question considered the correlation between maternal iron and zinc biochemical levels and multiple measures of toddler cognition. The second research question considered the correlation between infant Visual Information Processing (VIP) variables and multiple toddler cognition measures. Lastly, the third research question considered the correlation between maternal dietary iron and zinc intake and multiple toddler cognition measures.

Demographics

Table 1 summarizes maternal demographic results. When the toddlers were assessed, mothers were a mean of 32.0 years old with a standard deviation (SD) of 4.2 years. The majority of the mothers were employed, and all of the mothers were married. The median for family income was between \$51,000 to \$60,000

Table 1. Maternal Demographics Results

Maternal Employment (n = 36)		
Unemployed	7	(19%)
Employed Part Time	15	(42%)
Employed Full Time	14	(39%)
Marital Status (n = 36)		
Married First Time	32	(88.9%)
Remarried	4	(11.1%)
Maternal Education (n = 36)		
Less than high school diploma	1	(2.78%)
High school graduate	1	(2.78%)
Some college	5	(13.89)
College graduate	16	(44.44%)
Post graduate or above	13	(36.11%)
Family Income (n = 34)*		
Under $10,000	1	(2.94%)
$11,000 to 20,000	1	(2.94%)
$21,000 to 30,000	2	(5.88%)
$31,000 to 40,000	5	(14.71%)
$41,000 to 50,000	6	(17.65%)
$51,000 to 60,000	3	(8.82%)
$61,000 to 70,000	2	(5.88%)
$71,000 to 80,000	3	(8.82%)
$81,000 to 90,000	2	(5.88%)
$91,000 to 100,000	6	(17.65%)
Over $100,000	3	(8.82%)
* Two participants chose to not disclose information for family income		

Table 2 presents the toddler demographics. The toddlers were between 2.5 to four years old with a mean of 3.16 years old and a SD of 0.5 years. The overwhelming majority of the sample was white and was disproportionately female.

Table 2. Toddler Demographic Results

Toddler Gender (n = 36)		
Female	22	(61.1%)
Male	14	(38.9%)
Toddler Race (n = 36)		
Native American	3	(8.3%)
Hispanic	1	(2.8%)
Asian	1	(2.8%)
White	31	(86.1%)

Maternal Iron and Zinc Status

An independent samples t-test was conducted for maternal blood values between participants in the previous study who did not return (n = 82) and participants in the current study (n = 36). These results indicate the participants in the original study and the participants in the current study do not differ for maternal iron and zinc levels.

Table 3. Maternal Iron and Zinc Independent Sample t-test

	Mean ± SD Previous Participants n = 82	Mean ± SD Current Participants n = 36	Significance
Serum Fe	0.93 ± 0.28	0.90 ± 0.31	*p* = 0.916
Ferritin	31.73 ± 22.94	37.79 ± 30.34	*p* = 0.176
sTfR	5.93 ± 2.26	5.29 ± 2.64	*p* = 0.283
Hgb	13.71 ± 0.80	13.71 ± 0.80	*p* = 0.814
Serum Zn	0.84 ± 0.15	0.85 ± 0.17	*p* = 0.621

Table 4 summarizes maternal iron (Fe) and zinc (Zn) status when measured at the three-month postpartum visit. Due to a probable hemolyzed sample, serum iron and serum zinc were unable to be properly analyzed for one sample. The means from maternal serum iron, ferritin, Hgb, and serum zinc levels were within the recommended ranges. The mean for soluble transferrin receptors (sTfR) was not within the recommended range (Mei et al., 2011; Skinke, 2008). Levels less than 4.4 mg/dL indicate adequate iron status while levels between 4.4 to 8.0 mg/dL indicate iron insufficiency, and levels greater than 8.0 mg/dL indicate poor iron status. Maternal blood values for soluble transferrin receptor (sTfR) indicated only 41.7 % (n = 15) of mothers had an adequate sTfR level (Mei et al., 2011; Skinke, 2008). Moreover, 47.2 % (n = 17) of mothers had inadequate sTfR levels, and 11.1 % (n = 4) of mothers had poor sTfR levels (Mei et al., 2011; Skinke, 2008). Hgb levels for women are recommended to be between 12.3 and 15.3 g/dL (NLM, 2014). All of the mothers had a hemoglobin (Hgb) level above 12 g/dL, but 11.1% (n = 4) had an Hgb level less than the recommended 12.3 g/dL (NLM, 2014).

Table 4. Maternal Iron and Zinc Status at Three Months Postpartum

	n	Mean	SD	Reference Value
Serum Fe (mg/L)	35*	0.90	0.31	0.6 to 1.7 **
Ferritin (μg/L)	36	37.79	30.34	12 to 150 **
sTfR (mg/L)	36	5.29	2.64	< 4.4 ***
Hgb (g/dL)	36	13.71	0.80	12.3 to 15.3 **
Serum Zn (mg/L)	35*	0.85	0.17	0.6 to 1.0 ****

* One sample could not be analyzed because of possible contamination by intracellular minerals from hemolyzed red blood cells
** NLM, 2014
*** Mei et al., 2011
**** IOM, 2001

Toddler Cognitive Measures

Table 5 summarizes the toddler cognition measures.

Table 5. Toddler Cognitive Results when measured from 2.5 to four years old

	n	Mean	SD
PPVT Standard Scores *	36	111	13.3
Imitation **	34	11	3.5
Distractibility**			
1ST Look (seconds)	34	17.9	21.26
Number of Looks	34	18	15.5
Total Looking Time (seconds)	34	68.2	60.48
MacArthur Bates CDI Raw Scores ***			
Vocabulary	36	67	20.8
Sentences	36	9	3.0
Usage	36	10	2.0
MLU	36	9	4.8

* Mean and SD are from standardized scores with mean of 100 and a SD of 15
** 34 for Imitation and Distractibility because two toddlers did not cooperate for the activity
*** Mean and SD are from the raw scores

Hypothesis I – Relation between Maternal Iron and Zinc Levels and Toddler Cognition

The next three tables present information for the partial correlations testing the first hypothesis. Partial correlations were conducted between maternal iron and zinc levels and toddler cognition. Toddler age and gender were controlled. Results presented in Table 6 indicated the PPVT standard scores and the Imitation task did not have statistically significant relations to maternal iron and zinc levels.

Table 6. Results of correlations between Maternal Iron and Zinc Levels and PPVT Standard Score and Imitation when controlled for toddler age and gender (n = 33)

	PPVT*	Imitation**
Serum Fe	*r* = -0.137 *p* = 0.434	*r* = 0.125 *p* = 0.511
Ferritin	*r* = -0.062 *p* = 0.718	*r* = 0.025 *p* = 0.894
sTfR	*r* = 0.001 *p* = 0.995	*r* = -0.194 *p* = 0.306
Hgb	*r* = 0.102 *p* = 0.553	*r* = 0.093 *p* = 0.626
Serum Zn	*r* = -0.122 *p* = 0.485	*r* = -0.129 *p* = 0.497
* Bivariate correlations were conducted as the standardized score already accounts for age and gender ** Toddler age and gender were controlled using partial correlations		

Table 7 presents a positive significant correlation (*p* = 0.042) between maternal sTfR when toddler Distractibility was measured as total looking time. The other two Distractibility measures, first look and number of looks, did not indicate a significant relation to maternal iron and zinc levels.

Table 7. Results of correlations between Maternal Iron and Zinc Levels and Toddler Distractibility when controlled for toddler age and gender (n = 33)

	1st Look	Number of Looks	Total Looking Time
Serum Fe	*r* = -0.054 *p* = 0.778	*r* = -0.177 *p* = 0.351	*r* = -0.205 *p* = 0.277
Ferritin	*r* = -0.101 *p* = 0.595	*r* = -0.106 *p* = 0.578	*r* = -0.196 *p* = 0.298
sTfR	*r* = 0.048 *p* = 0.799	*r* = 0.223 *p* = 0.237	***r* = 0.373** ***p* = 0.042**
Hgb	*r* = -0.249 *p* = 0.184	*r* = -0.127 *p* = 0.505	*r* = 0.018 *p* = 0.925
Serum Zn	*r* = -0.187 *p* = 0.323	*r* = -0.150 *p* = 0.428	*r* = -0.157 *p* = 0.407

Table 8 presents positive significant correlations between maternal Hgb and the MacArthur Bates CDI for toddler vocabulary (*p* = 0.036) and toddler mean length of utterance (MLU) (*p* = 0.014). However, there was not a significant relation between maternal Hgb and toddler sentences or toddler usage. Other measures of maternal iron levels as well as serum zinc were also not significantly related to toddler vocabulary.

Table 8. Results of correlations between Maternal Iron and Zinc Levels and MacArthur Bates CDI Raw Scores when controlled for toddler age and gender (n = 33)

	Vocabulary	Sentences	Usage	MLU
Serum Fe	*r* = -0.154 *p* = 0.418	*r* = -0.025 *p* = 0.894	*r* = 0.138 *p* = 0.467	*r* = 0.033 *p* = 0.862
Ferritin	*r* = 0.149 *p* = 0.432	*r* = -0.257 *p* = 0.170	*r* = -0.224 *p* = 0.234	*r* = -0.099 *p* = 0.602
sTfR	*r* = 0.149 *p* = 0.431	*r* = -0.048 *p* = 0.800	*r* = 0.146 *p* = 0.441	*r* = 0.181 *p* = 0.339
Hgb	***r* = 0.385** ***p* = 0.036**	*r* = 0.150 *p* = 0.428	*r* = 0.228 *p* = 0.225	***r* = 0.445** ***p* = 0.014**
Serum Zn	*r* = 0.079 *p* = 0.680	*r* = -0.312 *p* = 0.093	*r* = -0.207 *p* = 0.272	*r* = 0.026 *p* = 0.892

Hypothesis II – Relation Between Infant Visual Information Processing (VIP) and Toddler Cognition

Similar to maternal iron and zinc levels, an independent t-test was conducted for infant Visual Information Processing (VIP) between participants in the previous study who did not return and participants in the current study. These results indicate participants from the original study do not differ from participants in the current study for infant VIP.

Table 9. Infant VIP Independent Sample t-test

	Mean ± SD Previous Participants n = 71	Mean ± SD Current Participants n = 36	Significance
Longest Look 9 months	10.1 ± 4.6	12.0 ± 7.6	*p* = 0.188
Longest Look Change	-49.1 ± 81.4	-78.5 ± 185.5	*p* = 0.376

Table 10 presents descriptive information on the VIP longest look at nine months old and the longest look change from three months old to nine months old. The negative value of the longest look change indicates infants at nine months old took less time to focus and recall the stimulus than infants at three months old.

Table 10. VIP Longest Look at nine months old and VIP Longest Look Change from three months old to nine months old

	n	Mean	SD
Longest Look 3 months (seconds)*	35**	90.1	185.0
Longest Look 9 months (seconds)*	36	10.1	4.60
Longest Look Change (seconds)	35**	-78.5	185.53
* Longest Look used is the log transformed value ** Missing one of the three month old longest look			

The following tables (Tables 11 to 13) present information regarding partial correlations with infant VIP and toddler cognition when controlled for toddler age and gender. Infant VIP was measured at nine months old, and toddler cognition was measured at 2.5 to four years old. Infant VIP did not significantly correlate to toddler cognition measures.

Table 11. Results of correlations between Infant VIP and Toddler PPVT Standard Score and Imitation when controlled for toddler age and gender (n = 33)

	Longest Look*	Longest Look Change
PPVT Standard Score**	r = - 0.128 p = 0.458	r = 0.113 p = 0.445
Imitation***	r = -0.041 p = 0.828	r = 0.035 p = 0.856
*Longest Look used is the log transformed value **Bivariate correlations were conducted as the standardized score already accounts for age and gender ***Toddler age and gender were controlled using partial correlations		

Table 12. Results of correlations between Infant VIP and Toddler Distractibility when controlled for toddler age and gender (n = 33)

	Longest Look*	Longest Look Change
1st Look	r = -0.110 p = 0.561	r = 0.155 p = 0.413
Number of Looks	r = 0.203 p = 0.283	r = 0.142 p = 0.453
Total Looking Time	r = 0.053 p = 0.782	r = 0.200 p = 0.290
*Longest Look used is the log transformed value		

Table 13. Results of correlations between Infant VIP and MacArthur Bates CDI Raw Scores when controlled for toddler age and gender (n = 33)

	Longest Look*	Longest Look Change
Vocabulary	r = 0.179 p = 0.345	r = -0.022 p = 0.909
Sentences	r = 0.005 p = 0.981	r = 0.162 p – 0.391
Usage	r = 0.165 p = 0.383	r = 0.008 p = 0.966
Mean Length of Utterance	r = 0.162 p = 0.394	r = -0.004 p = 0.983
*Longest Look used is the log transformed value		

Hypothesis III – Relations Between Maternal Diet and Toddler Cognition

Table 14 describes the maternal intake of iron and zinc as assessed by the Dietary History Questionnaire (DHQ). The DHQ, completed by the women at the three months postpartum visit, measured dietary intake for one year, which included the duration of pregnancy until three months postpartum. Mean total iron and mean total zinc intake estimates both exceeded the Estimated Average Requirement (EAR) for iron and zinc during pregnancy. For total iron intake, 48.5% (n = 16) of mothers met the EAR for pregnancy and 97.0% (n = 32) of mothers met the EAR for lactation. (IOM, 2001) Moreover, 42.4% (n = 14) of mothers met the Recommended Dietary Allowance (RDA) for iron for pregnancy and 97.0% (n = 32) of mothers met the RDA for lactation (NIH, 2014a). For total zinc intake, only 6.1% (n = 2) of mothers did not meet the EAR for pregnancy and for lactation (IOM, 2001) or the RDA for pregnancy and for lactation

(NIH, 2014b). Results for partial correlations between maternal iron and zinc levels and maternal iron and zinc intake are presented in Table 18 and Table 19 in the Appendix on page 85.

Table 14. Maternal Dietary Iron and Zinc at Three Months Postpartum

	n	Mean	SD	Pregnancy EAR	Lactation EAR
Fe (mg/d)	33*	16.6	6.78	22	6.5
Fe – Supp (mg/d)	33*	9.18	8.57	22	6.5
Fe – Total (mg/d)	33*	25.8	11.42	22	6.5
Zn (mg/d)	33*	12.3	3.89	9.5	10.4
Zn – Supp (mg/d)	33*	6.6	4.79	9.5	10.4
Zn – Total (mg/d)	33*	18.9	6.31	9.5	10.4
*Three mothers did not complete the Dietary History Questionnaire (DHQ)					

The following tables (Tables 15 to 17) include correlations for maternal dietary intake and toddler cognition. The results indicated maternal dietary iron and zinc intake does not have a significant relation to toddler cognition.

Table 15. Results of correlations between Maternal Dietary Iron and Zinc and Toddler PPVT Standard Score and Imitation when controlled for toddler age and gender (n = 31)

	Iron	Iron – Total	Zinc	Zinc – Total
PPVT*	*r* = -0.078 *p* = 0.882	*r* = -0.245 *p* = 0.170	*r* = -0.027 *p* = 0.882	*r* = -0.087 *p* = 0.631
Imitation**	*r* = 0.233 *p* = 0.233	*r* = 0.092 *p* = 0.641	*r* = -0.022 *p* = -0.910	*r* = -0.050 *p* = 0.801
* Bivariate correlations were conducted as the standardized score already accounts for age and gender ** Toddler age and gender were controlled using partial correlations				

Table 16. Results of correlations between Maternal Dietary Iron and Zinc and Toddler Distractibility when controlled for toddler age and gender (n = 31)

	Iron	Iron – Total	Zinc	Zinc – Total
1st Look	*r* = 0.103 *p* = 0.603	*r* = -0.077 *p* = 0.696	*r* = 0.168 *p* = 0.392	*r* = -0.072 *p* = 0.718
Number of Looks	*r* = -0.201 *p* = 0.304	*r* = 0.000 *p* = 0.999	*r* = -0.279 *p* = 0.151	*r* = -0.080 *p* = 0.684
Total Looking Time	*r* = -0.090 *p* = 0.649	*r* = 0.159 *p* = 0.420	*r* = -0.154 *p* = 0.434	*r* = 0.082 *p* = 0.678

Table 17. Results of correlations between Maternal Dietary Iron and Zinc and MacArthur Bates CDI when controlled for toddler age and gender (n = 33)

	Iron	Iron – Total	Zinc	Zinc – Total
Vocabulary	$r = 0.050$ $p = 0.799$	$r = 0.113$ $p = 0.568$	$r = 0.184$ $p = 0.348$	$r = 0.277$ $p = 0.154$
Sentences	$r = 0.152$ $p = 0.440$	$r = -0.102$ $p = 0.606$	$r = 0.279$ $p = 0.150$	$r = 0.066$ $p = 0.741$
Usage	$r = 0.191$ $p = 0.331$	$r = 0.121$ $p = 0.540$	$r = 0.277$ $p = 0.154$	$r = 0.215$ $p = 0.272$
MLU	$r = 0.245$ $p = 0.209$	$r = 0.101$ $p = 0.608$	$r = 0.313$ $p = 0.105$	$r = 0.135$ $p = 0.493$

CHAPTER V

DISCUSSION

Overview

The focus of maternal nutritional health in relation to long-term offspring development is based on the Fetal Origins Hypothesis (Barker, 2012). The first aim of the current project was to analyze the relation between maternal iron and zinc levels and toddler cognition. The second aim was to analyze the relation between infant Visual Information Processing (VIP) and toddler cognition. Lastly, the third aim was to analyze the relation between maternal dietary iron and zinc and toddler cognition. Assessing maternal iron and zinc levels as well as maternal dietary iron and zinc with toddler cognition are based on the Fetal Origins Hypothesis.

The current sample was composed of 36 mother/toddler pairs as part of a follow up study, and the original study included 132 mother/infant pairs. All of the infants were born apparently healthy from apparently healthy mothers and were predominantly breastfed until at least three months old. Both the original study and the current study considered the relation between maternal nutritional status and offspring outcomes, such as anthropometry, diet, and cognition.

The majority of participants included in the current study were from families who were white, had mothers who were well educated and married, and had a household annual income of greater than $50,000. Due to the location of the study, the Native American participation (n = 3, 8.3%) was greater than the general US population of 0.9% in 2010 (United States Census Bureau, 2011).

The mean of the mothers' total iron intake and total zinc intake met the Estimated Average Requirement (EAR) for both pregnancy and lactation (IOM, 2001). The mean maternal dietary iron intake did not meet the EAR for pregnancy, but it met the EAR for lactation. Dietary intake without supplementation below the EAR for pregnancy was indicated by increased soluble transferrin receptor (sTfR) levels (Skinke, 2008). On the other hand, mean maternal dietary zinc intake was above the EAR for both pregnancy and lactation. The results concerning the hypothesis testing are outlined below.

Research Questions

I. Toddler cognition will be positively correlated with maternal iron and zinc status as assessed when the children were three months of age.

A positive statistically significant relation was indicated for maternal blood levels and two different toddler cognition measures. A positive relation was indicated between maternal sTfR and Distractibility Total Looking Time (*p*=0.042). In addition, a positive relation was found between maternal Hgb and MacArthur Bates Vocabulary (*p*=0.036) and MacArthur Bates MLU (*p*=0.014). Other maternal iron and zinc measures including serum iron, ferritin, and serum zinc did not have significant relations with the toddler cognitive test results (Tables 5 to 7). Therefore, due to the three significant relations found, the results partially support the hypothesis.

II. Toddlers who showed shorter looking times during the Visual Information Processing test at nine months old, which is indicative of faster processing speed, will have better performance on cognitive tests when measured at 2.5 to four years old.

Infant VIP longest look at nine months old and longest look change from three months old to nine months old did not have a significant relation to multiple measures of toddler cognition (Tables 9 to 11). Therefore, the results failed to reject the null hypothesis.

III. Toddlers' increased cognition will be related to higher levels of maternal iron and zinc dietary intake during pregnancy and three months postpartum.

Maternal dietary iron and zinc did not have a positive significant relation to multiple measures of toddler cognition (Tables 13 to 15). Maternal dietary iron was measured as dietary iron, supplemental iron, and the sum of dietary and supplemental iron as total iron. Equally, maternal dietary zinc was measured as dietary zinc, supplemental zinc, and total zinc. None of the three variables, dietary, supplemental, and total, indicated any significant results in relation to toddler cognition. Therefore, the results failed to reject the null hypothesis.

Relation of Findings to the Literature

The results will be discussed according to each hypothesis. The three significant results were all found within the context of the first hypothesis. These results share similarities with previous research both for human studies as well as for animal studies, and these similarities as well as the differences will be discussed. The tests of the second hypothesis did not find a significant relation between infant VIP and toddler cognition despite a previous finding that infant VIP predicts long-term cognition (Rose et al. 2003). Therefore, the results in relation to previous research will be considered. Similarly, the tests of the third null hypothesis did not find any significant relations. However, the results indicated interesting conclusions considering maternal dietary iron and zinc intake. Overall, the first hypothesis was supported, but the researchers for the current project failed to reject the null hypothesis for the second and third hypotheses.

Hypothesis I – Maternal Iron and Zinc Levels and Toddler Cognition

Three significant correlations were identified between maternal three-month postpartum iron levels and multiple measures of toddler cognition. The life span of red blood cells (RBC) is approximately 120 days or about four months (IOM, 20001). Therefore, assessing three-month maternal postpartum biochemical levels extends beyond the average life of RBCs. Consequently, three-month maternal postpartum levels indicate what was available to the fetus prenatally for fetal development. This supports the Fetal Origins Hypothesis since maternal nutritional status was related to long-term offspring development (Barker, 2012). Maternal sTfR levels were positively related to toddler Distractibility total looking time, which indicated increased sTfR was positively related to more distractible toddlers. The MacArthur Bates CDI was related to maternal Hgb for Vocabulary and MLU, which indicated increased Hgb was positively related to better vocabulary and sentence length in the toddlers. Maternal Hgb levels were generally adequate (NLM, 2014). The remaining maternal iron measures, serum iron and ferritin, as well as serum zinc were not significantly related to toddler cognition.

Maternal sTfR and Toddler Distractibility

Less than half of mothers in the current study had sTfR within the recommended ranges at three months postpartum (Mei et al, 2001). Increased sTfR levels are indicative of low iron in the tissues since receptors increase as iron levels decrease. Perinatal sTfR impacted toddler Distractibility, yet it did not have a significant relation to other measures of toddler cognitive development. This may be because maternal iron in the tissue impacts this specific domain of cognitive development. Moreover, the timing in pregnancy of inadequate or poor sTfR levels may have also impacted this specific domain of cognitive development. Therefore, the results suggest maternal perinatal iron in the tissues may be more important for toddler focus than for other domains of cognitive development. Toddlers with inadequate or poor maternal sTfR levels suggest a disruption in toddler attention. Maternal sTfR levels may be related to toddler brain development. Iron is important for brain development, and the cerebellum helps control attention (Gower-Winter & Levenson, 2012). Mihaila (2011) restricted iron in rodents during pregnancy. The rodent pups who were iron restricted prenatally and during the first week of pregnancy showed decreased cerebellum tissue. Moreover, the prenatally iron restricted rodent pups also had decreased neural development. Therefore, inadequate or poor sTfR levels may be related to cerebellum development. Likewise, decreased cerebellum development may explain the disruption in toddler attention.

sTfR is not commonly used in the research, and recent PubMed searches using the key words sTfR and children did not include articles for toddler or child outcomes for cognition. However, previous research has been conducted between iron levels and attention deficit hyperactivity disorder (ADHD) in children (Konofal et al., 2008). Konofal et al. (2008) examined children five to eight years old with ADHD and low ferritin levels (<30 ng/mL) but normal Hgb levels. The research included two groups; children were supplemented (80 mg/day ferrous sulfate) or children were given a placebo. Children in the supplementation group showed improved scores for hyperactivity/impulsivity and inattentiveness compared to the placebo group. Overall, the positive correlation for Distractibility total looking time and maternal sTfR levels may indicate a concern for attention disturbances, such as attention deficit disorder (ADD) or ADHD, as the participants continue to grow and develop.

Maternal Hgb and Toddler Vocabulary

Higher levels of maternal Hgb in the current study were associated with better vocabulary and increased sentence length in toddlers despite the generally adequate maternal perinatal Hgb levels. The majority of mothers (n = 32, 88.9%) met recommendations for Hgb levels (NLM, 2014). Language for the MacArthur Bates CDI was measured as expressive language (Fenson et al., 2000). The positive significant difference in the relation to MacArthur Bates Vocabulary and

MLU and maternal Hgb may also be related to cerebellum development, similar to Distractibility and maternal sTfR, since the cerebellum also helps to controls language (Mihaila et al., 2011).

The current project shares similarities with the previous research (Black, 2012; Lozoff, 2006; Radlowski & Johnson, 2013). As reviewed by Black (2012) and Lozoff (2006) the literature examining the relation between perinatal iron and cognition is lacking unless the mother was ID or IDA. Perinatal ID exposure was related to an increased risk of low-test scores for educational achievement in children (Radlowski & Johnson, 2013). The findings in the current study indicate the higher the Hgb levels, the better the outcomes for vocabulary and sentence length in toddlers. Therefore, results in the current study suggest maternal Hgb may be important not only in ID and IDA, but also in healthy individuals.

On the other hand, vocabulary was also measured by the PPVT. The PPVT Standard Score and maternal iron levels did not indicate a positive significant relation, but this may be due to the above average scores of the toddlers or the generally adequate blood levels for most of the maternal iron measures. Another explanation for may be because the PPVT assessed toddler receptive language (Hoffman et al., 2012) while the MacArthur Bates CDI assessed toddler expressive language (Fenson et al., 2007). Therefore, the finding of a significant correlation between the significance between maternal iron and the MacArthur Bates CDI for vocabulary and MLU but the lack of a similar correlation between maternal iron and the toddler PPVT may be due to the differences between the two vocabulary measures.

The current study shares similarities to research conducted in rural China (Chang et al., 2013) since both used perinatal maternal Hgb levels in relation to toddler cognition. Yet, the China project used prenatal Hgb while the current project used postpartum Hgb. The participants were different in terms of maternal health, toddler age, ethnicity, and socioeconomic status (SES). Chang et al. (2013) found significant results between maternal IDA Hgb levels and cognitive development at 12, 18, and 24 months old. However, Chang et al. (2013) measured cognitive development with the Bayley Scales of Infant Development (BSID), and the current study measured cognitive development with more specific measures of language. Both projects suggest maternal Hgb levels are associated with the cognitive development of toddlers.

Maternal Iron Levels and Toddler Imitation

Significant relations were not seen between maternal iron levels and the toddler Imitation task. The Imitation task measured learning and working memory, also known as short-term memory or remembering information related to specific facts (Frethman, et al., 2011). Although the current study did not show a significant relation between toddler Imitation and maternal iron levels, the literature included showed different results (Fretham et al, 2011; Osendarp et al., 2010). Osendarp (2010) concluded that decreased dopamine impacted working memory, and iron plays a role for dopamine synthesis. Children who were ID had decreased scores for imitation (Fretham et al., 2011). Iron deficiency (ID) in humans from late gestation to approximately two to three years old was related to decreased hippocampal development. Therefore, maternal and child ID impacted memory in children (Fretham et al, 2011; Osendarp et al., 2010), yet generally sufficient iron levels in the current study did not impact toddler working memory. Consequently, working memory may be protected by iron levels in the normal range and may not be impacted by iron unless iron is inadequate or ID.

Maternal Zinc Levels and Toddler Cognition

Perinatal maternal zinc levels in the current study did not show significant correlations to any of the multiple measures of toddler cognition. Zinc sufficiency in the current project may explain the lack of significant relations. This may be because serum zinc is not an ideal indicator for zinc status since serum zinc is highly regulated by the body, and zinc is primarily located in skeletal muscle and bone (IOM, 2001). Therefore, it is not surprising that zinc levels were sufficient since they are controlled by the body. Assessment of other pools of zinc besides serum zinc, such as hair or nail zinc, or urinary zinc, may have resulted in different findings since they may be more sensitive to changes in zinc status (Vale et al., 2014).

Zinc research lacks a consensus in relation to brain development (Adamo & Oteiza, 2010; Bhatnagar & Taneja, 2001; Grower-Winter & Levenson, 2012; Shah & Sachdev, 2006). According to Grower-Winter and Levenson (2012) zinc, like iron, is important for cerebellum development. Adamo and Oteiza's (2010) showed that prenatal zinc deficiency was related to fetal brain malformations, decreased neurotransmission, and decreased cell signaling. Bhantnagar and Taneja (2001) found a correlation between zinc deficiency in pre-school aged children and decreased attention in pre-school aged children. On the other hand, research reviewed by Shah and Sachdev (2006) concluded maternal zinc status does not play a role in neurobehavioral development in infants. Although Shah and Sachdev (2006) did not find a relation between zinc status and cognition in humans, rodent studies indicate a relation between zinc deficiency and delayed brain development (Sandstead, et al., 2000). Although there are inconsistencies in the literature, more research than not indicated the importance for zinc and brain development in both humans and animals (Adamo & Oteiza, 2010; Bhatnagar & Taneja, 2001; Grower-Winter & Levenson, 2012; Shah & Sachdev, 2006). However, a relation between zinc and cognition was found in zinc deficiency Adamo & Oteiza, 2010; Bhatnagar & Taneja, 2001), and non-significant findings between zinc and toddler cognition in the current study may be due to the fact that mothers were zinc sufficient.

Fetal Origins Hypothesis

The relation between maternal nutritional status and offspring outcomes supports the Fetal Origins Hypothesis (Barker, 2012). Previous research in relation to the Fetal Origins Hypothesis considered under-nutrition and/or low birth weight individuals (Barker, 2001). However, the current sample consisted of full-term infants/toddlers from healthy mothers. Therefore, the current project suggests the origins of toddler cognitive development are related to *in utero* development when fetal conditions were not energy restricted and individuals were born full-term.

Yet, fetal development is complex (Barker, 2001). There are multiple steps for fetal nourishment: Nutrients must be transferred to maternal blood, the uterus, and the placenta before reaching the fetus. This complex line of fetal nourishment for fetal development could have been influenced by the mothers' fetal development, and the current project did not consider the mothers' health when they were born (Barker, 2003). An additional complexity is fetal gender (Barker, 2001). Fetuses respond differently to maternal nutrition if they are male or female. It is suspected this may be related to different gender growth rates. For instance, early in gestation female fetuses have a slower growth rate than male fetuses. This makes females less susceptible to undernourishment than males. However, the current project controlled for toddler gender.

Previous research for the Fetal Origins Hypothesis considered adult diseases, such as type two diabetes, hypertension, and coronary heart disease (Barker, 2003). Higher birthweight was related to increased risk of disease. Yet, the current project suggests fetal development impacts individuals much earlier in life. Moreover, the current project consisted of infants with healthy birthweights. Therefore, an individuals' birthweight may not be as relevant for cognitive development as the research shows it is relevant for adult disease (Barker, 2003). The impact earlier in life is interesting since the theory also incorporates developmental plasticity. The idea for developmental plasticity considers responding and adapting to different environmental conditions during development. However, the current study showed toddler vocabulary and distractibility may not be as adaptable as toddler working memory.

More specifically, the current project showed significant results between two maternal iron levels and toddler cognitive development. Overall, the majority of mothers in the current study had inadequate or poor sTfR levels, which is related to the constraint concept (Ellison, 2005). Inadequate or poor maternal sTfR levels lead to decreased development for toddler

Distractibility. Consequently, the fetus did not develop to its full potential *in utero,* and this may have been what impacted long-term toddler Distractibility. On the other hand, the current study had mothers with generally adequate Hgb levels (NLM, 2014); however, the theory focuses on inadequate nutrition. Therefore, the current project brings an interesting interpretation of the theory. The current study suggests maternal nutritional status, whether adequate or inadequate, may relate to long-term offspring development.

The non-significant relation between maternal iron and toddler Imitation may also relate to the Fetal Origins Hypothesis (Barker, 2012). The fetus may have adapted *in utero* to any iron inadequacy in maternal nutritional status (Ellison, 2005). Through adaptation the fetus could have had physiologically normal development. Hence, there may be a relation to the theory despite the non-significant results.

Overall, the Fetal Origins Hypothesis is supported by the current project. Previous research considered adult physical diseases rather than toddler cognitive development (Barker, 2001; Barker, 2003). Therefore, the current project adds an interesting perspective to the existing research. Findings in the current project suggest fetal development impact toddler cognitive development in relation to vocabulary and distractibility. Moreover, the impacts of fetal development are not only relevant in under-nutrition but also in full-term, healthy birthweight individuals from well-nourished mothers.

Summary

In conclusion, the sample included mothers and toddlers who were at a decreased risk for nutritional concerns. The participants were from middle SES families and with educated mothers who were all married. Mean maternal perinatal levels for sTfR were increased outside of the recommended range, but mean levels were adequate for serum iron, ferritin, and Hgb as well as for serum zinc (Skinke, 2008; IOM, 2001). The generally inadequate or poor sTfR levels were related to toddler distractibility. Despite generally adequate Hgb levels, increased Hgb was related to outcomes for toddler vocabulary and sentence length. Hgb recommendation levels for women may be adjusted to be more similar to the recommendation levels for men because of our findings that increased maternal perinatal Hgb was related to improved toddler outcomes (NLM, 2014). The Fetal Origins Hypothesis is supported (Barker, 2003) since maternal sTfR and Hgb levels were related to toddler cognitive development. This is interesting since previous research considered under-nourished mothers (Barker, 2001), and mothers in the current project were generally well nourished.

Overall, serum zinc is more regulated in the body than iron indicators (IOM, 2001). This may explain the non-significant results in relation to serum zinc and toddler cognition. Moreover, previous research indicated significant results between zinc and cognition in zinc deficiency (Adamo & Oteiza, 2010; Bhatnagar & Taneja, 2001), and the current sample was zinc sufficient. Therefore, cognitive development may be impacted by zinc only in terms of inadequate or deficient zinc status.

Hypothesis II – Infant Visual Information Processing (VIP) and Toddler Cognition

Infant VIP at nine months old and longest look change from three months old to nine months old did not have significant relations to multiple measures of toddler cognition. The infant VIP measured processing speed, which was not measured by any of the toddler cognitive measures in the current study (Colaizzi et al., 2014). Therefore, the lack of a significant relation between infant VIP and toddler cognition may be due to different variables measured for cognition between the infants and the toddlers. This is an interesting result since visual recognition memory in infants has been found to be related to IQ and language in later childhood (Rose et al., 2003). Yet, the current study did not find significant relations for vocabulary as measured by the PPVT and the MacArthur Bates CDI. The lack of significance in the current study may be because the participants in the previous research were four to seven years old (Rose et al., 2003). Therefore, the toddlers in the current study may not have been old enough for significance to be seen. In addition, language in previous research was measured with different methods than the current study, such as the Reynell Scales of Language Development and the Test of Language Development-Primary. In summary, infant VIP in the current study was not correlated to toddler vocabulary, which may be because the participants were younger than participants in the previous studies, the research used different methods, and the mothers in the current study were well educated.

Visual recognition memory in infants was shown in previous research to be related to memory and to recognition in childhood and adolescence (Rose et al., 2003). Similar to the other variables, memory and recognition in previous research was measured in older children at 11 years old. The age difference between the previous research and the current study may explain why significant relations were not seen in the current study for memory as measured by Imitation in the toddlers. Correlations for the current study were not significant for infant VIP and toddler Imitation. The infant VIP measured short-term memory as familiarization while the toddler Imitation task measured more long-term memory as measured by the ability to recall specific actions (Bauer et al., 2000). Therefore, both infant VIP and the toddler Imitation task analyzed memory, yet different forms of memory were considered between the two measures.

Hypothesis III – Maternal Dietary Iron and Zinc and Toddler Cognition

Maternal dietary iron and zinc intake in relation to long-term offspring outcomes is sparse since it is limited to observational studies (Mei et al., 2011). Furthermore, research conducted generally involves participants who were from low-income backgrounds. This is dissimilar to the current study as participants were from middle-income families. Yet, it is interesting that a significant correlation was not found for dietary iron and toddler cognition since significant correlations were found for maternal Hgb and MacArthur Bates CDI Vocabulary and MLU and for sTfR and Distractibility Total Looking Time. Significant results may not have been seen between maternal dietary iron and multiple measures of toddler cognition because the participants in the current study were not ID or IDA due to generally good total iron intake and maternal iron levels at three months postpartum. Subar (2001) validated the Dietary History Questionnaire (DHQ), and it was shown to be more reliable than other food frequency questionnaires (Subar et al., 2001). However, using dietary intake has concerns. For instance, it is self reported and the diet software may have a difference between food in the software and food that was consumed by the participant. Therefore, using the biomarkers is more reliable than using the DHQ.

Iron supplementation in the literature has controversial results (Aukett et al., 1986; Metallinos-Katsaras et al., 2004; Sachdev et al., 2004). Supplemental iron was beneficial in children in relation to cognitive development (Aukett et al., 1986). Yet, other research indicated iron supplementation in toddlers did not improve cognitive development (Metallinos-Katsaras et al., 2004; Sachdev et al., 2004). The controversies for iron supplementation may be due to the differences in age, SES, and iron status. In addition, iron supplements are not readily bioavailable and are poorly absorbed (IOM, 2001). In the current study mean dietary iron levels did not meet the EAR for pregnancy. Yet, when iron supplementation was combined with dietary iron, mean total iron was greater than the EAR for pregnancy. Therefore, the current project suggests iron supplementation in combination with dietary iron may be more beneficial than dietary iron or iron supplementation alone.

In the current study, significant relations were not found between maternal dietary zinc and toddler cognition. This may be due to the overwhelming majority of mothers with adequate dietary intake and adequate serum zinc levels (IOM, 2001). Although the current project is different from previous research in terms of maternal health, maternal education, SES, and offspring age (Hamandani et al., 2002; Tsenenobu et al., 2003), both the current project as well as the previous research indicated null results for relations between maternal dietary zinc and toddler cognition. This may be due to the high regulation of zinc in the body despite variations in zinc intake (IOM, 2001).

Overall, results were not significant between maternal dietary iron and zinc and toddler cognition. This may be because the mean total iron and zinc intake met the EAR for pregnancy and lactation (IOM, 2001). In addition, iron and zinc biochemical levels were generally adequate (Mei et al., 2011; NLM, 2014). Results in previous research were found in participants with deficiencies (Bhatnagar & Taneja, 2001; Fretham et al., 2011; Grantham-McGreggor & Ani, 2001; Lozoff et al., 200). Pregnant women who were IDA had infants with decreased developmental scores and toddlers with decreased scores for cognitive function tests (Grantham-McGreggor & Ani, 2001). Moreover, toddlers and pre-school aged children with zinc deficiency had decreased attention, activity, and motor development (Bhatnagar & Taneja, 2001). Therefore, it is not surprising significant results were not seen in the current project since iron and zinc intake met the EAR in the mothers, toddlers generally meet the EAR, and maternal iron and zinc levels were generally adequate (IOM, 2001; Butte et al., 2010).

Limitations

Though the sample size was small, a strength of the study is it represented a homogenous population. Because of the similarity within the sample, potential confounding variables may not have distorted the results as much as in a more diverse sample. An additional strength of the study was that the sample was from a healthy population in a developed nation, which helps to fill a gap in the literature. Previous literature in relation to iron and zinc status was generally conducted in developing nations (Chang et al., 2013; Hamadanei et al., 2001; Tamura et al., 2000; Tran et al., 2013).

The 47 returned participant pairs were reduced to 36 participant pairs, which represented only 27% of the original sample. For each research question, participants were lost due to toddlers not cooperating in the cognitive measures, mothers not returning the MacArthur Bates CDI, and mothers not completing the food frequency questionnaire. Toddler cooperation is challenging to overcome since the participants are not familiar with the researchers. Likewise, the results may have been impacted due to nervous or shy toddler participants.

An additional limitation may be maternal dietary information was self reported and may have included human error. Similarly, the MacArthur Bates CDI was also self-reported by mothers, who may have over reported their toddler's abilities. A major limitation affecting the results may be the large standard deviation for the infant VIP for the longest look at three months old as well as the longest look change from three months old to nine months old.

Implications for Practice

The research from the current project may be used to provide information regarding recommendation levels for iron intake. Iron is important for cognitive development, and maternal iron plays an important role for the health and well being of toddlers. For instance, significant correlations were seen in the current project for two maternal iron measures. Maternal sTfR was significantly related to toddler distractibility, and maternal Hgb was significantly related to MacArthur Bates CDI for vocabulary and MLU. sTfR levels are a sensitive marker for iron status because they are not influenced by inflammation like ferritin (Skinke, 2008). However, sTfR levels were increased above the reference range for the majority of the sample (58.3 %, n = 21). This is an interesting result since the mean iron levels for serum iron, ferritin, and Hgb met recommendations (Mei et al., 2011; NLM, 2014). Therefore, the current project suggests maternal iron intake recommendations may need to be increased above the current recommendations for maternal iron sTfR levels to be adequate.

Moreover, Hgb met recommendations in 88.9 % (n = 32) of the mothers (NLM, 2014). Yet, a significant positive relation was seen between Hgb and the MacArthur Bates CDI for

Vocabulary and MLU. Despite maternal mean total iron intake meeting the EAR for pregnancy and lactation, the mean was below the Recommended Dietary Allowance (RDA) (IOM, 2001). Therefore, the current study could indicate recommendations for perinatal Hgb levels should be increased above the current recommendation.

Yet, it is important that iron dietary recommendations not be increased too much because they may exceed iron overload (Fretham et al., 2011). Iron overload may generate reactive oxygen species (ROS) and may damage cells. Iron overload and ROS may be more harmful than helpful in relation to learning and memory (Lozoff, 2007). It is a current practice to recommend iron supplements independently or in a multivitamin, yet it is known iron in a supplemental form is not readily bioavailable (IOM, 2001). Therefore, practitioners need to be cautious when recommending iron supplements since there are consequences for both ID and iron overload.

As a dietitian, the current study indicates it may be beneficial to educate mothers and give educational materials to mothers for iron rich foods since mean maternal dietary iron without supplementation did not meet the EAR for pregnancy. Educational materials may be the most beneficial when given prior to conception since it is known iron levels decrease as pregnancy progresses (Mei et al., 2011). Therefore, user-friendly educational materials need to be understandable to mothers and readily available to pre-conception women and pregnant women. This is particularly important to the general public since the participants for this project were at a decreased risk since they were highly educated and with a mean annual income greater than 50,000 dollars. Since the sample was at a low risk, the general public may not be as well aware of the importance of nutrition in prenatal care as the participants in this study. Thus, educating pre-conception and pregnant women is important due to ID in 9% of women in their childbearing years (CDC, 2014a). Furthermore, maternal perinatal sTfR levels and Hgb levels related to toddler cognitive development, which supports the Fetal Origins Hypothesis (Barker, 2012). As a practitioner it may be important to consider perinatal nutritional status when assessing toddlers and children for health concerns.

Future Research

Toddler cognition measures used in the current project looked at multiple cognitive domains. No significant relations between infant VIP and toddler cognition were found, which may have been attributed to the different cognitive domains measured for each method. Therefore, for future research it may be beneficial to measure cognitive domains of the same nature in both infants and toddlers to see a correlation.

Furthermore, previous research tended to focus on infants less than two years old due to the rapid cognitive development during the first two years of life (Lozoff, 2007). On the other hand, perinatal iron research is lacking because it is believed there is protection from the mother. Toddler protection from the maternal iron levels is important, yet results were seen in the current project when maternal iron levels were adequate. Therefore, the results from the current project suggest maternal protection from iron may not last long-term. This finding supports the Fetal Origins Hypothesis since maternal nutritional status was related to long-term development of the offspring (Barker, 2012). Previous research in Ethiopia has indicated iron status in children is related to prenatal maternal iron status, which also supports the Fetal Origins Hypothesis (Habte et al., 2013). Therefore, perinatal iron levels are important not only for infants less than two years old, but also for toddlers older than two years old. The current project suggests there is a relation between maternal iron levels and toddler cognitive development, and future research may consider a relation between maternal iron levels and child cognitive development.

It would have been uncomfortable to toddlers to complete biochemical tests, but it may have been helpful in examining the importance of iron and zinc levels in relation to cognitive development. Furthermore, it would have been interesting to see if there was a correlation between maternal sTfR and toddler sTfR as seen previously in the research with Hgb (Habte et al., 2013).

Conclusion

Maternal perinatal nutrition is important for long-term cognitive development in the offspring. Maternal sTfR levels were related to increased toddler distractibility. Consequently, different levels of iron may be related to different domains of cognitive development as well as fetal brain development. The current study found significant relations between maternal iron and toddler vocabulary, sentence length, and distractibility. Therefore, as indicated by Gower-Winter and Levenson (2012) iron may be important for fetal cerebellum development since the cerebellum controls language and attention. The iron results from the current study add to the body of evidence concerning the importance of iron for neurological development (Fretham et al., 2011). Yet, maternal zinc levels were not related to toddler cognitive development. This may be because serum zinc is a poor indicator of zinc status (Vale et al., 2014). On the contrary, increased maternal Hgb levels were related to increased toddler vocabulary and sentence length. The current project supports the Fetal Origins Hypothesis since maternal iron measures were related to toddler cognitive development for vocabulary, sentence length, and distractibility (Barker, 2012). Therefore, maternal health is not only important for somatic development but also for cognitive development

On the other hand, evidence was not found for the second or the third hypotheses. The second hypothesis for Infant VIP and toddler cognition did not find any significant relations. Infant VIP in the current study did not relate to toddler cognitive development despite previous evidence for VIP and long-term outcomes (Rose et al., 2003). Lastly, the third hypothesis for maternal dietary iron and zinc and toddler cognitive development also did not find any significant relations. This may be because mean total iron and zinc intake was above the EAR for pregnancy

(IOM, 2001). Despite the lack of significant results for the second and third hypotheses, it is thought provoking to conduct research and see the similarities and differences between the current project and previous research.

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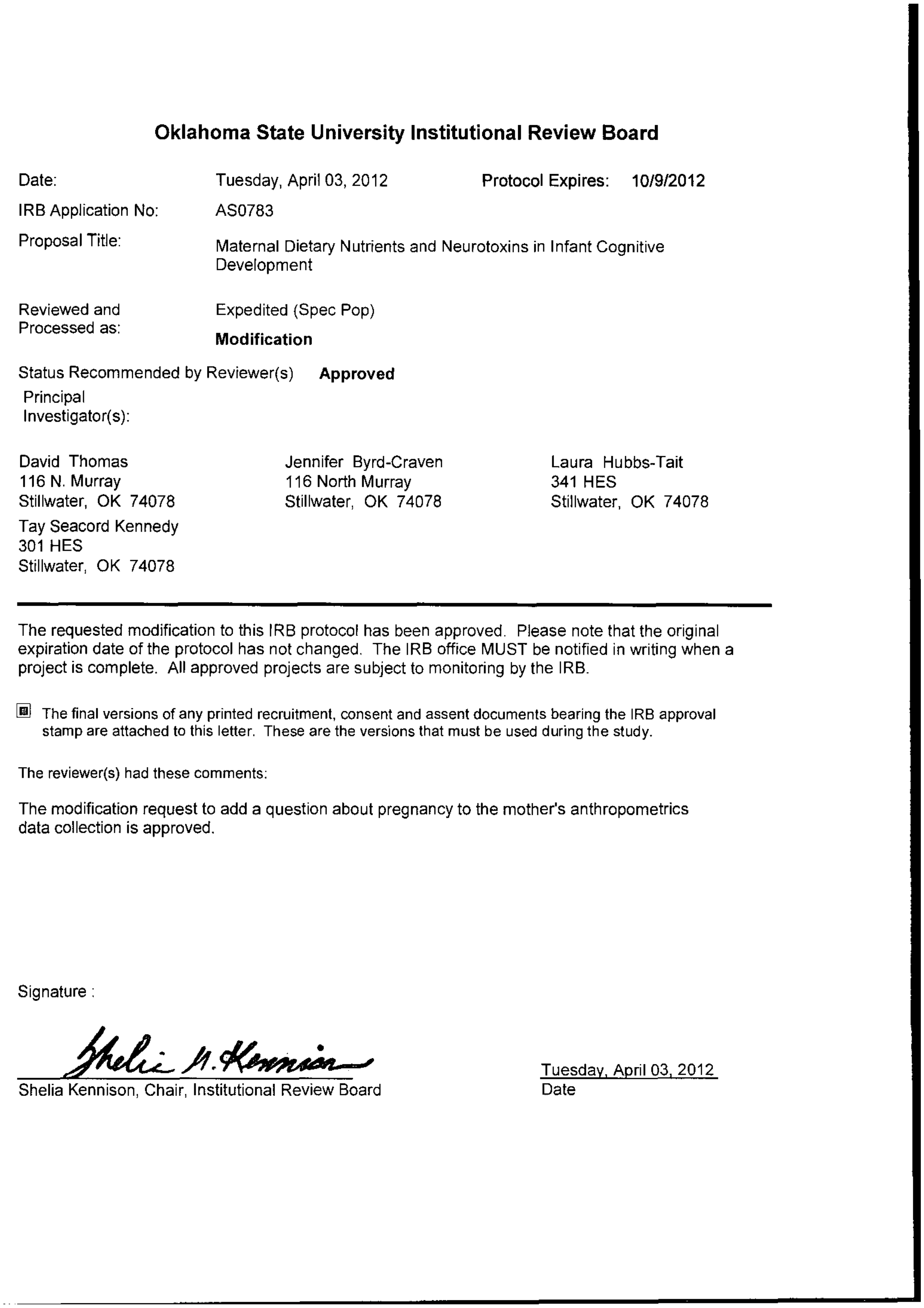
APPENDICES



Oklahoma State University Institutional Review Board

Date: Tuesday, April 03, 2012 **Protocol Expires: 10/9/2012**

IRB Application No: AS0783

Proposal Title: Maternal Dietary Nutrients and Neurotoxins in Infant Cognitive Development

Reviewed and Processed as: Expedited (Spec Pop)

Modification

Status Recommended by Reviewer(s) **Approved**

Principal Investigator(s):

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The requested modification to this IRB protocol has been approved. Please note that the original expiration date of the protocol has not changed. The IRB office MUST be notified in writing when a project is complete. All approved projects are subject to monitoring by the IRB.

☒ The final versions of any printed recruitment, consent and assent documents bearing the IRB approval stamp are attached to this letter. These are the versions that must be used during the study.

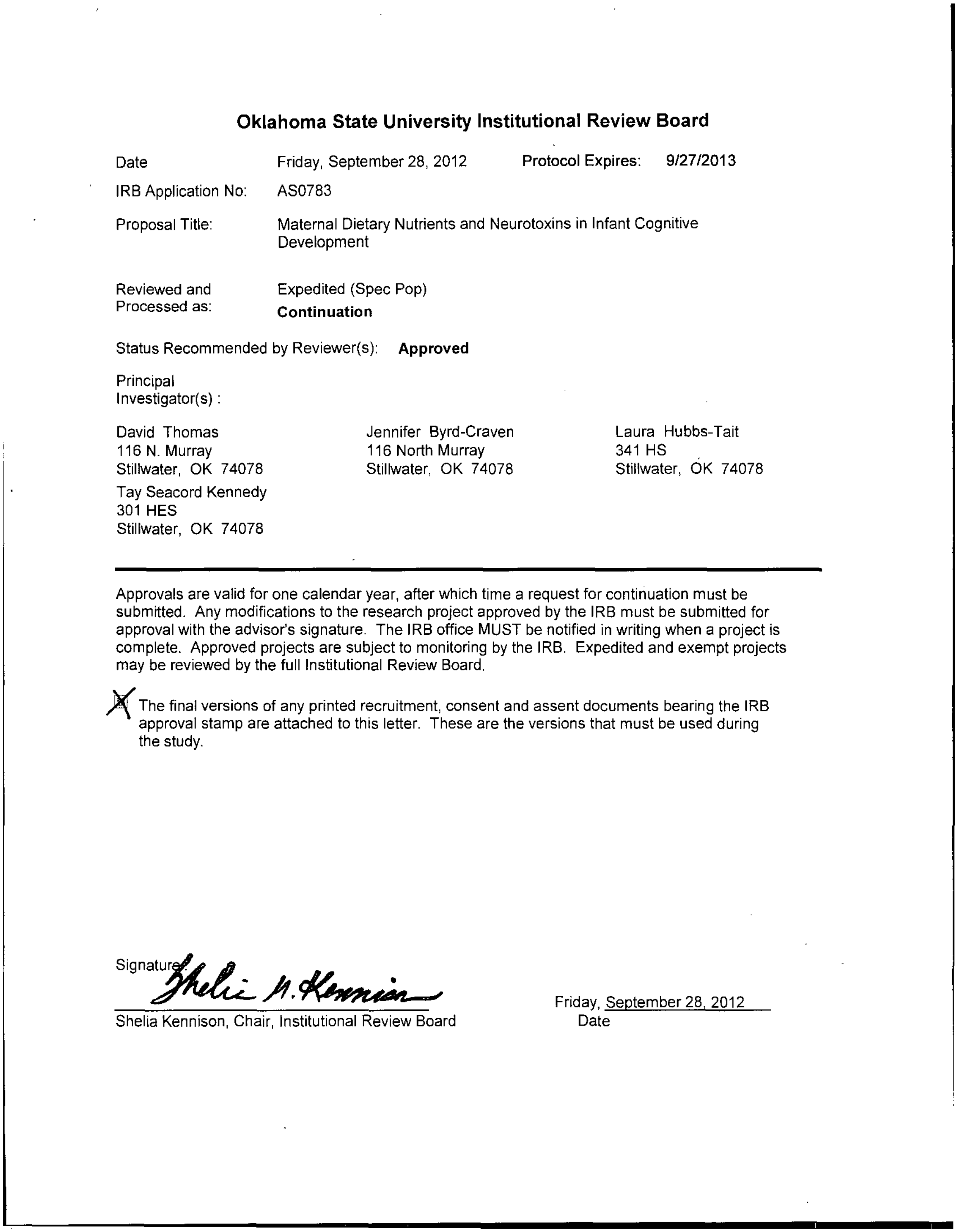
The reviewer(s) had these comments:

The modification request to add a question about pregnancy to the mother's anthropometrics data collection is approved.

Signature :

Shelia M. Kennison
Shelia Kennison, Chair, Institutional Review Board

Tuesday, April 03, 2012
Date



Oklahoma State University Institutional Review Board

Date: Friday, September 28, 2012 — **Protocol Expires:** 9/27/2013

IRB Application No: AS0783

Proposal Title: Maternal Dietary Nutrients and Neurotoxins in Infant Cognitive Development

Reviewed and Processed as: Expedited (Spec Pop)
Continuation

Status Recommended by Reviewer(s): **Approved**

Principal Investigator(s) :

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Approvals are valid for one calendar year, after which time a request for continuation must be submitted. Any modifications to the research project approved by the IRB must be submitted for approval with the advisor's signature. The IRB office MUST be notified in writing when a project is complete. Approved projects are subject to monitoring by the IRB. Expedited and exempt projects may be reviewed by the full Institutional Review Board.

☒ The final versions of any printed recruitment, consent and assent documents bearing the IRB approval stamp are attached to this letter. These are the versions that must be used during the study.

Signature: Shelia M. Kennison
Shelia Kennison, Chair, Institutional Review Board

Friday, September 28, 2012
Date

Table 18. Results of correlations between Maternal Iron and Zinc Levels and Maternal Iron Dietary Intake

	n	Dietary Fe	Supp Fe	Total Fe
Serum Fe	32*	***r* = 0.514** ***p* = 0.003**	*r* = 0.162 *p* = 0.376	***r* = 0.419** ***p* = 0.017**
Ferritin	33**	*r* = -0.023 *p* = 0.897	*r* = 0.217 *p* = 0.225	*r* = 0.149 *p* = 0.408
sTfR	33**	*r* = -0.033 *p* = 0.856	*r* = -0.069 *p* = 0.704	*r* = -0.071 *p* = 0.694
Hgb	33**	*r* = 0.290 *p* = 0.102	*r* = 0.235 *p* = 0.188	***r* = 0.349** ***p* = 0.047**
Serum Zn	32*	*r* = 0.181 *p* = 0.323	*r* = 0.257 *p* = 0.156	*r* = 0.304 *p* = 0.091

* One sample could not be analyzed because of possible contamination by intracellular minerals from hemolyzed red blood cells

** Three mothers did not complete the Dietary History Questionnaire (DHQ)

Table 19. Results of correlations between Maternal Iron and Zinc Levels and Maternal Zinc Dietary Intake

	n	Dietary Zn	Supp Zn	Total Zn
Serum Fe	32*	***r* = 0.418** ***p* = 0.017**	*r* = 0.034 *p* = 0.855	*r* = 0.285 *p* = 0.114
Ferritin	33	*r* = -0.119 *p* = 0.510	***r* = 0.440** ***p* = 0.010**	*r* = 0.261 *p* = 0.143
sTfR	33	*r* = 0.062 *p* = 0.734	*r* = -0.130 *p* = 0.471	*r* = -0.061 *p* = 0.736
Hgb	33	*r* = 0.305 *p* = 0.084	***r* = 0.369** ***p* = 0.035**	***r* = 0.468** ***p* = 0.006**
Serum Zn	32*	*r* = 0.135 *p* = 0.461	*r* = 0.110 *p* = 0.550	*r* = 0.169 *p* = 0.354

* One sample could not be analyzed because of possible contamination by intracellular minerals from hemolyzed red blood cells

** Three mothers did not complete the Dietary History Questionnaire (DHQ)

VITA

MONICA HAKES

Candidate for the Degree of

Master of Science

Thesis: THE RELATION OF MATERNAL IRON AND ZINC STATUS TO COGNITIVE DEVELOPMENT IN TODDLERS

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John Milner Cancer Prevention Practicum 2015 Attendee
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Oklahoma Academy of Nutrition and Dietetics
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