Assessing the Relationship Between Prenatal DHA Status on Offspring Body Composition

By
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ABSTRACT

Background: As childhood obesity rates continue to rise, it is crucial to discover more effective prevention strategies. Most obesity prevention treatment strategies are implemented in school-age children. However, it may be necessary to intervene during pregnancy to prevent offspring obesity. Maternal docosahexaenoic acid (DHA) status throughout pregnancy may promote improved infant growth, thus helping prevent obesity. Furthermore, maternal DHA status may improve adiposity of infants born to mothers with excessive gestational weight gain (GWG).

Objective: The purpose of this study was to determine if maternal DHA status influences infant percentage body fat (% fat), fat mass (FM), and fat-free mass (FFM). Furthermore, this study examined if maternal DHA status improves body composition of infants born to mothers with excessive GWG.

Design: Pregnant women were randomized to receive 200 or 800 mg DHA per day from 12-20 weeks gestation to birth. Maternal blood was collected at baseline and at delivery to determine maternal red blood cell phospholipid (RBC-PL) DHA status. Infant body composition was measured at 1 month and 4 months of age using two-compartment air-displacement plethysmography (ADP). Change in infant fat body weight, FM, FFM, and %fat was calculated. A median split was created to represent high vs. low for the change in DHA status from early pregnancy to late in pregnancy (32 weeks). Maternal GWG was categorized as excessive vs. not-excessive based on clinical guidelines. ANCOVA examined the main effects for differences in infant body composition between groups based on the change in maternal DHA status (high vs. low) and GWG (excessive vs. not-excessive). An interaction between maternal DHA status and GWG category was assessed.

Results: Maternal DHA status was not associated with the change in infant body composition, regardless of GWG. However, infants born to mothers with excessive GWG had a smaller change in FFM compared to infants born to mothers who did not gain excessively ($p=0.016$).

Conclusions: Maternal DHA status was not related to the change in infant body composition in mothers who gained excessive or not excessive during pregnancy. More studies are needed to demonstrate the effects maternal DHA status may have on offspring adiposity.

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INTRODUCTION

According to data collected from the National Health and Nutrition Examination Survey (NHANES), almost 20% of the nation's youth were obese in 2015-2016, compared to 13.9% in 1999-2000 (1). As childhood obesity rates continue to rise, it is crucial to discover more effective prevention strategies. Current strategies are targeted towards school-age children and adolescents (2). However, it may be necessary to implement prevention strategies starting before conception and during pregnancy. A clear relationship exists between pre-pregnancy body-mass index (BMI), GWG and offspring adiposity. Current statistics show that 26.5% of women are overweight and 40.4% of women are obese (1). Additionally, it is estimated that almost 50% of women gain excessive weight during pregnancy (3). Women who are overweight or obese before pregnancy are more likely to have infants with excess adiposity (4-9). Furthermore, excessive GWG is related to obesity of offspring (10-13). Infants with greater FM are at risk for developing obesity during childhood and children who are obese are more likely to become obese adults (14-20). Obesity is associated with numerous health complications such as impaired pulmonary, cardiac and pancreatic function (21-26). Obesity is also a risk factor for heart disease, cancer and type 2 diabetes (27, 28).

DHA (DHA) may be a nutrient that can attenuate the effects of excessive GWG and promote favorable fat deposition in infants, thus decreasing risk of obesity in childhood and adulthood. DHA is thought to prevent excess fat deposition in utero by inhibiting adipogenesis (29). However, the majority of pregnant women do not consume the recommended amount of DHA, so it is unclear whether improved DHA status can promote favorable fat deposition during infancy (30). Therefore, the purpose of this study was to determine if maternal DHA status influences offspring body composition. Furthermore, this study examined if maternal DHA status improves body composition of infants born to mothers with excessive GWG.

Research Questions

1. Does maternal DHA status impact FM, %fat and FFM of offspring at 1 and 4 months of age?
2. Does maternal DHA status improve FM, %fat and FFM of 1 and 4 month old infants born to mothers who gained excessive weight during pregnancy?

REVIEW OF LITERATURE

Recommendations for Gestational Weight Gain

GWG is important to monitor during pregnancy because it is related to maternal and offspring health during gestation and postnatally (10-13, 31-33). In 2009, the National Academy of Medicine (NAM: formerly the Institute of Medicine) published the current GWG guidelines, which are based on pre-pregnancy BMI (34). Women who are pregnant with one child and are underweight before pregnancy (BMI <18.5 kg/m^2) are recommended to gain 28-40 pounds; women who are normal weight (BMI 18.5-24.9 kg/m^2) should gain 25-35 pounds; women who are overweight (BMI 25.0-29.9 kg/m^2) should gain 15-25 pounds; and women who are obese (BMI $\geq$ 30.0 kg/m^2) should gain 11-20 pounds (34).

Pregnant women are encouraged to develop a plan to ensure appropriate weight gain. Strategies to promote appropriate GWG include tracking weight regularly, eating a nutrient-dense diet, limiting foods with added sugars and solid fats, adhering to caloric needs, and following a regular exercise routine (34). Women who do not follow strategies for appropriate weight gain during pregnancy are at risk for excessive weight gain. Additional factors that predict excessive GWG are overweight and obesity at conception, lower maternal education level and poor diet and exercise habits (34-38). However, little is known about other variables that may contribute to excessive GWG.

Rates of inadequate, appropriate and excessive GWG are well documented by the CDC. The National Vital Statistics System in 2015 indicated that 32% of pregnant women gained appropriately, 48% of women gained excessively, and 21% gained inadequately (3). This trend held true when GWG was categorized by pre-pregnancy weight status. Thirty-nine percent of women with a normal pre-pregnancy BMI gained appropriately, while only 26% of overweight women and 24% of obese women gained appropriately (3). Furthermore, 61% of overweight

women and 55% of obese women gained excessively during pregnancy compared to 37% of women with a normal pre-pregnancy BMI (3).

Developmental Programming Hypothesis

The association between pre-pregnancy weight status and GWG in relation to offspring body composition and obesity is documented in numerous studies. Pre-pregnancy BMI largely predicts offspring adiposity in all life stages. Two cross-sectional studies (4, 5), three prospective cohort studies (6-8) and one longitudinal study (9) showed that offspring born to mothers who were overweight or obese before pregnancy were more likely to have greater total body fat and %fat with less FFM compared to offspring born to mothers with normal BMIs at conception. Only one prospective cohort study (39) found no association between pre-pregnancy BMI and offspring adiposity.

GWG also predicts offspring body composition at different life stages. Two prospective cohort studies (6, 7) and one cross-sectional study (40) found that infants born to mothers with excessive GWG had greater FM, %fat and birth weight compared to infants born to mothers who gained appropriately. Furthermore, four prospective cohort studies (8, 39, 41, 42) and two cross-sectional studies (10, 43) showed that children born to mothers with excessive GWG had higher BMIs, FM, %fat, waist circumference and skinfold thickness compared to children born to mothers who gained appropriately. The same results were seen in adolescence and adulthood in two cross-sectional studies (44, 45) in that excessive GWG was positively associated with greater adiposity in adolescence and higher %fat, waist circumference and BMI in adulthood.

Because excessive GWG can predict offspring adiposity, it likely also determines risk of overweight and obesity in childhood and adulthood. Multiple studies found that mothers who experience excessive GWG are more likely to have children that are overweight or obese (10-13). The associations between pre-pregnancy BMI and GWG with offspring adiposity and risk of

overweight and obesity is linked to the intrauterine environment. Epidemiological evidence from animal models shows that exposure to certain intrauterine stimuli, including altered maternal nutrition and metabolic inflammation associated with overweight and obesity genetically predisposes the offspring to obesity (46-48). When the developing fetus is exposed to these stimuli during critical periods of development, adaptations in metabolism occur (49-52). For example, maternal and fetal inflammation stimulate increased adipogenesis in the fetus, resulting in greater offspring adiposity leading to obesity (48, 53-55). These metabolic alterations also manifest later in life in the form of obesity and other metabolic diseases (51, 56).

Childhood Obesity

Current data from NHANES indicates that 18.5% of youth age 2-19 years were obese in 2015-2016, as defined by World Health Organization Standards (BMI>95th percentile) (1). There are numerous undesirable health outcomes related to pediatric obesity both in childhood and adulthood. Children who are obese experience metabolic problems such as high blood pressure, high cholesterol, impaired glucose tolerance, insulin resistance and type 2 diabetes (21-23). They also have impaired pulmonary function, joint pain, fatty liver disease, gastroesophageal reflux disease and gallstones (24-26). If obesity develops in childhood, it is likely to persist into adulthood, with greater severity of obesity related illnesses (20). This puts the child at an increased risk for heart disease, type 2 diabetes, and cancer in the future compared to children with healthy BMIs (27, 28).

When compared to data from 1999-2000, obesity in youth increased from 13.9%, suggesting that current prevention strategies are not effective (1). The American Academy of Pediatrics provides evidence based prevention strategies that are targeted towards school-age and adolescent children (2). The recommendations do not address prevention strategies that could be implemented during pregnancy, infancy or early childhood, although certain prevention

strategies may be more effective when implemented early in life or even prenatally (57, 58). Maternal factors such as poor nutritional status, excessive GWG, and high blood sugar levels are known to predict childhood obesity (59, 60). Prevention programs that address these factors may contribute to decreasing rates of childhood obesity (57, 61, 62).

Infant Adiposity and Risk of Obesity

Infant adiposity is another risk factor for development of childhood obesity. Multiple studies have examined the relationship between infant growth and risk of obesity in childhood. Two longitudinal studies (14, 15) three prospective cohort studies (16-18) and one cross-sectional study (19) found that greater weight gain and rapid growth in infancy were associated with an increased prevalence of obesity at different ages during childhood. Rapid growth in infancy is linked with obesity beyond childhood and into adolescence and adulthood, though few studies have examined the relationship between infant adiposity and adult obesity (63-66). Results from these studies were largely based on weight-for-length and growth rates during infancy and BMI in childhood and adulthood. It remains unknown whether other markers of infant adiposity such as location of FM, skinfold thickness, and abdominal circumference are associated with obesity in childhood and adulthood.

There are several methods available for measuring infant growth and adiposity. Dual X-ray absorptiometry (DXA) assesses the location of fat as well as bone and muscle mass. Many studies have confirmed the reliability of DXA scans for assessing infant body composition (67-70). However, the majority of these studies used animal models. Furthermore, no reference data exists for healthy infants (71, 72). Even so, DXA is validated for use in pediatric populations.

Another method of body composition analysis in infants is ADP. The Pea Pod® is specifically designed to measure infant body composition by using body weight and volume to calculate body fat. Numerous studies have validated the precision of the Pea Pod® in calculating

infant body composition (73-76). The Pea Pod® cannot assess body composition of infants weighing more than 6 kilograms and like DXA, limited comparative standard data exist.

Infant skinfolds of the thigh, triceps, bicep, suprailiac and subscapular regions can also provide valuable information regarding location of infant fat. Few studies have compared infant skinfold thickness with other validated measures of body composition, but findings suggest that analyzing skinfold thickness is a non-invasive and reliable way to measure infant adiposity (77). The World Health Organization (WHO) provides growth charts for plotting triceps skinfold-for-age for boys and girls from 3 months to 5 years (78). Growth charts and reference ranges for other areas that skinfolds may be completed do not currently exist.

Weight, length, weight-for-length, and head circumference are more commonly used techniques to measure infant growth. Clinicians use the WHO growth standards to track growth of infants and to compare plotted percentiles or z-scores to the general population. However, these methods do not indicate total body composition or FM location.

Definition of Docosahexaenoic Acid and Status During Pregnancy

DHA is an essential polyunsaturated n-3 fatty acid that cannot be synthesized by the body and must be obtained from the diet. The polyunsaturated fatty acids (PUFA) n-3 and n-6 work against each other to stimulate or prevent excess fat deposition in utero. Fetal n-6 exposure increases adipocyte maturation and fat deposition whereas fetal n-3 exposure inhibits adipocyte differentiation, thus preventing excess fat deposition (29, 79). Therefore, it is hypothesized that improved prenatal DHA status through DHA supplementation promotes favorable fat deposition in infancy.

During pregnancy, women should consume 200 mg of DHA per day (80, 81). The 2015-2020 Dietary Guidelines for Americans recommends pregnant women consume 8-12 ounces of seafood per week (82). Pregnant women are encouraged to choose varieties of fatty fish that are

low in mercury content but higher in DHA and EPA such as salmon, trout, herring and sardines (82). However, women are also counseled to avoid fish with high mercury content during pregnancy, which may contribute to a decreased intake for all fish types (83-85). Other food sources of DHA include DHA enriched eggs and DHA fortified foods and beverages. These foods are often deficient in the westernized diet, especially during pregnancy (30, 86). Furthermore, not all prenatal vitamins contain DHA and not all women take a prenatal vitamin consistently (83, 87, 88). For these reasons, it is estimated that pregnant women only consume an average of 60 mg DHA per day (30).

A common method of assessing maternal and infant DHA status is by RBC-PL analysis. This method is validated and measures DHA as a percentage of total fatty acids in a controlled diet and comparing dietary DHA with RBC-PL DHA (89-91). Maternal and infant DHA status is also assessed by diet recalls or food frequency questionnaires, although these methods are less accurate as they are subject to bias and variability of food sources (92). Currently, it is difficult to classify maternal and infant DHA status. Although methods of assessing maternal and infant DHA exist, an optimal level of maternal and infant RBC-PL DHA is not established. In most studies that assessed maternal and infant DHA status, participants were categorized into high- and low- DHA groups based on the RBC-PL DHA group median.

Maternal DHA Status and the Effect on Offspring Body Composition

Because of DHA's proposed role in promoting appropriate fetal fat deposition, numerous studies assessed maternal PUFA status throughout different stages of pregnancy and the effects on offspring body composition in infancy, childhood and early adulthood. Only results specific to DHA will only be reported. Of 8 prospective cohort studies identified, RBC-PL DHA and dietary DHA intake measured maternal DHA status and methods such as DXA, ADP, skinfolds and BMI assessed offspring body composition.

Two studies found that maternal DHA status was negatively related to body composition in infancy. Specifically, Sanz et al. measured maternal and newborn DHA status in relation to total and abdominal fat assessed by DXA (93). Abdominal adiposity was negatively associated with newborn DHA status at 2 weeks, but not at 4 months of age. Similarly, O'Tierney-Ginn et al. also analyzed maternal and fetal PUFA status in relation to infant body composition (94). Length, weight, BMI, skinfold thickness and chest, abdominal, head and arm circumference were assessed at 1 day and 6 months of age. Maternal DHA status was not associated with infant body composition at either time point but infant DHA status was negatively related to skinfold thickness at birth and BMI z-score at 6 months of age. O'Tierney-Ginn et all did not use DXA or ADP to measure body fat so it was unknown whether maternal DHA status was associated with total infant body fat.

In studies that measured offspring body composition during childhood, Donahue et al found that maternal DHA+EPA status mid-pregnancy was negatively related to triceps and subscapular skinfolds and risk of obesity of offspring at 3 years of age (95). However, maternal DHA status was only reported as DHA and eicosapentaenoic acid (EPA) concentrations combined so the role of DHA alone could not be confirmed. Furthermore, Vidakovic et al. measured maternal DHA status mid pregnancy and offspring BMI, FM percentage, and android: gynoid fat ratio between 5 and 9 years old (96). DXA indicated that better maternal DHA status was related to lower %fat and android:gynoid fat ratio of offspring. Android:gynoid fat ratio is of specific concern because a lower ratio suggests that there is less fat stored viscerally. Moon et al measured maternal DHA status in late pregnancy and found an that better maternal DHA status was associated with higher lean-mass of offspring at 4 and 6 years of age as assessed by DXA (97). However, maternal DHA status was not associated with weight or FM of offspring at either time point. Because maternal DHA status was only assessed in late pregnancy, this may be the

reason that no associations were found for other measures of body composition. The studies that observed positive effects of maternal DHA status on offspring body composition and adiposity only measured these variables in infancy and childhood. Therefore, it cannot be confirmed that maternal DHA status impacts offspring body composition into adolescence or adulthood.

Three other studies that were identified found no relationship between maternal DHA status and offspring adiposity. de Vries et al. measured maternal DHA status at 4 timepoints throughout pregnancy but did not find a relationship between weight, waist and hip circumference and skinfold measurements at 7 years of age (98). Other valid measures of adiposity such as DXA or ADP were not used to analyze body composition so it could not be determined if maternal DHA status protected against visceral fat accumulation. Bernard et al. measured offspring body composition from birth to 5 years in relation to maternal DHA status measured mid-pregnancy (99). Maternal DHA status was associated with offspring height from birth to 5 years but not with measures of adiposity as assessed by abdominal circumference and skinfolds. Again, fetal exposure to DHA was only measured in late pregnancy so it is unknown whether maternal DHA status at earlier time points impacted offspring adiposity. Lastly, Stratakis et al. analyzed offspring body composition from birth to early adulthood in relation to DHA status measured from blood taken from the umbilical vein at birth (100). Weight, height and BMI were measured at 9 time points from birth to 23 years of age but no associations between early DHA status and BMI were observed. The population in this study included mothers with lower BMIs at study entry, which may have impacted results due to the link between maternal and offspring BMI. Furthermore, location of FM was not measured so it is unknown whether early DHA status was related to offspring adiposity at the time points measured. Based on the conflicting results from these observational studies, it remains unclear whether maternal DHA status is related to offspring body composition.

Maternal High-Dose DHA Supplementation and Impact on Offspring Adiposity

In efforts to better understand whether maternal DHA status impacts offspring body composition, many studies have assessed the effects of high-dose prenatal DHA supplementation on markers of adiposity. The majority of these studies were randomized, blinded trials and their follow-up studies. Furthermore, the primary aim of many of pilot studies was not offspring adiposity. Even so, results from the follow-up studies provide valuable information on the impact of prenatal DHA supplementation on offspring body composition.

Of 8 studies identified, 3 found that high-dose DHA supplementation was associated with offspring adiposity. In these trials, DHA dosage ranged from 200 mg/day to 800 mg/day. Bergmann et al. supplemented mothers with 200 mg DHA per day and analyzed maternal DHA status at 21 and 37 weeks during pregnancy (101). Offspring growth was measured at birth, 1 month, 3 months and 21 months though weight, length, head circumference and BMI. Maternal DHA supplementation was associated with a lower BMI and weight of offspring at 21 months of age compared to offspring whose mothers did not receive supplementation. These results suggest that risk of childhood obesity was reduced in infants who were exposed to DHA prenatally. Foster et al. provided 800 mg DHA per day starting between 25-29 weeks gestation and measured weight, height, skinfolds, arm circumference and waist circumference of offspring at 2 and 4 years of age (102). Children born to mothers who were supplemented with DHA had lower BMI z-scores compared to children whose mothers were not supplemented, again suggesting that prenatal DHA supplementation may lower risk of childhood obesity. Results from these studies would be more meaningful if other methods had been used to assess offspring body composition such as DXA or ADP. Only one study identified measured offspring adiposity through means of ADP. Hidaka et al. supplemented mothers with 600 mg DHA per day during pregnancy and measured offspring adiposity at 5 years of age with ADP (103). DHA supplementation was

associated with higher FFM of offspring but not with total FM, %fat or BMI. In each of these studies, maternal RBC-PL DHA levels were significantly higher in mothers who received supplementation.

In contrast, 5 of the studies identified did not find a relationship between maternal DHA supplementation and offspring adiposity. Two studies conducted follow-up measurements on offspring of participants in the Impact of Nutritional Fatty Acids During Pregnancy and Lactation on Early Adipose Tissue Development (INFAT) study (104, 105) and two articles published follow-up results from participants in the DHA to Optmise Maternal Infant Outcomes (DOMInO) study (54, 106, 107). One additional study conducted follow-up measurements on participants from a previous different study (108). In studies that did not observe a relationship between prenatal DHA supplementation and offspring body composition, prenatal DHA dosage ranged from 200-1020 mg/day. One study did not report the specific DHA dose provided in the supplement (108).

Hauner et al. and Brei et al. followed offspring of participants in the INFAT study in which mothers were supplemented with 1020 mg DHA starting at 15 weeks of gestation (104, 105). Hauner et al. reported on measures of weight, length, BMI, skinfold thickness and abdominal and preperitoneal fat assessed with ultrasonography at different time points during the first year of life. Brei et al. followed the children from 2 to 5 years of age and assessed the same variables. Additionally, Brei et al measured abdominal adipose tissue through use of MRI in a subgroup of children at 5 years of age. Neither team observed differences in body composition of children born to mothers who received DHA supplementation compared to children born to mothers who were not supplemented. The average pre-pregnancy BMI of mothers who participated in the INFAT study was 22 kg/m^2, which is classified as normal. This may have largely confounded results because the children were genetically less likely to have excess

adiposity. Furthermore, ultrasonography as a means to assess abdominal adiposity is not a validated method. Similarly, Muhlhausler et al and Wood et al. followed children whose mothers participated in the DOMInO study (106, 107). Pregnant mothers were supplemented with 800 mg DHA per day starting in the second half of pregnancy. Mulhausler et al. assessed weight, height, BMI and waist, head and hip circumference of offspring as well as total FM and FFM with bioelectrical impedance spectroscopy (BIA) at 3 and 5 years of age. Wood et al. took the same measurements at 7 years of age. However, ADP was used to measure total FM and FFM instead of BIA. Again, neither group found differences in body composition of offspring born to mothers who were supplemented with DHA compared to offspring whose mothers were not supplemented. A major reason for these null findings could be that maternal DHA status may have already been adequate. The researchers reported that ~70% of pregnant women in the area the study was conducted consumed supplements that contained DHA. If this held true for women who were included in the DOMInO trial, it would be impractical to expect significant results. Additionally, few overweight and obese women were included in the trial, which could further confound results.

Only one study identified assessed the effect maternal PUFA supplementation on offspring adiposity beyond childhood. Rytter et al. followed children born to mothers who were prenatally supplemented with 2.7 g fish-oil starting in the third trimester (108). The specific dose of DHA provided in the supplement was not defined. Height, weight, BMI and waist circumference of offspring were measured at 19 years of age in participants who chose to participate in the lab visit. However, fish oil supplementation during pregnancy did not appear to be associated with these variables. Because of the long follow-up time of this study, attrition rate was large, which may have impacted results. Furthermore, participants who did not participate in

the lab visit were allowed to self-report their height and weight, introducing the possibility of bias.

Due to limitations in the studies presented, it cannot be established that prenatal DHA supplementation improves offspring body composition. Therefore, additional high-quality studies that address these limitations are needed to confirm the proposed relationship.

Maternal DHA Status, Excessive GWG and the Impact on Infant Body Composition

It is evident that a positive relationship exists between excessive GWG and infant adiposity but strategies to attenuate these effects are not well-established. Prenatal DHA supplementation may promote desirable fat distribution in infancy and it is proposed that prenatal DHA supplementation could also improve adiposity of infants born to mothers with excessive GWG. However, few known studies have examined the relationship between maternal DHA status and infant adiposity specific to mothers with excessive GWG. Preliminary evidence from Hull et al. suggests that lower maternal DHA status is related to higher FM in infants born to mothers with excessive GWG. With data lacking, it is important that more studies be conducted to confirm these results.

METHODS

Overview of Parent Study

The Prenatal autonomic Neuro-Developmental Assessment (PANDA) Study is an ongoing double-blind, Phase III randomized controlled trial examining the effects of prenatal DHA supplementation on newborn DHA levels as well as fetal and infant growth and neurodevelopment. Women are enrolled when they are 12-20 weeks pregnant and are randomized to take 200 or 800 mg DHA per day throughout the remainder of the pregnancy. Prenatal visits occur at enrollment, 32 weeks, 36 weeks and birth. Maternal-Fetal magnetocardiography is measured at 32 and 36 weeks gestation. Postnatal visits happen when the infants are 1 month, 4 months, 6 months and 12 months. Infant electroencephalogram occurs when the infant is 1 month, 6 months and 12 months. Infant body composition is measured at 1 month and 4 months. The higher dose of DHA is hypothesized to improve fetal and infant DHA levels and promote favorable growth and neurodevelopment.

Recruitment

Pregnant women were recruited from the University of Kansas Medical Center (KUMC) Obstetrics and Gynecology clinic. PANDA flyers were also displayed in other OBGYN clinics in the Kansas City area. Additionally, the PANDA website was shared with mother and baby groups online. Eligible women were required to be 18 years or older and English speaking. Participants must have agreed to take the DHA capsules throughout the remainder of the pregnancy to participate. Furthermore, participants were required to be available to reach by telephone to be included. Women were excluded if they were underweight (BMI <18.5) or weighed >250 lbs at enrollment. Women who had serious illness, type 1 diabetes or hypertension were also excluded. Women expecting multiple infants or infants with congenital cardiac defects

or brain malformations were not eligible to participate. No minority groups were excluded from participating in this study.

Visits and procedures occurred at 6 time points from enrollment at 12-20 weeks until the infant turned 4 months. Pre-pregnancy visits were at enrollment, 32 weeks, 36 weeks and birth. Postnatal visits occurred when the infant was 1 month and 4 months.

Population

Eligible women were invited to enroll in the study during the second trimester when they were 12-20 weeks pregnant. Enrollment commenced in June 2016 and concluded in May 2018. Demographic information was collected including race, ethnicity, education level and household income. Women were also asked to report their maternal health history and pre-pregnancy and alcohol use and smoking habits during pregnancy. 102 women and their infants who had completed each prenatal visit and each postnatal visit to at least 4 months were included in this data set.

Ethics

This study was approved by the University of Kansas Medical Center Institutional Review Board and all study procedures were ethically conducted in accordance with the principles outlined in the Declaration of Helsinki. All subjects were given written informed consent before any study procedures were performed.

Blood Sample Collection and Analysis

To analyze maternal and infant fatty acid status of participants, 8 mLs of maternal blood were collected by venipuncture at enrollment, 32 weeks and delivery. 8 mLs of cord blood were also collected from the infant at delivery. Samples were immediately placed on ice, centrifuged and then stored at -80 degrees until processing. Maternal and fetal red blood cells (RBC) were analyzed for fatty acid content through gas chromatography. Individual peaks were identified by

comparing to standards (PUFA 1 and PUFA 2; Sigma Aldrich). A weighed standard mixture (Supelco 37 Component FAME mix; Sigma Aldrich) was used to adjust fatty acids for area/weight to calculate a final percentage weight of total fatty acids. RBC-PL DHA was reported as a weight percentage of total fatty acids present in the blood (109).

Gestational Weight Gain

Maternal pre-pregnancy weight was self-reported at the enrollment visit. GWG was calculated by subtracting the maternal pre-pregnancy weight from the last prenatal appointment weight. Excessive GWG was classified according to the mother's pre-pregnancy weight status as determined by her BMI. Excessive GWG was considered weight gain >35 lbs in normal weight women, >25 lbs in overweight women and >20 lbs in obese women.

Infant Body Composition

Infant body composition was assessed at the 1 month and 4 month visits using two-compartment ADP. Specifically, the Pea Pod® (COSMED) was used to measure body volume and density. The Pea Pod® was calibrated before each test using a phantom calibration cylinder with a known volume. The Pea Pod® scale was calibrated at least once every two weeks using a 5000 gram weight.

Prior to testing, infants were undressed to the diaper and length was measured from crown to heel with a length board. The infant was laid on the board with the head touching the head piece and the foot board was placed flush against the child's flexed foot at least twice. A third measurement was performed if first two measurements were not within 10% of each other. Length was recorded to the nearest 0.1 centimeter. The infant was then undressed and a wig cap was fitted on the infant's head. The infant was placed on the Pea Pod® scale to measure body weight to the nearest 0.0001 kilogram. The infant's gender, date of birth, gestational age at birth,

study ID and average length were entered into the Pea Pod® computer system for the two-step calibration to occur before body volume testing.

Once the two-step calibration was complete, the infant was placed inside the Pea Pod® chamber for measurement of body volume. Parents were instructed to not touch the machine during the measurement, which took approximately 2 minutes. After the body volume measurement was complete, body density was converted to percentage of fat (%fat) using gender specific equations so that FM and FFM could be calculated.

Head circumference and abdominal circumference were measured in centimeters at 1 and 4 month visits using a flexible measuring tape. Head circumference was measured by placing the tape smoothly across the frontal bones of the skull and over the occipital prominence, perpendicular to the long axis of the face and above the ears. The tape was tightened around the head at the maximal circumference and two measurements were recorded. If the first two measurements were not within 10% of each other, a third measurement was taken. Abdominal circumference was measured while the infant was in the supine position. The tape was wrapped around the infant's waist at the level of the umbilicus. A second measurement was taken for accuracy and if the first two measurements were not within 10% of each other, a third measurement was taken.

Statistics

Group means and standard deviations were calculated for all maternal and infant descriptive characteristics. ANOVA and Pearson Chi-Square analyzed differences between maternal and infant variables. The change in infant % fat, FM and FFM between the 1 and 4 month visits was also calculated. The change in maternal DHA status was quantified by subtracting the baseline RBC-PL DHA from the 32-week RBC-PL DHA. Groups were then calculated by creating a median split to determine high (>50th percentile) vs. low DHA (≤50th

percentile) status during pregnancy. Four groups were created based on the median split for DHA status and weight gain status (excessive vs. not excessive): prenatal high DHA status with either excessive or not-excessive GWG and prenatal low DHA status with either excessive or not excessive GWG. Analysis of covariance (ANCOVA) examined mean differences in body composition between these four groups. The analysis assessed the main effects of prenatal DHA status (low vs. high) and maternal weight gain (excessive vs. not-excessive) on the change in offspring body composition between 1 and 4 months. The analyses were controlled for the following covariates: GWG, pre-pregnancy BMI, maternal race, maternal alcohol intake, maternal smoking status, infant gender, gestational age at birth, change in maternal n-6:n-3 ratio from baseline to postpartum, and the infant characteristic at 1 month (e.g., % fat, FM, or FFM). An interaction was also assessed between the change in maternal DHA status and GWG category. Microsoft SPSS version 24 was used for all statistical analysis and $p = 0.05$ was considered significant.

RESULTS

Maternal characteristics of participants with data on fatty acids, GWG, and infant body composition at 1 and 4 months are described in table 1a. Characteristics are listed in the tables based on maternal DHA status. One-hundred and one maternal and infant groups had all variables of interest measured. Fifty women had low change in DHA during pregnancy (change <3.34 %DHA) and 51 women had a high change in DHA status during pregnancy (change ≥ 3.35 %DHA). Of the total sample, 83.3% were white, 8.8% were African American, 2.9% were Asian and 4.9% identified as another race. Approximately ten percent of the sample was Hispanic. The average maternal age at enrollment was 29.7 ± 4.4 years. In women with a low change in DHA status, the average pre-pregnancy BMI was 28.7 ± 5.5 kg/m^2, with 33.3% classified as and 39.2% classified as obese. The average pre-pregnancy BMI of women with a high change in DHA status was 24.9 ± 4.7 kg/m^2, with 25.5% classified as overweight and 17.6% classified as obese. Of the total cohort, 62.4% of women gained excessively during pregnancy, which is above the national average of 48%. In women with a low change in DHA status, 68.6% gained excessive weight during pregnancy compared to 54.9% of women with a high change in DHA status. Significant differences were found between groups for pre-pregnancy weight, pre-pregnancy BMI and BMI categories, and last prenatal weight ($p<0.05$). Baseline RBC-PL DHA in women with a low change in DHA status was 6.95 ± 1.6%, which increased by a mean of 1.4 ± 1.2%, compared to a baseline RBC-PL DHA of 6.6 ± 1.5% in women with a high change in DHA status, which increased by a mean of 5.6 ± 1.7%. Postpartum RBC-PL DHA in women with a low change in DHA status was 8.4 ± 1.7%, compared to postpartum RBC-PL DHA of 12.2 ± 2.0% in women with a high change in DHA status. Significant differences were found between groups for 32-week RBC-PL DHA, postpartum RBC-PL DHA, postpartum RBC-PL omega-6, postpartum RBC-PL omega-3, and change in n-3 n-6:n-3 ratio ($p<0.05$).

Infant characteristics divided by maternal DHA status are included in tables 1b-d. The average gestational age of the total sample was 39.2 ± 1.1 weeks. Forty-nine percent of the total sample was male. Infants born to mothers with a low DHA status during pregnancy had a RBC-PL DHA of 9.6 ± 1.7% measured in cord blood, which was significantly lower compared to 11.6 ± 2.0 % in infants born to mothers with a high DHA status during pregnancy ($p<0.001$). No other differences were found between groups for unadjusted infant characteristics.

Table 2 lists the differences for the change in infant body mass and body composition based on maternal DHA status. No differences were found by maternal DHA status for the change in infant body mass or body composition. Table 3 lists the differences for the change in infant body mass and body composition based on maternal GWG status. A difference was found for the change in infant FFM ($p=0.016$). Infants exposed to excessive GWG gained 289 g less of FFM. Confounding variables included for both models were GWG, pre-pregnancy BMI, maternal race, maternal alcohol intake, maternal smoking status, infant gender, gestational age at birth, change in maternal DHA status from baseline to postpartum, maternal baseline DHA, change in maternal n-6:n-3 ratio from baseline to postpartum, infant weight at 1 month (baseline).

Table 4 presents the model to understand the main effects and interactions of maternal DHA status and GWG status on the changes in infant body mass and infant body composition. No significant interactions were detected. Confounding variables included for both models were GWG, pre-pregnancy BMI, maternal race, maternal alcohol intake, maternal smoking status, infant gender, gestational age at birth, change in maternal DHA status from baseline to postpartum, maternal baseline DHA, change in maternal n-6:n-3 ratio from baseline to postpartum, infant weight at 1 month (baseline).

DISCUSSION

While this exploratory analysis did not find a significant interaction between maternal DHA status and infant body composition, excessive GWG was associated with lower change in infant FFM from 1 to 4 months compared to infants born to women who did not gain excessively, as has been previously established (4-6, 8, 40). The association between GWG and the change in infant body mass approached significance in that infants born to mothers who did not gain excessively had a higher change in body mass from 1 to 4 months, likely due to their increased FFM. We did not find differences between groups for change in infant FM or %fat from 1 month to 4 months, which is different from current literature that suggests excessive GWG is associated with increased FM of offspring. In literature, excessive GWG was associated with increased FM of offspring from infancy to adulthood (10, 12, 39, 41-45).

In contrast with many studies, we did not find an association between improved maternal DHA status and markers of offspring body composition. These findings do agree with outcomes measured in other studies. Similar to our study, Bernard et al. did not find an association between maternal DHA status and infant adiposity, as assessed by ADP (99). de Vries et al. measured offspring adiposity and also found that maternal DHA status was not related to offspring adiposity at 7 years of age, as assessed by sum of skinfolds (98). Stratakis et al. followed offspring growth from infancy to young adulthood and found that maternal DHA status did not predict offspring BMI at any time point (100). However, many studies have found an association between maternal DHA status and offspring growth and adiposity. Sanz et al. found that fetal DHA exposure was associated with improved abdominal circumference of infants at 2 weeks of age (93). Similarly, O'Tierney-Ginn et al found that maternal DHA status was negatively associated with skinfold thickness at birth and BMI z-score at 6 months of age (94). In studies that measured growth and adiposity of older offspring, Vidakovic et al. measured offspring

adiposity at 5 and 9 years of age and found that better maternal DHA status was associated with decreased % fat and better android:gynoid fat ratio at both time points (96). Moon et al. also reported that better maternal DHA was correlated with improved lean-mass accumulation of offspring at 4 and 6 years of age as assessed by DXA (97).

This study is one of the first to investigate the interaction of maternal DHA status and GWG with infant body composition. No significant interactions between maternal DHA status and GWG on change in infant body mass or body composition were detected. Although no between groups differences were detected for any markers of infant body composition, higher powered trials may find associations due to the established effect of GWG on offspring body composition, and the hypothesized effect of DHA. Furthermore, studies that supplement DHA prenatally with the primary aim of detecting differences in body composition of offspring may be more likely to find differences. Several researchers have demonstrated the effects prenatal DHA supplementation on offspring growth and adiposity. Bergmann et al. found that growth of offspring born to mothers who were supplemented with 200 mg DHA per day during pregnancy was better than the un-supplemented group, as evidenced by improved BMI of offspring measured at 2 years of age. Foster et al. also studied growth of offspring born to mothers who were supplemented with 800 mg DHA per day and found that offspring had lower BMI z-scores at 2 and 4 years of age. Finally, Hidaka et al. found that mothers who were supplemented with 600 mg DHA per day during pregnancy had offspring with higher FFM at 5 years of age. Due to the relationship between excessive GWG and offspring adiposity, it can be speculated that prenatal DHA supplementation may attenuate these effects by promoting desirable fat and lean-mass distribution in the offspring. However, with data lacking, more studies are needed to observe this possibility. It is crucial that the effect of prenatal DHA supplementation on infant adiposity be further explored, especially in offspring born to mothers with excessive GWG.

There may be potential reasons for null results. In this study, the PeaPod® was used to measure infant body composition at 1 and 4 months. This analysis was nested within an ongoing clinical trial, so it was possible that differences a were not observed because infant adiposity was not measured between birth and one month, where a significant amount of fat and lean mass accumulation occurs. Furthermore, infants were only tracked at two timepoints in early infancy. If offspring growth and adiposity had been followed for a longer period, results may have been more likely to be observed. In this study, maternal baseline RBC-PL DHA status was better compared to other studies, suggesting this population did not have a deficient DHA status at baseline. In this study, mean baseline RBC-PL DHA was 6.95 ± 1.6% in the group that had low change in DHA status and 6.6 ± 1.5% in the group that had high change in DHA status. In three other supplemental DHA studies conducted in the Kansas City area, baseline DHA levels ranged from 4.3-5.0% (109-111). Because DHA status of this cohort was already adequate, the effect of improved maternal DHA status throughout pregnancy would be less likely to improve infant body composition. Further limitations include the relatively small sample size. Only 101 mother-infant pairs could be included. A larger sample size would allow for more power to detect differences between groups. Lastly, because this analysis was nested within an ongoing clinical trial, DHA supplementation compliance could not be controlled for in the analysis. However, the statistical analysis only examined the change in maternal DHA status throughout pregnancy to form a median split rather exploring than the main effects of prenatal DHA supplementation on infant body composition.

This study has many strengths. The blinded nature of this study minimized observer bias. Sampling bias was also minimal due to the study design that required mothers to consent to postnatal visits. Furthermore, the biological marker of RBC-PL DHA and omega-3 were used to assess maternal DHA status. Although dietary omega-3 and DHA intake were not controlled for,

blood status of omega-3 and DHA are the best indicator of intake (112). Use of ADP technology to assess infant body composition allowed for analysis of FM, %fat and FFM. Other studies have used skinfold thickness, which only estimates regional adiposity rather than total body fatness. Furthermore, studies that only measured BMI of offspring in relation to maternal DHA status could not verify location or amount of body fat.

Further research that observes the effects of prenatal DHA supplementation on infant adiposity may help guide DHA recommendations for pregnant women to help promote favorable growth and body composition in offspring, especially for infants born to mothers who gained excessive weight in pregnancy. Studies that measure offspring growth and adiposity from birth and over a longer period could help determine how maternal DHA supplementation impacts offspring fat and lean-mass accumulation throughout childhood. Future research should include genetic and mechanistic studies that examine the impacts of placental fatty acid transporter gene expression to see if these would be related to infant body composition. Soluble receptors for advanced glycation end products (sRAGE) may also determine the effects of DHA status on inflammation. Studies that consider dietary PUFA intake in lactation could contribute to current knowledge regarding DHA as a nutrient that inhibits excess fat accumulation during infancy.

Childhood obesity prevention strategies are currently targeted towards school-age children, but it may be necessary to implement strategies earlier such as in pregnancy. Although this exploratory analysis did not find an association between maternal DHA status and infant body composition, many studies have detected a positive impact of fetal DHA exposure on offspring fat accumulation. DHA may be a nutrient to start early obesity prevention when supplemented throughout pregnancy. Improved maternal DHA status may inhibit excess offspring fat accumulation, thus possibly preventing obesity in childhood and adulthood. These effects may be even more apparent in offspring born to mothers with excessive GWG.

REFERENCES

1. Hales CM, Carroll MD, Fryar CD, Ogden CL. Prevalence of Obesity Among Adults and Youth: United States, 2015-2016. NCHS data brief 2017(288):1-8.
2. Barlow SE. Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. Pediatrics 2007;120 Suppl 4:S164-92. doi: 10.1542/peds.2007-2329C.
3. QuickStats: Gestational Weight Gain Among Women with Full-Term, Singleton Births, Compared with Reccommendations - 48 States and the District of Columbia, 2015. MMWR Mor Mortal Wkly Rep 2016:65:1121. http://dx.doi.org/10.15585/mmwr.mm6540a10.
4. Hull HR, Dinger MK, Knehans AW, Thompson DM, Fields DA. Impact of maternal body mass index on neonate birthweight and body composition. American journal of obstetrics and gynecology 2008;198(4):416.e1-6. doi: 10.1016/j.ajog.2007.10.796.
5. Carlsen EM, Renault KM, Norgaard K, Nilas L, Jensen JE, Hyldstrup L, Michaelsen KF, Cortes D, Pryds O. Newborn regional body composition is influenced by maternal obesity, gestational weight gain and the birthweight standard score. Acta paediatrica (Oslo, Norway : 1992) 2014;103(9):939-45. doi: 10.1111/apa.12713.
6. Sauder KA, Kaar JL, Starling AP, Ringham BM, Glueck DH, Dabelea D. Predictors of Infant Body Composition at 5 Months of Age: The Healthy Start Study. The Journal of pediatrics 2017;183:94-9.e1. doi: 10.1016/j.jpeds.2017.01.014.
7. Starling AP, Brinton JT, Glueck DH, Shapiro AL, Harrod CS, Lynch AM, Siega-Riz AM, Dabelea D. Associations of maternal BMI and gestational weight gain with neonatal adiposity in the Healthy Start study. The American journal of clinical nutrition 2015;101(2):302-9. doi: 10.3945/ajcn.114.094946.
8. Castillo H, Santos IS, Matijasevich A. Relationship between maternal pre-pregnancy body mass index, gestational weight gain and childhood fatness at 6-7 years by air displacement plethysmography. Maternal & child nutrition 2015;11(4):606-17. doi: 10.1111/mcn.12186.
9. Andres A, Hull HR, Shankar K, Casey PH, Cleves MA, Badger TM. Longitudinal body composition of children born to mothers with normal weight, overweight, and obesity. Obesity (Silver Spring, Md) 2015;23(6):1252-8. doi: 10.1002/oby.21078.
10. Dello Russo M, Ahrens W, De Vriendt T, Marild S, Molnar D, Moreno LA, Reeske A, Veidebaum T, Kourides YA, Barba G, et al. Gestational weight gain and adiposity, fat distribution, metabolic profile, and blood pressure in offspring: the IDEFICS project. International journal of obesity (2005) 2013;37(7):914-9. doi: 10.1038/ijo.2013.35.
11. Sridhar SB, Darbinian J, Ehrlich SF, Markman MA, Gunderson EP, Ferrara A, Hedderson MM. Maternal gestational weight gain and offspring risk for childhood overweight or obesity. American journal of obstetrics and gynecology 2014;211(3):259.e1-8. doi: 10.1016/j.ajog.2014.02.030.
12. Ensenauer R, Chmitorz A, Riedel C, Fenske N, Hauner H, Nennstiel-Ratzel U, von Kries R. Effects of suboptimal or excessive gestational weight gain on childhood overweight and abdominal adiposity: results from a retrospective cohort study. International journal of obesity (2005) 2013;37(4):505-12. doi: 10.1038/ijo.2012.226.
13. Mourtakos SP, Tambalis KD, Panagiotakos DB, Antonogeorgos G, Arnaoutis G, Karteroliotis K, Sidossis LS. Maternal lifestyle characteristics during pregnancy, and the

risk of obesity in the offspring: a study of 5,125 children. BMC pregnancy and childbirth 2015;15:66. doi: 10.1186/s12884-015-0498-z.
14. Taveras EM, Rifas-Shiman SL, Sherry B, Oken E, Haines J, Kleinman K, Rich-Edwards JW, Gillman MW. Crossing growth percentiles in infancy and risk of obesity in childhood. Archives of pediatrics & adolescent medicine 2011;165(11):993-8. doi: 10.1001/archpediatrics.2011.167.
15. Gunnarsdottir I, Thorsdottir I. Relationship between growth and feeding in infancy and body mass index at the age of 6 years. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 2003;27(12):1523-7. doi: 10.1038/sj.ijo.0802438.
16. Wang G, Johnson S, Gong Y, Polk S, Divall S, Radovick S, Moon M, Paige D, Hong X, Caruso D, et al. Weight Gain in Infancy and Overweight or Obesity in Childhood across the Gestational Spectrum: a Prospective Birth Cohort Study. Scientific reports 2016;6:29867. doi: 10.1038/srep29867.
17. Taveras EM, Rifas-Shiman SL, Belfort MB, Kleinman KP, Oken E, Gillman MW. Weight status in the first 6 months of life and obesity at 3 years of age. Pediatrics 2009;123(4):1177-83. doi: 10.1542/peds.2008-1149.
18. Stettler N, Zemel BS, Kumanyika S, Stallings VA. Infant weight gain and childhood overweight status in a multicenter, cohort study. Pediatrics 2002;109(2):194-9.
19. Sacco MR, de Castro NP, Euclydes VL, Souza JM, Rondo PH. Birth weight, rapid weight gain in infancy and markers of overweight and obesity in childhood. European journal of clinical nutrition 2013;67(11):1147-53. doi: 10.1038/ejcn.2013.183.
20. Gordon-Larsen P, The NS, Adair LS. Longitudinal trends in obesity in the United States from adolescence to the third decade of life. Obesity (Silver Spring, Md) 2010;18(9):1801-4. doi: 10.1038/oby.2009.451.
21. Freedman DS, Katzmarzyk PT, Dietz WH, Srinivasan SR, Berenson GS. Relation of body mass index and skinfold thicknesses to cardiovascular disease risk factors in children: the Bogalusa Heart Study. The American journal of clinical nutrition 2009;90(1):210-6. doi: 10.3945/ajcn.2009.27525.
22. Cote AT, Harris KC, Panagiotopoulos C, Sandor GG, Devlin AM. Childhood obesity and cardiovascular dysfunction. Journal of the American College of Cardiology 2013;62(15):1309-19. doi: 10.1016/j.jacc.2013.07.042.
23. Bacha F, Gidding SS. Cardiac Abnormalities in Youth with Obesity and Type 2 Diabetes. Current diabetes reports 2016;16(7):62. doi: 10.1007/s11892-016-0750-6.
24. Mohanan S, Tapp H, McWilliams A, Dulin M. Obesity and asthma: pathophysiology and implications for diagnosis and management in primary care. Experimental biology and medicine (Maywood, NJ) 2014;239(11):1531-40. doi: 10.1177/1535370214525302.
25. Pollock NK. Childhood obesity, bone development, and cardiometabolic risk factors. Molecular and cellular endocrinology 2015;410:52-63. doi: 10.1016/j.mce.2015.03.016.
26. Africa JA, Newton KP, Schwimmer JB. Lifestyle Interventions Including Nutrition, Exercise, and Supplements for Nonalcoholic Fatty Liver Disease in Children. Digestive diseases and sciences 2016;61(5):1375-86. doi: 10.1007/s10620-016-4126-1.
27. Jensen MD, Ryan DH, Apovian CM, Ard JD, Comuzzie AG, Donato KA, Hu FB, Hubbard VS, Jakicic JM, Kushner RF, et al. 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The

Obesity Society. Journal of the American College of Cardiology 2014;63(25 Pt B):2985-3023. doi: 10.1016/j.jacc.2013.11.004.
28. Bass R, Eneli I. Severe childhood obesity: an under-recognised and growing health problem. Postgraduate medical journal 2015;91(1081):639-45. doi: 10.1136/postgradmedj-2014-133033.
29. Kim HK, Della-Fera M, Lin J, Baile CA. Docosahexaenoic acid inhibits adipocyte differentiation and induces apoptosis in 3T3-L1 preadipocytes. The Journal of nutrition 2006;136(12):2965-9. doi: 10.1093/jn/136.12.2965.
30. Brenna JT, Lapillonne A. Background paper on fat and fatty acid requirements during pregnancy and lactation. Annals of nutrition & metabolism 2009;55(1-3):97-122. doi: 10.1159/000228998.
31. McClure CK, Catov JM, Ness R, Bodnar LM. Associations between gestational weight gain and BMI, abdominal adiposity, and traditional measures of cardiometabolic risk in mothers 8 y postpartum. The American journal of clinical nutrition 2013;98(5):1218-25. doi: 10.3945/ajcn.112.055772.
32. Al Mamun A, Mannan M, O'Callaghan MJ, Williams GM, Najman JM, Callaway LK. Association between gestational weight gain and postpartum diabetes: evidence from a community based large cohort study. PloS one 2013;8(12):e75679. doi: 10.1371/journal.pone.0075679.
33. Macdonald-Wallis C, Tilling K, Fraser A, Nelson SM, Lawlor DA. Gestational weight gain as a risk factor for hypertensive disorders of pregnancy. American journal of obstetrics and gynecology 2013;209(4):327.e1-17. doi: 10.1016/j.ajog.2013.05.042.
34. Institute of M, National Research Council Committee to Reexamine IOMPWG. The National Academies Collection: Reports funded by National Institutes of Health. Edtion ed. In: Rasmussen KM, Yaktine AL, eds. Weight Gain During Pregnancy: Reexamining the Guidelines. Washington (DC): National Academies Press (US)
National Academy of Sciences., 2009.
35. Olson CM, Strawderman MS. Modifiable behavioral factors in a biopsychosocial model predict inadequate and excessive gestational weight gain. Journal of the American Dietetic Association 2003;103(1):48-54. doi: 10.1053/jada.2003.50001.
36. Stuebe AM, Oken E, Gillman MW. Associations of diet and physical activity during pregnancy with risk for excessive gestational weight gain. American journal of obstetrics and gynecology 2009;201(1):58.e1-8. doi: 10.1016/j.ajog.2009.02.025.
37. Gaillard R, Durmus B, Hofman A, Mackenbach JP, Steegers EA, Jaddoe VW. Risk factors and outcomes of maternal obesity and excessive weight gain during pregnancy. Obesity (Silver Spring, Md) 2013;21(5):1046-55. doi: 10.1002/oby.20088.
38. Huynh M, Borrell LN, Chambers EC. Maternal education and excessive gestational weight gain in New York city, 1999-2001: the effect of race/ethnicity and neighborhood socioeconomic status. Maternal and child health journal 2014;18(1):138-45. doi: 10.1007/s10995-013-1246-5.
39. Jacota M, Forhan A, Saldanha-Gomes C, Charles MA, Heude B. Maternal weight prior and during pregnancy and offspring's BMI and adiposity at 5-6 years in the EDEN mother-child cohort. Pediatric obesity 2017;12(4):320-9. doi: 10.1111/ijpo.12145.
40. Hull HR, Thornton JC, Ji Y, Paley C, Rosenn B, Mathews P, Navder K, Yu A, Dorsey K, Gallagher D. Higher infant body fat with excessive gestational weight gain in overweight women. American journal of obstetrics and gynecology 2011;205(3):211.e1-7. doi: 10.1016/j.ajog.2011.04.004.

41. Crozier SR, Inskip HM, Godfrey KM, Cooper C, Harvey NC, Cole ZA, Robinson SM. Weight gain in pregnancy and childhood body composition: findings from the Southampton Women's Survey. The American journal of clinical nutrition 2010;91(6):1745-51. doi: 10.3945/ajcn.2009.29128.
42. Kaar JL, Crume T, Brinton JT, Bischoff KJ, McDuffie R, Dabelea D. Maternal obesity, gestational weight gain, and offspring adiposity: the exploring perinatal outcomes among children study. The Journal of pediatrics 2014;165(3):509-15. doi: 10.1016/j.jpeds.2014.05.050.
43. Badon SE, Dyer AR, Josefson JL. Gestational weight gain and neonatal adiposity in the Hyperglycemia and Adverse Pregnancy Outcome study-North American region. Obesity (Silver Spring, Md) 2014;22(7):1731-8. doi: 10.1002/oby.20742.
44. Oken E, Rifas-Shiman SL, Field AE, Frazier AL, Gillman MW. Maternal gestational weight gain and offspring weight in adolescence. Obstetrics and gynecology 2008;112(5):999-1006. doi: 10.1097/AOG.0b013e31818a5d50.
45. Reynolds RM, Osmond C, Phillips DI, Godfrey KM. Maternal BMI, parity, and pregnancy weight gain: influences on offspring adiposity in young adulthood. The Journal of clinical endocrinology and metabolism 2010;95(12):5365-9. doi: 10.1210/jc.2010-0697.
46. Barker DJ. The developmental origins of adult disease. Journal of the American College of Nutrition 2004;23(6 Suppl):588s-95s.
47. Gluckman PD, Hanson MA, Beedle AS. Early life events and their consequences for later disease: a life history and evolutionary perspective. American journal of human biology : the official journal of the Human Biology Council 2007;19(1):1-19. doi: 10.1002/ajhb.20590.
48. Shankar K, Harrell A, Liu X, Gilchrist JM, Ronis MJ, Badger TM. Maternal obesity at conception programs obesity in the offspring. American journal of physiology Regulatory, integrative and comparative physiology 2008;294(2):R528-38. doi: 10.1152/ajpregu.00316.2007.
49. Catalano PM, Presley L, Minium J, Hauguel-de Mouzon S. Fetuses of obese mothers develop insulin resistance in utero. Diabetes care 2009;32(6):1076-80. doi: 10.2337/dc08-2077.
50. Samuelsson AM, Matthews PA, Argenton M, Christie MR, McConnell JM, Jansen EH, Piersma AH, Ozanne SE, Twinn DF, Remacle C, et al. Diet-induced obesity in female mice leads to offspring hyperphagia, adiposity, hypertension, and insulin resistance: a novel murine model of developmental programming. Hypertension (Dallas, Tex : 1979) 2008;51(2):383-92. doi: 10.1161/hypertensionaha.107.101477.
51. Han J, Xu J, Epstein PN, Liu YQ. Long-term effect of maternal obesity on pancreatic beta cells of offspring: reduced beta cell adaptation to high glucose and high-fat diet challenges in adult female mouse offspring. Diabetologia 2005;48(9):1810-8. doi: 10.1007/s00125-005-1854-8.
52. Murabayashi N, Sugiyama T, Zhang L, Kamimoto Y, Umekawa T, Ma N, Sagawa N. Maternal high-fat diets cause insulin resistance through inflammatory changes in fetal adipose tissue. European journal of obstetrics, gynecology, and reproductive biology 2013;169(1):39-44. doi: 10.1016/j.ejogrb.2013.02.003.
53. Yan X, Zhu MJ, Xu W, Tong JF, Ford SP, Nathanielsz PW, Du M. Up-regulation of Toll-like receptor 4/nuclear factor-kappaB signaling is associated with enhanced

adipogenesis and insulin resistance in fetal skeletal muscle of obese sheep at late gestation. Endocrinology 2010;151(1):380-7. doi: 10.1210/en.2009-0849.
54. Muhlhausler BS, Duffield JA, McMillen IC. Increased maternal nutrition stimulates peroxisome proliferator activated receptor-gamma, adiponectin, and leptin messenger ribonucleic acid expression in adipose tissue before birth. Endocrinology 2007;148(2):878-85. doi: 10.1210/en.2006-1115.
55. Yang QY, Liang JF, Rogers CJ, Zhao JX, Zhu MJ, Du M. Maternal obesity induces epigenetic modifications to facilitate Zfp423 expression and enhance adipogenic differentiation in fetal mice. Diabetes 2013;62(11):3727-35. doi: 10.2337/db13-0433.
56. Howie GJ, Sloboda DM, Kamal T, Vickers MH. Maternal nutritional history predicts obesity in adult offspring independent of postnatal diet. The Journal of physiology 2009;587(Pt 4):905-15. doi: 10.1113/jphysiol.2008.163477.
57. Gregory EF, Goldshore MA, Henderson JL, Weatherford RD, Showell NN. Infant Growth following Maternal Participation in a Gestational Weight Management Intervention. Childhood obesity (Print) 2016;12(3):219-25. doi: 10.1089/chi.2015.0238.
58. Mameli C, Mazzantini S, Zuccotti GV. Nutrition in the First 1000 Days: The Origin of Childhood Obesity. International journal of environmental research and public health 2016;13(9). doi: 10.3390/ijerph13090838.
59. Li S, Zhu Y, Yeung E, Chavarro JE, Yuan C, Field AE, Missmer SA, Mills JL, Hu FB, Zhang C. Offspring risk of obesity in childhood, adolescence and adulthood in relation to gestational diabetes mellitus: a sex-specific association. International journal of epidemiology 2017;46(5):1533-41. doi: 10.1093/ije/dyx151.
60. Hivert MF, Rifas-Shiman SL, Gillman MW, Oken E. Greater early and mid-pregnancy gestational weight gains are associated with excess adiposity in mid-childhood. Obesity (Silver Spring, Md) 2016;24(7):1546-53. doi: 10.1002/oby.21511.
61. Robinson SM, Crozier SR, Harvey NC, Barton BD, Law CM, Godfrey KM, Cooper C, Inskip HM. Modifiable early-life risk factors for childhood adiposity and overweight: an analysis of their combined impact and potential for prevention. The American journal of clinical nutrition 2015;101(2):368-75. doi: 10.3945/ajcn.114.094268.
62. Patel N, Godfrey KM, Pasupathy D, Levin J, Flynn AC, Hayes L, Briley AL, Bell R, Lawlor DA, Oteng-Ntim E, et al. Infant adiposity following a randomised controlled trial of a behavioural intervention in obese pregnancy. International journal of obesity (2005) 2017;41(7):1018-26. doi: 10.1038/ijo.2017.44.
63. Stettler N, Kumanyika SK, Katz SH, Zemel BS, Stallings VA. Rapid weight gain during infancy and obesity in young adulthood in a cohort of African Americans. The American journal of clinical nutrition 2003;77(6):1374-8. doi: 10.1093/ajcn/77.6.1374.
64. Tienboon P, Wahlqvist ML. A prospective study of weight and height going from infancy to adolescence. Asia Pacific journal of clinical nutrition 2002;11(1):42-7.
65. Monteiro PO, Victora CG, Barros FC, Monteiro LM. Birth size, early childhood growth, and adolescent obesity in a Brazilian birth cohort. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 2003;27(10):1274-82. doi: 10.1038/sj.ijo.0802409.
66. Charney E, Goodman HC, McBride M, Lyon B, Pratt R. Childhood antecedents of adult obesity. Do chubby infants become obese adults? The New England journal of medicine 1976;295(1):6-9. doi: 10.1056/nejm197607012950102.

67. Fields DA, Gunatilake R, Kalaitzoglou E. Air displacement plethysmography: cradle to grave. Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition 2015;30(2):219-26. doi: 10.1177/0884533615572443.
68. Godang K, Qvigstad E, Voldner N, Isaksen GA, Froslie KF, Notthellen J, Henriksen T, Bollerslev J. Assessing body composition in healthy newborn infants: reliability of dual-energy x-ray absorptiometry. Journal of clinical densitometry : the official journal of the International Society for Clinical Densitometry 2010;13(2):151-60. doi: 10.1016/j.jocd.2010.01.121.
69. Koo WW, Hammami M, Hockman EM. Validation of bone mass and body composition measurements in small subjects with pencil beam dual energy X-ray absorptiometry. Journal of the American College of Nutrition 2004;23(1):79-84.
70. Henche SA, Torres RR, Pellico LG. An evaluation of patterns of change in total and regional body fat mass in healthy Spanish subjects using dual-energy X-ray absorptiometry (DXA). European journal of clinical nutrition 2008;62(12):1440-8. doi: 10.1038/sj.ejcn.1602883.
71. Fomon SJ, Haschke F, Ziegler EE, Nelson SE. Body composition of reference children from birth to age 10 years. The American journal of clinical nutrition 1982;35(5 Suppl):1169-75. doi: 10.1093/ajcn/35.5.1169.
72. Butte NF, Hopkinson JM, Wong WW, Smith EO, Ellis KJ. Body composition during the first 2 years of life: an updated reference. Pediatric research 2000;47(5):578-85.
73. Ellis KJ, Yao M, Shypailo RJ, Urlando A, Wong WW, Heird WC. Body-composition assessment in infancy: air-displacement plethysmography compared with a reference 4-compartment model. The American journal of clinical nutrition 2007;85(1):90-5. doi: 10.1093/ajcn/85.1.90.
74. Eriksson B, Lof M, Forsum E. Body composition in full-term healthy infants measured with air displacement plethysmography at 1 and 12 weeks of age. Acta paediatrica (Oslo, Norway : 1992) 2010;99(4):563-8. doi: 10.1111/j.1651-2227.2009.01665.x.
75. Urlando A, Dempster P, Aitkens S. A new air displacement plethysmograph for the measurement of body composition in infants. Pediatric research 2003;53(3):486-92. doi: 10.1203/01.Pdr.0000049669.74793.E3.
76. Ma G, Yao M, Liu Y, Lin A, Zou H, Urlando A, Wong WW, Nommsen-Rivers L, Dewey KG. Validation of a new pediatric air-displacement plethysmograph for assessing body composition in infants. The American journal of clinical nutrition 2004;79(4):653-60. doi: 10.1093/ajcn/79.4.653.
77. Schmelzle HR, Fusch C. Body fat in neonates and young infants: validation of skinfold thickness versus dual-energy X-ray absorptiometry. The American journal of clinical nutrition 2002;76(5):1096-100. doi: 10.1093/ajcn/76.5.1096.
78. WHO Child Growth Standards based on length/height, weight and age. Acta paediatrica (Oslo, Norway : 1992) Supplement 2006;450:76-85.
79. Korotkova M, Gabrielsson B, Lonn M, Hanson LA, Strandvik B. Leptin levels in rat offspring are modified by the ratio of linoleic to alpha-linolenic acid in the maternal diet. Journal of lipid research 2002;43(10):1743-9.
80. Fats and fatty acids in human nutrition. Report of an expert consultation. FAO food and nutrition paper 2010;91:1-166.
81. Koletzko B, Lien E, Agostoni C, Bohles H, Campoy C, Cetin I, Decsi T, Dudenhausen JW, Dupont C, Forsyth S, et al. The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus

recommendations. Journal of perinatal medicine 2008;36(1):5-14. doi: 10.1515/jpm.2008.001.
82. U.S. Department of Health and Human Services and U.S. Department of Agriculture 2015-2020 Dietary Guidelines for Americans. 8th Edition. December 2015. Available at https://health.gov/dietaryguidelines/2015/guidelines/.
83. Nochera CL, Goossen LH, Brutus AR, Cristales M, Eastman B. Consumption of DHA + EPA by low-income women during pregnancy and lactation. Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition 2011;26(4):445-50. doi: 10.1177/0884533611406133.
84. Vardeman JE, Aldoory L. A qualitative study of how women make meaning of contradictory media messages about the risks of eating fish. Health communication 2008;23(3):282-91. doi: 10.1080/10410230802056396.
85. Oken E, Kleinman KP, Berland WE, Simon SR, Rich-Edwards JW, Gillman MW. Decline in fish consumption among pregnant women after a national mercury advisory. Obstetrics and gynecology 2003;102(2):346-51.
86. Troxell H, Anderson J, Auld G, Marx N, Harris M, Reece M, Allen K. Omega-3 for baby and me: material development for a WIC intervention to increase DHA intake during pregnancy. Maternal and child health journal 2005;9(2):189-97. doi: 10.1007/s10995-005-4908-0.
87. Branum AM, Bailey R, Singer BJ. Dietary supplement use and folate status during pregnancy in the United States. The Journal of nutrition 2013;143(4):486-92. doi: 10.3945/jn.112.169987.
88. Scholl TO, Hediger ML, Bendich A, Schall JI, Smith WK, Krueger PM. Use of multivitamin/mineral prenatal supplements: influence on the outcome of pregnancy. American journal of epidemiology 1997;146(2):134-41.
89. Ma J, Folsom AR, Shahar E, Eckfeldt JH. Plasma fatty acid composition as an indicator of habitual dietary fat intake in middle-aged adults. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. The American journal of clinical nutrition 1995;62(3):564-71. doi: 10.1093/ajcn/62.3.564.
90. Vidgren HM, Agren JJ, Schwab U, Rissanen T, Hanninen O, Uusitupa MI. Incorporation of n-3 fatty acids into plasma lipid fractions, and erythrocyte membranes and platelets during dietary supplementation with fish, fish oil, and docosahexaenoic acid-rich oil among healthy young men. Lipids 1997;32(7):697-705.
91. Arterburn LM, Hall EB, Oken H. Distribution, interconversion, and dose response of n-3 fatty acids in humans. The American journal of clinical nutrition 2006;83(6 Suppl):1467s-76s. doi: 10.1093/ajcn/83.6.1467S.
92. Kuratko CN, Salem N, Jr. Biomarkers of DHA status. Prostaglandins, leukotrienes, and essential fatty acids 2009;81(2-3):111-8. doi: 10.1016/j.plefa.2009.05.007.
93. Sanz N, Diaz M, Lopez-Bermejo A, Sierra C, Fernandez A, de Zegher F, Ibanez L. Newborns with lower levels of circulating polyunsaturated fatty acids (PUFA) are abdominally more adipose. Pediatric obesity 2014;9(3):e68-72. doi: 10.1111/j.2047-6310.2013.00200.x.
94. O'Tierney-Ginn PF, Davina D, Gillingham M, Barker DJP, Morris C, Thornburg KL. Neonatal fatty acid profiles are correlated with infant growth measures at 6 months. Journal of developmental origins of health and disease 2017;8(4):474-82. doi: 10.1017/s2040174417000150.

95. Donahue SM, Rifas-Shiman SL, Gold DR, Jouni ZE, Gillman MW, Oken E. Prenatal fatty acid status and child adiposity at age 3 y: results from a US pregnancy cohort. The American journal of clinical nutrition 2011;93(4):780-8. doi: 10.3945/ajcn.110.005801.
96. Vidakovic AJ, Gishti O, Voortman T, Felix JF, Williams MA, Hofman A, Demmelmair H, Koletzko B, Tiemeier H, Jaddoe VW, et al. Maternal plasma PUFA concentrations during pregnancy and childhood adiposity: the Generation R Study. The American journal of clinical nutrition 2016;103(4):1017-25. doi: 10.3945/ajcn.115.112847.
97. Moon RJ, Harvey NC, Robinson SM, Ntani G, Davies JH, Inskip HM, Godfrey KM, Dennison EM, Calder PC, Cooper C. Maternal plasma polyunsaturated fatty acid status in late pregnancy is associated with offspring body composition in childhood. The Journal of clinical endocrinology and metabolism 2013;98(1):299-307. doi: 10.1210/jc.2012-2482.
98. de Vries PS, Gielen M, Rizopoulos D, Rump P, Godschalk R, Hornstra G, Zeegers MP. Association between polyunsaturated fatty acid concentrations in maternal plasma phospholipids during pregnancy and offspring adiposity at age 7: the MEFAB cohort. Prostaglandins, leukotrienes, and essential fatty acids 2014;91(3):81-5. doi: 10.1016/j.plefa.2014.04.002.
99. Bernard JY, Tint MT, Aris IM, Chen LW, Quah PL, Tan KH, Yeo GS, Fortier MV, Yap F, Shek L, et al. Maternal plasma phosphatidylcholine polyunsaturated fatty acids during pregnancy and offspring growth and adiposity. Prostaglandins, leukotrienes, and essential fatty acids 2017;121:21-9. doi: 10.1016/j.plefa.2017.05.006.
100. Stratakis N, Gielen M, Margetaki K, Godschalk RW, van der Wurff I, Rouschop S, Ibrahim A, Antoniou E, Chatzi L, de Groot RHM, et al. Polyunsaturated fatty acid levels at birth and child-to-adult growth: Results from the MEFAB cohort. Prostaglandins, leukotrienes, and essential fatty acids 2017;126:72-8. doi: 10.1016/j.plefa.2017.09.004.
101. Lucia Bergmann R, Bergmann KE, Haschke-Becher E, Richter R, Dudenhausen JW, Barclay D, Haschke F. Does maternal docosahexaenoic acid supplementation during pregnancy and lactation lower BMI in late infancy? Journal of perinatal medicine 2007;35(4):295-300. doi: 10.1515/jpm.2007.085.
102. Foster BA, Escaname E, Powell TL, Larsen B, Siddiqui SK, Menchaca J, Aquino C, Ramamurthy R, Hale DE. Randomized Controlled Trial of DHA Supplementation during Pregnancy: Child Adiposity Outcomes. Nutrients 2017;9(6). doi: 10.3390/nu9060566.
103. Hidaka BH, Thodosoff JM, Kerling EH, Hull HR, Colombo J, Carlson SE. Intrauterine DHA exposure and child body composition at 5 y: exploratory analysis of a randomized controlled trial of prenatal DHA supplementation. The American journal of clinical nutrition 2018;107(1):35-42. doi: 10.1093/ajcn/nqx007.
104. Hauner H, Much D, Vollhardt C, Brunner S, Schmid D, Sedlmeier EM, Heimberg E, Schuster T, Zimmermann A, Schneider KT, et al. Effect of reducing the n-6:n-3 long-chain PUFA ratio during pregnancy and lactation on infant adipose tissue growth within the first year of life: an open-label randomized controlled trial. The American journal of clinical nutrition 2012;95(2):383-94. doi: 10.3945/ajcn.111.022590.
105. Brei C, Stecher L, Much D, Karla MT, Amann-Gassner U, Shen J, Ganter C, Karampinos DC, Brunner S, Hauner H. Reduction of the n-6:n-3 long-chain PUFA ratio during pregnancy and lactation on offspring body composition: follow-up results from a randomized controlled trial up to 5 y of age. The American journal of clinical nutrition 2016;103(6):1472-81. doi: 10.3945/ajcn.115.128520.

106. Wood K, Mantzioris E, Lingwood B, Couper J, Makrides M, Gibson RA, Muhlhausler BS. The effect of maternal DHA supplementation on body fat mass in children at 7 years: follow-up of the DOMInO randomized controlled trial. Prostaglandins, leukotrienes, and essential fatty acids 2017. doi: 10.1016/j.plefa.2017.09.013.
107. Muhlhausler BS, Yelland LN, McDermott R, Tapsell L, McPhee A, Gibson RA, Makrides M. DHA supplementation during pregnancy does not reduce BMI or body fat mass in children: follow-up of the DHA to Optimize Mother Infant Outcome randomized controlled trial. The American journal of clinical nutrition 2016;103(6):1489-96. doi: 10.3945/ajcn.115.126714.
108. Rytter D, Bech BH, Christensen JH, Schmidt EB, Henriksen TB, Olsen SF. Intake of fish oil during pregnancy and adiposity in 19-y-old offspring: follow-up on a randomized controlled trial. The American journal of clinical nutrition 2011;94(3):701-8. doi: 10.3945/ajcn.111.014969.
109. Carlson SE, Colombo J, Gajewski BJ, Gustafson KM, Mundy D, Yeast J, Georgieff MK, Markley LA, Kerling EH, Shaddy DJ. DHA supplementation and pregnancy outcomes. The American journal of clinical nutrition. 2013;97(4):808-15. doi:10.3945/ajcn.112.050021.
110. Smuts CM, Huang M, Mundy D, Plasse T, Major S, Carlson SE. A randomized trial of docosahexaenoic acid supplementation during the third trimester of pregnancy. Obstetrics and gynecology. 2003;101(3):469-79.
111. Gustafson KM, Carlson SE, Colombo J, Yeh HW, Shaddy DJ, Li S, Kerling EH. Effects of docosahexaenoic acid supplementation during pregnancy on fetal heart rate and variability: a randomized clinical trial. Prostaglandins, leukotrienes, and essential fatty acids. 2013;88(5):331-8. doi:10.1016/j.plefa.2013.01.009.
112. Browning LM, Walker CG, Mander AP, West AL, Madden J, Gambell JM, Young S, Wang L, Jebb SA, Calder PC. Incorporation of eicosapentaenoic and docosahexaenoic acids into lipid pools when given as supplements providing doses equivalent to typical intakes of oily fish. The American journal of clinical nutrition 2012;96(4):748-58. doi:10.3945/ajcn.112.041343.

APPENDIX

Table 1a: Maternal Characteristics shown by DHA status

	N	Low Change in DHA Status	N	High Change in DHA Status
Age at Enrollment	51	29.1 ± 4.7	51	30.2 ± 4.2
Race (%)	51		51	
White		40 (78.4)		45 (88.2)
Black		6 (11.8)		3 (5.9)
Asian		2 (3.9)		1 (2.0)
Other		3 (5.9)		2 (3.9)
Ethnicity (%)	51		51	
Non-Hispanic		46 (90.2)		46 (90.2)
Hispanic		5 (9.8)		5 (9.8)
Annual Income (%)	51		51	
<$10k		1 (2.0)		0 (0)
$10-15k		2 (3.9)		0 (0)
$15-25k		5 (9.8)		1 (2.0)
$25-50k		6 (11.8)		9 (17.6)
$50k-100k		20 (39.2)		21 (41.2)
$100k-150k		10 (19.6)		12 (23.5)
$150k-200k		6 (11.8)		2 (3.9)
>$200k		1 (2.0)		6 (11.8)
Education Level (%)	51		51	
High School or Less		3 (5.9)		5 (9.8)
Post-Secondary to less than graduate		34 (66.7)		30 (58.8)
Graduate degree or more		14 (27.5)		16 (31.4)
Smoked in Pregnancy (%)	51		51	
Yes		5 (9.8)		1 (2.0)
No		46 (90.2)		50 (98.0)
Alcohol in Pregnancy (%)	51		51	
Yes		7 (13.7)		4 (7.8)
No		44 (86.3)		47 (92.2)
Pre-pregnancy weight (kg)	51	77.8 ± 14.1	51	68.0 ± 13.5*
Height (cm)	51	164.7 ± 5.1	51	165.0 ± 6.1
Pre-pregnancy BMI (kg/m^2)	51	28.7 ± 5.5	51	24.9 ± 4.7*
Normal n (%)		14 (27.5)		29 (56.9)*
Overweight n (%)		17 (33.3)		13 (25.5)*
Obese n (%)		20 (39.2)		9 (17.6)*
Last Prenatal Weight (kg)	50	93.7 ± 14.4	51	83.0 ± 14.2*
Gestational Weight Gain (kg)	50	16.1 ± 6.1	51	15.0 ± 5.8
Gained Excessively	50		51	
Excessive n (%)		35 (68.6)		28 (54.9)
Not Excessive n (%)		15 (29.4)		23 (45.1)
Baseline RBC DHA %	51	6.95 ± 1.6	51	6.6 ± 1.5

32 week RBC DHA %	47	8.6 ± 1.7	49	11.6 ± 2.0*
Postpartum RBC DHA %	51	8.4 ± 1.7	51	12.2 ± 2.0*
Change in RBC DHA % Baseline to Postpartum	51	1.4 ± 1.2	51	5.6 ±1.7*
Baseline RBC n-3	51	9.9 ± 2.0	51	9.5 ±1.7
Postpartum RBC n-3	51	10.7 ± 1.8	51	14.3 ±2.0*
Baseline RBC n-6	51	40.8 ± 3.4	51	39.8 ± 2.9
Postpartum RBC n-6	51	38.8 ± 3.1	51	36.8 ± 2.7*
Baseline RBC n-6:n-3	51	4.3 ± 0.9	51	4.3 ± 0.9
Postpartum RBC n-6:n-3	51	3.7 ± 0.8	51	2.6 ± 0.5*

Values are presented as unadjusted means ± standard deviations.
***Denotes significant difference from the low change in DHA status group ($p \leq 0.05$)**
RBC DHA% = red blood cell docosahexaenoic acid as a percent of total red blood cell fatty acids; RBC n-3= total omega-3 fatty acid of red blood cell; RBC n-6 = total omega-6 fatty acid content of red blood cell; RBC n-6:n-3 = ratio of omega-6 to omega-3 fatty acids in red blood

Table 1b: Birth characteristics by DHA status

		Low Change in DHA Status		**High Change in DHA Status**
	N		N	
Gender n (%) **Male** **Female**	51	 22 (43.1) 29 (56.9	51	 28 (54.9) 23 (45.1)
Gestational Age	51	39.4 ± 1.0	51	39.1 ± 1.1
Birth Weight (g)	51	3334.39 ± 474.95	51	3330.71 ± 409.97
Birth Length (cm)	49	50.4 ± 2.5	51	50.6 ± 2.1
Birth Head Circumference (cm)	50	34.5 ± 1.2	49	34.2 ± 1.3
Cord Blood RBC DHA %	51	9.6 ± 1.7	51	11.6 ± 2.0*
Cord Blood RBC n-3	51	10.7 ± 1.8	51	12.7 ± 2.2
Cord Blood RBC n-6	51	39.6 ± 3.0	51	38.9 ± 2.7
Cord Blood RBC n-6:n-3	51	3.8 ± 0.7	51	3.2 ± 0.6
Values are presented as unadjusted means ± standard deviations. ***Denotes significant difference from the low change in DHA status group ($p \leq 0.05$)**				

Table 1c: Infant characteristics at 1 month by DHA status

		Low Change in DHA Status		**High Change in DHA Status**
	N		N	
Age (wks)	51	5.1 ± 1.3	51	5.3 ± 1.1
Length (cm)	51	54.3 ± 2.1	51	54.1 ± 2.0
Head Circumference (cm)	51	37.6 ± 1.0	51	37.6 ± 1.1
Abdominal Circumference (cm)	50	36.2 ± 2.2	51	36.6 ± 2.0
Body weight (g)	51	4269.9 ± 454.0	51	4385.2 ± 511.2
Percentage body fat (%fat)	51	17.5 ± 4.8	51	18.7 ± 4.5
FM (g)	51	759.7 ± 255.1	51	829.5 ± 258.6
FFM (g)	51	3510.2 ± 310.5	51	3555.8 ± 363.5
Values are presented as unadjusted means ± standard deviations.				

Table 1d: Infant characteristics at 4 months by DHA status

		Low Change in DHA Status		**High Change in DHA Status**
	N		N	
Age (wks)	51	18.0 ± 1.1	51	18.4 ± 2.5
Length (cm)	51	62.5 ±2.2	51	63.4 ± 2.1*
Head Circumference (cm)	51	41.6 ± 1.2	51	41.2 ± 1.2
Abdominal Circumference (cm)	51	40.6 ±2.4	50	40.7 ± 2.4
Body Weight (g)	51	6495.4 ± 761.4	51	6559.0 ± 778.5
Percentage body fat (%fat)	51	25.1 ± 4.9	51	25.9 ± 5.4
Fat mass (g)	51	1639.1 ± 413.8	51	1733.3 ± 509.8
Fat-free mass (g)	51	4856.3 ± 582.3	51	4884.3 ± 457.4
Values are presented as unadjusted means ± standard deviations. ***Denotes significant difference from the low change in DHA status group ($p \leq 0.05$)**				

Table 2: Change in infant body composition from 1 month to 4 months of age based on the change in maternal DHA status

DHA Status	Δ Body mass (g)	P value	Δ Percentage body fat (% fat)	P value	Δ Fat mass (g)	P value	Δ Fat-free mass (g)	P value
Low Change in DHA Status N=50	2139.0 ± 827.3	0.468	21.1 ± 6.2	0.973	843.0 ± 544.5	0.512	1290.0 ± 530.3	0.496
High Change in DHA Status N=51	2287.0 ± 821.3		21.2 ± 6.1		931.0 ± 535.6		1380.0 ± 528.5	
Difference	-149.0		-0.052		-88.0		-90.0	

Values are presented as adjusted means ± standard deviations.
Covariates included in the model: gestational weight gain, pre-pregnancy BMI, maternal race, maternal alcohol intake, maternal smoking status, infant gender, gestational age at birth, change in maternal DHA status from baseline to postpartum, maternal baseline DHA, change in maternal n-6:n-3 ratio from baseline to postpartum, infant weight at 1 month (baseline)

Table 3: Change in infant body composition from 1 month to 4 months of age based on maternal weight gain

GWG Status	Δ Body mass (g)	P value	Δ Percentage body fat (% fat)	P value	Δ Fat mass (g)	P value	Δ Fat-free mass (g)	P value
Not Excessive N=38	2429 ± 795.2	0.069	21.3 ± 6.0	0.848	933.0 ± 524.0	0.559	1516.0 ± 505.5	0.016
Excessive N=63	2084 ± 706.4		21.1 ± 5.3		860.0 ± 468.3		1227.0 ± 452.4	
Difference	345.0		0.27		72.0		289.0	

Values are presented as adjusted means ± standard deviations.
Covariates included in the model: gestational weight gain, pre-pregnancy BMI, maternal race, maternal alcohol intake, maternal smoking status, infant gender, gestational age at birth, change in maternal DHA status from baseline to postpartum, maternal baseline DHA, change in maternal n-6:n-3 ratio from baseline to postpartum, infant weight at 1 month (baseline)

Table 4: Change in infant body composition from 1 month to 4 months of age based on maternal weight gain and DHA status.

Infant Variable		Weight gain status				P value for interaction
	DHA Status	Not Excessive	Difference in change: High - low	Excessive	Difference in change: High - low	
Δ Body mass (g)	Low Change in DHA Status	2276.0 ± 720.4	285.0 gm	2033.0 ± 846.0	-88.0 gm	0.417
	High Change in DHA Status	2561.0 ± 844.1		2121.0 ± 751.4		
Δ Percentage body fat (% fat)	Low Change in DHA Status	20.9 ± 5.5	0.7%	21.2 ± 6.5	-0.3%	0.579
	High Change in DHA Status	21.6 ± 6.4		20.9 ± 5.6		
Δ Fat mass (g)	Low Change in DHA Status	850.0 ± 480.2	155.0 gm	829.0 ± 567.9	55 gm	0.535
	High Change in DHA Status	1005.0 ± 561.1		884.0 ± 492.1		
Δ Fat-free mass (g)	Low Change in DHA Status	1419.0 ± 460.9	179.0 gm	1196.0 ± 538.4	54 gm	0.411
	High Change in DHA Status	1598.0 ± 532.3		1250.0 ± 470.9		
<50th %ile/Not Excessive: N=15; ≥ 50th %ile/Not Excessive: N=23 <50th %ile/Excessive: N=35; ≥ 50th %ile/Excessive: N=28						

Values are presented as adjusted means ± standard deviations.

Covariates included in the model: gestational weight gain, pre-pregnancy BMI, maternal race, maternal alcohol intake, maternal smoking status, infant gender, gestational age at birth, change in maternal DHA status from baseline to postpartum, maternal baseline DHA, change in maternal n-6:n-3 ratio from baseline to postpartum, infant weight at 1 month (baseline)

Informed Consent

RESEARCH CONSENT FORM

Prenatal Docosahexaenoic Acid (DHA) & Neurofunctional Development (PANDA Study)
Sponsor: National Institute of Child Health and Development R01 HD086001

Kathleen M. Gustafson, PhD
University of Kansas Medical Center
913-588-0065

INTRODUCTION

You are being asked to join a research study. You are being asked to take part in this study because you are a pregnant woman who is between 12 and 20 weeks of gestation. You do not have to participate in this research study. The main purpose of research is to create new knowledge for the benefit of future patients and society in general. Research studies may or may not benefit the people who participate.

Research is voluntary, and you may change your mind at any time. There will be no penalty to you if you decide not to participate, or if you start the study and decide to stop early. Either way, you can still get medical care and services at the University of Kansas Medical Center (KUMC).

This consent form explains what you have to do if you are in the study. It also describes the possible risks and benefits. Please read the form carefully and ask as many questions as you need to, before deciding about this research.

You can ask questions now or anytime during the study. The researchers will tell you if they receive any new information that might cause you to change your mind about participating.

This research study will take place at the University of Kansas Medical Center with Kathleen M. Gustafson, PhD as the researcher. About 340 women will be in the study at KUMC.

BACKGROUND

Docosahexaenoic acid (DHA) is an essential nutrient. Our bodies make DHA from the foods we eat. If we eat foods with a lot of DHA, like fatty ocean fish, we have more DHA in our bodies. DHA is found in all cells of the body but is especially high in nerve cells of the brain and eye. Babies get DHA from the mother when they're in the womb. After birth, they can get DHA from breast milk or infant formulas. Many studies have shown that DHA in the infant's diet may help vision development, attention, and ability to learn. DHA may also be important before babies are born, while the nervous system is developing.

We studied a small number of women who took DHA supplements during their pregnancy and found that babies were still not getting enough. If a woman has enough DHA in her body, her baby will have the same amount, or slightly less than she does. Research suggests that when babies don't get enough DHA, it can limit their development. This is why we are asking you to take part in a study that will provide DHA to all women, at two different doses. Current over-the-counter DHA capsules for pregnant women range from about 50 mg to 200 mg per day. We don't know what the best dose is and we don't know how the foods women eat alter how much DHA is available to the baby.

PURPOSE

By doing this study, we hope to learn how much DHA to give to mothers in order to provide enough to the baby. We also want to learn if there are differences in fetal development at 32 and 36 weeks. After your baby is born, we want to learn if there are differences in infant body composition, how they pay attention in different sounds and sights and how their brain functions the first year of life.

Since we don't know what dose is best or how mother's diet affects how much DHA a baby gets, we are testing two different doses of DHA supplements (200 mg or 800 mg) and are asking what women eat before and during their pregnancy. This will help us find out what dose is best for the developing baby.

PROCEDURES

If you are eligible and decide to participate in this study, your participation will last from the time you enroll (12-20 weeks of pregnancy) until your baby is 12 months old. If you choose to enroll in this study, the investigators will get some information from your medical record about your pregnancy and medical history and will continue to check your medical records throughout your pregnancy. They will also ask you questions about foods that you usually eat, alcohol intake, smoking history, and your demographics (such as race and ethnicity, education, household income). You will be asked for a phone number where you can be reached during the day. Below is a description of study procedures..

SCHEDULE OF EVENTS	BEFORE BIRTH VISITS			AFTER BIRTH VISITS				
Study Procedure	Enroll 12-20 wks	32 wks	36 wks	Birth	1 mo	4 mo	6 mo	12 mo
Informed Consent and Capsule Assignment	●							
Maternal Blood Samples	●	●		●				
Diet History Questionnaire (DHQ-II)	●	●						
Maternal-Fetal MCGs, Ultrasound		●	●					
Record Birth Weight, Length				●				
Newborn Cord Blood				●				
Infant EEG					●		●	●
PeaPod (infant body composition)					●	●		
Infant Visual Attention Tasks						●	●	
Infant Still Face Procedure (Optional)							●	
Infant Temperament Questionnaire (Optional)							●	
Maternal Height	●							
Maternal Weight and Blood Pressure	●	●	●	●				● (weight only)
Estimated Time for Each Study Visit	1.5 hrs	1.5 hrs	1 hr	0	1.5 hrs	1 hr	1.5 hrs	1.5 hrs
Subject Compensation	$50	$75	$50		$75	$50	$75	$75

DHA Capsule Assignment

You will be randomly assigned (like flipping a coin) to capsules with DHA-oil of either 200mg or

800 mg per day. You will take these capsules as long as you are pregnant.

Following DHA capsule assignment, you will be asked to not take any additional DHA from other dietary supplements. You should continue to take any other vitamin and mineral supplements that do not contain DHA as recommended by your doctor.

You will be given enough capsules each month to take 4 capsules each day. If you consume all 4 capsules, you will be getting either 200 mg or 800 mg of DHA. You do not need to take the capsules at any specific time of day. You do not have to take all 4 at once, but you should take 4 each day.

Neither you nor the people in the research study will know which capsules you have been assigned. 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 capsules), you will receive another bottle of capsules in the mail. AT THAT TIME, WE WILL ASK THAT YOU PLACE THE FIRST BOTTLE WITH ANY REMAINING CAPSULES IN THE POSTAGE-PAID ENVELOPE AND PUT IT INTO THE MAIL. You will get a letter each month that explains what to do.

This process will be repeated each month until your baby is born and you will continue to take 4 capsules per day until your baby is born.

The investigators will contact you by phone at least once per month. They will ask about how you are doing on the capsules and how many you are taking each day. 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 913-588-3140 AND LEAVING A MESSAGE.

BEFORE BIRTH VISITS

MCG/Ultrasound Measurement- (32 weeks and 36 weeks of pregnancy)

The heart naturally sends out an electrical signal, we can record it with a special machine. The machine we use is called a biomagnetometer (MCG). We will record your heart rate and the heart rate of your baby using the MCG at 32 and 36 weeks of pregnancy.

You should eat and drink a normal meal 1 ½ hours prior to testing. This is done to increase the chance of seeing your baby move during the testing. We will record your body weight, heart rate, and blood pressure before the MCG recording.

We will ask you to change into scrubs (a cotton pullover top and slacks with a loose waistband) and remove any jewelry. You should wear a sports bra or something similar with no underwire for the MCG recording. After you change you will be taken to a testing room and asked to sit in an adjustable, reclining chair designed to support pregnant women. Every effort will be made to make you comfortable in the chair. You should not proceed with testing if you are uncomfortable. Prior to starting the MCG, we will do an ultrasound examination to help position the machine. We will get a few basic measurements that will tell us how big your baby is and how it is lying in your womb. The biomagnetometer will be positioned to lightly touch your abdomen. After this is

completed we will leave the room and close the door. Someone may stay in the room with you if you like. We will be able to see you with a video camera. We can hear you and talk to you through a sound system. We will come in at any time if you become tired or uncomfortable.

When the testing begins, we will ask you to hold still for a few minutes. We will first try to find the signals coming from your baby's heart. We do not expect the MCG recording to take longer than 45 minutes to an hour.

Collection of Blood Samples – (Enrollment, 32 weeks and after delivery)
Blood samples will be collected from your arm when you sign up for the study, at your 32 week visit and on the morning after your baby is born. We will draw about 4.5 teaspoons at enrollment and 1.5 teaspoons of blood at all other times. The blood will be used to measure DHA in your blood as well as other nutrients. In addition, we will test for genes that control how a person makes DHA in their body. This will be described in a separate consent. Some people have a gene that can make DHA well and some do not. This difference in genes can make a difference on how much DHA is in the blood sample when people are taking the same supplements and will help us interpret the study results. Any remaining sample will be stored for possible analysis of other nutrients and indicators in the blood that may help us understand how environmental exposures may influence pregnancy or to predict pregnancy outcomes (for example preterm labor). These are not clinical tests but are part of the research study so that we may learn how DHA affects these indicators in the blood. Your baby's blood sample (1.5 teaspoons) will be taken from the umbilical cord after your baby is born.

Diet Survey- (Enrollment and at 32 weeks of pregnancy)
We will ask you to fill out a diet survey (DHQ-II) at enrollment and at the 32 week visit. The survey can be completed online or by a paper copy. The survey asks about the food you normally eat and what types of supplements you might use. We will also give you a short survey asking what types of fish you might eat. The surveys can be completed in about 30-45 minutes, however, you can take as much time as you like. You will be compensated $25 for completing the diet survey at enrollment and the diet survey at 32 weeks.

DELIVERY
At your 36 week visit, we will give you a phone number to call when you are admitted to have your baby, to let the study team know you are about to deliver. Your baby's blood sample, taken from the umbilical cord at birth, will be given to the investigators. A hospital nurse will also draw a small blood sample (1.5 teaspoons) from you while you are in the hospital.

AFTER BIRTH VISITS
Pea Pod – Infant Body Composition (1 month and 4 month)
At the 1 month and 4 month visits, we will ask if you are breast-feeding or formula feeding your baby. To measure body composition, the infant will be placed on its back in a special incubator called a Pea Pod. The amount of volume (space) occupied by the infant will be measured and used to calculate lean mass (muscle) and total body fat. The staff and parent are able to monitor the child during the test through the transparent top. Weight, length, head circumference, abdominal circumference and crown-rump length will be measured. The test takes 15 to 20 minutes to perform and will take place at the Hoglund Brain Imaging Center.

The study will pay for all study-related research services provided during this study. These services include the capsules, and all of the research measures described in this consent form.

FOR NON-UNIVERSITY OF KANSAS HOSPITAL PATIENTS:
Any medical visits or procedures you have outside of the study due to other standard of care treatments or health issues are billable to you or your insurance through normal billing practices. Standard of care means necessary for the care of a medical issue as determined by your doctor and not necessary for this study.

FOR UNIVERSITY Of KANSAS HOSPITAL PATIENTS:
Your insurance may not cover some or all of the standard care services if you are part of a research study. You may want to talk to your insurance company and review your specific benefits and coverage before deciding to participate. You will be responsible for normal co-pays, deductibles and non-covered services that are not the responsibility of the study. Some procedures require Pre-Certification from your insurance company. Pre-Certification is not a guarantee of payment.

You can still be in the study even if your insurance denies coverage for your standard of care treatment or if you are uninsured. The hospital has a financial assistance program which it makes available to all patients who qualify. If your insurance denies coverage and you do not qualify for the financial assistance, you will be charged for all bills that are not the responsibility of the study. The study staff will be able to provide more information to you.

PAYMENT TO SUBJECTS
You will be given a ClinCard, which works like a debit card. You will receive $50 when you enroll in the study ($25 for the blood draw and history plus $25 after completing the diet survey). You will receive $50 each for the shorter 36 week and 4 month visit. You will receive $50 for the 32 week visit plus $25 for completing the 32 week diet survey for a total of $75. You will receive $75 for each of the longer 1 month, 6 month and 12 month visits for a total of up to $450. If you do not complete all study visits then you will only receive payment for the study visits you have completed. This is to cover the costs of transportation and to partially compensate you for the time required to participate in the study.

After a study visit, payment will be added onto your card by computer. The money will be available within 1 business day. You can use the ClinCard at an ATM or a store. No one at KUMC will know where you spend the money. You will be given one card during the study. If your card is lost or stolen, please call (866) 952-3795.

The KUMC Research Institute will be given your name, address, social security number, and the title of this study to allow them issue a ClinCard for your study payments. Study payments are taxable income. A Form 1099 will be sent to you and to the Internal Revenue Service if your study payments are $600 or more in a calendar year.

IN THE EVENT OF INJURY
If you are harmed or experience other problems during this study, you should seek appropriate medical care and contact Dr. Gustafson at 913-588-0065 or Dr. Christifano at 913-588-3140. If

it is after 5:00 p.m., a holiday or a weekend, you should call Dr. Gustafson at 913-703-6525

If you have a bodily injury as a result of participating in this study, treatment will be provided for you at the usual charge. Treatment may include first aid, emergency care and follow-up care, as needed. Claims will be submitted to your health insurance policy, your government program, or other third party, but you will be billed for the costs that are not covered by the insurance. You do not give up legal rights by signing this form.

INSTITUTIONAL DISCLAIMER STATEMENT

If you think you have been harmed as a result of participating in research at the University of Kansas Medical Center (KUMC), you should contact the Director, Human Research Protection Program, Mail Stop #1032, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160. Under certain conditions, Kansas state law or the Kansas Tort Claims Act may allow for payment to persons who are injured in research at KUMC.

CONFIDENTIALITY AND PRIVACY AUTHORIZATION

The researchers will protect your information, as required by law. Absolute confidentiality cannot be guaranteed because persons outside the study team may need to look at your study records. The researchers may publish the results of the study. If they do, they will only discuss group results. Your name will not be used in any publication or presentation about the study. Your health information is protected by a federal privacy law called HIPAA. By signing this consent form, you are giving permission for KUMC to use and share your health information. If you decide not to sign the form, you cannot be in the study.

The researchers will only use and share information that is needed for the study. To do the study, they will collect health information from the study activities and from your medical records. You may be identified by information such as name, address, phone, date of birth, social security number, or other identifiers. Your health information will be used at KU Medical Center by Dr. Gustafson, members of the research team, the KUMC Research Institute, the KUMC Human Subjects Committee and other committees and offices that review and monitor research studies. Study records might be reviewed by government officials who oversee research, if a regulatory review takes place.

All study data that is sent outside KU Medical Center will have your name and other identifying characteristics removed, so that your identity will not be known. Because identifiers will be removed, your study data will not be re-disclosed by outside persons or groups and will not lose its federal privacy protection.

Your permission to use and share your health information will not expire unless you cancel it.

Certificate of Confidentiality

This research is covered by a Certificate of Confidentiality from the National Institutes of Health (NIH). This protects the researchers from being forced to give out personal information about you in response to a court order. This does not stop you from voluntarily releasing information about yourself or your participation in this research.

One exception to the Certificate is if you agree that we can give out research information with your name on it. This includes any purposes described in this consent form.

Other exceptions are information we must report if we learn about child abuse or neglect or if we think you might harm yourself or others.

QUESTIONS

Before you sign this form, Dr. Gustafson or other members of the study team should answer all your questions. You can talk to the researchers if you have any more questions, suggestions, concerns or complaints after signing this form. If you have any questions about your rights as a research subject, or if you want to talk with someone who is not involved in the study, you may call the Human Subjects Committee at (913) 588-1240. You may also write the Human Subjects Committee at Mail Stop #1032, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160.

A description of this clinical trial is available on ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this website at any time.

SUBJECT RIGHTS AND WITHDRAWAL FROM THE STUDY

You may stop being in the study at any time. Your decision to stop will not prevent you from getting treatment or services at KUMC. The entire study may be discontinued for any reason without your consent by the investigator conducting the study.

You have the right to cancel your permission for researchers to use your health information. If you want to cancel your permission, please write to Kathleen M. Gustafson, PhD. The mailing address is Kathleen M. Gustafson, PhD University of Kansas Medical Center, Hoglund Brain Imaging Center, MS 1052, 3901 Rainbow Boulevard, Kansas City, KS 66160. If you decide to withdraw from the study then we may ask permission to collect you and your baby's health information at delivery. If you cancel permission to use your health information, you will be withdrawn from the study. The research team will stop collecting any additional information about you. The research team may use and share information that was gathered before they received your written cancellation.

CONSENT

Dr. Gustafson or the research team has given you information about this research study. They have explained what will be done and how long it will take. They explained any inconvenience, discomfort or risks that may be experienced during this study.

By signing this form, you say that you freely and voluntarily consent to participate in this research study. You have read the information and had your questions answered.
You will be given a signed copy of the consent 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

In the future, we may want to contact you because you took part in this study. You can initial the statement below to let us know whether or not you would be interested in participating in future studies:

I am interested in being contacted by Dr. Gustafson or her colleagues to receive information about participating in future studies (please initial one) Yes_____ No_____

OPTIONAL GENETIC RESEARCH CONSENT

Purpose
You are being asked to allow us to use some of your blood and the cord blood (your baby's blood) to study genes that are related to the status of DHA or other nutrients.

The cells in your body contain deoxyribonucleic acid, or DNA for short. DNA is passed down from your parents. It carries the genes that determine how you look and how your body works. Differences in genes may help explain why some people have trouble making DHA.

The study of DNA is called genetic research. Your entire genetic makeup will not be determined from this testing. Your DNA will only be used for research to understand your requirement for DHA or other nutrients.

By studying these samples, researchers hope to learn if some individuals need to consume more DHA than others because their body cannot make DHA as well.

What is involved?
You will not be required to do anything more than you agreed to in the consent form for the primary study. If the sample of blood we took is too small, we could ask your permission to swab cells from the inside of your or your child's mouth.

How will information about me be kept private?

- Samples will be stored in a freezer in a locked room. The samples will be labeled with your participant ID, date sample was obtained, and study visit. The samples maybe stored for up to 10 years.
- KUMC will keep the list that links the participant ID to your name separate from your sample and information.
- Qualified researchers can submit a request to use the stored samples. A committee will review each request. There will also be an ethics review to ensure that the study is necessary and proper. Researchers will not be given your name or any other information that could identify you.
- You may withdraw your consent to use the remaining samples and associated health information at any time by contacting Dr. Kathleen Gustafson at 913-588-0065 or the research nurse, Danielle Christifano at 913-588-3140. In this case, the sample will be destroyed. Samples or related information that have already been used by researchers cannot be returned or destroyed.
- The information about the uses and disclosures of your health information for the main study also applies to this and future research.
- Reports about research done with your samples will not be given to you or your doctor. These reports will not be put into your medical record. The research will not have an effect on your care.
- Your samples will only be used for research purposes.

If results are published, your name and other personal information will not be given.

What are possible risks?

If a swab of cells from your or your child's mouth is required there is a very slight risk of scrapes or bruising of the inside of the cheek. Study staff will be careful when collecting the cheek cell samples to minimize this risk.

The main risk of this optional research is possible loss of privacy and confidentiality. We will take reasonable precaution to reduce this risk.

There is a small risk that if people other than the researchers were given your genetic information, they could misuse them. If genetic information was given to employers or insurers it could affect your ability to get a job or be insured. Misuse could cause problems for family members. To minimize these risks, your genetic information will be kept confidential as discussed in this form.

GINA

A federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways:

- Health insurance companies and group health plans may not request your genetic information that we get from this research.
- Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums.
- Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment. The GINA protections do not help you if you work for a company with less than 15 employees.

Be aware that this federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

This study has safeguards to protect your confidential genetic information. It is extremely unlikely that your identity could be connected to results of current or future genetic studies. However, it is possible that this information could be discovered by someone who is not authorized to have access to it.

Research methods are rapidly changing. In the future, researchers may develop methods that allow your samples to be linked back to you.

If a commercial product is developed from this research, the profits will belong to the study sponsor. There are no plans to provide financial payment to you should this occur.

Consent

The choice to participate in the genetic research is completely voluntary. You can decide not to have your samples used and still participate in the main study. Please mark your choice "Yes" or "No" below. If you have any questions you can talk to the investigator or the study team.

You give permission that your blood, your baby's cord blood, and/or cheek swab samples may be used for genetic research as described above.

☐YES ☐NO

_____
Print Participant's Name

_____ _____ _____
Signature of Participant Time Date

_____
Print Name of Person Obtaining Consent

_____ _____
Signature of Person Obtaining Consent Date

OPTIONAL STILL FACE PROCEDURE CONSENT

You have enrolled in the University of Kansas PANDA Study (HSC#00003792) where we hope to learn how much DHA to give to mothers in order to provide enough to the baby and how DHA affects infant development. As part of the study, we are interested in learning how DHA affects infant temperament. To learn about your baby's temperament, we have a tool called the "Still Face" procedure. If you participate, you will be asked to place your infant in a car seat or carrier at the end of the 6 month visit. Three small foam tabs with wires will remain on your infant's abdomen to record heart beat while they take the test. To begin the test, you will be asked to play with your child as you normally would, without toys, for two minutes. You will then be asked to maintain a blank, neutral expression ("Still Face") and to not touch or interact with your baby for two minutes. You will be asked to complete five series of play and still face, each lasting two minutes, for a total of 10 minutes. The study team will use a stopwatch and let you know when to play and when to use a still face. You will also be asked to complete a questionnaire about your baby's temperament. To complete the Still Face procedure and infant temperament questionnaire it will take you about 30 minutes. There are no known risks for completing the Still Face procedure or temperament questionnaire. All of the data we collect will be kept confidential just as we have with all your personal information. If you decide to participate this will not change the financial compensation you receive. Research is voluntary. If you decide not to participate, you still can participate from all other parts of PANDA study (HSC #00003792) and you can still receive care at the University of Kansas Medical Center.

CONSENT
If you agree to participate in the Still Face procedure and temperament questions, please check the "yes" box; if you do not agree, please check the "no" box. A signed copy of this consent form will give to you to keep for your records.

☐YES ☐NO

_____
Print Participant's Name

_____ _____ _____
Signature of Participant Time Date

_____
Print Name of Person Obtaining Consent

_____ _____
Signature of Person Obtaining Consent Date