# MATERNAL FACTORS INFLUENCING INFANT TOTAL BODY IRON AT BIRTH AND FOUR MONTHS OF AGE

by

Sarah C. Scroggs RD

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Committee Members

Susan Carlson Ph.D, Chairperson

_____
Chair Person Susan E. Carlson, Ph.D.

_____
Linda Griffith, Ph.D. RD, CNSC

_____
Adrienne Baxter, MS, RD

Date Defended: _____

The Thesis Committee for Sarah Scroggs

certifies that this is the approved version of the following thesis:

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Chairperson Susan E. Carlson, Ph.D.

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**ABSTRACT**

Iron is necessary in fetal development, however little research has been conducted to assess factors that affect both maternal and fetal total body iron. The objective of this research is to investigate infant iron status at birth and 4 months of age in relation to the factors affecting maternal iron status.

Pregnant subjects (n=350) between the ages of 16 and 35.99 and a BMI < 40 were recruited between 8 and 20 weeks gestational age to consume DHA or placebo capsules. This analysis is secondary to the primary research. Post-partum and cord blood samples were collected at birth and an additional sample was collected from the infant at 4 months of age. Transferrin receptor and ferritin were analyzed from all blood samples obtained. Maternal and infant medical records were also followed along with subject report to gather information on factors that may affect iron status. Correlations between maternal body iron and cord blood; and maternal body iron and 4 month infant body iron were obtained through bivariate correlations. Linear regression assessed covariates that affect cord blood and infant iron status.

Smoking before pregnancy, high blood pressure during pregnancy, and infant gender were all found to significantly affect the infant total body iron in the cord blood sample ($p<0.05$). Infant total body iron at birth was also correlated with 4 month infant body iron ($p<0.05$). This study reaffirms previously published research that infant and maternal iron status are affected by smoking, high blood pressure, and infant being male gender.

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TABLE OF CONTENTS

LIST OF TABLES AND FIGURES VII IN-TEXT TABLES

# Chapter 1

## INTRODUCTION

Iron is necessary in fetal development of brain tissue, auditory and visual systems, and the immune system. Research shows that fetuses receive iron preferentially from the mother (1). However, little research exists concerning factors relating to maternal iron status and relationship to infant iron status. The present body of literature focuses on factors that affect maternal iron status or iron supplementation and infant iron status alone. Zhou et al. (2) reported that maternal iron supplementation was not correlated with infant iron status at either six months or 4 years of age. Iron status of infants whose mothers were supplemented with iron did not differ from those whose mothers received a placebo. However, these researchers found a positive correlation between iron supplementation and decreased incidence of iron deficiency and iron deficiency anemia in mothers (2).

Hemoglobin and hematocrit are frequently used indicators of iron status that can determine iron deficiency anemia only after iron deficiency has developed (3). These are the primary indicators of iron status that have been measured in studies of infants and mothers. Body iron can be determined calculated by using both plasma transferrin receptor and ferritin and both are the currently accepted means of identifying iron status (4). Physiologically, serum transferrin receptor increases as stored iron decreases, while serum ferritin is used to store and transport iron and decreases when iron stores are low (5). Few studies have measured iron status in infants and mothers using the body iron method (6)

Many factors are involved in the iron status of pregnant women. The major factors associated with variable iron status are ethnicity, BMI, smoking, and parity. Increases in blood volume during pregnancy make physiologic anemia and iron deficiency anemia difficult to determine, particularly in late pregnancy (7).

Even though it is well known that the mother is the source of iron for the fetus, the evidence linking maternal iron status to the newborn infant is weak. Published data do not demonstrate strong relationships between newborn iron status and factors affecting maternal iron status. Much of the published research used hemoglobin and hematocrit to assess iron status and these are not specific measures of iron status. Studies comparing maternal and newborn body iron might better indicate a relationship between maternal and newborn iron status.

## Statement of Purpose

The purpose of this research is to investigate infant iron status at birth and 4 months of age in relation to maternal iron status and factors that affect maternal iron status.

## Research Questions

1. What is maternal body iron, newborn (cord blood) and infant (4-month-old) body iron in the cohort enrolled in the Kansas University DHA Outcomes Study?
2. What are the relationships among maternal body iron at delivery, newborn (cord blood) and infant (4-month-old) body iron?
3. Are the relationships between maternal and newborn or maternal and infant body iron influenced by mothers' baseline hemoglobin, postpartum body iron, ethnicity, BMI, smoking, parity, and postnatal feeding?

# Chapter 2

# LITERATURE REVIEW

## Prevalence of Iron Deficiency

Iron deficiency is a global problem. It is estimated that 1.2 billion people worldwide are iron deficient; however, iron deficiency is less common in the United States (8). Thirty to fifty percent of pregnancies worldwide are iron deficient and the high prevalence of iron deficiency may be related to lasting developmental effects in infants (9). It is recommended by the World Health Organization that food be supplemented with ferrous sulfate, ferrous fumarate, ferric pyrophosphate, or electrolytic iron powder because they have the best bioavailability (10). US National Health and Nutrition Examination Survey (NHANES) data show that iron deficiency is greatest in children one to two years of age (7%) and in females of child bearing age (12-49 years). The prevalence of iron deficiency is two times higher among US African Americans and Hispanic-American females than non-Hispanic whites (8). The recommended dietary allowance is 27 mg per day of iron for pregnant women to prevent iron deficiency, (11). NHANES data suggests that the mean iron intake among pregnant women is 15mg per day (8).

## Iron Absorption

Iron absorption is primarily regulated by body iron stores with the duodenum being the primary site of absorption. When the body's demand for iron is increased,

absorption is also increased (12). Iron homeostatsis is regulated by hepcidin produced in liver and adipose tissue (13).

There are two forms of iron from the diet. Heme iron is the most bioavailable source of iron and is only available in animal products. Vitamin C is believed to aid absorption of non heme iron by facilitating extraction of iron from food and preserving it as a soluble, low molecular weight form. Iron then must be converted to reduced ferrous iron for absorption in the intestine to occur. Factors that aid this conversion include: gastric pH and organic acids, mucus, redox reactions, and intestinal transit time. Dietary factors that inhibit iron absorption include: phytates, polyphenols, calcium, and some proteins (casein, whey, egg white, and soy) (12).

Ninety percent of the body's daily iron needs are met by recycling red blood cells; the remaining 10% must come from the diet or supplements. During pregnancy, additional exogenous iron is needed due to fetal demands and plasma volume changes (13). During the total pregnancy, 300 mg of iron is needed for the fetoplacental unit. Maternal hemoglobin mass expansion requires about 800 mg of iron; and about 200 mg of iron is lost from the gut, urine, and skin during the total duration of pregnancy (14). A majority of the iron is needed during the third trimester of pregnancy due to rapid growth of the fetus (9).

**Fetal iron acquisition**

There are mixed data relating to the ability of the fetus to obtain adequate iron stores with mild to moderate maternal iron deficiency. It is known that the fetus

receives most nutrients preferentially. Nevertheless, O'Brien et al. (1) reported that iron transfer was related to maternal stores, suggesting that increased maternal iron stores would correlate with better fetal iron status (1).

Maternal iron is bound to transferrin and transferred to the fetus by transferrin receptors on the apical surface of the placental cells. The iron that is taken up is transferred to apotransferrin on the fetal side of the placenta and from there to fetal circulation as halotransferrin (10). In a study by O'Brien et al (1), 41 pregnant women ages eighteen to thirty-five, received radioactive iron tracer or a placebo. Amounts of tracer were then measured in the infant cord blood and serum. It was found that the transfer of non-heme iron to the fetus was significantly greater if the mother did not receive iron supplementation. The study also suggested that dietary iron was transferred rapidly to the fetus and little iron tracer was remobilized and transferred to the fetus from maternal stores. A significant positive correlation was seen with higher maternal iron concentration and the increased transfer of oral iron tracer. Serum ferritin indicated maternal iron stores and was a strong inverse predictor of transfer of oral iron tracer to the fetus; there was a significantly greater amount of fetal iron tracer in infants born to iron deficient mothers. Poorer iron status in the fetus as evidenced by lower cord ferritin and hematocrit was associated with more tracer transferred to infants (1). This finding demonstrates that iron transfer to the fetus is dependant on maternal iron status.

Iron needs for the fetus increase during the third trimester. Approximately 1-1.5 mg of iron is required per day before pregnancy and this is increased to greater

than 6 mg per day in the third trimester. To meet these needs, it is usually necessary to provide supplemental iron (1). If the fetus does not receive adequate iron, iron decreases first in the reticuloendothelial system (placenta and fetus), and then in the heart and brain (9).

The total body iron content of full term infants is approximately 75 mg/kg with 60% of the iron accumulated in the third trimester. About 75-80% of body iron is found in hemoglobin, 10% in tissues as iron containing proteins, and the remaining 10-15% stored in ferritin and hemosiderin.

**Assessment of iron status**

There is not a single laboratory test to assess iron status in all compartments (red blood cell, transport, functional, and storage) (9). It is common to use hemoglobin or hematocrit. Serum ferritin is a more specific measure because it shows anemia caused by iron rather than other nutrients (15). The "gold standard" for iron status is stained aspirate of bone marrow for hemosiderin. However, this technique is invasive and is not used to screen for iron deficiency in pregnant women (5). It is becoming more common to use total body iron calculation using serum transferrin receptor and ferritin Both are specific measures of iron status (16).

Many techniques used to assess iron status in pregnancy are not accurate measures of iron status. Hemoglobin is only a measure of the degree of iron deficiency once anemia has developed. It is also unreliable during pregnancy due to hypervolemia and changes in red blood cell mass. Total iron binding content and

immunochemical transferrin are good measurements of iron status; but they may not be appropriate measurements during pregnancy due to inflammation. Mean corpuscular volume is not an accurate measure of iron status because it can be influenced by other nutrients/factors and therefore is not a sensitive measurement. Serum iron is not a quality measurement of iron status due to diurnal variation (5).

Zinc protoporphyrin (ZPP) can be used to measure iron status and increased ZPP levels suggest incomplete incorporation of iron into protoporphyrin. ZPP is regarded as a sensitive iron indicator. Serum ferritin has been found to be the best single indicator, but it can be artificially elevated by inflammation. It is best to use a mixture of values including ferritin, transferrin receptor, and reticulocyte hemoglobin which are true measures of iron status (5).

Non-anemic women can have normal ferritin values between 20-300 μg/L. Women with anemia can have levels less than 20 μg/L, however serum ferritin values as high as 37 μg/L have been observed in anemic women. When assessed in comparison to hemosiderin, den Broek (17) reported that the optimal cut-off point for serum ferritin to assess anemia in pregnant women was 30 μg/L or less(17).

Total body iron calculation is an accurate and non-invasive way to calculate iron stores. This method is calculated using a ratio of transferrin receptor to ferritin, the equation is as follows: body iron (mg/kg)=[log(R/F ratio)-2.8229]/0.1207. Total body iron calculation is a reliable method to detect mild iron deficiency in a healthy population where chronic infection, inflammation, and liver disease does not exist

(16). Cook et. al has demonstrated iron deficiency anemia occurs when total body iron goes below -4 mg/kg (18).

Scientific reports indicate the use of different indicators to determine iron deficiency. Siddappa used a serum ferritin of less than or equal to 34 μg/L for evaluating suspected brain iron deficiency (19). Algarin defined iron deficiency anemia as having two of the three iron identifiers, mean corpuscular volume less than 70fL, erythrocyte protoporphyrin greater than 100 μg/L, red blood cell less than 1.77 μM, serum ferritin less than 12 μg/L, hemoglobin less than or equal to 10 mg/dL at six months or less; or 11 mg/dL at 12 & 18 months of age (3).

**Physiological functions of iron**

In iron deficiency anemia, microcytic, hypochromic red blood cells and low hemoglobin are evident. If the diet does not supply adequate iron, iron is taken from ferritin or storage iron. During this time, ferritin decreases and transferrin receptors increase. Next, iron is taken from hemoglobin and iron metalloenzyme causing a decrease in these values. When the body is using ferritin stores, hemoglobin may remain in the normal range, called iron deficiency without anemia. After a prolonged period of time, hemoglobin synthesis is slowed and hemoglobin concentration will decrease. There are many changes in hemoglobin after birth, for this reason, age-specific values need to be used to define anemia. After birth, the infant hemoglobin decreases by 30-50% related to decreased erythropoiesis, lysis of senescent fetal red blood cells, and expansion of vascular volume. Preterm infants miss the normal

accumulation of fetal iron and are at risk for iron deficiency (9). Hemoglobin can drop to less than 10 g/dL in infants and is still considered in normal range (20).

During pregnancy, there are changes in plasma volume, increased erythropoiesis, and increased demands of the fetoplacental unit for iron (17). Iron deficiency is more common than iron deficiency anemia in the later stages of pregnancy and even occurs in women who begin pregnancy with adequate iron stores. Hemoglobin is an indicator of iron deficiency anemia, hemoglobin is typically microcytic and hypochromic. This is because a majority of iron transfer to the fetus occurs after 30 weeks of gestation when maternal iron absorption is most efficient (10).

Maternal hemoglobin and hematocrit decrease throughout the first and second trimesters and reach the lowest point early in the third trimester. Both values normally rise closer to term. Risk for anemia may need to be detected early in pregnancy because physiologic anemia and iron deficiency anemia are difficult to discern in late pregnancy (4). Iron supplementation during pregnancy significantly improves maternal hemoglobin when iron deficiency is present (7), however supplementation has not been found to affect fetal body iron (21).

**Iron and cognitive and intellectual development**

The liver and brain have the highest concentration of iron during the first trimester. This is a critical time of brain development in the fetus, and the increase suggests an important role of iron in cognitive development (22).

A study of Indian women showed that lower maternal hemoglobin concentrations were correlated with lower infant Apgar scores and higher risk of infant asphyxia (10). A study comparing full-term infants with anemic infants reported that the infants with better iron status scored 6-15 points higher on mental development tests. Toddlers at 3-4 years of age continued to have lower mental development if they were iron deficient in infancy (23).

Effects of iron deficiency during development can be found beyond infancy. Neurocognitive abnormalities have been found in previously iron deficient infants at 6-24 months of age (24). Adolescents (11-14 years of age) who were treated for severe iron deficiency as infants tested lower in math, writing achievement, and motor function. These adolescents had behaviors that were viewed as problematic including: anxiety/depression, social problems, and attention problems. In a state-wide Florida study, anemia during infancy based on hemoglobin screening in Women Infants and Children (WIC), was related to special education placement at ten years of age (23).

**Iron and auditory and visual development**

Iron is required for normal auditory development. One theory is that impaired myelination caused by iron deficiency may result in delayed auditory recognition as suggested by the correlation with the time of deficiency and period of rapid myelin production (19). Siddappa et al. (19) observed that neonatal auditory recognition memory processing was lower in iron deficient infants than iron sufficient newborns.

This study determined iron deficiency as a neonatal serum ferritin concentration of less than 34 μg/L (19). At four years of age, the children who had iron deficient anemia at birth had longer auditory brainstem response and lower visual evoked potentials compared to infants without iron deficiency anemic (3).

**Infection**

Iron plays a vital role in the immune system. It is well known that infants and children are prone to illness, particularly upper respiratory infections. Having good iron stores is most likely beneficial in preventing infection. Mullick et al. (25) found that iron deficiency anemia correlated with decreased T-lymphocytes when compared with non-anemic children. With supplementation of iron, T-lymphocyte counts significantly improved, thus implying the children's immune system and resistance to infection improved (25).

In an observational study by Levy et al (15), iron deficient anemic children were found to have a significant increase incidence of diarrhea and respiratory infection when compared to non-anemic children (15). It can be inferred that since iron status is related to the incidence of infection and the immune system in children, iron status also plays a role in the immune system of infants.

**Maternal iron status and infant outcomes**

Maternal iron status is normally assessed with hemoglobin and hematocrit. It was once thought that deleterious effects of iron deficiency did not occur unless

anemia was present. It is now known that many organs show morphological, physiological, and biochemical changes before a drop in hemoglobin is observed (22). Infants are at risk for iron deficiency because of rapid growth and limited dietary sources of iron (3).

Studies have shown if women enter pregnancy with low iron stores, iron supplements may not prevent iron deficiency anemia (10). Infants born to iron deficient mothers are at risk for iron deficiency throughout infancy, even if it appears the infant is consuming enough iron. It has been found that maternal hemoglobin less than 8.5 mg/dL is related to a decrease in fetal iron stores (9).

**Relation of maternal iron status to infant status**

Two different studies, in France and Turkey, found that serum ferritin at two months of age was associated with maternal iron status (10). Most literature examines hemoglobin rather than body iron. Many studies with diverse populations have demonstrated that maternal hemoglobin is not correlated with fetal cord blood hemoglobin. Decreased hemoglobin in the second and third trimester is related to increases in plasma volume throughout pregnancy rather than iron deficiency (4). A study in Britain found that infants born to non-anemic mothers compared to infants with anemic mothers had more positive outcomes such as higher blood volume, red blood cell volume, and circulating hemoglobin mass (10).

**Maternal iron status related to infant outcomes**

Iron deficiency anemia early in pregnancy has been correlated with increased risk for small for gestation age (SGA) and preterm delivery. Risk for preterm delivery was increased when iron deficiency anemia occurred early in gestation or the first trimester; but not when it occurred in the second or third trimester (4). In a study with non-anemic women, incidence of low infant birth weight was reduced with routine iron supplementation (5).

**Breast feeding and infant iron status**

Iron content is highest in human milk during early transitional milk (0.97 mg/dL) and decreases as the infant grows, reaching about 0.3 mg/dL by five months of age. At one month, infants ingest about 0.075 mg/kg iron, 0.055 mg/kg at two months, and 0.048 mg/kg at three months. Breast milk iron concentration does not appear to be affected by mothers iron status or maternal supplementation. Iron deficiency anemia has been observed in 10% of infants who weighed greater than 3kg, 50% in infants who weighed less than 3kg who were breast fed (26). Children born without iron deficiency or iron deficiency anemia who are exclusively breast fed have better iron status at four months of age (95% GI:0.04, 0.90) (5). Based on calculations, it is expected that human milk provides adequate iron until six months of age in a healthy weight infant (1). The relationship between maternal iron status and infant iron status is more difficult to determine when infants are fed iron fortified

formula/food from an early age (10). The American Academy of Pediatricians recommends iron supplementation at 1 mg/kg by four to six months in breast fed infants (27).

**Ethnicity in relation to maternal iron deficiency**

African American and Hispanic women are at the greatest risk for iron deficiency. The NHANES data show that Hispanic women of childbearing age are 2.8 times more likely to be iron deficient and 2.9 times more likely to have iron deficiency anemia respectively. The study used the markers of serum ferritin concentration, transferrin saturation, erythrocyte protoporphyrin, and hemoglobin. Mexican American females were also more likely to have lower mean hemoglobin, transferrin saturation, and serum ferritin while mean erythrocyte protoporphyrin was significantly higher. It should be noted that Mexican American and non Hispanic American women had similar intakes of iron and that both groups had intake below the recommended intake levels for the United States (16).

Similar findings have been demonstrated in African-American women (8). Young low income black women in the United States were three times more likely to have small for gestational age (SGA) and preterm delivery if they were iron deficient in the first trimester of pregnancy (10).

**BMI in relation to maternal iron deficiency**

Obesity and overweight during pregnancy are also associated with risk for poor iron status during pregnancy (28, 29). A mechanism for why BMI may have an effect on iron stores in pregnancy has not been extensively researched. Differences in dietary intake have been implicated by Laraia et al. (30) and found that as BMI increased, the women who did not meet the estimated adequate intake for iron also increased (30).

**Smoking and iron status**

Maternal iron deficiency may be masked in smoking mothers because hemoglobin may be normal due to the increased need for hemoglobin to supply adequate oxygen to cells. Rao et al. (9) reported that women who smoke need extra iron in addition to iron requirements for pregnancy. Smoking can cause intrauterine fetal hypoxia, and changed erythropoiesis requiring additional iron (9). The relationship to smoking and decreased iron status has been well researched; however current studies are observational because it is unethical to prescribe smoking.

**Parity and iron status**

The number of prior pregnancies is inversely related to iron stores and the chance of iron deficiency increases with each pregnancy. This may be because maternal iron stores are used during pregnancy to support fetal development. Mothers with no previous pregnancies were found to have higher serum ferritin than mothers with previous pregnancies. As the number of pregnancies increased, mean

serum ferritin decreased (31). Compared to women with no previous pregnancy, those with two or more children were three to four times more likely to develop iron deficiency (7).

## Conclusion

Iron deficiency is still a problem among U.S women even though iron supplementation is practiced. Pregnant women are at greater risk for iron deficiency because of increased need. Iron deficiency is detrimental to cognitive, auditory, visual, and immune function in the developing fetus. Better maternal iron status correlates with better fetal iron stores and improved infant development.

Many factors may be related to maternal iron status including ethnicity, BMI, smoking status, and parity. Evidence is still inconclusive regarding maternal iron stores and their affect on infant iron status, especially later in development of the infant and child. More research is necessary to assess the greatest risk factors affecting maternal iron status and its impact on infant body iron.

# Chapter 3

# METHODS

## Overview

The primary purpose of the Kansas University DHA Outcomes Study was to investigate the effects of DHA supplemented during pregnancy on the cognitive and visual development of children. The research below is a secondary analysis that will examine the relationship between factors that affect maternal iron status and body iron in newborn and four month old infants.

## Setting

The study was conducted at the University of Kansas Medical Center and all subjects lived in the Kansas City Metro area. The study duration was from January 2006 and follow-up is still being performed on children. Subjects were recruited from three hospital sites including St. Luke's Plaza, Truman Medical Center, and the University of Kansas Medical Center. Subjects were also recruited through word of mouth. Interested participants recruited from St. Luke's Plaza, Truman Medical Center, and word of mouth were required to come to the University of Kansas Medical Center to enroll in the study. Mothers recruited from the University of Kansas Medical Center could enroll on the spot in the obstetrics and gynecology clinic.

Study personnel would visit mothers receiving prenatal care at University of Kansas Medical Center in the OB clinic. Subjects were not visited at antenatal

appointments if they received prenatal care from another clinic. Contact was maintained with all subjects through phone, email, and written correspondence.

**Sample**

Subjects (n=350) were enrolled if they met the inclusion criteria for a study on the effects of DHA on pregnancy outcome. Recruiting took place from January 2, 2006 thru November 17, 2009. Signs explaining the study were in waiting areas for mothers to view. Recruiters screened mothers based on age, BMI, gestation duration, and medical history. Pregnant women were enrolled if they were between 16-35.99 years of age with a BMI less than 40 and between 8 and 20 weeks gestation at enrollment. Women were excluded if they did not speak English, had pre-existing diabetes or hypertension, or were carrying more than one fetus. Women were also excluded if they had a chronic illness that could affect the planned outcomes of the primary clinical trial. If a woman was eligible, she was asked for permission to be told about the study and decided if she wished to enroll.

Subjects from the primary research study were excluded for this investigation if a postpartum blood sample was not obtained. Also, to be included in the analysis the subject had to have at least one combination of pairs; postpartum maternal blood and infant cord blood or postpartum maternal blood and 4 month infant blood samples.

This research investigation was submitted to the Human Subjects Committee and the secondary analysis is covered under the primary research study. All subjects signed a consent form to participate in the investigation (Appendix A).

**Procedures**

Recruiters reviewed the consent form in detail with the subject before signing the consent form. Information concerning the subjects' pregnancy history, smoking and alcohol intake, medical history and medication intake, supplement use, and a food frequency questionnaire created for the investigation was gathered at enrollment. Height, weight, and blood pressure were gathered from the subject's medical records. Information from medical records and patient reports was recorded in the study database. Medical records were followed throughout pregnancy and post delivery until the infant reached 18 months of age.

At enrollment, a baseline blood sample was collected by a trained phlebotomist. Blood was drawn from the anticubical vein in an EDTA tube and placed on ice. Blood was separated to plasma and red blood cells by centrifugation within 24 hours of the blood draw. Red blood cells and plasma were separated and plasma was frozen and then later thawed to measure DHA levels.

Blood samples were collected at birth from the umbilical cord and from the mother, then later from the infant at four months of age. DHA in plasma was analyzed as described above for baseline blood samples. The remaining plasma was refrozen and then later analyzed to measure transferrin receptor and serum ferritin levels. Transferrin receptor was measured using Ramco Laboratories, Inc. TFR-94 and ferritin was measured with Ramco Laboratories, Inc. T-13 testing supplies.

Procedures for transferrin receptor are described in Appendix B and ferritin is described in Appendix C.

All transferrin receptor and ferritin analysis were conducted between April 21, 2010 and September 8, 2010. Seventeen samples were run in total and serum transferrin receptor and ferritin were tested in duplicate on each sample.

**Materials**

Data were gathered through the case report (Appendix D) form and from medical records. Transferrin receptor and serum ferritin were measured with kits from Ramco laboratories described above.

**Statistical analysis**

Statistical analysis was performed with SPSS 18.0 software. The correlations between maternal body iron and cord blood and maternal body iron and 4 month infant body iron were obtained through bivariate correlations. Factors that affect maternal iron status were examined using linear regression on maternal body iron and cord blood; and 4 month old infant body irons and the covariates. Pearson's correlations of greater than 0.20 were considered significant. Otherwise a significance factpr of $p<0.05$ was considered significant.

# Chapter 4

# RESULTS

## Demographics

The mean age of subjects was 25.8 years of age with a mean gestational age of 14.6 weeks at enrollment. At the time of enrollment, this was the first pregnancy for 38% of subjects. It was the second pregnancy for 30%, third pregnancy for 17%, fourth pregnancy for 7%, fifth pregnancy for 5%, sixth pregnancy for 2% of the subjects, and 2% of subjects it was their seventh or eighth pregnancy. Women had a mean BMI of 26.9 at enrollment indicating the overweight category for BMI. A total of 31% participants were African American, 67.7% were Caucasian, 6.8% Hispanic, and 1.2% classified themselves as other (see **Table 1**).

**TABLE 1**

Parity and ethnicity of subjects

| Parity | N (%) |
|---|---|
| Gravid 1 | 92 (38%) |
| Gravid 2 | 73 (30%) |
| Gravid 3 | 42 (17%) |
| Gravid 4 | 16 (7%) |
| Gravid 5 | 12 (5%) |
| Gravid 6 | 4 (2%) |
| Gravid 7 | 2 (1%) |
| Gravid 8 | 1 (1%) |
| Ethnicity | |
| African American | 76 (31%) |
| Caucasian | 149 (68%) |
| Hispanic | 14 (7%) |
| Other | 3 (1%) |

At the time of delivery, the mean gestational age was 39.43 weeks with a mean birth weight of 3362.8 g. Five percent of the subjects had preterm deliveries (<37 weeks gestational age) (n=12). Information concerning breastfeeding was gathered at infant follow-up visits, see **Table 2** for breastfeeding characteristics of subjects. No statistically significant correlations were found with breast feeding versus formula feeding in infants total body iron at four months of age.

**TABLE 2**

Breastfeeding

| | N (%) |
|---|---|
| 0-6 days BF | 22 (31%) |
| 7-42 days BF | 14 (20%) |
| 43-120 days | 8 (11%) |
| 121+ days BF | 26 (37%) |

**Body iron status**

The mean maternal, cord blood, and 4 month infant body iron are displayed in **Table 3**. Seventeen percent of women (n=43) had negative total body iron at delivery. Of these forty-three subjects, eight may have had iron deficiency anemia because they had a total body iron of <-4 mg/kg. One infant had a negative total body iron from the cord blood sample (-0.0336 mg/kg). At four months of age, 2 infants (2%) had a negative total body iron, total body iron was -3.763 mg/kg and -0.483 mg/kg for these infants. Maternal body iron was not found to be correlated to the infant's body iron at birth or at four months of age ($p > 0.05$), however, a statistically

significant correlation was found between the cord blood and the infants body iron at four months of age (p=0.017). Smoking prior to pregnancy, high blood pressure during pregnancy, and infant gender were correlated with total body iron in infant cord blood (see **Table 4**). The following variables were not found to affect infant cord blood body iron: parity, smoking during pregnancy, alcohol consumption, BMI, preeclampsia, gestational diabetes, ethnicity, hemoglobin, birth weight, or gestational age at delivery (p>0.05) (see **Table 4 & 5**). No significant correlations of the above factors were found to affect four month body iron (p>0.05) with the exception of infant gender (p>0.001).

**TABLE 3**

Mean body iron of subjects

| | Mean±SE | Range | N | R-Value |
|---|---|---|---|---|
| Maternal Body Iron (mg/kg) | 4.35±4.04 | -6.85 - 14.95 | 242 | 1 |
| Cord Blood Body Iron (mg/kg) | 8.25±2.64* | -0.03 - 14.86 | 164 | 0.036 |
| 4 month Body Iron (mg/kg) | 6.27±2.69* | -3.76 - 12.42 | 100 | -0.141 |

* Statistically significant at 0.017

**TABLE 4**

Maternal factors and the correlation to maternal and cord blood or maternal and 4 month infant iron

| | Maternal & Cord Blood Pairs | | | | Maternal & 4mo. Infant Pairs | | | |
|---|---|---|---|---|---|---|---|---|
| | N | Total N | R-value | P-value | N | Total N | R-value | P-value |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Smoking during pregnancy | 77 | 163 | -0.123 | 0.116 | 45 | 95 | 0.102 | 0.314 |
| Smoking before pregnancy | 64 | 163 | -0.153 | .050 | 35 | 95 | -0.004 | 0.967 |
| Alcohol use before pregnancy | 90 | 163 | -0.152 | 0.052 | 62 | 95 | -0.093 | 0.356 |
| Alcohol use during pregnancy | 2 | 163 | -0.028 | 0.722 | 0 | 95 | --- | --- |
| High Blood Pressure | 28 | 162 | -0.227 | 0.003* | 19 | 94 | 0.038 | 0.710 |
| Preeclampsia current pregnancy | 2 | 163 | -0.007 | 0.926 | 3 | 95 | 0.047 | 0.643 |
| Preeclampsia previous pregnancy | 3 | 163 | -0.024 | 0.760 | 2 | 95 | 0.150 | 0.136 |
| Gestational Diabetes | 8 | 163 | 0.048 | 0.545 | 4 | 94 | 0.073 | 0.472 |
| Ethnicity | n/a | 163 | 0.158 | 0.043* | n/a | 95 | 0.154 | 0.127 |
| Iron Supplementation | 47 | 163 | -0.053 | 0.498 | 26 | 95 | 0.031 | 0.763 |
| Iron Supplement Taken | 33 | 163 | -0.067 | 0.396 | 20 | 95 | 0.121 | 0.229 |
| Prenatal Vitamin During Pregnancy | 160 | 163 | 0.056 | 0.478 | 92 | 95 | 0.011 | 0.917 |
| Vitamin Prior to Pregnancy | 59 | 163 | 0.121 | 0.121 | 40 | 95 | 0.079 | 0.434 |
| Infant Gender (male) | 70 | 163 | 0.188 | 0.016** | 50 | 95 | 0.338 | 0.001*** |
| Preterm Delivery (<37 weeks) | 7 | 163 | 0.120 | 0.126 | 5 | 95 | 0.000 | 0.997 |
| Breastfeeding Duration | n/a | n/a | 0.072 | n/a | 48 | 94 | 0.005 | 0.961 |

* Statistically significant at p<0.01
** Statistically significant at p<0.05
*** Statistically significant at p<0.001

**TABLE 5**

Maternal factors and the correlation to maternal and cord blood or maternal and 4 month infant iron

| | Maternal & Cord Blood Pairs | | | | Maternal & 4mo. Infant Pairs | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean±SD | N | R-value | P-value | Mean±SD | N | R-Value | P-value |
| Parity (Gravid) | 2.26±1.43 | 163 | 0.088 | 0.262 | 2.1±1.26 | 95 | -0.065 | 0.52 |
| Maternal BMI ($kg/m^2$) | 26.9±5.16 | 163 | 0.023 | 0.774 | 27.3±4.85 | 95 | 0.035 | 0.731 |
| Initial Hemoglobin (mg/dL) | 12.6±0.98 | 162 | -0.032 | 0.69 | 12.6±0.89 | 94 | -0.038 | 0.709 |
| Gestational Age at Delivery (# weeks) | 39.4±1.43 | 163 | -0.093 | 0.234 | 39.4±1.52 | 95 | 0.132 | 0.19 |
| Weight Gain (pounds) | 27.7±12.67 | 163 | 0.079 | 0.079 | 28.53±13.45 | 95 | 0.806 | 0.806 |
| Birth Weight (g) | 3328.4±491.50 | 162 | -0.059 | 0.452 | 3349.2±479.81 | 95 | 0.183 | 0.068 |

Parity was correlated with maternal body iron at birth. High blood pressure, initial hemoglobin, iron supplementation, prenatal vitamin intake, vitamin intake prior to pregnancy, and weight gain were all correlated with maternal body iron at birth, but not infant total body iron at birth ($p<0.05$).

# Chapter 5

## DISCUSSION

Few studies have examined the relationship between maternal and infant total body iron. As demonstrated in this investigation as well as other research, hemoglobin was not related to maternal or cord blood total body iron at birth. These results show that hemoglobin is not a good indicator of iron status in pregnant women and total body iron needs to be used. This investigation found that smoking before pregnancy, high blood pressure during pregnancy, ethnicity, and infant gender are significantly correlated to both maternal and infant cord blood body iron assessment. These are not novel findings nor were they found to be related to infant body iron at four months of age (16), (8). The only factor that remained significantly correlated to 4 month total body iron was the infant gender ($p<0.001$).

Cord blood total body iron was correlated to 4 month infant body iron ($p=0.017$). This finding demonstrates that infant iron status at birth has lasting effects into infancy. Factors such as iron fortified formulas and breast feeding may not change the infants iron status at 4 months of age (9, 20).

Some observations were made that were nearing significance. Maternal weight gain was nearing significance at $p=0.079$. Maternal alcohol use before pregnancy neared significance at $p=0.052$. These results were likely not significant because the study was not designed or powered to answer the secondary research

questions. Previous studies have found maternal weight gain and alcohol use before pregnancy to be negatively correlated with infant iron status (9).

Gestational age, high blood pressure during pregnancy, baseline hemoglobin, prenatal vitamin intake during pregnancy, vitamin intake prior to pregnancy, and maternal weight gain during pregnancy were all found to be significantly correlated to maternal total body iron at delivery ($p<0.05$ for all variables). Previous research has correlated these variables to maternal iron status (8).

One limitation of this study is that the study was not designed to investigate maternal and infant iron status as the primary outcomes. It would be beneficial to have maternal total body iron at baseline to compare to postpartum and infant total body iron. Research has shown that iron status in the first trimester is a better predictor of infant iron status (4). Also, this study was observational and placing all mothers on an iron supplement or a specific PNV may better assess iron status. Subjects were allowed to take a PNV of their choosing.

Implications of this study are that total body iron is a more sensitive assessment of iron status than hemoglobin and hematocrit. Physicians should use transferrin receptor or ferritin alone in particular when assessing iron status in pregnant women. Total body iron should be used to assess maternal iron status when prescribing iron to pregnant women. More research needs to be done to investigate maternal factors that affect iron status and their affect on infant total body iron. Suggestions for further research include a study specifically designed to answer the research question. Having equal groups of iron deficient and iron replete subjects

would better assess factors that may affect iron status. Another suggestion for research would be to assess dietary intake of iron through dietary recalls throughout the study.

# Chapter 6

## SUMMARY

Total body iron is a sensitive indicator of iron status in pregnant women and infants and should be used in preference to hemoglobin and hematocrit. Research has shown many factors affect maternal iron status. However the fetus receives iron preferentially (1).

The observational investigation demonstrated that maternal and infant iron status can be correlated with infant gender and high blood pressure during pregnancy ($p<0.05$). Women were followed between 8 and 20 weeks of gestation until the infant was four months of age. During this time information was collected from subjects including known risk factors that affect iron status. Medical records were also monitored. It was expected that parity, smoking during or before pregnancy, alcohol consumption, BMI, preeclampsia, gestational diabetes, ethnicity, hemoglobin, birth weight, and gestational age at delivery would be correlated with maternal and infant total body iron. These factors were most likely not correlated with maternal or infant body iron in this investigation because the study was not designed or powered to investigate total body iron.

Most of the current body of literature does not measure total body iron to assess iron status. More research needs to be conducted concerning total body iron and factors that affect maternal iron status. This study provides analysis to support that further investigation needs to be done to assess factors that affect maternal and

infant iron status using total body iron. Parity, smoking during or before pregnancy, alcohol consumption, BMI, preeclampsia, high blood pressure, gestational diabetes, ethnicity, hemoglobin, birth weight, or gestational age at delivery may be used in the future as screening tools to further investigate maternal iron status. Since total body iron is a more sensitive iron indicator, it should be used to assess iron status before iron supplementation is recommended.

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# APPENDIX A

**CONSENT FORM FOR PARTICIPATION IN A RESEARCH STUDY**

The Effects of DHA on Pregnancy and Infant Outcome
**(Kansas University DHA Outcomes Study or KUDOS)**

Sponsor: NIH (1R01 HD047315)

**INTRODUCTION**

As a pregnant woman who is between 8 and 20 weeks of gestation, you are being invited to enroll in a research study of a nutrient (DHA) that is a component of normal brain and important for brain development. The centers involved in the study are the University of Kansas Medical Center in Kansas City, Kansas, Truman Medical Center in Kansas City, Missouri and St. Luke's Hospital in Kansas City, Missouri. If you decide to enroll in this study, your baby will participate in research procedures at the University of Kansas Medical Center. Dr. Susan Carlson is the main investigator for this study. A total of 350 pregnant women will be enrolled in this study between October 2005 and January 2010.

You do not have to participate in this research study. It is important that before you make a decision to participate, you read the rest of this form. You should ask as many questions as you need to understand what will happen if you participate in the study.

**BACKGROUND**

*Docosahexaenoic acid (DHA) is a fat that is found in very large amounts in the brain. DHA is important for how my baby sees and learns. Breast milk and, since 2002, US formulas contain DHA. Many studies have shown that DHA in the diet helps the baby's vision, attention, and ability to learn. In this way, DHA is considered an important nutrient for babies after they are born.*

*DHA may also be important before babies are born. Four studies found that women's DHA during pregnancy was related to higher infant/child function. These studies are called observational studies, meaning that the women's normal DHA status was studied in relation to development of the baby/child. There is*

*only one study that gave women DHA during pregnancy and measured development of their babies/children. That study showed higher IQ at 4 years of age in children whose mothers took fish oil capsules during the last 6 months of pregnancy. (Fish oil contains a lot of DHA). However, because women in the study also consumed DHA while they were breastfeeding they provided more DHA to their babies after they were born. Therefore, the study does not prove that giving DHA before babies are born will help their development. There are no studies that have varied DHA intake only during pregnancy. You and your child are being asked to participate in such an experimental study.*

**PURPOSE**

*The purpose of this study is to determine if a dietary supplement of DHA during pregnancy will help babies be born at the right time and help their development. If you decide to be in the study, you will have a 50-50 chance of receiving capsules with the supplement of DHA or ordinary food oil, which does not contain any DHA.*

**PROCEDURES**

If you choose to enroll yourself and your infant in this study, the investigators will record some information from your medical record about your pregnancy and medical history. They will also ask you a few questions about foods that you usually eat. You will have a blood sample collected from a vein in your arm. One-half teaspoon of blood will be drawn. The blood will be used to measure DHA in your blood as well as other nutrients. You will be asked to provide a current address and phone number where you can be contacted.

**During pregnancy:** You will be randomly assigned (like flipping a coin) to capsules with DHA-oil or ordinary food oil (which does not contain any DHA). The DHA-oil is the same oil that is used in US infant formulas and has been fed safely to millions of infants.

You will be given enough capsules each month to take 3 capsules each day and you agree to try to consume all 3 capsules. If you consume all 3 capsules, you will consume 600 mg of DHA. The capsules are relatively small and you should find them easier to swallow than many nutrient supplements. They are orange-flavored, so if you burp (common in pregnancy and in the first week of taking any nutrient supplement), the taste should not be unpleasant. You do not need to take the capsules at any specific time as they are a nutrient and not a drug. However, you should decide upon a regular time to take them so that taking the

capsules will become a habit and you won't forget. For example, you might wish to take them just before you go to bed or when you have your first beverage of the day.

Neither you nor the investigators will know which capsules you have been assigned to. On the day you enroll for the study, we will send you home with your first bottle of capsules. About 30 days later (early enough so that you do not run out of capsule), you will receive another bottle of capsules in the mail. AT THAT TIME, YOU AGREE TO PLACE THE FIRST BOTTLE WITH ANY REMAINING CAPSULES IN THE ENVELOPE AND DROP IT INTO THE MAIL.

This process will be repeated each month until your baby is born and you will continue to take 3 capsules per day until your baby is born. Each time you receive a new bottle, you will mail back the bottle that you have been using and that day will open and begin using the new bottle.

The investigators will contact you by phone at least once per month. They will ask about capsule intake and they will ask how you are doing. Maintaining contact with our study personnel on a monthly basis is very important.

IF YOUR PHONE NUMBER OR ADDRESS CHANGES AT ANY TIME DURING THE STUDY, YOU WILL LET THE INVESTIGATORS KNOW BY CALLING 913-588-3781 AND LEAVING A MESSAGE.

**Delivery:** After you are admitted to the hospital to deliver, you should telephone study personnel or ask the person at admitting to telephone them. You will be given a cell phone number today to call. Once you deliver your baby, the investigators will visit you in the hospital to collect data about your delivery and your baby's health. A sample of your baby's cord blood will be collected after delivery by nurses at the hospital and given to the investigators. A nurse will also draw a small blood sample (one-half teaspoon) from you while you are in the hospital. The blood samples will be used to measure DHA and other nutrients. The investigators will visit you, and give you an appointment for your baby's first follow-up visit at KUMC.

***Visit 1 (6 weeks of age)****:* The investigators will measure how your baby sees using a test that involves placing 3 electrodes directly on your baby's head. The process involves cleaning the area then placing a small amount of paste similar to toothpaste on the head. The electrodes are placed on top of the paste. The

electrodes will be used to record your baby's brain waves while he/she is looking at pictures. Your child's weight, height and head circumference will be measured again and you will be asked questions about what your baby eats. If you are breastfeeding your baby, you will be asked to provide a teaspoon of breast milk to the investigator. The sample will be frozen and analyzed for fats that are found in the capsules. The visit should last about 40 minutes. You should arrive on time and allow that amount of time for the visit.

**Visit 2 (4 months of age)**: The investigators will measure how your baby sees using the same test as before and another vision test. Your baby will wear a pair of plastic glasses during the second test. In another test, your child will be given an object to look at several times. The investigator will measure how long he/she looks at the object and how quickly he/she stops looking at the object. Your child will be video recorded during the test. Your baby's heart rate will be measured during the test. Your baby's height, weight and head circumference will be measured and you will be asked about what food your baby eats. Your baby will have a blood sample collected by either heel stick or drawn from a vein. If it is necessary to use a heel stick, the investigator may use a cream or spray that will numb the area before obtaining the sample. One-half teaspoon of blood will be drawn. The blood will be used to measure DHA and other nutrients. You should let the investigator know if your baby has been sick or not acting well since his/her last visit. The visit will take 60-90 minutes.

**Visit 3 (6 months of age)**: The investigators will measure how your baby sees using the test that requires him/her to wear a pair of plastic glasses. In another test, he/she will be given an object to look at several times (just like at 4 months of age). The investigator will measure how long he/she looks at the object and how quickly he/she stops looking at the object. Your child will be video recorded during the test. Your baby's heart rate will be measured during the test. Your baby's height, weight and head circumference will be measured. You will be asked questions about what your baby eats. You should let the investigator know if your baby has been sick or not acting well since his/her last visit. The visit should take 40 -60 minutes.

**Visit 4 (9 months of age)**: Your baby will have both tests that measure how he/she sees. In another test, your child will be given an object to look at several times (just like at 4 and 6 months of age). The investigator will measure how long he/she looks at the object and how quickly he/she stops looking at the object and your baby's heart rate will be measured during the test. Your child will be video recorded during the test. Your baby's height, weight and head

circumference will be measured. You will be asked questions about what your baby eats. You should let the investigator know if your baby has been sick or not acting well since his/her last visit. The visit should take about 40-60 minutes.

**Visit 5 (10 months of age)**: During this visit your baby will be placed on your lap in front of a small table. A test will be completed with a small toy, foam block and 2 cloths that will be placed in front of your child. You will also take a short language test. The small toy will be given to your child to keep. In another test, your baby will be asked to take turns with the researcher building fun toys. After your baby has played for a moment with the pieces, the researcher will show him or her how to build the toy. Then, your baby will be given a turn to put the toy together. Your baby's turn will happen either immediately or after 10-minutes of play with other things. Your child will be video recorded during the tests. You should let the investigator know if your baby has been sick or not acting well since his/her last visit. You will be asked questions about what your baby eats. The entire 10-month visit should last 65 - 70 minutes.

**Visit 6 (12 months of age)**: The investigators will measure how your baby sees using both vision tests. Your child will be video recorded while playing with an interesting toy and the investigator will use the recording to measure some aspects of attention. Your child's height, weight and head circumference will be measured. You will be asked questions about what your baby eats. You should let the investigator know if your child has been sick or not acting well since his/her last visit. The visit should take about 2 hours. It is important that your child be rested before the testing at this visit. If for some reason your baby cannot finish the tests that day – this may happen if he/she is unusually fussy or tired – you will be asked to return to finish the remaining tests within 7 days.

**Visit 7 (18 months of age)**: The investigators will measure how your child sees using the test that he/she had while wearing plastic glasses. Your child will be video recorded while playing with an interesting toy and the investigator will use the recording to measure some aspects of attention. Your child will also be given a standardized test to measure mental and physical development. Your child's height, weight and head circumference will be measured. You will be asked questions about what your baby eats. You will be asked questions about the words your child uses and understands. You should let the investigator know if your child has been sick or not acting well since his/her last visit. The visit should take about 2 hours. It is important that your child be rested before the testing at this visit. If for some reason your child cannot finish the tests that day – this may

happen if he/she is unusually fussy or tired – you will be asked to return to finish the remaining tests within 7 days.

**RISKS**

Some redness, soreness, or bruising may occur at the site of blood sampling. There is also a very slight risk of infection.

You may experience burping from the capsules and find this unpleasant

There are no known risks of consuming the amount of DHA you will be provided if you receive the DHA. Even if you forget to take your capsules for one or two days, there is no known risk of deciding to "catch up" on the third day. The amount is smaller than pregnant women in many countries eat every day. Nevertheless, you could develop a problem that has not been observed before.

**NEW FINDINGS STATEMENT**

You will be informed if any significant new findings develop during the course of the study that may affect your willingness to participate or to allow your child to participate in this study.

**BENEFITS**

You and your child may or may not benefit from participating in this study**.** If you receive the supplement, it may help your baby to be born at the right time and your baby's/child's development**.** If you will not get the supplement, your baby and you will not be getting any of those benefits. It is also possible that all infants/children will get some benefit from being followed closely with developmental testing. It is hoped that additional information gained in this research study may be useful in understanding if DHA can help your baby be born at the right time and help your baby's vision, attention, and learning as he or she grows. You will receive a video recording of your infant doing the 4, 6, and 9 month looking test when the 12 month visit is complete.

**ALTERNATIVES**

You do not have to participate in this study to be able to take DHA supplements while you are pregnant. You may purchase capsules containing DHA at local stores without a prescription (for example, Osco, Costco, Wal-Mart). There are also several brands of prenatal supplements with DHA available by prescription

or over the counter. The prenatal capsules typically contain 200 mg of DHA each and are marketed to take one capsule/day as a DHA supplement.

**COSTS**

Capsules containing either DHA or food oil will be provided to you at no cost while you are participating in this study. You will not incur any costs because of your or your child's participation

**PAYMENT TO SUBJECTS**

If study investigators are able to communicate with you each month you will be given 2 bonus gift cards to either Wal-Mart or Target of $25 each. The first gift card will be given to you half way through your treatment phase if communication is maintained at least one time each month during the first half of your treatment. The second gift card will be given at delivery if communication maintained at least one time each month during the second half of your treatment.

Additionally, if the study investigators are called after you are admitted for delivery you will be given your choice of a bonus gift card worth $50 from either Wal-Mart or Target. You may make the call yourself or have someone else call for you. Study personnel will give you the gift card when they come to the hospital after your baby is born.

Once your baby is born, you will receive a check for $50 after your baby completes each of the following visits: 6 weeks, 4 months, 6 months, 9 months, and 10 months. You will receive a check for $100 after your child completes each of the following visits: 12 and 18 months. The reimbursements are to cover the costs of transportation and to partially compensate you for your time required to participate in the study.

Your name, address, social security number, and the title of this study will be given to the KUMC Research Institute. This is done so that the Research Institute can write a check for study payments. Payments are taxable income.

**IN THE EVENT OF INJURY**

In the event you experience any serious health problem (hospitalization, life-threatening illness, or death) for any reason during your pregnancy, you should immediately seek treatment or help in the way you normally would as if you

were not in a study. You should let Susan Carlson, Ph.D. know about any of these problems as soon as possible by calling her office (913-588-5359) or the study office (913-588-3781). A message may be left at both numbers. Dr. Carlson may also be reached at home (816-960-1805).

**INSTITUTIONAL DISCLAIMER STATEMENT**

Although the University of Kansas Medical Center does not provide free medical treatment or other forms of compensation to persons injured as a result of participating in research, such compensation may be provided under the terms of the Kansas Tort Claims Act. If you believe you or your child has been injured as a result of participating in research, you should contact the Office of Legal Counsel, University of Kansas Medical Center, Kansas City, KS 66160-7101. You do not give up any of your or your child's rights by signing this form.

Truman Medical Center (TMC) will provide medical attention to you if you suffer any injury or harm as a direct result of participating in this research project. TMC, your study doctor, and the sponsor of this study will decide, in their discretion, who should pay for the medical care. TMC will provide treatment for you in the event of any medical emergency while present at TMC, whatever the cause. Moreover, you will have the benefit of the coverage of any existing health insurance you own. Truman Medical Center shall not be required to bill third party payers for any expenses related to this research study. No other compensation of any type will be provided by TMC or the sponsor.

Participation in this research study does not take the place of routine physical examinations or clinic visits to your personal physician. If you believe you have been injured as a result of participating in this study you are encouraged to contact the study investigator, Dr. Susan Carlson, at her work number, 913-588-5359.

The University of Missouri-Kansas City appreciates the participation of people who help it carry out its function of developing knowledge through research. Although it is not the University's policy to compensate or provide medical treatment for persons who participate in studies, if you think you have been injured as a result of participating in this study, please call the investigator, Dr.

Susan Carlson, at her work number, 913-588-5359. or the IRB Administrator of UMKC's Adult Health Sciences Institutional Review Board at 816-235-6150.

**CONFIDENTIALITY AND PRIVACY AUTHORIZATION**

Names of subjects or information identifying subjects will not be released without written permission unless required by law. Videotapes of your baby when he/she is looking at pictures and playing with toys will be used only by the investigators and their students and to make a videotape copy for you. The videotapes will be secured under lock and key like all other information that could be linked directly to your child. The videotape of your child will not be shown without specific permission from you and even then would not identify your child by name. Efforts will be made to keep you and your child's personal information confidential. Researchers cannot guarantee absolute confidentiality. If the results of this study are published or presented in public, information that identifies you and/or your baby will be removed.

The privacy of you and your child's health information is protected by a federal law known as the Health Insurance Portability and Accountability Act (HIPAA). If you choose to participate in this study, you will be asked to give permission for researchers to use and disclose your and your baby's health information that is relevant to the study.

To perform this study, researchers will collect health information about me and my child from his/her and my medical records and from the study activities that are listed in the Procedures section of this consent form. My and my baby's study-related health information will be used at KU Medical Center by Dr. Carlson, members of the research team, Truman Medical Center, St. Luke's Hospital andthe KU Hospital Medical Record Department. The KUMC Research Institute as well as officials at Truman Medical Center and St. Luke's Hospital that oversee research, including the KUMC Human Subjects Committee, the IRB that governs Truman Medical Center and St. Luke's Hospital and other committees and offices that review and monitor research studies, may also see my and my baby's study-related health information

Dr. Carlson and her team may share information about me and my baby with representatives of Martek Biosciences, the monitoring company who verifies study data, the laboratory that processes study lab samples, other business partners who help with the study, the U.S. Food and Drug Administration (FDA), and U.S. agencies that govern human research (if and when regulatory compliance issues arise). Martek Biosciences (Columbia, MD) donated the capsules for this study that is otherwise supported by the National Institute of Child Health and Human Development.

Some of the persons or groups that receive my and my baby's study information may not be required to comply with HIPAA privacy laws. My and my child's information may lose its federal protection if those persons or groups disclose it.

Permission granted on this date to use and disclose my health information remains in effect indefinitely. By signing this form I give permission for the use and disclosure of my and my child's information for purposes of the study at any time in the future.

If I enroll in the study, the investigators cannot tell me what capsule I was assigned to until the study ends. This may be after I have stopped taking the capsules.

**QUESTIONS**

I have read the information in this form. Dr. Carlson or her associates have answered my question(s) to my satisfaction. I know if I have any more questions after signing this I may contact Dr. Carlson or one of her associates at (913) 588-5359. If I have any questions about my or my child's rights as a research subject, I may call (913) 588-1240 or write the Human Subjects Committee, University of Kansas Medical Center, 3901 Rainbow Blvd. MSN 1032, Kansas City, KS 66160.

**SUBJECT RIGHTS AND WITHDRAWAL FROM THE STUDY**

My and my child's participation in this study is voluntary and the choice to not participate or to quit at any time can be made without penalty or loss of benefits. Not participating or quitting will have no effect upon the medical care of treatment my child receives now or in the future at the University of Kansas

Medical center. The entire study may be discontinued for any reason without my consent by the investigator conducting the study, by the sponsor of the study, or the FDA. My child's participation can be discontinued by the investigator or by the sponsor if it is felt to be in my child's best interest or if I do not follow the study requirements. If I choose to withdraw before my child is 18 months of age, I may be asked to answer questions about the study on the telephone.

If I want to cancel permission to use my or my child's health information, I should send a written request to Dr. Carlson. The mailing address is Susan Carlson, Ph.D., Dept. of Dietetics and Nutrition, MS 4013, 4019 Delp, University of Kansas Medical Center, 3901 Rainbow Boulevard, Kansas City, KS 66160. If I cancel permission to use my child's health information, the research team will stop collecting any additional information about me and my child.

Should the study be terminated prior to the completion of my pregnancy, neither the investigator nor the University of Kansas Medical Center will be under any obligation to provide me with DHA capsules used in the study.

**CONSENT**

Dr. Carlson or her associates have given me information about this research study.

They have explained what will be done and how long it will take. They explained the inconvenience, discomfort and risks that may be experienced during this study.

By signing this form, I give my permission for my and my child's health information to be used and disclosed for the purposes of this research study. If I choose not to sign this form, my child and I will not be able to participate in the study.

I voluntarily consent to my and my child's participation in this research study. I have read the information in this form and have had an opportunity to ask questions and have them answered. ***I will be given a copy of the signed form to keep for my records.***

_____

Type/Print Subject's Name

_____ _____

Signature of Subject Time Date

_____

Type/Print Name of Person Obtaining Consent

_____ _____

Signature of Person Obtaining Consent Date

_____

Type/Print Name of Principal Investigator

_____ _____

Signature of Principle Investigator Date

May the investigators contact you after the study is over to ask if you interested in continuing your child's participation? If you agree to be contacted, the investigators would explain any new study to you later and you would have the chance to decide if you wanted to participate at that time (please circle your response).

Yes

No

_____
Type/Print Subject's Name

_____ _____
Signature of Subject Time Date

_____
Type/Print Name of Person Obtaining Consent

_____ _____
Signature of Person Obtaining Consent Date

_____
Type/Print Name of Principal Investigator

_____ _____
Signature of Principle Investigator Date

APPENDIX B

Procedure taken from insert of Enzyme Immunoassay for quantifying Human Transferrin Receptors in Serum or Plasma (Catalog #TFR-94)

Ramco Laboratories, Inc

4507 Mt. Vernon

Houston, TX 77006

1. Remove plasma samples from -80ºC freezer and allow to thaw

2. Remove the necessary strips from the packet and place in Microwell Frame (any unused strips must be returned to and resealed in the ziplock foil packet, returned to 2-8ºC and used in 6 weeks)

3. Prepare a 1:100 dilution of each patient sample and Control by dispensing 1ml of Sample Dilutent into a test tube and adding 10µl of patient serum or plasma or Control. Mix thoroughly. DO NOT dilute standards

4. Pipette 50µl of each diluted patient sample and control, in duplicate, into individual wells

5. Pipette 150µl of HRP-conjugate into all individual wells containing samples, controls, and standards

6. Seal the microwell strips with the self-adhesive plastic film and place on a rotating table and mix for 10 minutes at 190nm or rotate by hand, pressing the frame firmly against the counter to avoid sloshing, to ensure mixing of the HRP-conjugate with the samples, controls, and standards. Allow reaction to proceed for an additional 2 hours (no rotation) at room temperature upon completion of mixing

7. Prepare the substrate solution: calculate the amount of substrate solution needed by multiplying 0.2ml by the number of wells in the assay run plus 0.5-1ml dead volume. Mix equal volumes of TMB substrate solution A and TMB substrate solution B just prior to addition of the microwells. This should be used within 30 minutes of preparation.

8. After the 2 hours incubation, remove plastic film, invert the plate, and dump the contents of the microwell strips. Tap the plate dry on absorbent pad or paper towels. Wash the wells with the prepared washing solution (wash solution 5X in 250ml graduated cylinder QS to 250ml) using a plate washing device or by using the wash bottle to flood the wells and then invert and dump the contents of the wells. The wells should be washed a total of 3 times, tapping dry between each wash. After the final wash, make sure the wells are completely empty

9. Pipette 200µl of substrate solution into each well containing samples, controls, and standards, and incubate in the dark at room temperature for 30 minutes. A blue color should develop in those wells containing TfR

10. Stop the color reaction by pipetting 50µl of acid stop solution into the wells and mix briefly to remove any air bobbles

11. Read the absorbance of each well at 450nm using a microplate reader. It is recommended that the reader be zeroed using blanks prepared with 200µl of substrate solution and 50µl acid stop solution

APPENDIX C

# FER-IRON II

An Immunoradiometric Procedure

For The Quantitative Analysis of Serum Ferritin.

Catalog Number: T-13

## SUMMARY

The serum ferritin concentration is proportional to the amount of iron in stores in the human body. The measurement of serum ferritin enables the clinician to differentiate between the anemia caused by iron deficiency and other forms of anemia[1,2,3]. Serum ferritin concentration is a useful, noninvasive screening test for iron overload, which may allow the detection of idiopathic hemochromatosis in the precirrhotic stages[4].

## TEST PRINCIPLE

This is a one stage, 2-site immunoradiometric (sandwich) assay. Antibody to ferritin is coated on the surface of the plastic beads. Ferritin present in sera or calibrators binds to the antibody coated beads. Radiolabeled antibody in turn binds to the ferritin on the solid phase antibody (forming a "sandwich"). The solid phase is washed and counted in a gamma counter. The amount of radiolabel bound to the solid phase is directly proportional to the concentration of ferritin present in the sera or calibrator solutions.

## REAGENTS

**Prediluted Ferritin Calibrator Solutions**: 6 vials containing 0.3ml of human spleen ferritin diluted to concentrations of 6, 20, 60, 200, 600, and 2000 ng/ml in borate buffer containing bovine serum albumin, rabbit serum, sodium chloride, and inert coloring agents with sodium azide as a preservative. Store at 2 - 8C. **Do Not Use After Expiration Date.**

Note: *The ferritin concentration of the base stock of cadmium crystallized human spleen ferritin from which these calibrators were derived was established by a protein determination. As such, these calibrators have more immunologic reactivity than WHO calibrators. Values for the Control Sera and for the Patient Samples will be approximately 25.4% lower than those observed using WHO calibrators*.

**Solid Phase Antihuman Ferritin**: 128 plastic beads (Beads) coated with rabbit antihuman spleen ferritin and stored in a bottle containing borate buffer with bovine serum albumin, rabbit serum, and sodium chloride with sodium azide as a preservative. Store at 2 - 8C. **Do Not Use After Expiration Date**.

**Sample Diluting Buffer (Blue Solution)**: 1 bottle containing 30 ml of borate buffer with bovine serum albumin, rabbit serum, sodium chloride, ethylenediaminetetra-acetate, and inert coloring agents with sodium azide as a preservative. Store at 2 - 8C. **Do Not Use After Expiration Date on Bottle**.

**Radiolabeled ($^{125}$I) Antihuman Ferritin (Red Solution**): 1 bottle containing 27 ml of radiolabeled ($^{125}$I) rabbit antihuman spleen ferritin dissolved in borate buffer with bovine serum albumin, rabbit serum, sodium chloride, ethylenediaminetetra-acetate, inert coloring agents and sodium azide as a preservative. This kit contains less than 10 uCi (370 kBq) of radioactivity. Store at 2 - 8C. **Do Not Use After Expiration Date on Bottle.:**

**Reaction Trays and Grippers**: 4 Reaction Trays containing 32 Reaction Wells each plus 128 bead Grippers.

**Graph Paper**: 3 sheets of Standard Plot logit-log graph paper.

## PRECAUTIONS

*This Kit is Intended for In-vitro Diagnostic Use Only*

## **WARNING** - RADIOACTIVE MATERIAL

1. Do not pipette radioactive material by mouth.

2. Use only in a designated area.

3. Store in the original container in a designated area.

4. Clean up spills with paper towels and dispose of in accordance with approved regulations.

5. Used radioactive solutions and solids should be disposed in accordance with approved regulations (see 10 CRF part 20).

**WARNING -** The human ferritin in the calibrator solutions has been tested and found to be nonreactive for Hepatitis B Antigen by RIA, and antibody to HTLV III by ELISA. The calibrator solutions as well as the patient specimens and control sera should be considered potentially hazardous and handled in accordance with approved laboratory procedures and regulations.

**WARNING -** Reagents in this kit contain sodium azide. Contact with copper or lead drain pipes may result in the formation of explosive azide deposits. It is important during disposal to flush drains with copious amounts of water to prevent azide accumulation. Plumbing that may be contaminated with azides can be flushed with 10 percent sodium hydroxide solution.

### Other Precautions:

- Avoid splashing or generating aerosols.
- Follow kit recommendations for incubation times and temperatures to avoid possibly erroneous results.
- Microbial contamination of reagents may cause erroneous results.

- Do not use reagents with those from other lots or manufacturers.
- Do not use kit reagents after the expiration date.

## SAMPLE COLLECTION AND PRESERVATION

Collect 5 ml of venous blood aseptically. Allow the blood to coagulate and separate the serum from the clot by centrifugation. Plasma may also be used for ferritin analysis. Moderate hemolysis will not interfere with the assay. If the assay will be performed within 7 days, store the serum refrigerated. If more than 7 days will elapse before the test is performed, the serum specimen should be frozen. Serum specimens may be stored frozen for 4 months without change in the ferritin content.

## MATERIALS REQUIRED BUT NOT PROVIDED

- Deionized or distilled water.
- Precision pipettes capable of delivering volummes of 10 and 200 ul.
- 12 X 75 mm glass or plastic test tubes.
- Gamma counter capable of detecting $^{125}I$.
- Clinical rotator table or vibrator table

## GENERAL INFORMATION ON PROCEDURE

Serum ferritin levels up to 2000 ng/ml may be measured without prediluting serum specimens. If precision is required for higher concentrations, samples may be diluted with sample diluting buffer and reassayed. Precision techniques are necessary for accurate and reproducible results. All solutions should be dispensed directly into the bottom of the reaction tube.

## ASSAY PROCEDURE

Determine the number of Solid Phase Antihuman Ferritin Beads needed for the assay and label the Reaction Trays and Grippers accordingly. Allow reagents, sera, and patient samples to reach room temperature before performing the assay.

1. Beginning with Reaction Well A3 (skip Wells A1 and A2), pipette 10 ul of Prediluted Ferritin Calibrator Solution and sample, in duplicate, into separate Wells. Reaction Wells A1 and A2 measure non specific binding and only contain the Radiolabeled Antihuman Ferritin and Antihuman Ferritin Coated Beads.

2. Pipette 200 ul of Radiolabeled Antihuman Ferritin (red solution) into each Well and place the tray(s) on a clinical rotator table for 5 minutes at 200 rpm to assure mixing.

3. Add 1 Bead to each Reaction Well and place the numbered Grippers into the Reaction Wells. Push down firmly to attach the beads.

4. Incubate on a clinical rotator (200 rpm) or vibrator table for 2 hours at room temperature. (For those requiring a more rapid assay the incubation time can be reduced to 1 hour at room temperature.)

5. Wash Beads under running deionized water while still attached to Grippers. Shake to remove excess water.

6. Remove each Bead or Bead plus Gripper to a counting tube.

7. Count in a gamma counter for 1 minute.

8. Calculate results. (See CALCULATION section below.)

**NOTE**: A rotating table or vibrator table must be used for a 1 or 2 hour incubation period. Extend the incubation period to overnight (14-18 hours) at room temperature if a mixing device is not used. Steps 1 through 8 are otherwise unaltered.

## SUMMARY OF ASSAY PROCEDURE

### Procedure Flow Sheet:

| Reaction Well | Calibrator (ul) | Patient Sera (ul) | Radiolabeled Antibody (ul) | Incubation |
|---|---|---|---|---|
| A1 & A2 | **(0 ng) | -- | 200 | |
| A3 & A4 | 10 (6 ng) | -- | 200 | INCUBATE FOR |
| A5 & A6 | 10 (20 ng) | -- | 200 | 2 HOURS ON A |
| A7 & A8 | 10 (60 ng) | -- | 200 | SHAKING OR |
| A9 & A10 | 10 (200 ng) | -- | 200 | VIBRATING |
| A11 & A12 | 10 (600 ng) | -- | 200 | TABLE |
| B1 & B2 | 10 (2000 ng) | -- | 200 | |
| B3 & B4 | -- | 10 | 200 | |
| etc | -- | 10 | 200 | |

**The 0 ng/ml calibrator consists of the 200 ul radiolabeled antiferritin only and is used to measure nonspecific binding (NSB).

After the incubation period, wash each Bead under running deionized water, and shake to remove excess fluid.

Count Beads in gamma counter for 1 minute.

## CALCULATIONS

Calculate the mean total count for each calibrator and patient serum. Determine the net count by subtracting the mean count of the 0 calibrator (NSB) from each.

### I. ESTIMATION OF MAXIMUM BINDING (MB)

Maximum Binding (MB) now must be determined. Either of the following techniques are satisfactory:

**METHOD I**: Maximum Binding (MB) can be estimated using the formula:

$$MB = C_2(2C_1C_3 - C_1C_2 - C_2C_3) / [(C_1C_3) - (C_2)^2]$$

Where: $C_1$ = net counts of 20 ng calibrators

$C_2$ = net counts of 200 ng calibrators

$C_3$ = net counts of 2000 ng calibrators

Example using Example Data Table:

$$MB = [30493\ (2(3736 \times 76797) - (3736 \times 30493) - (30493 \times 76797))] / [(3736 \times 76797) - (30493)^2]$$

$$MB = -5.738374 \times 10^{13} / -6.429095 \times 10^{8} = 89256$$

**METHOD II**: The net counts of the 2000 ng/ml calibrator multiplied by the factor on the quality control sheet enclosed in the kit closely approximates MB.

Example: $76797 \times 1.15 = 88317$

### II. CALCULATION OF PERCENT BOUND (PB)

Once (MB) has been derived, the Percent Bound (PB) must be determined for each calibrator and unknown.

PB = 100 x Net counts for each cal. or unknown / MB

Example for Normal Serum from Example Data Table:

PB = 100 x 19685/89256 = 22%

## III. CONSTRUCTION OF THE CALIBRATION CURVE

Construct a best fit line on the Logit-Log paper supplied with the kit, plotting the Percent Bound of each calibrator versus its ferritin concentration in ng/ml. Read the concentration of each unknown directly from the graph of the calibrator response curve.

## IV. OTHER METHODS OF DATA REDUCTION

Other methods of data reduction may be used to analyze the 2-site IRMA assay of ferritin. These include the plots of:

1. Counts bound (B) versus the log of the ferritin concentration.

2. Counts per minute versus ferritin concentration on a semi-log graph. It has been demonstrated that the data conforms closely to a Scatchard plot.

3. Percent Bound:

Bound / Total × 100 versus the log of the ferritin concentration

4. Automated Methods: Available on most multi-sample gamma counters. Refer to the instrument's operating manual for details.

Alternatively, the serum concentration of ferritin can be calculated from a regression equation. If you desire to use this form of calculation, call Ramco Laboratories at 800-231-6238 or 281-313-1250.

### EXAMPLE DATA

(For Demonstration Purposes Only)

COUNT TIME = 1 MINUTE

| ng/ml | TOTAL COUNTS | MEAN | -NSB** | PB* | CONC. ng/ml |
|---|---|---|---|---|---|
| NSB* | 275<br>293 | 284 | | | 0 or |
| 6 | 1390<br>1211 | 1301 | 1017 | 1.1 (1.2) | |
| | 3958 | | | | |

| | | | | | |
|---|---|---|---|---|---|
| 20 | 4082 | 4020 | 3736 | 4.2 (4.2) | |
| 60 | 11015<br>9950 | 10483 | 10199 | 11.4 (11.5) | |
| 200 | 29064<br>32489 | 30777 | 30493 | 34.1 (34.5) | |
| 600 | 54018<br>53946 | 53982 | 53689 | 60.1 (60.8) | |
| 2000 | 78000<br>76161 | 77081 | 76797 | 86.0 (87.0) | |
| Normal Serum | 19875<br>20063 | 19969 | 19685 | 22.0 (22.3) | 112 (114) |
| Iron Deficient Serum | 2482<br>2484 | 2483 | 2199 | 2.5 (2.5) | 12 (12) |

ESTIMATED MAXIMUM BINDING (MB) = 89256 (88317)

*PERCENT BOUND (PB) was estimated using Method I calculation for MB, the results in parenthesis were estimated using Method II calculation for MB.

**0 ng/ml calibrator measures nonspecific binding (NSB).

Concentrations for normal and iron deficient serum were determined using the Example data Logit-Log Graph.

## LIMITATIONS OF THE PROCEDURE

Moderate hemolysis has no effect upon the reproducibility or accuracy of the procedure. No drugs or other administered substances have been found to produce any effect on tests. Anticoagulants have not been shown to influence the test so long as they do not result in dilution of the plasma. Strict adherence to precise laboratory procedure is essential for maximum accuracy of the final results.

## EXPECTED VALUES

Normal values are age and sex dependent. Serum ferritin concentrations greater than 300 ng/ml may indicate increased iron stores as seen in idiopathic hemochromatosis.

The serum ferritin concentration reflects the amount of iron in stores. In iron deficiency, the stores are gone and the serum ferritin is very low (less than 20 ng/ml). In other kinds of anemia iron stores are higher than normal and the serum ferritin values are usually over 100 ng/ml. Values between 20 and 100 ng/ml in anemic patients may suggest a combination of iron deficiency with some cause of anemia. Serum ferritin concentrations greater than 300 ng/ml are elevated and may indicate increased iron stores as seen in idiopathic hemochromatosis.

## PERFORMANCE CHARACTERISTICS

### Intra-Assay Precision:

| SAMPLE | NUMBER OF SAMPLES | MEAN ng/ml | +/- 1 SD | C.V. |
|---|---|---|---|---|
| 1 | 8 | 18.1 | 0.23 | 1.2% |
| 2 | 8 | 62.9 | 1.19 | 1.9% |
| 3 | 8 | 228.0 | 7.48 | 3.3% |

### Inter-Assay Precision:

| SAMPLES | NUMBER OF ng/ml | MEAN +/- 1 SD | C.V. | SAMPLE |
|---|---|---|---|---|
| 1 | 12 | 10.6 | 0.69 | 6.5% |
| 2 | 12 | 54.2 | 3.19 | 5.9% |
| 3 | 12 | 202.0 | 13.20 | 6.5% |

## SENSITIVITY

Sensitivity is defined as the smallest value of ferritin which can be distinguished from the zero standard with a 95% confidence limit (+/- two standard deviations). Using the 10 ul sample size specified in the assay procedures, the smallest concentration of ferritin that can be distinguished from zero is 0.23 ng/ml.

## This Kit Contains Ramco Calibrators

*The concentrations of the Ramco Calibrators contained in this kit was established by a protein determination of the base stock of cadmium crystallized human spleen ferritin. As such, these Calibrators have more immunologic reactivity than WHO calibrators. Values for the Control Sera and for the Patient Samples will be 13.0% lower than those observed using WHO Calibrators (3rd International Standards for Ferritin, Recombinant NIBSC code: 94/572; National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire, EN6 3QG, UK).*

*To convert the values for the Control Sera and Patient Samples to equivalent WHO values, multiply the values obtained using these Ramco Calibrators by 1.34.*

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Date Issued: February 2, 1987

Revised: August 10, 2001; May 26, 2005;

APPENDIX D

# CASE REPORT FORMS

# FOR PREGNANCY PORTION OF STUDY

**PATIENT INVESTIGATIONAL NUMBER: _____**

**PATIENT INITIALS: _____**

**DATE OF PATIENT CONSENT: _____**
**(MMM/DD/YYYY)**

## INCLUSION / EXCLUSION CRITERIA

| PATIENT NUMBER | PATIENT INITIALS | DATE |
|---|---|---|
| | | |

**Inclusion:**

**Pregnant female age 16 – 35.99 yrs:** o yes o no

**Gestation between 8 and 20 weeks:** o yes o no

**Signed Informed Consent** o yes o no

**Date: _____** (mmm/dd/yyyy)

**Time: _____** (24 hour clock hh:mm)

**BMI <40** o yes o no

**Willing to consume 3 small capsules/day** o yes o no

**English speaking** o yes o no

**Exclusion:**

## MEDICAL HISTORY

| PATIENT NUMBER | PATIENT INITIALS | DATE |
|---|---|---|
| | | |

**Blood Pressure at Enrollment: _____/_____**

Systolic / Diastolic

| | YES | NO | If Yes Please Specify |
|---|---|---|---|
| **Reproductive History** | ■ | ■ | **Gravida ___ Para __ __ __ __**<br>**T PT A L** |
| **Smoking Before Pregnancy** | | | **PPD_____ Years _____**<br>**Pack Years _____**<br>**(# of PPD X Years Smoked)** |
| **Smoking During Pregnancy** | | | **# of cigarettes per day: _____**<br>**If was a smoker, date stopped: ____/____/____**<br>**mmm/dd/yyyy** |
| **Alcohol Intake Before Pregnancy** | | | **Amount per day: _____**<br>**(# of standard drinks/day – drinks/day from source and amount of alcohol))** |
| **Alcohol Intake During Pregnancy** | | | **Amount per day: _____**<br>**(# of standard drinks/day – drinks/day from source and amount of alcohol)** |
| **Mother's Date of Birth** | ■ | ■ | **____/____/____**<br>**mmm/dd/yyyy** |
| **Other relevant history:** | | | **Current Medications:** |

## PHYSICAL EXAMINATION

| PATIENT NUMBER | PATIENT INITIALS | DATE |
|---|---|---|
| | | |

| | |
|---|---|
| **Maternal Height** | **Inches** |
| **Maternal Weight at Enrollment** | **_____ Pounds Pre-pregnancy Wt_____** |
| **Body Mass Index at Enrollment** | **BMI** |
| **Last Menstrual Period**<br>**_____/_____/_____ [ ] Unknown**<br>**mmm/dd/yyyy**<br>**EDD based on LMP: _____/_____/_____** | **Ultrasound on _____/____/____**<br>mmm/dd/yyyy<br>**Approximate Gestational Age: _____**<br>**EDD based on U/S: _____/_____/_____** |
| **FINAL Expected Date of Delivery**<br>***Notes:*** | **_____/_____/_____**<br>**mmm/dd/yyyy** |
| **Maternal years of education** | |
| **Average frequency of fish intake during pregnancy** | **_____**<br>**If yes, kind of fish eaten: _____** |
| **Preterm Birth in previous pregnancy**<br>**If yes, number of times and wks gestation:** | **o yes o no**<br>**_____** |
| **Blood pressure ≥140/≥90 mm Hg during pregnancy?**<br>**24-hour urine protein loss (if done)** | **o yes o no**<br>**_____** |
| **Preeclampsia in current pregnancy**<br>**Preeclampsia in previous pregnancy** | **o yes o no**<br>**o yes o no** |
| **Gestational Diabetes in previous pregnancy. If yes, number of pregnancies:** | **o yes o no**<br>**_____** |
| **One hour load glucose after 26-28 wk 50 g screen (mg/dl at 1 hr)** | **_____**<br>**Date: GA:** |
| **3 Hr GTT results (if done) (mg/dl at 0,1,2 and 3 hrs)** | **_____**<br>**Date: GA:** |
| **Gestational Diabetes diagnosed** | **o yes o no** |

## SUPPLEMENT INTAKE DATA

| PATIENT NUMBER | PATIENT INITIALS | DATE |
|---|---|---|
| | | |

| | |
|---|---|
| **Ethnicity**<br>**(Check one box)** | o **African-American**<br>o **Asian-American**<br>o **Hispanic**<br>o **European-American (other than Hispanic):**<br>o **Other_____** |
| **Initial Hgb (at 1st OB appt):_____**<br>**2nd Hgb (at 28 week lab):_____** | **Date:_____**<br>**Date:_____** |
| **Was an additional iron supplement prescribed *during* pregnancy?** | o **yes** o **no**<br>**Date:_____** |
| **Separate iron supplements taken *during* pregnancy** | o **yes** o **no**<br>**If yes, frequency: _____** |
| **Prenatal Vitamins taken *during* pregnancy**<br>*if just prescribed, complete at subsequent visit or by phone interview | o **yes** o **no**<br>**If yes, name of supplement: _____**<br>**Approximate date started: _____**<br>**Gestational Age at start of intake:_____**<br>**Intake frequency: _____** |
| **Was an additional DHA supplement taken *during* pregnancy?** | o **yes** o **no**<br>**If yes, name of supplement: _____**<br>**Dose:_____ Start Date:_____** |
| **Vitamin supplements *prior to* pregnancy** | o **yes** o **no**<br>**If yes, what: _____** |
| **Other nutritional supplements *prior to* pregnancy** | o **yes** o **no**<br>**If yes, what: _____** |

**Date enrolled: _____/_____/_____**
**mmm/dd/yyyy**

**Age Group – (check one)**

**16 – 25.99 Group I _____** **26 – 35.99 Group II _____**

**Date of Initial Capsule Dispensing: ____/_____/_____**
**mmm/dd/yyyy**

# TEST PRODUCT ACCOUNTABILITY

| PATIENT NUMBER | PATIENT INITIALS | DATE |
|---|---|---|
| | | |

| | Week 0 | Week 4 | Week 8 | Week 12 | Week 20 | Week 24 | Week 30 | Week ____ | Total |
|---|---|---|---|---|---|---|---|---|---|
| **Date Delivered to Patient (mmm/dd/yyyy)** | | | | | | | | | |
| **Number of Capsules** | **100** | **100** | **100** | **100** | **100** | **100** | **100** | **100** | |
| **Date Returned from Patient** | | | | | | | | | |
| **Number of Capsules Returned** | | | | | | | | | |
| **Total Capsules Consumed By Count** | | | | | | | | | |
| | | | | | | | | | |
| ***Total Capsules Consumed Interview** | | | | | | | | | |

***To be completed by study personnel ongoing or at completion of pregnancy. The Investigational Pharmacy will complete the remaining info on an identical form with the same subject identifier**

**# of Weeks in Treatment Phase:_____ (Date of Enrollment:_____ to Date of Delivery:_____)**

***Total Study Capsule Intake: _____**

***Average Number of Capsules Consumed per Week: _____**

# LABORATORY

| PATIENT NUMBER | PATIENT INITIALS | DATE |
|---|---|---|
| | | |

**Maternal Blood at Enrollment:** o yes o no

**Date & Time:** _____/_____/_____ _____
mmm/dd/yyyy hh:mm

**Maternal Blood Postnatally:** o yes o no

**Date & Time:** _____/_____/_____ _____
mmm/dd/ yyyy hh:mm

**Cord Venous Blood** o yes o no

**Date & Time:** _____/_____/_____ _____
mmm/dd/yyyy hh:mm

**Maternal RBC Phospholipid DHAat Enrollment:** _____
**(grams per 100 grams of total fatty acids)**

**Materal RBC Phospholipid DHA Postnatal :** _____
**(grams per 100 grams of total fatty acids)**

**Cord RBC Phospholipid DHA** _____
**(grams per 100 grams of total fatty acids)**

## DELIVERY

| PATIENT NUMBER | PATIENT INITIALS | DATE |
|---|---|---|
| | | |

**Date of Delivery: ____/____/_____ Time of Delivery: _____**
**mmm/dd/yyyy) (hh:mm)**

**Gestational Age at Delivery: _____ Maternal Weight at Delivery: _____Pounds**

**Weight gain enrollment to delivery _____ Pounds**

**Delivery Type: o C-Section o Vaginal**

**If C-Section, reason: _____**

**If vaginal: o Spontaneous o Augmented o Induced**

**Did patient go into labor on her own? o Yes o No**

**Estimated Blood Loss recorded in labor and delivery chart: _____cc**

**Infant gender:_____ Apgar at 1 min.: _____ Apgar at 5 min.: _____**

**Birth Weight: _____ (g) Length: _____ cm**

**Head Circumference: _____cm**

**Low Birth Weight (< 2500 grams): o Yes o No**

**Size for Gestational Age: o SGA o AGA o LGA**

**Preterm (< 37 weeks): o Yes o No GA at delivery:_____**

**Meconium in Amniotic Fluid: o Yes o No**

**Infant transfer to NICU: o Yes o No**

*****Full Contact Log to be printed at time of delivery from G:\users\Scarlson\ Research Group\DHA Pregnancy Study\Contact Logs and Contact Info *****

## PATIENT CONTACT INFORMATION

| PATIENT NUMBER | PATIENT INITIALS | DATE |
|---|---|---|
| | | |

| Scheduled Week | Date/Time | Telephone Comments (include all nonroutine contacts by phone/letter in same 4 wk-period and denote as NR) |
|---|---|---|
| **Week 4** | _____/_____/_____ (mmm/dd/yyyy) _____ (24 hour clock hh:mm) | |
| **Week 8** | _____/_____/_____ (mmm/dd/yyyy) _____ (24 hour clock hh:mm) | |
| **Week 12** | _____/_____/_____ (mmm/dd/yyyy) _____ (24 hour clock hh:mm) | |
| **Week 16** | _____/_____/_____ (mmm/dd/yyyy) _____ (24 hour clock hh:mm) | |
| **Week 20** | _____/_____/_____ (mmm/dd/yyyy) _____ (24 hour clock hh:mm) | |
| **Week 24** | _____/_____/_____ (mmm/ddyyyy) _____ (24 hour clock hh:mm) | |
| **Week 30** | _____/_____/_____ (mmm/dd/yyyy) _____ (24 hour clock hh:mm) | |

## CONCOMITANT MEDICATIONS

| PATIENT NUMBER | PATIENT INITIALS | DATE |
|---|---|---|
| | | |

| Medication (Generic or Trade Name) | Date | Reason |
|---|---|---|
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☐ Check this box if this is last page of Concomitant Medications

# ADVERSE EXPERIENCES

| PATIENT NUMBER | PATIENT INITIALS | DATE |
|---|---|---|
| | | |

**Did the patient have any adverse experiences since consuming study supplement? o Yes o No**

| Adverse Event Code by Body System | Adverse Event Description<br>(One event per line)<br>(Underlying conditions only;<br>do not list procedures) | Was Adverse Event Serious?<br>1=Yes<br>2=No | Date of Onset<br>___/___/_____<br>mm dd yyyy | Date Stopped<br>___/___/_____<br>mm dd yyyy | ONGOING | Intensity<br>1=Mild<br>2=Moderate<br>3=Severe | Relationship To Treatment<br>1=Not Related<br>2=Possibly Rel<br>3=Probably Rel<br>4=Definitely Related | Action Taken<br>1=No Action Taken<br>2=Regimen Adjusted/Interrupted<br>3=Regimen Discontinued<br>4=Concomitant Medication<br>5=Non-Drug Therapy | Outcome<br>1=Ongoing<br>2=Resolved<br>3=Death<br>4=Unknown |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | ☐ | | | | |
| | | | | | ☐ | | | | |
| | | | | | ☐ | | | | |

_____ _____/_____/_____

Investigator's Signature Date (mmm/dd/yyyy)

☐ Check this box if this is last page of Adverse Experiences

**INVESTIGATOR CONFIDENCE AND SIGN-OFF**

| PATIENT NUMBER | PATIENT INITIALS | DATE |
|---|---|---|
| | | |

# To be Answered At Completion of Study

**Has the patient been employed within the past year?**

**If YES, occupation: _____**

**Does patient intend to return to work or begin work before baby is 18 months of age?**

**Have any phone numbers or address changed? If so, get new info**

**Phone number and name of person who can always contact them**

**How long has person had this phone number (if only a short time seek another contact)**

**Did the patient have any concerns about the study or product? If so, what were the concerns?**

## INVESTIGATOR SIGN-OFF

**I have reviewed the data in the Case Report Form.**
**By signing below, I confirm that it is accurate and complete.**

**Investigator Signature** **Date (mmm/dd/yyyy)**

## FOOD AND SUPPLEMENTS INTAKE QUESTIONNAIRE

| PATIENT NUMBER | PATIENT INITIALS | DATE |
|---|---|---|
| | | |

**How often and what amount does the patient eat of the following foods?**

| FOOD (ENTER AMOUNT IN APPROPRIATE COLUMN) | Never | 1 time per Month | 2-3 times per Month | 1 time per Week | 2-3 times per Week | 3-4 times per Week | 5-6 times per Week | Every day | 2 or more times per Day |
|---|---|---|---|---|---|---|---|---|---|
| **Eggs** | | | | | | | | | |
| **Oysters** | | | | | | | | | |
| **Shellfish (shrimp, scallops, crab)** | | | | | | | | | |
| **Tuna, salmon, or other ocean fish (including sardines or herring)** | | | | | | | | | |
| **Catfish or lake fish (state kind)** | | | | | | | | | |
| **Fish sandwich** | | | | | | | | | |
| **Tuna salad or casserole** | | | | | | | | | |
| **Chicken or beef liver or liverwurst** | | | | | | | | | |
| **Beef alone** | | | | | | | | | |
| **Beef as part of a mixed dish** | | | | | | | | | |
| **Chicken** | | | | | | | | | |
| **Chicken as part of a mixed dish** | | | | | | | | | |
| **Pork** | | | | | | | | | |
| **Pork as part of a mixed dish** | | | | | | | | | |

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