Cover Page for Protocol

Official study title:	Growth and Adiposity in Newborns: The Influence of Prenatal DHA Supplementation
NCT number:	NCT03310983
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Summary of Protocol Changes

Page 15: Extension of target visit window
Justification: 46 study visits are projected to occur between March 16, 2020 to May 16, 2020. Due to COVID-19 participant contact restrictions, we will not be able to collect growth measurements for these visits unless the target visit window is extended.

Page 16: Remote data collection
Justification: In normal circumstances, the questionnaires are administered in person at the study visit. If a parent is not able to attend the visit, or if world events prevent the study visit from happening on time, a member of the study team will call the parent to obtain this data as close to the visit target date as possible. The study team will ensure that both parents have been consented prior to obtaining this data. Note: This protocol addition shall remain in effect after the COVID-19 restrictions are lifted.

SPECIFIC AIMS

Approximately 55% of US pregnant women have excessive gestational weight gain (GWG), and their offspring have greater total fat mass (FM) at birth compared to women who gain appropriately. Infant FM is directly linked to childhood FM, and to overweight and obesity, a characteristic of ~33% of US children. Childhood overweight, obesity, and central FM are linked to cardiovascular disease and type 2 diabetes in adulthood; and their prevalence has led to the prediction of a generational decline in life expectancy. Our working hypothesis is that the stress of excessive GWG on the developing fetus programs obesity risk. **Our long-term goal** is to understand if improving nutrition can protect against higher fetal adipose tissue (AT) accumulation in pregnancies with excessive GWG. **Our immediate goal is** to test the hypothesis that the nutrient, docosahexaenoic acid (DHA), generally deficient in US women, can mitigate fetal AT accumulation in pregnancies with excessive GWG. In order to achieve this goal, we propose to make efficient use of a cohort of infants born to women enrolled in an RCT (R01 HD083292, ClinicalTrials.gov ID: NCT02626299) to test the effect of an ecologically valid amount of DHA (200 mg/d, an amount similar to that found in many prenatal supplements) and 1000 mg/d (an amount predicted to result in a more optimal DHA status) on reduction of birth before 34 weeks.

Our central hypothesis is that the higher DHA supplement provided in the parent RCT will protect against excessive FM accumulation in the first 2 years of life when compared to the lower DHA supplement in offspring whose mothers have excessive GWG. Observational studies associate greater maternal DHA levels with lower offspring FM at 3-7 years of age; and lower visceral AT in 5-7 year olds. However, data are lacking from RCT prenatally supplementing DHA and directly measuring infant body composition and AT distribution. Our preliminary data from another cohort of pregnant women found that FM increased more (1499 g vs 1026 g, p=0.038) in just the first 3 months in infants exposed to excessive GWG if their mothers had a DHA status below compared to above the median at the time of delivery (mean RBC DHA 3.8 vs. 6.4% of total fatty acids). We take this as preliminary evidence that low maternal DHA status combined with excessive GWG during pregnancy leads to higher fat accumulation in the offspring after birth, because DHA status was unrelated to change in FM in the offspring of women with appropriate GWG.

Our project objective is to enroll n=360 subjects from the Kansas City cohort of NCT02626299 and follow their body composition and AT distribution using dual energy x-ray absorptiometry (DXA) at 2 weeks and 6, 12 and 24 months. Only 1 RCT has studied FM as the primary aim and reached a null conclusion, however, DXA was not used as proposed here, the only precise technique for evaluating amount and location of body fat. Data on maternal weight status and GWG as well as other details of the pregnancy will be shared by PIs of the parent trial, Carlson and Gajewski. Both are co-Is on this ancillary proposal; *see section C for details*). Our specific aims are:

***Aim 1*: To determine how the dose of DHA (1000 mg vs 200 mg/day) during pregnancy interacts with GWG (excessive vs appropriate) to influence total infant FM at 24 months of age**. We propose a novel nutritional approach to test the effect of DHA on infant FM. In the parent trial, pregnant women are randomized to daily capsules that provide 1000 or 200 mg DHA/day. All of these pregnant women will experience GWG, some at the appropriate level and some at the excessive level. Offspring exposed to excessive GWG have greater FM than offspring exposed to appropriate GWG. We believe there is an opportunity for DHA to influence FM in infants exposed to excessive GWG. Preliminary data suggest that DHA may protect against excess infant FM. We will test if the mean difference in total infant FM at 24

months of age is greater in infants exposed to excessive GWG and high vs. low DHA compared to infants exposed to appropriate GWG and high vs. low DHA.

***Aim 2*: To explore how dose of DHA (1000 mg vs 200 mg/day) may interact during pregnancy to influence central FM at 24 months of age**. We will explore the interaction with variables including GWG and gender.

Impact: This data will address a gap in knowledge about how improving maternal DHA status might mitigate excess offspring fat accumulation in response to the stress of excessive GWG. The results of this proposal could lead to evidence-based guidelines for DHA requirement in pregnancy.

RESEARCH STRATEGY

A) **SIGNIFICANCE**

A1) **Solutions are needed**: The obesity statistics stabilized over the last decade but remain high with a detrimental health impact. Currently 35% of adults are overweight and 35% are obese and 17% of 2-19 year olds are obese[1]. Maternal obesity is related to greater central FM in newborns[2,3]. FM location in adults[4] and children[5,6] is an important driver of disease risk and development. Centrally located fat, especially visceral adipose tissue (AT), in adults is greater in individuals with type 2 diabetes when compared to those without diabetes[7] whereas AT located in the femoral-gluteal region (hips and legs) is cardioprotective[8]. Interventions to prevent the evolution of obesity, including improvements in nutrient intake that result in a cardioprotective AT pattern are desperately needed.

A2) **Early steps in obesity development:** A growing body of literature links the prenatal period to offspring obesity development and disease risk. Overall, 55% of women gain excessive gestational weight[9,10]. Excessive gestational weight gain (GWG) is associated with higher infant birth weight[11,12], increased infant fat mass (FM)[9,13] and increased FM in childhood[14,15] and adulthood[16]. Research shows a strong relationship between maternal excessive GWG and offspring obesity development[17,18], diabetes, and cardiovascular disease[19,20]. Infant FM is directly linked to childhood FM[14]. Overweight and obesity in childhood are related to the development of metabolic syndrome, diabetes, and cardiovascular disease that track from childhood into adulthood[21]. With the current child and adult obesity epidemics, it is critical to understand if improving low DHA status in US pregnant women can favorably influence FM accumulation and adipose tissue (AT) distribution as these are important drivers of obesity occurrence, disease risk, and severity of disease development. Interventions to blunt the impact of excessive GWG on the offspring phenotype are needed.

A3) **Promoting a healthy start**: Ideally, women would enter pregnancy at an appropriate body weight. However pre-conception interventions to encourage appropriate weight at the time of pregnancy have yet to be tested, proven viable, or successful. Primary care physicians rarely counsel patients on weight loss, diet, and exercise because they do not have adequate resources and feel ineffective[22]. Pregnancy is a time when many women are more likely to adopt healthy behaviors for the well-being of their baby[23]. Several behavioral lifestyle interventions have been tested to prevent excessive GWG with varying success. Four meta-analyses[24-27] and five systematic reviews[25,28-31] show limited success. For interventions including diet and physical activity (PA), three[32-34] found improvement in rate of adherence to IOM guidelines while ten[35-44] did not see improvements. Studies compared outcomes by BMI groups and found they were more likely to be successful in normal weight compared to overweight/obese participants[45,46], however, women who are overweight/obese are more likely to gain excessively than normal weight women[9,10]. We need effective interventions with high compliance and adherence that provide a meaningful improvement in outcomes for the mother and her offspring for all pregnancies with excess GWG. Dr. Carlson has achieved high compliance in her prior prenatal DHA supplementation RCT trials, including her most recent NICHD trial[47]. A prenatal supplement, if effective in modifying FM and AT distribution, could be an ideal intervention method.

A4) **Importance of PUFAs**: Early nutrition is a modifiable behavior that can protect or promote disease development[48]. Two essential polyunsaturated fatty acids (PUFA) are related in opposing ways to promote or prevent obesity development: α-linolenic acid (ALA, 18:3n-3 or omega-3) and linoleic acid (LA. 18:2n-6 or omega-6) are the precursors for long chain polyunsaturated fatty acids (LCPUFA) of the n-3 and n-6 fatty acid families. The difference in their chemical structure is a single double bond that cannot be inserted by mammalian

desaturases. These two 18 carbon fatty acids compete for the enzymes required for their elongation and desaturation to n-3 and n-6 LCPUFA with 20 and 22 carbons and multiple double bonds[49]. In addition, they and their fatty acid products compete for incorporation into plasma lipid fractions and cell membranes and have very different effects on the development of obesity[50-53].

A5) **Effects of DHA**: Docosahexaenoic acid (DHA) is an LCPUFA member of the n-3 fatty acid family synthesized from ALA by elongation and desaturation. DHA is required for optimal offspring nervous system development and cognitive function[47,54]. In adults, DHA has anti-inflammatory effects and is beneficial for treatment of inflammatory diseases[55-57]. Additionally, PUFA are hypothesized to influence fetal growth and AT deposition. Increased prenatal exposure to n-6 fatty acids is thought to promote adipocyte maturation while increased exposure to DHA prevents adipocyte maturation[58-61]. Rats supplemented prenatally with n-3 PUFA had offspring with lower body weight and adipose tissue levels when compared to offspring exposed prenatally to a maternal diet high in n-6 PUFA or low in n-3 PUFA[58,59]. It would thus appear that the n-6 to n-3 balance of fatty acids is an early programmer of body composition. Limited human data are available, especially from RCT, however, it is thought that the general number of adipocytes is set sometime in childhood and remains stable during adulthood[62].

A6) **DHA intake during pregnancy in US**: On average, US women consume ~60 mg DHA/day[63] and synthesize little DHA from ALA (18:3n-3) they consume in other foods[64,65]. DHA is found in animal foods with the richest sources being certain varieties of ocean fish[66]. DHA intake among US women is lower than other Western populations[63]. Two commonly used indicators of DHA status: 1) red blood cell phospholipid (RBC-PL) DHA as a percent of total membrane fatty acids[47,67] and 2) human milk DHA as a percent of total fatty acids[68] are lower in US women than in other developed countries. For example, baseline RBC-PL-DHA means ranged from 4.3-5.0 % in Dr. Carlson's last 3 Kansas City pregnancy cohorts[47,69,70] compared to greater than 6% RBC-PL-DHA reported by others[71-73].

A7) **DHA and offspring body composition**: Observational studies have measured maternal DHA status during pregnancy and looked for differences in body composition and AT deposition in the offspring. Sanz et al.[74] measured maternal red blood cell (RBC) DHA at 2-days post-partum and assessed infant body composition using dual energy x-ray absorptiometry (DXA) at 2 weeks and 4 months. At 2 weeks but not at 4 months, they found an inverse correlation between maternal DHA levels and offspring abdominal FM ($r=-0.51$; $p=0.003$). Donahue et al.[75] measured maternal RBC fatty acids late in pregnancy and offspring skinfolds at 3 years old in the Project Viva cohort. They found an inverse association between maternal n-3 LCPUFA status (DHA and eicosapentaenoic acid (EPA, 20:5n-3) and the sum of offspring triceps and subscapular skinfolds. Data from the Southampton Women's Survey[76] measured maternal RBC PUFA status late in pregnancy and measured offspring body composition using DXA at 4 and 6 years old. They found a positive correlation between maternal RBC total n-3 and offspring FFM at 4 and 6 years old ($\beta=0.11$; $p=0.06$ and $\beta=0.14$; $p=0.02$, respectively). Data were not reported for AT distribution. Vidakovic et al.[77] measured maternal RBC PUFA status mid-pregnancy and used DXA to measure offspring body composition and distribution at 6 years old. They associated greater maternal DHA with lower offspring FM and android to gynoid FM ratio (a measure of central to peripheral FM). Even though relationships were found with FM, no relationships were found between maternal DHA and offspring BMI. This suggests BMI is not a sensitive marker of fat deposition early in development.

A8) **DHA RCTs where body composition is not the primary outcome**: Multiple RCTs have been completed where DHA is prenatally and/or postnatally supplemented and the

primary outcome is not offspring growth. Two systematic reviews[78,79] in humans and one meta-analysis[79] have been published on this topic. Six studies provided prenatal DHA supplementation and continued supplementation into the postnatal period[75,80-84] and three trials provided prenatal supplementation only (400 mg/day-920 mg/day DHA supplementation)[85-88]. Of the six trials, two studies found DHA supplementation reduced offspring BMI[75,83], one found DHA supplementation increased offspring BMI[84], and three found no effect[75,81,82]. In the trials providing prenatal DHA supplementation only, two found no difference in offspring adiposity measures between groups[87,88]. The third trial found differences at 18 months[85] but not at 5 years[86].

Muhlhausler et al.[89] (published 2016) followed offspring from the DOMInO trial (DHA to Optimize Mother Infant Outcome) where the primary outcome was maternal depression and offspring neurodevelopment[90]. The mean maternal pre-BMI was 26 kg/m^2 with a range that had few obese women (23.2-30.5 kg/m^2). Women were prenatally supplemented with either 800 mg/d DHA (n=770) or vegetable oil capsules (n=761). Offspring body composition using bioelectrical impedance was assessed at 3 and 5 years old and BMI z scores were calculated. No between groups differences were found for body composition or BMI at 3 or 5 years old. Many maternal pregnancy variables were included as confounding variables, however maternal GWG was not included, because it was not collected in the primary study[90]. Maternal GWG, especially when excessive, is strongly related to offspring FM in infancy[9,13], at 3 years old, 5 years old[14], 10 years old[15] and in adulthood[16]. Further, the study states ~70% of the population where the sample was drawn consume nutritional supplements that provide DHA. No data were presented on maternal baseline DHA status and the change in maternal DHA when taking the supplement[89,90]. If the population already had adequate DHA status or were consuming DHA supplements, an effect of DHA on offspring body composition would not have been expected. Nevertheless, data from this trial do suggest DHA supplementation provided a protective effect in those with a high BMI percentile at 3 yrs old (>85th). The proportion with a high BMI was less in the DHA group vs. the control group (33.2% vs. 37.7%; p=0.10).

A9) **DHA interventions to modify offspring body composition**: Only one prenatally supplemented RCT has been reported in which the primary outcome was offspring body composition[80]. The study examined the effect of reducing the n-6/n-3 ratio on offspring body composition. Women with a pre-pregnancy BMI between 18.0-30.0 kg/m^2 were randomized to receive 1,200 mg/day DHA coupled with a reduction in n-6 intake (n=104) compared to a control group (n=104). The sum of 4 skinfolds was used to assess infant FM and ultrasound assessed abdominal subcutaneous AT. The average maternal pre-pregnancy BMI was very lean (22.4±3.0 kg/m^2). No differences were found at birth or through 1 year old in any measures. It is highly likely the maternal leanness contributed to the null findings as fewer normal weight women have excessive GWG. Published data from Dr. Hull and others show a positive relationship between maternal pre-pregnancy BMI and offspring adiposity, therefore, a greater maternal pre-pregnancy BMI is related to greater offspring FM[13,91,92]. The offspring may have already had low FM therefore DHA provided no additional benefit. Further, though the study purpose was to assess differences in AT growth, direct measures of FM were not obtained. While skinfold measures are attractive to use due to ease in measurement, they are best used to estimate regional fatness rather than relative fatness[93]. The acceptability of ultrasound to assess regional AT depots remains to be proven and accepted. The methodology used and the lean maternal population may have contributed to the null findings. Our proposed study will overcome limitations from these studies by using direct measures of body FM and accepted techniques to detect differences in AT distribution, and will enroll offspring born to women with a pre-BMI >17.0 kg/m^2. In the ADORE cohort enrolled thus far, the average maternal pre-pregnancy BMI is 28.5±7.1 kg/m^2 with a range of 17.4-50.1 kg/m^2.

There are several major limitations in the discussed prior studies that support the ***scientific premise*** for this proposal. The first major limitation is the use of BMI to estimate offspring adiposity during infancy and childhood. BMI in adults is related to clinical outcomes, however the predictive value and usefulness in infants, children, and adolescents is less clear[93]. The use of BMI in infants, children, and adolescents is further complicated by the inter-individual variability among children for periods of rapid growth for which BMI cannot distinguish changes in FM or lean mass. In infants and children, there is a twofold range of variation in fatness for a given BMI value[94]. However, in studies using sophisticated methods to directly assess offspring body composition and AT distribution, relationships between maternal DHA and offspring body composition are supported[77].

Another limitation is that information or variability on maternal pre-pregnancy BMI and GWG are limited or lacking in the Muhlhausler[89] and Hauner[80] et al. studies. The Muhlhauser[89] study has a mean maternal pre-BMI of 26 kg/m^2 with a range that had few obese women (23.2-30.5 kg/m^2) and GWG was not collected and not included in the analysis. The sample from the Hauner et al. study was even leaner (22.3±3.0 kg/m^2) and GWG was ~15.5±5.0 kg with no report on percentage excessive GWG. The ADORE cohort from which we will recruit in Kansas City so far has a mean pre-pregnancy BMI of 28.5±7.1 kg/m^2 with a wide pre-pregnancy BMI range (17.4-50.1 kg/m^2). GWG data are not yet available in the ADORE cohort, however, in Dr. Carlson's prior DHA study (KUDOS) in Kansas City, the mean GWG was 16.4±7.1 kg with GWG classification of 62% excessive. Our study will provide important data from a representative cohort to understand when controlling for excess maternal weight and excess GWG, what effect DHA has on offspring body composition.

A10) **Regulation of gene expression**: The n-3 and n-6 fatty acids direct gene expression for fatty acid metabolism and inflammation[95,96]. To regulate gene expression, fatty acids act like hormones and bind to receptors (example: peroxisome proliferator-activated receptors (PPARs))[97]. The activated receptor binds to the promoter region of the gene thereby increasing or decreasing transcription. Arachidonic acid, an n-6 fatty acid with 20 carbons and 4 double bonds, is a potent adipogenic fatty acid that upregulates PPAR leading to adipogenesis[98-102]. PUFAs can also regulate transcription factors inside the cell nucleus[103]. Examples include NFκB, which regulates genes involved with inflammation, and SREBP-1, which regulates *de novo* lipogenesis and PUFA synthesis. The n-3 fatty acid family suppresses genes involved in lipogenesis (fatty acid synthase, lipoprotein lipase, and stearoyl-CoA desaturase-1) and increases the expression of genes involved with β oxidation (acetyl Co-A oxidase)[51,52]. The overall net effect of n-3 fatty acids is a decrease in AT deposition.

Innovation: This proposal is innovative for the following reasons. First, we are examining the interaction between GWG and DHA in a new way. Prior studies have looked at the effect of prenatal DHA supplementation on offspring body composition without considering that DHA may protect against the effect of excessive GWG on infant FM. Maternal excessive GWG is related to greater offspring FM[9,13-16] however it is unknown if DHA protects against the effect of excessive GWG to prevent greater FM accumulation, especially during a period of rapid growth such as infancy. Second, published data have not explored if maternal DHA supplementation influences offspring AT distribution. Data show a reduction in central FM with greater intake of LCPUFA in children[104] and LCPUFA supplementation decreased central FM in adult subjects with T2DM[105,106]. Third, we are capitalizing on the unique resource of an NIH funded RCT trial where safety, efficacy, and purity of the supplement have been documented (IND with the FDA). Finally, this proposal could result in a recommendation for prenatal DHA supplementation by our Institute of Medicine (DHA is currently not recognized in the US as an essential nutrient)

and lead to evidence-based guidelines for DHA supplementation during pregnancy where none currently exist.

This study will assess the impact of prenatal DHA supplementation on programming offspring body composition and AT distribution during a critically identified time frame; the first 1,000 days post conception[107]. With the increased rates of obesity, it is important to identify interventions that may protect against the adverse programming effect of excess GWG on the offspring phenotype so as to decrease the long term risk of disease development. Prenatal supplement interventions are well received by mothers as many are open to behavioral changes that are shown to positively impact the development and well-being of their child. DHA is a nutrient with a low risk and high potential benefit to favorably program the offspring phenotype, particularly in the face of excess GWG. GWG currently occurs in 55% of US pregnancies and at this time, has no viable means of prevention.

B) **Significance of the expected research contribution**: Data are lacking on the national prevalence of prenatal nutritional supplement use. In Dr. Hull's pregnancy health study (n=82), only 62.1% (n=41) reported taking a prenatal vitamin in the first trimester which is similar to other reports that found between 63%-83% women consumed a prenatal supplement in the first trimester[108-110]. From Dr. Hull's data of those taking a prenatal, only 22% (n=9) reported taking a prenatal with DHA or a separate DHA supplement. Nochera et al.[110] examined the consumption of DHA+EPA during pregnancy and found only 1 woman took a prenatal with DHA. The total DHA+EPA was 1.18 g/month, well below the recommended 6-9 g/month suggested by several expert recommending bodies. Reported barriers of DHA consumption is the cost of DHA rich foods[111] and advice to avoid consuming mercury containing fish[110]. This advice decreases maternal fish consumption during pregnancy[112,113]. Published NHANES data show in non-pregnant women of reproductive age, women are consuming only 59 mg/day of DHA from food or supplements[114], well below the Dietary Reference Intake (DRI) of 200 mg/d of DHA during pregnancy[115]. Therefore, women are entering pregnancy with low intakes of DHA and not consuming recommended levels during pregnancy. These data suggest the tremendous potential an increase in the recommendation for DHA supplementation could have on the offspring if our hypothesis is correct.

The innovation of this application is in the unique utility of the potential findings. There is clearly a gap in knowledge and mixed directionality regarding the role of DHA to influence offspring body composition. This study will inform this critical gap and recommendations to women for DHA consumption. In addition, this grant proposal is innovative by proposing to solve a problem in new ways. The role of DHA in cognitive development is clear but DHA's role in obesity prevention is unknown. This proposal will move the body of knowledge forward and could ***change*** recommendations for the dose of prenatal DHA supplementation.

C) **Overview of the parent trial (ADORE)**: Section C provides an overview for the parent trial. The parent study has already received NIH funding and Human Subject Committee approval (Title: "*Docosahexaenoic acid (DHA) supplementation in pregnancy to reduce early preterm birth*" (ADORE); R01 HD83292-01 R01). The next sections (C1a-C1f) provide information on the approved and funded study. Drs. Carlson and Gajewski are PIs on the parent study and both are Co-Is on this ancillary proposal. Study recruitment began June 2016 with the first delivery in November 2016. The proposed sample size is n=1200 with enrollment of 400 pregnant women at each of three sites (Kansas City, KS, Columbus, OH, and Cincinnati, OH). In this ancillary proposal, we are planning to follow offspring at the Kansas City site only. The primary purpose of the parent trial is to determine if prenatal DHA supplements of 1000 mg/d vs. 200 mg/d during the last two trimesters of pregnancy can reduce early pre-term birth (ePTB;

Specific Aim 1) and to conduct a secondary pregnancy efficacy analysis to determine if there is a subset of pregnancies most likely to benefit from DHA supplementation (Specific Aim 2). To obtain information about the effects of DHA on inflammation, sRAGE will be measured, which is plausibly influenced by DHA supplementation based on a murine model of LPS-induced inflammation (Specific Aim 3). Maternal and cord blood will be banked and available for future evaluations beyond fatty acid analysis. Budget constraints prevented including analysis in this ancillary proposal to explore the potential mechanisms for the influence of DHA on offspring body composition. However, the study team plans to use stored samples to conduct these studies.

C1a) Subject recruitment of the parent trial: Women who are 18 years of age and older and are between their 12th to 20th week of gestation (based upon 2014 American Congress of Obstetricians and Gynecologists (ACOG) guidelines) are eligible for enrollment. Pregnant women who receive prenatal care in obstetrics clinics of all 3 participating centers (University of Cincinnati, Ohio State University, and the University of Kansas Medical Center) receive an ultrasound assessment at approximately 12 wk gestation. The estimated date of delivery (EDD) determined by the 2014 ACOG guidelines are fixed as the EDD for assessment of gestation duration in the study. Women must be able to read or orally understand the study in English or Spanish and sign an informed consent form. They must agree at enrollment to consume the capsules assigned them from then until they are delivered. Women expecting multiple infants are excluded, because multiple fetuses increase risk of preterm and low birth weight delivery for reasons other than hypothesized reduction in inflammation with DHA. Availability by telephone is necessary for optimal coordination in both phases of the study. We routinely obtain additional phone numbers of friends and family who have a stable address. The complete inclusion and exclusion criteria are listed in **Table 1**.

Table 1: Inclusion and Exclusion Criteria for the parent RCT	
Inclusion Criteria	
1.	Pregnant females 18.0 years and older who are 12 to 20 weeks gestation at study entry
2.	Agree to consume study capsules and a typical prenatal supplement of 200 mg DHA
3.	Available by telephone
Exclusion Criteria	
1.	Less than 18 years of age at enrollment
2.	Expecting multiple infants
3.	Gestational age at baseline <12 weeks or >20 weeks
4.	Unable or unwilling to agree to consume capsules until delivery
5.	Unwilling to discontinue use of another prenatal supplement with DHA that contains ≥ 200 mg DHA
6.	Women with allergy to any component of DHA product (including algae), soybean oil or corn oil

For the parent RCT, the goal is equal enrollment at the 3 centers with 400 subjects enrolled at each center (total n=1200) and the anticipated attrition is 11%. The goal enrollment at Kansas City is n=400 subjects. The expected enrollment pace is 8-10 subjects/month, with completed enrollment by late 2019 (~42 months) providing adequate time for assessment of the offspring to 24 months. Dr. Carlson's prior Phase III trial (KUDOS) at one site only (KUMC) enrolled 350 subjects in 42 months despite a relatively slow enrollment in the first 6 months of the study. The enrollment pace for the KUDOS trial at Kansas City was 8-12 subjects/month.

C1b) Placebo and DHA supplementation: A marine algae oil source of DHA (DSM, Columbia, MD) will be provided in capsules. Specific capsules to be used are equivalent to Spring Valley

Algal-900 DHA Dietary Supplement Softgels, 450/mg per capsule. The algal oil capsules in this study provides 800 mg DHA in 2 1-g capsules and the higher DHA group of subjects will be asked to consume 2 capsules per day. The placebo control group will receive 2 1-g capsules containing half soybean oil and half corn oil. The soybean and corn oil combination does not contain DHA. Two capsules provide 80 mg of α-linolenic acid, a precursor of DHA. On average, US adults consume ~1000 mg/d of α-linolenic acid but can make only about ~40 mg DHA/day. Both capsules will be prepared and provided with orange flavor to mask the taste if there is eructation. The placebo and masked DHA capsules will be provided in bottles of 100 capsules (a supply for 50 days).The capsules are donated by DSM. DSM also donated 200 mg DHA capsules to both groups for daily use. These are already available commercially as a prenatal supplement under several product names. The 200 mg capsules will be provided in bottles of 135 capsules (135-day supply) and will be marked with an expiration date. Other fatty acids found in the capsules do not contribute significantly to the amounts in the diets of US women. DHA is the only fatty acid expected to change in the RBC-phospholipids (PL) of the supplemented group. Capsule compliance was excellent in our KUDOS trial, on average 74% of capsules were consumed. The parent trial is under an IND from the FDA, and the IND requires ongoing assurance of capsule purity.

C1c) Capsule records and accountability: The Investigational Pharmacy at The University of Cincinnati sends capsules to each enrolled subject on a regular schedule until they give birth; and the bottles and any remaining capsules are returned by mail to the Investigational Pharmacy in a self-addressed envelope provided with the capsule mailing, the remaining capsules counted, the number of capsules remaining recorded and the capsules destroyed. Records of capsules mailed to and received back from subjects are entered into the study database with a flag to investigators at the subject's study site. Investigators at each site review the database and contact subjects who do not return their capsule bottle. Study personnel contact the subject by telephone early within the first month and monthly thereafter to determine if there are any problems and encourage compliance. The study investigator, study site staff and subjects do not know which capsules are being consumed by each patient. The Investigational Pharmacy at the University of Cincinnati receives all bottles of capsules directly from DSM, Columbia, MD and maintains packing receipts for study products.

C1d) Randomization for ADORE: Pregnant women are randomized to one of two arms (groups) with a maximum number of pregnant women n_{max}=1200 (we plan for 1355 enrollments due to expected dropout). Each study site location has a separate randomization code. Using a Bayesian Adaptive Design, at each interim analysis a decision is made. Depending upon the birth outcome the randomization structure is updated. The primary endpoint, percentage of ePTB, is used to drive the adaptive randomization. After we have 150 women in each group enrolled the data will be analyzed and an updated randomization schedule will be used. The arm that looks to be the best will get more pregnant women allocated to it in this subsequent randomization. A new adaptive randomization schedule is updated every 13 weeks, using up to date outcome data, until the trial is stopped.

C1e) Implementation: An initial allocation table would be generated (Master allocation table) with all the factors being considered (i.e. maximum sample size, endpoint etc), this allocation table would be attached to our eResearch tool. Once every patient gets an enrolled status they would be assigned to an ARM (Arm A, Arm B) using the randomization module within eResearch. After enrolling 150 patients, the latest data from the system would be analyzed to generate a new allocation table; this new table would be appended (ignoring all the unused assignments on the allocation table) to the existing allocation table that is already being used, this process would be repeated at every 13 weeks, until we reach the end of the trial.

C1f) Attrition: If a patient withdraws from the study prematurely, the assessments described at delivery that apply will be obtained if available and the subject has not requested her data not be obtained. This, and the requirement to obtain medical records for adverse events during pregnancy and following birth of the infant, will be explicit in the consent form subject to any provisos made by the Central IRB and HIPAA. If the subject is withdrawn due to an adverse event(s), the patient will be monitored until the adverse event has resolved or until the event is determined to be due to a stable or chronic condition. The reason for patient discontinuation will be documented. If a patient withdraws from the study, the patient's study number will not be reassigned.

D) **Approach/Preliminary studies to support the proposed study**

D1) Relevant experience of investigators: The unique interdisciplinary skills and experiences of this team provides *innovation* to the application. The research team has a history of successfully working together to conduct research and have worked collaboratively to address study design issues, recruiting and retaining subjects, administering a lifestyle intervention, and data collection and analysis. Dr. Hull has published multiple articles and has been PI on multiple pilot studies in pregnant women and following their offspring to explore how the maternal environment influences the health of the mother and her baby. Drs. Hull and Andres have published data examining the influence of maternal obesity on offspring body composition and AT distribution birth to 6 years using DXA. Drs. Hull, Sullivan, and Carlson are investigators on two completed studies to determine the effectiveness of interventions to promote appropriate GWG in pregnant women. Drs. Carlson and Gajewski are co-Is on the parent RCT. Dr. Sullivan has two decades of experience assessing diet in multiple NIH trials. Dr. Carlson has 35 years of clinical research study experience and has multiple successful RCTs recruiting and retaining pregnant women in clinical trials that involve DHA supplementation.

D2) **Preliminary data**: Included are preliminary data from two cohorts of infants. The first cohort represents data in a group of preterm infants where the mothers were supplemented with either high (1,000 mg/d) or low (200 mg/d) DHA during breastfeeding. Infant body composition was measured using the Pea Pod. The second infant cohort is from Dr. Hull's unsupplemented Pregnancy Health Study where offspring body composition was measured at birth and 3 months of age using the Pea Pod. Skinfolds assessed AT distribution at the same time points. All data support a protective role of DHA to program a favorable offspring body composition phenotype and to be protective in at risk populations (pre-term infants and infant exposed to excessive GWG).

Maternal DHA Supplementation protects against excessive FM accumulation in the preterm infant: Data shared by Drs. Christine Valentine and Lynette Rogers were presented at Experimental Biology in 2016[116]: Mothers of infants born <28 weeks were supplemented with either 200 or 1000 mg of DHA/day to influence milk DHA concentration during the equivalent of the 3rd intrauterine trimester. Mother/infant dyads were enrolled at the time of birth and randomized to either the 200 or 1000 mg/day of single source DHA for 8 weeks. A total of 18 mother/infant dyads completed the study; 8 were randomized to 200 mg/d and 10 were randomized to 1000 mg/d DHA supplementation. Offspring body composition was assessed using air displacement plethysmography, at term corrected gestational age or hospital discharge. Data were analyzed using Multivariate General Linear Regression with infant body composition as the dependent variable, DHA supplementation assignment as the independent variable, and age at analysis as a confounding variable. When compared to infants fed by mother receiving 200 mg/d DHA, infants fed by mothers receiving 1000 mg/d DHA had lower %fat (11.0 vs 12.4 %fat, p=0.030), FM (0.33 vs 0.38 kg, p=0.014), and body mass (2.90 vs 3.00 kg,

p=0.042). No differences were observed in FFM or length. The total number of infants in this study is small but the study does support the idea that DHA can reduce body fat accumulation when provided during the equivalent of the last trimester *in utero*.

Maternal DHA levels are related to lower change in infant FM: Infant body composition was measured using the Pea Pod (n=62) at birth and 3 months. No prenatal DHA supplementation was provided and maternal RBC DHA was measured at 36 weeks in pregnancy and categorized by the median as high (>50th percentile) or low (<50th percentile). We first explored the main effect of maternal DHA status on differences in the change in infant FM. Offspring born to mothers with a high DHA status had a lower change in FM when compared to offspring born to mothers with a low DHA status. With the known effect of excessive GWG on infant FM, next we explored the interaction of maternal DHA status and GWG category on the change in infant FM. Maternal GWG was categorized as appropriate or excessive based on the 2009 IOM guidelines. Using ANCOVA, we examined the main effects and interaction of maternal RBC DHA status and GWG category on the change in infant FM. Confounding variables included in the model were maternal pre-pregnancy BMI, GWG, 3rd trimester maternal measured DHA, father's BMI, maternal race, household income, gestational age at birth, infant gender, infant age at test, and baseline variable of interest (ie., infant FM). The interaction was significant (p=0.028). In post-hoc analysis, no difference was found by maternal DHA status in offspring exposed to appropriate GWG. However, in offspring exposed to excessive GWG, infants exposed to low maternal DHA status had a greater increase in offspring FM (Δ 1499.0 g) when compared to infants exposed to high maternal DHA status (Δ 1026.4 g; p=0.038). A similar effect was found for body mass, however, no difference for the change in FFM was found. Therefore, in the offspring exposed to excessive GWG and high DHA status, the lower change in body mass was due to a lower accrual in FM and not FFM. See **Table 2** for a summary of these results. These data are preliminary but promising considering no maternal prenatal DHA supplementation was provided.

Table 2. Change in infant body composition from birth to 3 months of age based on maternal GWG and DHA status.

Infant variable	*DHA status*	*Weight gain status*		*P value*
		Appropriate(n=25)	*Excessive (n=37)*	
Change in body mass (g)	<50th percentile	2943.1 ± 810.9	*3589.1 ± 965.3*	p-int=0.045; p=0.060 post-hoc for difference in excessive GWG by DHA status
	>50th percentile	3146.7 ± 926.9	*2961.7 ± 796.6*	
Change in %fat	<50th percentile	12.9 ± 6.2	15.5 ± 7.0	p-int=0.033; p=0.083 post-hoc for difference in excessive GWG by DHA status
	>50th percentile	15.2 ± 7.2	11.1 ± 6.1	
Change in FM (g)	<50th percentile	1189.5 ± 549.9	*1499.0 ± 622.4*	p-int=0.028; p=0.038 post-hoc for difference in excessive GWG by DHA status
	>50th percentile	1330.4 ± 640.7	*1026.4 ± 539.3*	
Change in FFM (g)	<50th percentile	1432.0 ± 435.3	*2049.8 ± 497.0*	p-int=0.292
	>50th percentile	1167.0 ± 519.2	*2034.8 ± 430.3*	

Values are presented at means ± standard deviations
P-int=P value for the interaction between DHA status and Weight Gain Category (appropriate vs. excessive)
Confounding variables included in the model: maternal pre-pregnancy BMI, GWG, 3rd trimester maternal measured DHA, father's BMI, maternal race, household income, gestational age at birth, infant gender, infant age at test, and baseline variable (ie., Body mass).

Maternal DHA levels and changes in central FM: Further analysis of Dr. Hull's un-supplemented pregnancy cohort are reported here. Skinfolds were measured on the central and peripheral part of the infant's body to assess AT distribution using standardized procedures. Using ANCOVA, we examined the main effects of maternal RBC status and infant gender on the change in central FM. The same confounding variables were included from the prior analysis. The p values for the main effects of DHA status and gender were p=0.282 and p=0.076, respectively. We next explored the interaction of maternal DHA status and gender on the change in central FM. This interaction approached significance (p=0.085). In post-hoc analysis, no difference was found by maternal DHA status in males but a clear difference was found in females. Females born to mothers with high DHA status had a lower change in central FM (Δ 3.6 mm) compared to females born to mothers with a low DHA status (Δ 5.7 mm; p=0.053). The main effect of gender detected in the first model appears to be driven by the difference found in females. No difference for the change in peripheral FM was found. See **Table 3** for a summary.

Table 3. Change in central FM from birth to 3 months of age.

DHA status	Change in central FM (mm)		*P value*
<50th percentile	4.5 ± 2.6		P=0.282
>50th percentile	3.6 ± 2.4		
	Male (n=36)	*Female (n=22)*	p-int=0.085; p=0.053 post-hoc for difference in females by DHA status
<50th percentile	3.7 ± 2.3	**5.7 ± 2.2**	
>50th percentile	3.6 ± 2.2	**3.6 ± 2.0**	

Values are presented at means ± standard deviations
P-int=P value for the interaction between DHA status and Gender (male vs. female)
Confounding variables included in the model: maternal pre-pregnancy BMI, GWG, 3rd trimester maternal measured DHA, father's BMI, maternal race, household income, gestational age at birth, infant gender, infant age at test, and baseline distribution variable (ie., Central FM).

What these data suggest: There are identified risk factors that are related to greater offspring FM; two of these risk factors are catch-up growth[117,118] and exposure to maternal excessive GWG[9,14]. Our preliminary data suggest a protective effect of DHA exposure for programming a favorable offspring body composition phenotype with excessive GWG during early growth. Infants born preterm and likely to experience high rates of weight gain were protected against excessive FM accumulation. In another cohort, infants exposed to excessive GWG had lower FM accumulation detected early, at 3 months old, when mothers had a high vs. low DHA status and were exposed to excessive GWG. At 3 months old we detect a 672 g difference in body weight that is largely accounted for by the change in FM of 472 g. Therefore, at 3 months old, there is already a more than 1-pound difference in FM between these groups and DHA appears to be mediating this difference.

It is known that FM location in adults[4] and children[5,6] is an important predictor of disease risk and development. Although the main effect of maternal DHA status on the change in central FM was not significant, it is important to note that women in our pilot study were a sample of convenience who were not being supplemented with DHA. No one has examined AT distribution in a cohort supplemented with high levels of DHA. Further, exploration of gender shows in female offspring born to mothers with a high DHA status, they had a lower change in central FM when compared to female offspring born to mothers with a low DHA status. No effect was found in males. Our preliminary data provide interesting differences detected early in infancy that warrant follow up in a larger sample exposed to high vs. low prenatal DHA supplementation.

D3) **Recruitment for the current proposal**: Our study will enroll subjects from the RCT; "*Docosahexaenoic acid (DHA) supplementation in pregnancy to reduce early preterm birth*" (NIH R01 HD83292-01; n=1200 at 3 study sites). The current ancillary grant proposes to follow offspring from the Kansas City site where the expected enrollment will reach ~n=600. The parent study partnered with Juntos to recruit Hispanic pregnant women and they have been highly successful in recruiting a large number of Hispanic pregnant women, whom only speak Spanish. The GAINS study must be able to recruit the offspring born to this population. Enrollment may occur at any of the 4 time points: 2 weeks, 6 months, 12 months, or 24 months. If parents decide not to participate when contacted by study personnel, but agree to be contacted when the infant is older, the study personnel will contact them at 24 months.

The parent study (ADORE) is a Phase III Double Blinded Randomized Controlled Clinical Trial where women will be randomized to either a low or high dose DHA (1,000 mg/d or 200 mg/d). The primary outcome of this RCT is to move early pre-term birth to late pre-term birth. No targeted recruitment of women at risk for pre-term birth will be done (see **Table 1** for inclusion/exclusion criteria). Anyone meeting the inclusion criteria will be enrolled. At enrollment of the parent RCT, all participants will be asked if they can be contacted for other related studies. Their decision is recorded in the consent and a database. Late in pregnancy, women enrolled in the RCT will be approached. Based on Dr. Carlson's prior KUDOS RCT pregnancy supplementation study, the number enrolled was 350 with 86% of the sample retained at birth for follow up in the postnatal period. The study team has full confidence the recruitment goal can be met (see c7a "Power calculations" and D9 "Study Challenges" for further discussion).

Recruitment process: Both parents must give their permission unless one parent is deceased, unknown, incompetent, or not reasonably available, or when only one parent has legal responsibility for the care and custody of the child. If only one parent is signing, the reason will be well documented in the subject's chart/source documentation. Both signatures will be

obtained prior to any research procedures taking place on the child. One or both parent signatures may be obtained via REDcap as long as:

a. A member of the study team calls the parent(s) and walks through the entire document over the phone, answering questions and making notes about the parent's (or parents') questions. (This will be well documented in the subject's chart/source documentation);
b. Time and date of the conversation are recorded; AND
c. The study team member obtaining consent writes a note on the consent form stating that the parent's (or parents') consent was obtained by phone/REDcap on the date the parent(s) signed.

At least one copy of the signed consent form (with both signatures) will be provided to one of the parents. If signatures are obtained via REDcap, then this signed consent form can be provided via mail or secured email.

Table 4. Current parent trial enrollment at Kansas City site

Month/year of visit	*Expected ADORE enrollment*	Actual # enrolled English	Actual # enrolled Spanish
Yr1: June 2016 - May 2017	108	70	28
June-17	9	8	4
July-17	9	3	14
August-17	9	4	8
September-17	9	11	10
October-17	9	5	10
November-17	9	6	10
December-17	9	2	7
January-18	9	9	3
February-18	9	5	5
March-18	9	9	7
April-18	9	7	6
May-18	9	11	6
June-18	9	14	7
July-18	9	6	3
Total Enrollment	**234**	**170**	**128**

Current recruitment from the parent RCT: At the time of this revised proposal, the trial has enrolled 298 pregnant women at the Kansas City site and 299 pregnant women at the other two sites combined. The Kansas City site is on track to recruit about 50% of the trial participants instead of the expected 33%. Of the 298 pregnant women at the Kansas City site, 43% (n=128) are Spanish speaking. We originally proposed to recruit n=180, accounting for a 33% attrition rate in the power analysis. The success of the parent RCT in recruiting Spanish speaking pregnant women allows us to answer our primary aim in a second group of women, but we must be able to recruit the offspring born to this population. Therefore, we are increasing our sample size by n=180 for a total sample of n=360.See **Table 4** for a summary of recruitment at the Kansas City site.

D4) **Standardized training and quality assurance**: Dr. Hull has considerable experience (over 10 years) assessing body composition in different populations using different systems. Dr.

Hull will provide initial and ongoing training for all Research Assistants regarding assessment of body composition. All Research Assistant's will undergo standardized body composition training and annually, re-fresher training will be completed. For quality control purposes, daily scans will be performed on the DXA system using the Quality Assurance block provided by the manufacturer to simulate fat and fat-free soft tissues, respectively[119,120]. Movement is not tolerated by the DXA system however total body scans in newborns and toddlers are relatively short (~3 minutes). Co-I Dr. Andres has considerable experience (n=1,540 scans from birth to 6 years old) obtaining DXA images in infants, toddlers, and children. She will be in charge of assisting Dr. Hull to develop protocols to ensure a valid DXA scan is captured at each age time point.

D5) **Assessments**: Infant body composition and AT distribution will be measured at 2 weeks and 6, 12, and 24 months using dual energy x-ray absorptiometry (DXA). Collection and analysis of maternal RBC will be completed as part of the parent RCT. Maternal blood will be collected at delivery and analysis will be completed after delivery. Upon completion of this protocol, Dr. Hull will have access to study assignment (1,000 mg/d or 200 mg/d). An overview of study visits is listed in **Table 5**.

Table 5. An outline of study visits and procedures.

Study procedure	2 wks	6 months	12 months	24 months
Enrollment & Consent	**Prior to any study visit**			
DXA scan	•	•	•	•
PeaPod	•	•		
Anthropometry & skinfolds	•	•	•	•
Diet recall & questionnaires	•	•	•	•
Incentives	**$125**	**$125**	**$125**	**$125**

D5a) Dual energy x-ray absorptiometry to measure total body fat and AT distribution: Dual energy x-ray absorptiometry (DXA; Prodigy, Madison, WI, encore software version 13.60) will be used to measure body composition and regional AT distribution. The DXA is located within the Dietetics & Nutrition Clinic inside the Smith West building at KUMC. Using specific anatomic landmarks as previously described, regions including the arms, legs and trunk will be demarcated[8]. Calculations for FM in each region and summed for regions comprising the central (trunk) and peripheral (arms plus legs) will be completed. If a child moves during the scan the scan may need to be re-done but no more than 8 total scans will be given over the duration of the study.

D5b) GWG: GWG will be calculated by subtracting the self-reported pre-pregnancy body weight from the last weighed measurement in the clinic (pulled from the EMR) prior to delivery. GWG will be classified according to 2009 IOM GWG guidelines as appropriate or excessive[121].

D5c) Anthropometry: Body weight will be assessed on the same calibrated scale throughout the study duration (Detetco Scales, Webb City, MO). Length will be measured using an infant length board (Shorr Productions) and at 24 months old, standing height will be measured using a wall mounted stadiometer (Accu-Hite, Seca Corp, Hanover, MD). Subjects will remove shoes and be centered on the stadiometer. Height will be recorded to the nearest 0.1 cm. Two measurements will be taken and the average will be recorded.

D5d) Skinfolds: Six skinfolds will be measured to represent AT distribution in order to compare to larger trials that do not have DXA. Dr. Hull has published using these methods to assess infant FM distribution[3] and it has been argued skinfolds are a valid metric of regional FM[74]. All measurements will be collected using standardized procedures to our Laboratory and will take place on the same day as the body composition assessment by DXA using standardized procedures. All skinfolds will be identified using anatomical landmarks and taken on the right side of the body using Lange calipers (Beta Technology, Santa Cruz, CA). Skinfolds will be taken in order from head to toe and then repeated in that same order. If two skinfold measurements differ by more than 1 mm, a third measurement will be taken. The two measurements within 1 mm will be averaged and used for analysis. Biceps and triceps skinfolds will be measured at the midline of the anterior and posterior surface of the arm, respectively, on the mid-point between acromial process of the scapula and olecranon process of the ulna. Subscapular skinfold will be measured at the lower angle of the scapula. Suprailiac skinfold will be measured anteriorly to the midaxillary line and superiorly to the iliac crest, along the natural cleavage of the skin. The thigh skinfold will be measured at the mid-point between patella (knee cap) and inguinal crease at the anterior surface of the thigh. Flank skinfold will be measured immediately above the iliac crest at the mid-axillary line. Central FM will be calculated by adding the subscapular, suprailiac and flank skinfolds and dividing by two. Peripheral FM will be calculated by adding the thigh, biceps and triceps skinfolds and dividing by three.

D5e) 24 hour dietary recall: One multiple-pass 24-hour dietary recalls will be collected by trained research staff at each visit to characterize energy and nutrient intake. 24-hour recalls accurately estimate dietary intake[122,123] and contain less reporting bias than diet records [122,124]. The recalls will be entered into the Nutrition Data System for Research (NDS-R, version 2018, Minneapolis, MN) for macro- and micronutrient analysis. At 6 and 12 months, a feeding questionnaire will be given to assess infant feeding practices (breastfeeding, formula, etc) and introduction of solids. Dr. Carlson has successfully used this method to assess children's diet in her prior RCT from birth to 5 years old.

D5f) Fatty acid analysis: Maternal blood taken at enrollment and birth, and cord blood will be analyzed a part of the parent NIH study. RBCs will be separated from plasma and buffy coat by centrifugation (3,000×*g*, 10 minutes; 4°C), frozen, and stored under nitrogen at −80°C until analysis. Phospholipids from erythrocytes will be isolated according to a modified Folch method[47], and fractionated by thin-layer chromatography, transmethylated with boron trifluoride-methanol, and the resulting fatty acid methyl esters (FAME) separated and quantified using a Varian 3900 gas chromatograph with an SP-2560 capillary column (100 m, Sigma Aldrich) and a Star 6.41 Chromatography Workstation for peak integration and analysis[47]. Individual peaks will be identified by comparison with qualitative standards (PUFA 1 and PUFA 2, Sigma Aldrich) and a weighed standard mixture (Supelco 37 Component FAME mix, Sigma Aldrich) will be used to adjust fatty acids for area/weight to calculate a final weight percent of total fatty acids. *The cost of DHA analysis is covered by the parent NIH study*.

D5g) Questionnaires: Questionnaires will be given at the baseline visit to assess maternal and familial characteristics. Examples of information collected include family history of disease, stress during pregnancy, other children in the home, and biological father's height and weight. Maternal health history information collected as part of the parent RCT will be reviewed.

D5h) Illness Assessment: In order to control for the potential impact of child's illness on growth, we will collect illness data. Medical records will be requested upon completion of study for each subject enrolled in the follow-up trial; medical records for any participants lost to follow-up will

be requested upon Release of Information form expiration. Medical records are reviewed for adverse events upon receipt and all adverse events are recorded by body system and coded by diagnosis within body system. For each adverse event, the following information is recorded: diagnosis, body system code for the diagnosis, whether the event was a serious adverse event, event start date, event ongoing dates if applicable, event stop date, treatment including names of medications, age of child in mos at the start of the event, and location of service for the event (ex. Hospital, pediatric clinic, urgent care clinic). Records are coded by one individual and then checked for accuracy by a second person.

D5i) Air Displacement Plethysmography to measure total fat mass and fat-free mass: Pea Pod (COSMED) will be used as an additional method to measure body composition at 2 weeks and 6 months. The Pea Pod assesses body volume and density to calculate fat mass and fat-free mass. The test will be conducted according to manufacturer guidelines.

D5j) Steps to maximize retention: At KUMC, we use the ClinCard system, which works like a debit/credit card. At study enrollment, subjects are given a card that looks like a debit/credit card with a number unique to each participant. At the completion of study visits with incentives, staff log in and the card is loaded with the respective stipend amount within 24-48 hours. The card can be used anywhere Visa/Mastercard is accepted. For each study visit completed, subjects will be paid $125 (total $500 if all visits are completed). If a DXA scan is unable to be acquired during any study visit, subjects may be asked to return at a later date and will receive an additional $50 for returning. Please refer to **Table 5** for the incentive schedule.

The ***scientific rigor*** of this application is high. We are following a cohort born to women participating in an already NIH funded and approved RCT that has been vetted for safety and efficacy which provides strength to this proposal. For the outcomes of this proposal, we are using DXA to assess body composition and distribution which is the only precise technique for evaluating amount and location of body fat.

D6) **Study timeline**: Listed in **Table 6** is the timeline for the study.

Table 6. Research timeline and activities.

Research Activities	2017	2018	2019	2020	2021	2022	2023
Hiring/train personnel	—						
Develop data collection protocols	—						
IRB submission	—						
Recruitment/screening	—	—	—	—	—	—	—
Data collection	—	—	—	—	—	—	—
Data entry	—	—	—	—	—	—	—
Data collection ends						—	
Data analysis and writing						—	—
Manuscript prep & submission						—	—

D7): **Statistical analyses**: The primary purpose of this study is to determine if there are differences in the offspring FM at 24 months when exposed prenatally to high vs. low DHA supplementation and if maternal GWG status impacts this relationship (appropriate or excessive GWG).

D7a) **Aim 1 analysis:** Using a two-way analysis of variance (ANOVA) on the 24 months FM, we will test for a statistical interaction between GWG group and DHA dose. The specific interaction from this ANOVA will test the contrast to **investigate if the supplement of 1000 mg DHA/day compared to 200 mg DHA/day during pregnancy can reduce infant FM at 24 months and specifically if this reduction is greater in offspring exposed to excessive GWG versus appropriate GWG**. A two sided t-test statistic (alpha=0.05) is calculated from the appropriate contrast from the two-way ANOVA (GWG and DHA).

D7b) **Aim 2 analysis:** A three-way analysis of variance (ANOVA) on the 2 year central FM, we will test for a statistical interaction between both GWG and DHA dose and offspring gender and DHA dose.

D7c) **Longitudinal analysis:** Additionally, we will perform a linear mixed model to explore differences between the high vs. low supplement groups for infant FM and distribution. The dependent variable will be infant FM or infant central FM and the independent variable will be DHA group (fixed effect) and time (4 visits: 2 wks, and 6, 12, and 24 months). The model will include the fixed effects of treatment, time and the treatment-time interaction, with the subject effect treated as random to account for the dependence among repeated observations. This analysis allows for unbalanced data (allows for missing data). We will use exploratory analyses to investigate, in a univariate fashion, covariates. GWG and infant gender will be included as well as other potential covariates including parity, maternal age, pre-pregnancy BMI, parity, education level, maternal diet, infant diet, infant age at test, gestational age at birth, and socioeconomic status. An auto-regressive covariance structure will account for unevenly spaced visits. For missing outcome data, the data vector will be used to impute the missing value. Infant gender will be explored both as a confounder and as an effect modifier to account for differences in growth rates. We will analyze the data without confounding variables and repeat the analysis with confounding variables.

D7d) **Power calculation:** We performed a power analysis based on pilot data reported in **Table 2**. We calculated a total of n=120 would be required to answer the primary aim. Of the total n=120, we anticipate n=54 will experience appropriate GWG (45%) and n=66 will experience excessive GWG (55%) with each approximately equally allocated to low and high DHA supplementation groups. For the given effect size (population mean differences of -168 vs 304 grams), the power is 0.815. This means that 81.5% of studies would be expected to yield a significant effect to detect an interaction, rejecting the null hypothesis that the two population means are equal. The test is two-sided. Allowing for 33% attrition, this increases the sample size to n=180. To answer the primary aim in a second group of Spanish speaking subjects, we will increase the sample size by n=180 for a total sample of n=360.

D8) **Study challenges**: We do not anticipate any major limitations as we developed strategies to successfully minimize issues that arose in the KUDOS follow-up, and applied these to the ADORE trial. The current proposal will follow the successful recruitment, retention, and data collection framework developed in the KUDOS trial and perfected in the ADORE trial. Recruitment of our study is reliant upon the successful recruitment of the parent RCT study. Co-I Dr. Carlson is the PI on the parent study we will be recruiting from. Dr. Carlson has successfully completed multiple RCT in pregnant women with high rates of monthly recruitment and retention rates. The average monthly recruitment for her KUDOS trial was 8-12 women/month, with n=350 recruited and n=301 were retained (86% retention rate). The study team has full confidence the recruiting goal of this project can be met. We anticipate some telephones being disconnected, but we obtain additional telephone numbers from subjects for relatives and friends. We expect that there will be attrition from the sample over time; however, our experienced team works to minimize this by adhering to principles that maximize retention of participants in longitudinal studies. These principles include the establishment of an identity for the study (there is a logo and an acronym that participants can readily recognize), maintenance of consistent contact with participants by the same team members, and provision of sufficient payments to participants in compensation for time, travel, and effort (participants will be paid $125 per visit). In all past RCTs with longitudinal follow-ups, we have been able to retain between 80% to 88% of the participants consented into the follow-up into the second year.

Acquiring DXA scans in infants and toddlers can be challenging due to the requirement for stillness. However, Dr. Andres has extensive experience in evaluating infants and toddlers using DXA scans with ≥75% success rate. The protocol used assesses infants and toddlers while sleeping or coaching them to remain still during the scanning process. If a child moves during the scan the scan may need to be re-done but no more than 8 total scans will be given over the duration of the study. A second potential downside of DXA scans are the exposure to radiation. However, radiation exposures for a full body DXA scan is minimal; 0.001 mSV, which is equal to natural background radiation of 3 hours or similar to a transcontinental flight. In comparison, a radiography chest x-ray is 0.1 mSv. We plan to minimize exposure by scanning the children only 4 times during the course of their first two years of life.

References

1. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011-2012. *JAMA.* 2014;311(8):806-814.
2. Hull HR, Thornton J, Paley C, Navder K, Gallagher D. Maternal obesity influences the relationship between location of neonate fat mass and total fat mass. *Pediatr Obes.* 2015;10(4):245-251.
3. Carlsen EM, Renault KM, Norgaard K, et al. Newborn regional body composition is influenced by maternal obesity, gestational weight gain and the birthweight standard score. *Acta Paediatr.* 2014;103(9):939-945.
4. Kannel WB, Cupples LA, Ramaswami R, Stokes J, 3rd, Kreger BE, Higgins M. Regional obesity and risk of cardiovascular disease; the Framingham Study. *Journal of clinical epidemiology.* 1991;44(2):183-190.
5. Freedman DS, Serdula MK, Srinivasan SR, Berenson GS. Relation of circumferences and skinfold thicknesses to lipid and insulin concentrations in children and adolescents: the Bogalusa Heart Study. *The American journal of clinical nutrition.* 1999;69(2):308-317.
6. Dai S, Fulton JE, Harrist RB, Grunbaum JA, Steffen LM, Labarthe DR. Blood lipids in children: age-related patterns and association with body-fat indices: Project HeartBeat! *Am J Prev Med.* 2009;37(1 Suppl):S56-64.
7. Gallagher D, Kelley DE, Yim JE, et al. Adipose tissue distribution is different in type 2 diabetes. *The American journal of clinical nutrition.* 2009;89(3):807-814.
8. Yim JE, Heshka S, Albu JB, Heymsfield S, Gallagher D. Femoral-gluteal subcutaneous and intermuscular adipose tissues have independent and opposing relationships with CVD risk. *J Appl Physiol.* 2008;104(3):700-707.
9. Hull HR, Thornton JC, Ji Y, et al. Higher infant body fat with excessive gestational weight gain in overweight women. *American journal of obstetrics and gynecology.* 2011;205(3):211 e211-217.
10. Badon SE, Dyer AR, Josefson JL, Group HSCR. Gestational weight gain and neonatal adiposity in the Hyperglycemia and Adverse Pregnancy Outcome study-North American region. *Obesity (Silver Spring, Md.* 2014;22(7):1731-1738.
11. Brown JE, Murtaugh MA, Jacobs DR, Jr., Margellos HC. Variation in newborn size according to pregnancy weight change by trimester. *The American journal of clinical nutrition.* 2002;76(1):205-209.
12. Lepercq J, Hauguel-De Mouzon S, Timsit J, Catalano PM. Fetal macrosomia and maternal weight gain during pregnancy. *Diabetes & metabolism.* 2002;28(4 Pt 1):323-328.
13. 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 e411-416.
14. Crozier SR, Inskip HM, Godfrey KM, et al. Weight gain in pregnancy and childhood body composition: findings from the Southampton Women's Survey. *The American journal of clinical nutrition.* 2010;91(6):1745-1751.
15. 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.

16. Reynolds RM, Osmond C, Phillips DIW, Godfrey KM. Maternal BMI, Parity, and Pregnancy Weight Gain: Influences on Offspring Adiposity in Young Adulthood. *J Clin Endocr Metab.* 2010;95(12):5365-5369.
17. Sridhar SB, Darbinian J, Ehrlich SF, et al. Maternal gestational weight gain and offspring risk for childhood overweight or obesity. *American journal of obstetrics and gynecology.* 2014;211(3):259 e251-258.
18. Mourtakos SP, Tambalis KD, Panagiotakos DB, et al. Maternal lifestyle characteristics during pregnancy, and the risk of obesity in the offspring: a study of 5,125 children. *BMC Pregnancy Childbirth.* 2015;15:66.
19. Reilly JJ, Armstrong J, Dorosty AR, et al. Early life risk factors for obesity in childhood: cohort study. *BMJ (Clinical research ed.* 2005;330(7504):1357.
20. Parsons TJ, Power C, Manor O. Fetal and early life growth and body mass index from birth to early adulthood in 1958 British cohort: longitudinal study. *BMJ (Clinical research ed.* 2001;323(7325):1331-1335.
21. Llewellyn A, Simmonds M, Owen CG, Woolacott N. Childhood obesity as a predictor of morbidity in adulthood: a systematic review and meta-analysis. *Obes Rev.* 2016;17(1):56-67.
22. Leverence RR, Williams RL, Sussman A, Crabtree BF, Clinicians RN. Obesity counseling and guidelines in primary care: a qualitative study. *Am J Prev Med.* 2007;32(4):334-339.
23. Phelan S. Pregnancy: a "teachable moment" for weight control and obesity prevention. *American journal of obstetrics and gynecology.* 2010;202(2):135 e131-138.
24. Thangaratinam S, Rogozinska E, Jolly K, et al. Effects of interventions in pregnancy on maternal weight and obstetric outcomes: meta-analysis of randomised evidence. *BMJ (Clinical research ed.* 2012;344:e2088.
25. Oteng-Ntim E, Varma R, Croker H, Poston L, Doyle P. Lifestyle interventions for overweight and obese pregnant women to improve pregnancy outcome: systematic review and meta-analysis. *BMC medicine.* 2012;10:47.
26. Gardner B, Wardle J, Poston L, Croker H. Changing diet and physical activity to reduce gestational weight gain: a meta-analysis. *Obes Rev.* 2011;12(7):e602-620.
27. Streuling I, Beyerlein A, von Kries R. Can gestational weight gain be modified by increasing physical activity and diet counseling? A meta-analysis of interventional trials. *The American journal of clinical nutrition.* 2010;92(4):678-687.
28. Muktabhant B, Lumbiganon P, Ngamjarus C, Dowswell T. Interventions for preventing excessive weight gain during pregnancy. *The Cochrane database of systematic reviews.* 2012;4:CD007145.
29. Tanentsapf I, Heitmann BL, Adegboye AR. Systematic review of clinical trials on dietary interventions to prevent excessive weight gain during pregnancy among normal weight, overweight and obese women. *BMC Pregnancy Childbirth.* 2011;11:81.
30. Campbell F, Johnson M, Messina J, Guillaume L, Goyder E. Behavioural interventions for weight management in pregnancy: a systematic review of quantitative and qualitative data. *BMC Public Health.* 2011;11:491.
31. Thangaratinam S, Rogozinska E, Jolly K, et al. Interventions to reduce or prevent obesity in pregnant women: a systematic review. *Health technology assessment.* 2012;16(31):iii-iv, 1-191.

32. Claesson IM, Sydsjo G, Brynhildsen J, et al. Weight gain restriction for obese pregnant women: a case-control intervention study. *BJOG.* 2008;115(1):44-50.
33. Mottola MF, Giroux I, Gratton R, et al. Nutrition and exercise prevent excess weight gain in overweight pregnant women. *Med Sci Sports Exerc.* 2010;42(2):265-272.
34. Hui A, Back L, Ludwig S, et al. Lifestyle intervention on diet and exercise reduced excessive gestational weight gain in pregnant women under a randomised controlled trial. *BJOG.* 2012;119(1):70-77.
35. Asbee SM, Jenkins TR, Butler JR, White J, Elliot M, Rutledge A. Preventing excessive weight gain during pregnancy through dietary and lifestyle counseling: a randomized controlled trial. *Obstetrics and gynecology.* 2009;113(2 Pt 1):305-312.
36. Guelinckx I, Devlieger R, Mullie P, Vansant G. Effect of lifestyle intervention on dietary habits, physical activity, and gestational weight gain in obese pregnant women: a randomized controlled trial. *The American journal of clinical nutrition.* 2010;91(2):373-380.
37. Huang TT, Yeh CY, Tsai YC. A diet and physical activity intervention for preventing weight retention among Taiwanese childbearing women: a randomised controlled trial. *Midwifery.* 2011;27(2):257-264.
38. Ilmonen J, Isolauri E, Poussa T, Laitinen K. Impact of dietary counselling and probiotic intervention on maternal anthropometric measurements during and after pregnancy: a randomized placebo-controlled trial. *Clinical nutrition (Edinburgh, Scotland).* 2011;30(2):156-164.
39. Jackson RA, Stotland NE, Caughey AB, Gerbert B. Improving diet and exercise in pregnancy with Video Doctor counseling: a randomized trial. *Patient education and counseling.* 2011;83(2):203-209.
40. Gray-Donald K, Robinson E, Collier A, David K, Renaud L, Rodrigues S. Intervening to reduce weight gain in pregnancy and gestational diabetes mellitus in Cree communities: an evaluation. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne.* 2000;163(10):1247-1251.
41. Olson CM, Strawderman MS, Reed RG. Efficacy of an intervention to prevent excessive gestational weight gain. *American journal of obstetrics and gynecology.* 2004;191(2):530-536.
42. Kinnunen TI, Aittasalo M, Koponen P, et al. Feasibility of a controlled trial aiming to prevent excessive pregnancy-related weight gain in primary health care. *BMC Pregnancy Childbirth.* 2008;8:37.
43. Shirazian T, Monteith S, Friedman F, Rebarber A. Lifestyle modification program decreases pregnancy weight gain in obese women. *American journal of perinatology.* 2010;27(5):411-414.
44. Dodd JM, Turnbull D, McPhee AJ, et al. Antenatal lifestyle advice for women who are overweight or obese: LIMIT randomised trial. *BMJ (Clinical research ed.* 2014;348:g1285.
45. Phelan S, Phipps MG, Abrams B, Darroch F, Schaffner A, Wing RR. Randomized trial of a behavioral intervention to prevent excessive gestational weight gain: the Fit for Delivery Study. *The American journal of clinical nutrition.* 2011;93(4):772-779.
46. Polley BA, Wing RR, Sims CJ. Randomized controlled trial to prevent excessive weight gain in pregnant women. *Int J Obes Relat Metab Disord.* 2002;26(11):1494-1502.

47. Carlson SE, Colombo J, Gajewski BJ, et al. DHA supplementation and pregnancy outcomes. *The American journal of clinical nutrition.* 2013;97(4):808-815.
48. Godfrey KM, Barker DJ. Fetal nutrition and adult disease. *The American journal of clinical nutrition.* 2000;71(5 Suppl):1344S-1352S.
49. Fickova M, Hubert P, Cremel G, Leray C. Dietary (n-3) and (n-6) polyunsaturated fatty acids rapidly modify fatty acid composition and insulin effects in rat adipocytes. *The Journal of nutrition.* 1998;128(3):512-519.
50. Schwinkendorf DR, Tsatsos NG, Gosnell BA, Mashek DG. Effects of central administration of distinct fatty acids on hypothalamic neuropeptide expression and energy metabolism. *Int J Obes (Lond).* 2011;35(3):336-344.
51. Clarke SD, Jump D. Polyunsaturated fatty acids regulate lipogenic and peroxisomal gene expression by independent mechanisms. *Prostaglandins, leukotrienes, and essential fatty acids.* 1997;57(1):65-69.
52. Jump DB, Clarke SD, Thelen A, Liimatta M. Coordinate regulation of glycolytic and lipogenic gene expression by polyunsaturated fatty acids. *Journal of lipid research.* 1994;35(6):1076-1084.
53. Amri EZ, Ailhaud G, Grimaldi PA. Fatty acids as signal transducing molecules: involvement in the differentiation of preadipose to adipose cells. *Journal of lipid research.* 1994;35(5):930-937.
54. Colombo J, Carlson SE, Cheatham CL, et al. Long-term effects of LCPUFA supplementation on childhood cognitive outcomes. *The American journal of clinical nutrition.* 2013;98(2):403-412.
55. Simopoulos AP. Omega-3 fatty acids in inflammation and autoimmune diseases. *J Am Coll Nutr.* 2002;21(6):495-505.
56. Proudman SM, Cleland LG, James MJ. Dietary omega-3 fats for treatment of inflammatory joint disease: efficacy and utility. *Rheumatic diseases clinics of North America.* 2008;34(2):469-479.
57. Balvers MG, Verhoeckx KC, Plastina P, Wortelboer HM, Meijerink J, Witkamp RF. Docosahexaenoic acid and eicosapentaenoic acid are converted by 3T3-L1 adipocytes to N-acyl ethanolamines with anti-inflammatory properties. *Biochimica et biophysica acta.* 2010;1801(10):1107-1114.
58. 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):1743-1749.
59. Korotkova M, Gabrielsson BG, Holmang A, Larsson BM, Hanson LA, Strandvik B. Gender-related long-term effects in adult rats by perinatal dietary ratio of n-6/n-3 fatty acids. *American journal of physiology. Regulatory, integrative and comparative physiology.* 2005;288(3):R575-579.
60. 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):2965-2969.
61. Gaillard D, Negrel R, Lagarde M, Ailhaud G. Requirement and role of arachidonic acid in the differentiation of pre-adipose cells. *Biochem J.* 1989;257(2):389-397.
62. Spalding KL, Arner E, Westermark PO, et al. Dynamics of fat cell turnover in humans. *Nature.* 2008;453(7196):783-787.

63. 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.
64. Burdge GC. Metabolism of alpha-linolenic acid in humans. *Prostaglandins, leukotrienes, and essential fatty acids.* 2006;75(3):161-168.
65. Emken EA, Adlof RO, Gulley RM. Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males. *Biochimica et biophysica acta.* 1994;1213(3):277-288.
66. *Committee on Nutrient Relationships in Seafood: Selections to Balance Benefits and Risks.* Washington, DC: INSTITUTE OF MEDICINE: Food and Nutrition Board;2007. p.
67. Parra MS, Schnaas L, Meydani M, Perroni E, Martinez S, Romieu I. Erythrocyte cell membrane phospholipid levels compared against reported dietary intakes of polyunsaturated fatty acids in pregnant Mexican women. *Public Health Nutr.* 2002;5(6A):931-937.
68. Yuhas R, Pramuk K, Lien EL. Human milk fatty acid composition from nine countries varies most in DHA. *Lipids.* 2006;41(9):851-858.
69. 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):469-479.
70. Gustafson KM, Carlson SE, Colombo J, et al. 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):331-338.
71. Kuipers RS, Luxwolda MF, Sango WS, Kwesigabo G, Dijck-Brouwer DA, Muskiet FA. Postdelivery changes in maternal and infant erythrocyte fatty acids in 3 populations differing in fresh water fish intakes. *Prostaglandins, leukotrienes, and essential fatty acids.* 2011;85(6):387-397.
72. Otto SJ, van Houwelingen AC, Badart-Smook A, Hornstra G. Changes in the maternal essential fatty acid profile during early pregnancy and the relation of the profile to diet. *The American journal of clinical nutrition.* 2001;73(2):302-307.
73. Lakin V, Haggarty P, Abramovich DR, et al. Dietary intake and tissue concentration of fatty acids in omnivore, vegetarian and diabetic pregnancy. *Prostaglandins, leukotrienes, and essential fatty acids.* 1998;59(3):209-220.
74. Sanz N, Diaz M, Lopez-Bermejo A, et al. Newborns with lower levels of circulating polyunsaturated fatty acids (PUFA) are abdominally more adipose. *Pediatr Obes.* 2014;9(3):e68-72.
75. 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):780-788.
76. Moon RJ, Harvey NC, Robinson SM, et al. 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.
77. Vidakovic AJ, Gishti O, Voortman T, et al. Maternal plasma PUFA concentrations during pregnancy and childhood adiposity: the Generation R Study. *The American journal of clinical nutrition.* 2016.

78. Rodriguez G, Iglesia I, Bel-Serrat S, Moreno LA. Effect of n-3 long chain polyunsaturated fatty acids during the perinatal period on later body composition. *Br J Nutr.* 2012;107 Suppl 2:S117-128.
79. Stratakis N, Gielen M, Chatzi L, Zeegers MP. Effect of maternal n-3 long-chain polyunsaturated fatty acid supplementation during pregnancy and/or lactation on adiposity in childhood: a systematic review and meta-analysis of randomized controlled trials. *European journal of clinical nutrition.* 2014;68(12):1277-1287.
80. Hauner H, Much D, Vollhardt C, 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):383-394.
81. Asserhoj M, Nehammer S, Matthiessen J, Michaelsen KF, Lauritzen L. Maternal fish oil supplementation during lactation may adversely affect long-term blood pressure, energy intake, and physical activity of 7-year-old boys. *The Journal of nutrition.* 2009;139(2):298-304.
82. Helland IB, Smith L, Blomen B, Saarem K, Saugstad OD, Drevon CA. Effect of supplementing pregnant and lactating mothers with n-3 very-long-chain fatty acids on children's IQ and body mass index at 7 years of age. *Pediatrics.* 2008;122(2):e472-479.
83. Lucia Bergmann R, Bergmann KE, Haschke-Becher E, et al. Does maternal docosahexaenoic acid supplementation during pregnancy and lactation lower BMI in late infancy? *J Perinat Med.* 2007;35(4):295-300.
84. Lauritzen L, Hoppe C, Straarup EM, Michaelsen KF. Maternal fish oil supplementation in lactation and growth during the first 2.5 years of life. *Pediatric research.* 2005;58(2):235-242.
85. Stein AD, Wang M, Martorell R, et al. Growth to age 18 months following prenatal supplementation with docosahexaenoic acid differs by maternal gravidity in Mexico. *The Journal of nutrition.* 2011;141(2):316-320.
86. Gonzalez-Casanova I, Stein AD, Hao W, et al. Prenatal Supplementation with Docosahexaenoic Acid Has No Effect on Growth through 60 Months of Age. *The Journal of nutrition.* 2015;145(6):1330-1334.
87. 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):701-708.
88. Campoy C, Escolano-Margarit MV, Ramos R, et al. Effects of prenatal fish-oil and 5-methyltetrahydrofolate supplementation on cognitive development of children at 6.5 y of age. *The American journal of clinical nutrition.* 2011;94(6 Suppl):1880S-1888S.
89. Muhlhausler BS, Yelland LN, McDermott R, et al. 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.
90. Makrides M, Gibson RA, McPhee AJ, et al. Effect of DHA supplementation during pregnancy on maternal depression and neurodevelopment of young children: a randomized controlled trial. *JAMA.* 2010;304(15):1675-1683.
91. 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):1252-1258.

92. Sewell MF, Huston-Presley L, Super DM, Catalano P. Increased neonatal fat mass, not lean body mass, is associated with maternal obesity. *American journal of obstetrics and gynecology.* 2006;195(4).
93. Wells JC, Fewtrell MS. Measuring body composition. *Archives of disease in childhood.* 2006;91(7):612-617.
94. Wells JC. A Hattori chart analysis of body mass index in infants and children. *Int J Obes Relat Metab Disord.* 2000;24(3):325-329.
95. Calder PC. Dietary modification of inflammation with lipids. *Proc Nutr Soc.* 2002;61(3):345-358.
96. Price PT, Nelson CM, Clarke SD. Omega-3 polyunsaturated fatty acid regulation of gene expression. *Current opinion in lipidology.* 2000;11(1):3-7.
97. Sampath H, Ntambi JM. Polyunsaturated fatty acid regulation of gene expression. *Nutrition reviews.* 2004;62(9):333-339.
98. Ren D, Collingwood TN, Rebar EJ, Wolffe AP, Camp HS. PPARgamma knockdown by engineered transcription factors: exogenous PPARgamma2 but not PPARgamma1 reactivates adipogenesis. *Genes & development.* 2002;16(1):27-32.
99. Rosen ED, Sarraf P, Troy AE, et al. PPAR gamma is required for the differentiation of adipose tissue in vivo and in vitro. *Molecular cell.* 1999;4(4):611-617.
100. Rosen ED, Hsu CH, Wang X, et al. C/EBPalpha induces adipogenesis through PPARgamma: a unified pathway. *Genes & development.* 2002;16(1):22-26.
101. Kubota N, Terauchi Y, Miki H, et al. PPAR gamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Molecular cell.* 1999;4(4):597-609.
102. Barak Y, Nelson MC, Ong ES, et al. PPAR gamma is required for placental, cardiac, and adipose tissue development. *Molecular cell.* 1999;4(4):585-595.
103. Jump DB, Tripathy S, Depner CM. Fatty acid-regulated transcription factors in the liver. *Annual review of nutrition.* 2013;33:249-269.
104. Cardel M, Lemas DJ, Jackson KH, Friedman JE, Fernandez JR. Higher Intake of PUFAs Is Associated with Lower Total and Visceral Adiposity and Higher Lean Mass in a Racially Diverse Sample of Children. *The Journal of nutrition.* 2015;145(9):2146-2152.
105. Kabir M, Skurnik G, Naour N, et al. Treatment for 2 mo with n 3 polyunsaturated fatty acids reduces adiposity and some atherogenic factors but does not improve insulin sensitivity in women with type 2 diabetes: a randomized controlled study. *The American journal of clinical nutrition.* 2007;86(6):1670-1679.
106. Mansoori A, Sotoudeh G, Djalali M, et al. Docosahexaenoic Acid-Rich Fish Oil Supplementation Improves Body Composition without Influence of the PPARgamma Pro12Ala Polymorphism in Patients with Type 2 Diabetes: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial. *J Nutrigenet Nutrigenomics.* 2015;8(4-6):195-204.
107. Black RE, Victora CG, Walker SP, Grp MCNS. Maternal and Child Nutrition Study Group. Maternal and child undernutrition and overweight in low-income and middle-income countries (vol 382, pg 427, 2013). *Lancet.* 2013;382(9890):396-396.
108. 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):486-492.
109. 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):134-141.

110. Nochera CL, Goossen LH, Brutus AR, Cristales M, Eastman B. Consumption of DHA + EPA by low-income women during pregnancy and lactation. *Nutr Clin Pract.* 2011;26(4):445-450.
111. Troxell H, Anderson J, Auld G, et al. 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):189-197.
112. Vardeman JE, Aldoory L. A qualitative study of how women make meaning of contradictory media messages about the risks of eating fish. *Health Commun.* 2008;23(3):282-291.
113. 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):346-351.
114. Papanikolaou Y, Brooks J, Reider C, Fulgoni VL, 3rd. U.S. adults are not meeting recommended levels for fish and omega-3 fatty acid intake: results of an analysis using observational data from NHANES 2003-2008. *Nutr J.* 2014;13:31.
115. *Dietary reference intakes for energy, carbohydrates, fiber, fat, fatty acids, cholesterol, protein and amino acids.* Institute of Medicine Panel on Macronutrients;2005. p.
116. Valentine CJ, Kleinman J, Dingess KA, Morrow AL, Rogers LK. Maternal docosahexaenoic acid (DHA) supplementation promotes lean body mass in the preterm infant. *Experimental Biology.* 2016;30(1 Supplement):432.435.
117. Meas T, Deghmoun S, Armoogum P, Alberti C, Levy-Marchal C. Consequences of being born small for gestational age on body composition: an 8-year follow-up study. *The Journal of clinical endocrinology and metabolism.* 2008;93(10):3804-3809.
118. Kramer MS, Martin RM, Bogdanovich N, Vilchuk K, Dahhou M, Oken E. Is restricted fetal growth associated with later adiposity? Observational analysis of a randomized trial. *The American journal of clinical nutrition.* 2014;100(1):176-181.
119. Park YW, Allison DB, Heymsfield SB, Gallagher D. Larger amounts of visceral adipose tissue in Asian Americans. *Obesity research.* 2001;9(7):381-387.
120. Vozarova B, Wang J, Weyer C, Tataranni PA. Comparison of two software versions for assessment of body-composition analysis by DXA. *Obesity research.* 2001;9(3):229-232.
121. *Weight gain during pregnancy: Reexamining the guidelines.* Washington (DC): Institute of Medicine, National Research Council, Committee to Reexamine IOM Pregnancy Weight Guidelines;2009. p.
122. Conway JM, Ingwersen LA, Vinyard BT, Moshfegh AJ. Effectiveness of the US Department of Agriculture 5-step multiple-pass method in assessing food intake in obese and nonobese women. *The American journal of clinical nutrition.* 2003;77(5):1171-1178.
123. Blanton CA, Moshfegh AJ, Baer DJ, Kretsch MJ. The USDA Automated Multiple-Pass Method accurately estimates group total energy and nutrient intake. *The Journal of nutrition.* 2006;136(10):2594-2599.
124. Hebert JR, Hurley TG, Chiriboga DE, Barone J. A comparison of selected nutrient intakes derived from three diet assessment methods used in a low-fat maintenance trial. *Public Health Nutr.* 1998;1(3):207-214.

PROTECTION OF HUMAN SUBJECTS

This study will recruit offspring of women who participated in prenatal DHA supplementation intervention. Offspring body composition and AT distribution will be assessed at 2 weeks and 6, 12, and 24 months using dual energy x-ray absorptiometry (DXA).

RISKS TO HUMAN SUBJECTS

Human Subjects Involvement, Characteristics, and Design

1. *Patient recruitment and informed consent.* All recruitment, consent, and data forms for the study proposal will be approved prior to any enrollment. Informed consent for continued testing is obtained by a trained research team member with NIH-approved Human Subjects' protection certification. Consent includes the standard elements: a study description, the potential risks, benefits and options for non-participation. All subjects are informed they are free to withdraw from the study without changes in their usual care. Consent is documented as a signed form and will be kept in a locked file at the study office. The study is conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB review includes a review of all appropriate study documentation in order to safeguard the rights, safety and well-being of the subjects. The protocol, informed consent, written information given to the subjects, safety updates, annual progress reports, and any revisions to these documents are provided to the IRB by the principal investigators. The method of obtaining and documenting the informed consent and the contents of the consent will comply with GCP and all applicable regulatory requirement(s).
2. *Describe the characteristics of the subject populations and identify criteria for inclusion and exclusion.* Subjects will consist of infants born to women who participated in a DHA supplementation RCT. A description of the women participating in the parent RCT is as follows: women will be between 12 and 20 wk gestation without medical conditions that put their pregnancy at higher than normal risk.
3. *Identify the sources of research material to be obtained from subjects.* Data to be acquired will be body composition and anthropometric measures, and information regarding the pregnancy from questionnaires (pre-pregnancy BMI, GWG, parity, infant diet, infant feeding practices, etc). All participants will be assigned a non-identifiable subject number. Data collected will be kept in Dr. Hull's locked research laboratory. Only study personnel and Dr. Hull have keys to access this area. Electronic data will be kept on a password protected computer in Dr. Hull's laboratory on the shared drive. Only personnel approved by HSC and on the protocol will have access to the data.
4. *Describe all potential risks.* No appreciable risk of physical, psychological, social, legal or other harm is expected. Assessment of infant body composition and skinfolds is very low risk. The DXA does emit radiation. Four total body DXA scans will be completed over 24 months. The amount of radiation exposure for one DXA scan is equal to 0.001 mSV or the equivalent of exposure to 3 hours of natural background radiation. As a comparison, one chest x-ray is equal to 0.1 mSV or 10 days of exposure to natural background radiation. Over the course of 24 months, the total radiation exposure will be under a day of background radiation exposure. This is minimal especially considered it is spread over 24 months.
5. *Describe the procedures for protecting against or minimizing any potential risks.* N/A. No known risks.
6. *Discuss why the risks are reasonable in relation to anticipated benefits.*
7. N/A. No known risks.
8. *Plan for monitoring and reporting problems.* Research staff will immediately report any adverse events to the study PI for review.
9. *Plan for handling study withdrawal/discontinuation.* Participants may discontinue study participation at any time in the study.

Adequacy of Protection Against Risks
Describe plans for the recruitment of subjects and the consent procedures to be followed.
Recruitment will be open to all pregnant women that participated in the parent study. The KUMC IRB will review and approve this study prior to implementation. Participant recruitment will be conducted by trained study staff under the supervision the PI. Consent forms and HIPPA disclosure information per IRBs will be given to all subjects and their signatures' witnessed. The consent forms will include a description of the study, nature of the data collection, the potential benefits and adverse reactions anticipated. The research team personnel will abide by all tenets of the University confidentiality policies, as well as the Privacy Protection for Research Subjects. All research staff will remain current in their NIH required Human Subjects protection and HIPAA certification.

Data monitors and auditors from the IRBs, and regulatory authorities will have access to the patient's original medical records for verification of data gathered on the case report forms and to audit the data collection process. Subjects will be made aware of persons who may see their protected health information in the informed consent document and may choose not to enroll in the study based on the information provided them in accord with 2003 HIPAA regulations. The patient's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

The investigators will conduct the study in compliance with the protocol given approval by the KUMC IRB. Any changes to the protocol will require written approval from these committees prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to patients or if the change(s) involves only logistical or administrative aspects of the trial. Any departures from the protocol will be fully documented in the case report form and source documentation. IRB review occurs each year and newly dates consent forms are issued. The Research Assistant will keep the study binder that includes all communication with the IRB and CVs for all study personnel, and study personnel approved roles. The ultimate responsibility for ensuring that the study binders are in order will fall to the PI (Hull).

Patient recruitment and informed consent. All recruitment, consent and data forms for the study proposal will be submitted to the KUMC IRB prior to enrollment using templates and educational materials formatted as previously IRB-approved in the pilot study. Informed consent will be obtained by trained research personnel who have completed the NIH-approved Human Subjects' protection certification. Consent includes the standard elements: a study description, the potential risks, benefits and options for non-participation. All participants will be informed that they are free to withdraw from the study without changes in their usual care. Consent will be documented as a signed form and will be kept in a locked file at the study office. The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the subjects. The protocol, informed consent, written information given to the subjects, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRBs by the investigator. The method of obtaining and documenting the informed consent and the contents of the consent will comply with GCP and all applicable regulatory requirement(s).

Only those women who consented to be contacted regarding additional studies in Dr. Carlson's RCT will be approached. At consent, women will be asked if they give permission for future contact and if yes, a database with willing names to be contacted will be created. These women will be approached regarding enrollment into this study. For each visit completed, subjects will

be paid $125. Therefore, if subjects complete all study visits, they will receive $500. The incentives to be given are reasonable for the amount of participant contact that the study entails.

Protection against risk. Although no appreciable risk of physical or mental harm is expected to result from the protocol, procedures for dealing with adverse effects are established. Subjects will be instructed to report immediately to the study coordinator if any health problem occurs and to call the PI (Hull) at any time if they have questions are concerns. In such an event, it is the PI responsibility to answer those concerns honestly and to reiterate to the subject that they should continue in the trial only if they feel entirely comfortable with it. Subjects who choose to withdraw from the study will be reassured if they indicate the desire to withdraw.

Subjects will be protected against the risk of breaking confidentiality by decoupling of names from databases. Each participant will be assigned a numerical study ID and informed consent forms that include the subject's signature will be stored separately in locked file cabinets. For ensuring confidentiality, these are generally acknowledged to be the best methods known for ensuring that names are not associated with data. Only selected research staff will have access to the subjects' data. Subjects' informed consent includes the HIPAA compliance documentation approved by the KUMC IRB. Research team personnel will abide by all tenets of the University confidentiality policies as well as the Privacy Protection for Research Subjects.

Potential Benefits of Proposed Research
Describe all potential benefits. There are no direct benefits for participation in the study outside of contributing to the knowledge base on this topic.

Data Safety and Monitoring Plan
Data security: All participants will be assigned a non-identifiable subject number. Data collected will be kept in Dr. Hull's locked research laboratory. Only study personnel and Dr. Hull have keys to access this area. Electronic data will be kept on a password protected computer in Dr. Hull's laboratory on the shared drive. Only personnel approved by HSC and on the protocol will have access to the data.

Data and safety monitoring plan for the current Observational follow up study: We are not providing any interventions but only monitor outcomes of the offspring born to women participating in the parent RCT. Monitoring will be handled by the PI and the research team. For this ancillary study, research staff will immediately report any adverse events to the study PI for review. The study PI will report to the study team and a decision will be made regarding any action that should be taken.

Safety Monitoring of the already approved and NIH funded Parent RCT trial
The parent RCT has already been vetted, approved, and deemed safe through rigorous peer review. Details for safety monitoring of the parent RCT are included below.

Adverse Events Analyses: Fisher's exact test will be used to compare the incidence of maternal and infant adverse events between treatment groups. All p values will be evaluated at the $\alpha = 0.05$ level. No adjustment for multiple comparisons will be made.

Definitions of an Adverse Event: *An adverse event (AE) is any reaction, side effect or other undesirable event that occurs in conjunction with the use of the test product, whether or not the event is considered related to the test product. New and worsening signs and symptoms of underlying or emerging disease will be recorded as an adverse event. Any patient complaint reported will be recorded as an adverse event. Signs and symptoms considered normal for*

pregnant or delivering patients will be collected as adverse events to ensure that even problems common to pregnant women such as headache, (lower) back pain, (lower) backache, abdominal pain, abdominal cramps and nausea are not influenced by consuming the DHA capsules.

Definition of a Serious Adverse Event: *Any adverse event occurring that results in the following is considered a serious adverse event (SAE): 1) death; 2) a life-threatening event; 3) inpatient hospitalization or prolonging of an existing hospitalization; 4) a persistent or significant disability/incapacity or 5) a congenital anomaly/birth defect. Medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.*

Safety Monitoring: A Data Safety Monitoring Board (DSMB) will monitor the study for safety. Dr. Ardythe Morrow (Professor in Biostatistics and Epidemiology Center at CCHMC), Dr. Kurt Schibler (Neonatologist and The Medical Director of Clinical research for the Neonatal Netwrok) and Alexander Vinks PhD (Pharmacology expert) will be the members of the DSMB in addition to Dr. Daniel Robinson, as the medical monitor. All adverse events that are not expected during pregnancy will be tracked from medical records and by periodic phone interview when the study coordinator calls to check for supplement compliance and tolerance. All adverse events will be documented in eResearch and in the study binder. SAEs will be reported to the Central IRB and the medical monitor (Dr. Robinson) within 2 days of our becoming aware of them. The DSMB will provide and independent review of the ongoing data, patient reports, adverse events at least yearly, making their deliberation about safety using data of adverse events from all 3 recruitment sites. Through its reviews of the study, the DSMB will determine whether cumulative data indicates the need to change the research design, to modify information presented to participants, or to terminate the project. Any action taken to suspend or terminate the project will be reported to the Central IRB, NIH Office of Sponsored Projects and the program director at NIH. The DSMB and medical monitor will evaluate the final study manuscript(s) and final reports to assure results are fairly presented and conclusions are appropriate.

INCLUSION OF WOMEN AND MINORITIES

Inclusion of Women: We are targeting pregnant women who have participated in a RCT designed study to test the impact of high versus low prenatal DHA supplementation on moving early pre-term birth to late pre-term birth. This study will continue to follow this cohort by studying the impact of high versus low prenatal DHA supplementation on offspring body composition and AT distribution.

Inclusion of Minorities: We are including all racial/ethnic groups in this study. There will be no exclusion criteria based on race or ethnicity.

INCLUSION OF CHILDREN

We are targeting pregnant women who have participated in a RCT designed study to test the impact of high versus low prenatal DHA supplementation. We will follow the offspring born to these women from birth to 2 years old.

Data Access, Dissemination, and Authorship

The data generated by this project will be entered into a system developed and managed by Dr. Gajewski's team in Biostatistics at KUMC. KUMC maintains an elaborate and secure digital networks for storage and remote access of data through encrypted and password-protected servers. Typically, with longitudinal projects like this one, decisions as to how and when to publish empirical reports is a difficult one. To resolve this issue, the research team will map out a preliminary dissemination plan that is principled yet flexible enough to allow for the clearest manner of presenting the results, and to determine the extent of authorship. This has been a satisfactory mechanism for the research team to address the issue of publication without conflict in their ongoing collaborations.