Omega Tots: A randomized, controlled trial of long-chain polyunsaturated fatty acid supplementation of toddler diets and developmental outcomes

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Erratum 03/18/2020: A typo was identified in the protocol document about the block sizes used in the randomization schedule. Dr. Turner prepared the randomization schedule using block sizes of 4 and 8, not 2 and 4.

STATEMENT OF COMPLIANCE

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46; 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312)
- ICH E6; 62 Federal Register 25691 (1997)
- NIH Clinical Terms of Award

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Site Investigator:*

Signed: _____ Date: _____

Name
Title

** The protocol should be signed by the local investigator who is responsible for the study implementation at his/her specific site; ie, if Investigational New Drug study, the individual who signs the Form FDA 1572.*

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LIST OF ABBREVIATIONS

AA	Arachidonic acid
AE	Adverse Event/Adverse Experience
ARASCO	ARASCO AA product
BITSEA	Brief Infant Toddler Social Emotional Assessment
Bayley-3	Bayley Scales of Infant and Toddler Development, 3rd edition
BPD	Bronchopulmonary dysplasia
BSID-II	Bayley Scales of Infant Development, 2nd edition
CCTS	Center for Clinical and Translational Science
CES-D	Center for Epidemiologic Studies-Depression Scale
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
CONSORT	Consolidated Standards of Reporting Trials
CFR	Code of Federal Regulations
CRF	Case Report Form
DHA	Docosahexaenoic acid
DHASCO	DHASCO DHA product
DHHS	Department of Health and Human Services
DSMB	Data and Safety Monitoring Board
ECBQ	Early Childhood Behavior Questionnaire
FDA	Food and Drug Administration
FFQ	Food Frequency Questionnaire
FWA	Federalwide Assurance
GCP	Good Clinical Practice
GRAS	Generally Recognized as Safe
HIPAA	Health Insurance Portability and Accountability Act
HRSA	Health Resources and Services Administration
HSFFQ	Harvard Service Food Frequency Questionnaire
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application

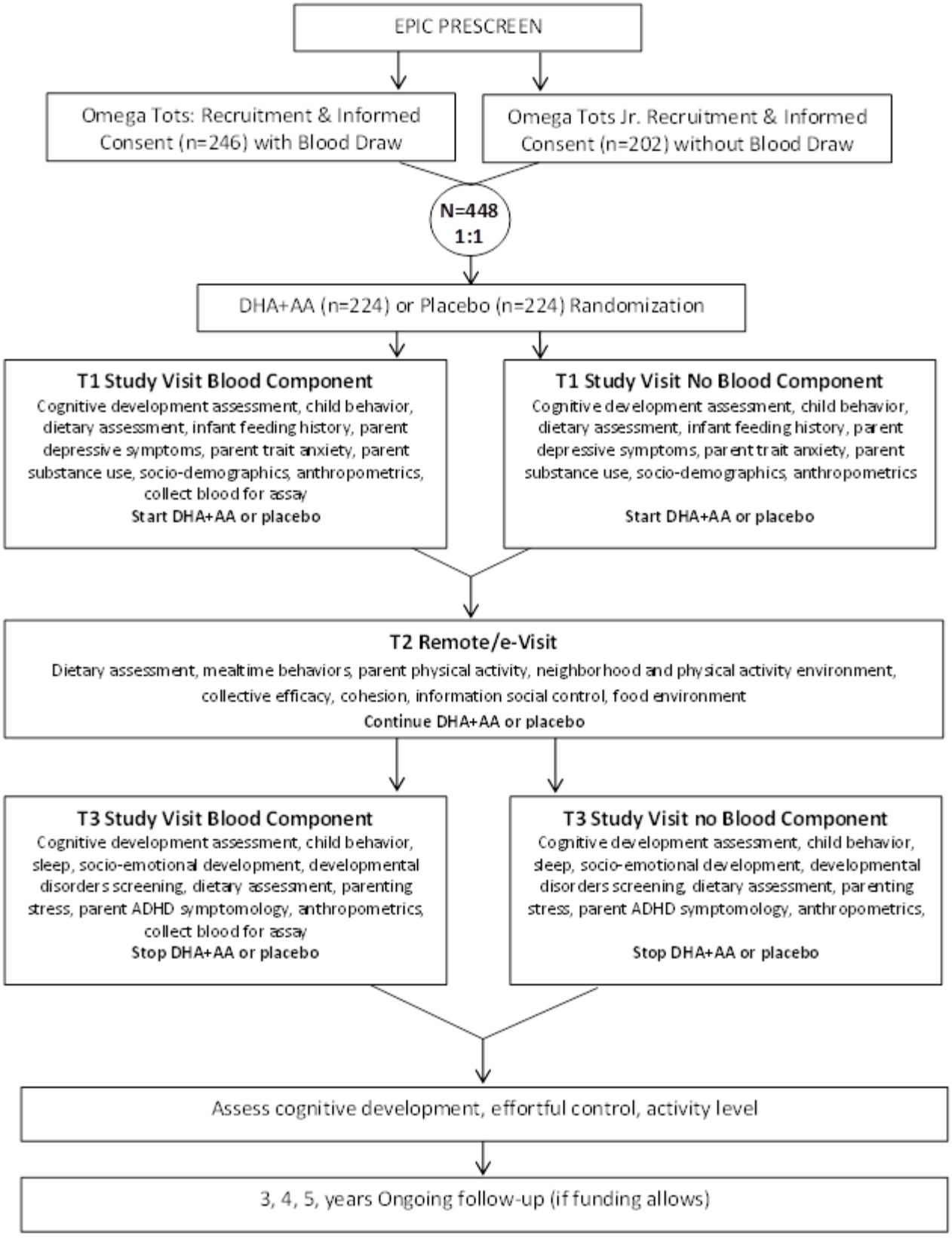
IRB	Institutional Review Board
ISM	Independent Safety Monitor
JAMA	Journal of the American Medical Association
LCPUFA	Long-chain Polyunsaturated Fatty Acid
MDI	Mental Development Index
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NCH	Nationwide Children's Hospital
NEJM	New England Journal of Medicine
NICU	Neonatal Intensive Care Unite
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research
OPRN	Ohio Perinatal Research Network
OSU	The Ohio State University
PHI	Protected Health Information
PI	Principal Investigator
PRR	Perinatal Research Repository
PSI	Parenting Stress Index
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
SAS	Statistical Analysis System software
SOP	Standard Operating Procedure
STAI-T	Spielberger State-Trait Anxiety Inventory – Trait scale
US	United States
WHO	World Health Organization

PROTOCOL SUMMARY

Title:	**Omega Tots: A randomized, controlled trial of long-chain polyunsaturated fatty acid supplementation of toddler diets and developmental outcomes**
Abstract:	Currently in the United States, 1 out of every 9 babies is born prematurely, and these children have an increased risk of cognitive deficits and learning disabilities. Previous studies have found dietary docosahexaenoic acid (DHA) supplementation *during infancy* enhances the cognitive development of preterm children. No studies have examined whether supplementation *during the second year of life* is beneficial although this is a critical window for development of higher level cognitive abilities and the average daily dietary intake of omega-3 fatty acids in this age group is low. We propose to conduct a randomized, placebo-controlled DHA+AA supplementation trial of 448 children 10-16 months old (corrected age) who were born at <35 weeks' gestation. Our aims are to: 1) Compare cognitive ability between the DHA+AA arm and placebo after 180 days of intervention and to 2) Compare measures of effortful control and activity level between the DHA+AA arm and placebo after 180 days of intervention.
Population:	Participants: 448 children (224 assigned to each arm) 10-16 months of age (corrected age) at enrollment who were born at less than 35 weeks' gestation.
Study Duration:	*7 years (2012-2018)*
Participation Duration:	*6 months*
Description of Agent or Intervention:	*200 mg DHA + 200mg AA, orally*
Objectives:	Our *long-term goal* is to find safe, effective, and low-cost interventions to improve cognitive and behavioral outcomes in children born preterm. *Our objective* here is to determine the efficacy of DHA+AA supplementation in improving cognitive performance and addressing early markers of behavior problems (i.e., poor effortful control, high activity level) in toddlers born at <35 weeks' gestation. Our *central hypothesis* is that children 10-16 months' corrected age randomized to DHA+AA supplementation for 6 months will exhibit better cognitive ability, greater effortful control, and less

	hyperactivity, compared to placebo. Our hypothesis has been formulated on the basis of our preliminary data from a pilot study of this intervention (using this protocol) that shows DHA levels are increased with supplementation in this population, as well as the evidence from the past trials involving infants (Makrides 2010; Clandinin 2005). The *rationale* for the proposed research is that, if proven efficacious, this intervention will become a low-risk, low-cost complement to early intervention programs, or a stand-alone supplement for children whose cognitive deficits are not clearly apparent but remain at increased risk for learning and behavior problems later in childhood.
Description of Study Design:	Randomized, double-blind, placebo-controlled trial with two treatment arms: 1) DHA+AA supplementation and 2) corn oil powder placebo. The DHA+AA arm will take 200mg DHA + 200mg AA powder daily and the placebo arm will take 400mg corn oil powder daily, for 180 days.
Estimated Time to Complete Enrollment (n=120):	*6 years*

Starting 4/1/15, 202 children who will not participate in the blood draw component of the study will be recruited. These participants will be screened, recruited, and followed in the same way, however, recruitment materials and conversations, as well as the informed consent process reflect the appropriate procedures for the respective group. For administrative purposes, study materials related to the 202 children who will not participate in the blood draw component are identified with the Omega Tots Jr. study title.


EPIC PRESCREEN
Omega Tots: Recruitment & Informed Consent (n=246) with Blood Draw
Omega Tots Jr. Recruitment & Informed Consent (n=202) without Blood Draw
N=448
1:1
DHA+AA (n=224) or Placebo (n=224) Randomization
T1 Study Visit Blood Component
Cognitive development assessment, child behavior, dietary assessment, infant feeding history, parent depressive symptoms, parent trait anxiety, parent substance use, socio-demographics, anthropometrics, collect blood for assay
Start DHA+AA or placebo
T1 Study Visit No Blood Component
Cognitive development assessment, child behavior, dietary assessment, infant feeding history, parent depressive symptoms, parent trait anxiety, parent substance use, socio-demographics, anthropometrics
Start DHA+AA or placebo
T2 Remote/e-Visit
Dietary assessment, mealtime behaviors, parent physical activity, neighborhood and physical activity environment, collective efficacy, cohesion, information social control, food environment
Continue DHA+AA or placebo
T3 Study Visit Blood Component
Cognitive development assessment, child behavior, sleep, socio-emotional development, developmental disorders screening, dietary assessment, parenting stress, parent ADHD symptomology, anthropometrics, collect blood for assay
Stop DHA+AA or placebo
T3 Study Visit no Blood Component
Cognitive development assessment, child behavior, sleep, socio-emotional development, developmental disorders screening, dietary assessment, parenting stress, parent ADHD symptomology, anthropometrics,
Stop DHA+AA or placebo
Assess cognitive development, effortful control, activity level
3, 4, 5, years Ongoing follow-up (if funding allows)

1 KEY ROLES

Individuals:

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Developmental consequences of prematurity

Currently in the United States, 1 out of every 9 babies is born prematurely. Children who are born preterm are at increased risk of cognitive deficits and learning disabilities compared with children born at term. The EPIPAGE study based on a geographic sample of births reported that 32% of children born before 32 weeks' gestation exhibited impaired mental processing (K-ABC mental processing score <85) at age 5 compared to only 11% of term children.[1] A study based on a British cohort born at less than 32 weeks' gestation (1991-1992) found an 11-point deficit in total IQ score at age 7-8 among preterm children compared with children born at term.[2]

LCPUFA supplementation in infancy and cognitive development: Available evidence

A number of randomized controlled trials have evaluated the effect of supplementation with certain long-chain polyunsaturated fatty acids (LCPUFA) during infancy on the cognitive development of children born preterm. Early supplementation with DHA, in particular, appears to provide a moderate benefit. A recent meta-analysis of randomized controlled trials of LCPUFA supplementation concluded that preterm infants fed LCPUFA supplemented formula had a Bayley Mental Development Index (BSID-II MDI) 3.44 points higher at 12-18 months of age than infants fed a control formula (95% confidence interval (CI): 0.57, 6.31, n=879), approximately one-quarter of a standard deviation difference.[3] A more recent study found a 4.5-point difference in MDI scores among girls 18 months old who were fed a high-DHA formula (1% of total fatty acids) to term corrected age.

Second year of life: Critical window for brain development

Despite the active brain development during the second year of life, previous research has not examined whether supplementation beyond infancy is beneficial for children born preterm. In the prefrontal cortex, the region responsible for executive function, synapses do not reach their maximum density until age 3.[4] DHA accumulates in particularly high concentration in the synaptosomal membranes.[5,6] Along a normal developmental trajectory, this life stage is characterized by key milestones reflecting increasingly higher-level cognitive ability such as the

beginning of intelligible language, gross motor milestones like walking, and increasingly complex social interactions with caregivers. Therefore, the time from 12 to 24 months of age is a critical window for intervention to improve long-term developmental outcomes for children born preterm and thus at higher risk for developmental delay.

DHA intake among toddlers is low

Simultaneously, the second year of life corresponds to a dramatic decrease in DHA intake as children transition from breast milk and DHA-supplemented formulas to cow's milk and other foods that contain very low levels of n-3 fatty acids including DHA. According to the 2008 Feeding Infants and Toddlers Study, the proportion of children who consume breast milk or formula daily decreases from 97% at 9-12 months of age to 12% at 15-18 months of age. Data from the 1999-2000 National Health and Nutrition Examination Survey indicate that the mean daily dietary DHA intake among U.S. children under 6 years of age who are no longer nursing is approximately 20mg per day, much less than the intake of most infants. (An infant consuming 800ml of breast milk per day containing 0.2g DHA/100 g total fatty acids takes in 61mg DHA per day, while an infant consuming 800ml of commercial infant formula takes in 44mg DHA per day). Only a small number of foods contain high levels of n-3 fatty acids in a portion size appropriate for toddlers. Fatty fish like salmon, tuna, and mackerel are high in DHA but are not often offered to toddlers because of lack of appeal or concerns about potential food allergies or the levels of methylmercury some fish contain.

Biological response to DHA supplementation

Toddlers fed DHA-supplemented formulas have been shown to incorporate the supplement into plasma and red blood cells (RBC) within weeks. In a recent study, toddlers (18-36 months) consuming a formula containing 130mg DHA per day experienced a 61% increase in total RBC DHA after 60 days.[7]

DHA+AA supplement

DHASCO® (life'sDHA™) and ARASCO® are DHA and AA-enriched microalgal oils manufactured by DSM, (formerly Martek Biosciences) (Columbia, MD) and are marketed in the U.S as over the counter supplements for individuals across the lifespan, including toddlerhood. DHASCO and ARASCO are classified as Generally Recognized As Safe (GRAS) by the FDA and is the source of DHA and AA in most U.S. infant formula products. We use a microencapsulated powder version of these oils to facilitate their addition to age-appropriate food and drink.

2.2 Rationale

If children born preterm benefit from DHA supplementation after 12 months of age, this may inform future dietary recommendations for this group. The current lack of research involving this developmental period makes it impossible to develop appropriate dietary recommendations. We propose to address this gap in knowledge by conducting a randomized, placebo-controlled trial of DHA+AA supplementation of children 10-16 months of age (adjusted for prematurity) who were born at less than 35 completed weeks' gestation.

Children born very or extremely preterm face significant cognitive deficits compared to their term peers (an average difference of 11 IQ points), and prematurity increases more than 2-fold the risk of Attention-Deficit Hyperactivity Disorder.[1,8,9,10] Early, sustained intervention is important for improving long-term developmental outcomes.[11,12] Interventions to help the most affected children often include intensive developmental or behavioral programs which are costly; children who are mildly impaired often receive no intervention.[13] Dietary supplementation with docosahexaenoic acid (DHA, an omega-3 long-chain polyunsaturated fatty acid (LCPUFA)) plus arachidonic acid (AA, an omega-6 fatty acid) *during infancy*, has been shown in several randomized controlled trials to improve cognitive development among infants born preterm,[14,15] and omega-3 fatty acids have shown promise *in school-age children* in treating ADHD.[16] DHA plays a crucial role in neurotransmitter function, signal transduction, gene expression, neurogenesis, and anti-inflammation. Children born preterm stand to benefit from supplementation because they miss the period of *in utero* development when the fetus accrues most of its DHA from the mother.[17] This is particularly troubling because the period from the third trimester to age 2 is a critical period for DHA accretion in the brain and neurodevelopment overall.[18] Although breast milk and infant formula supply DHA to infants, dietary intake of DHA during the second year of life is very low and may not meet the needs of the developing brain because children transition from breast milk and formula with DHA to foods with low omega-3 content. The benefits of DHA supplementation during early infancy to preterm infants are fairly well-established, but the question of whether supplementation *during the active period of neurodevelopment in the second year of life* would benefit these children remains unanswered. A test of this intervention among toddlers remains a critical need. Without such knowledge, children will continue to experience the current burden of significant cognitive deficits and behavior problems. Our *long-term goal* is to find safe, effective, and low-cost interventions to improve cognitive and behavioral outcomes in children born preterm. *Our objective* here is to determine the efficacy of DHA+AA supplementation in improving cognitive performance and addressing early markers of behavior problems (i.e., poor effortful control, high activity level) in toddlers born at <35 weeks' gestation. Our *central hypothesis* is that children 10-16 months'

corrected age randomized to DHA+AA supplementation for 6 months will exhibit better cognitive ability, greater effortful control, and less hyperactivity, compared to placebo. (Effortful control is "the ability to inhibit a dominant response to perform a subdominant response, to detect errors, and to engage in planning,…a major form of self-regulation."[19]) Our hypothesis has been formulated on the basis of our preliminary data from a pilot study of this intervention that shows DHA levels are increased with supplementation in this population, as well as the evidence from the past trials involving infants.[15,20] The *rationale* for the proposed research is that, if proven efficacious, this intervention will become a low-risk, low-cost complement to early intervention programs, or a stand-alone supplement for children whose cognitive deficits.

Eligible children were born at less than 35 weeks' gestation. Previous LCPUFA supplementation studies have been more consistent in showing that preterm infants benefit from supplementation, while studies of term infants have been equivocal.[21] This may be because preterm infants miss the most active period of LCPUFA transfer from mother to fetus in the third trimester. Children will be 10-16 months (corrected age) at the first study visit. This age corresponds to the time period when children transition from breast milk or formula to cow's milk and other foods with low omega-3 content.

Children in the DHA+AA supplementation group receive 200mg algal-DHA plus 200mg algal-AA orally daily in the form of a microencapsulated powder contained in small foil packets. AA is provided in addition to DHA because this combination of fatty acids mirrors the balance of what is found in infant formula and is the combination available in a powder form. Additionally, it may be important to provide a balance of omega-3 and omega-6 fatty acids to ensure proper child growth. Children in the placebo group receive 400mg of corn oil powder orally daily in the form of a microencapsulated powder contained in small foil packets. Parents are instructed to mix two packets of powder (either DHA+AA or placebo) per day into their child's drink or food. For an average 10 kg child this dose of DHA amounts to approximately 20 mg/kg/day and mirrors the intake recommendations of a 1994 report by the United Nations Food and Agriculture Organization.[22] Previous studies that began supplementation just after birth vary widely in the duration of supplementation, from a few weeks to 12 months. This intervention lasts for 6 months. No previous studies began supplementation around 12 months of age. We have chosen 6 months for the duration of the intervention because we expect the rate of DHA accretion to be potentially slower in this older age group and because we need to allow sufficient time developmentally for differences to become apparent. It is possible that no differences will be detectable after 6 months but will be detectable at a later time through ongoing follow-up. Participants will be asked if they are interested in participating (re-consent obtained for future research) in continued follow-up to age 5, which will be conducted if funding allows.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

All of the research related procedures, surveys, and questionnaires, are designed to meet the definition of “minimal risk” in the federal regulations [§45 CFR 46.102(i)] and to be reviewed by IRBs under §45 CFR 46.404 “Research not involving greater than minimal risk.” Minimal risk as defined in the federal regulations means “that the probability and magnitude of harm or discomfort anticipated in the research are not greater, in and of themselves, than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests.” In addition, the Study Staff is committed to minimizing risks even when the risks are minimal.

Experienced research nursing staff or phlebotomists perform biospecimen collections on 246 of the 448 participants. This may involve minimal discomfort or pain. All staff involved in collection of biospecimens will be credentialed accordingly. The physical risks of drawing blood by placing a needle in a vein may cause pain, lightheadedness, bleeding, bruising, or swelling at the puncture site. Infection is a rare possibility. No alternatives to blood collection exist to reliably assess fatty acid status in humans.

The informed consent process takes place in private. Questionnaires are structured to avoid creating discomfort for the research participants; and participants are reminded at each data collection encounter that participation is voluntary, they have the right to withdraw from the research project at any time, and they may refuse to answer or may skip any question.

The risks of collecting and storing linked clinical data and biospecimens are primarily psycho-social. Developing coding strategies to mitigate these risks is a fundamental ethical requirement of the study. Potential harm could result from a breach of confidentiality. One of the primary concerns is that employment and insurance discrimination might result from exposure of information about health history, genetic makeup, or familial predisposition to disease. The risk is minor, especially since unique participant identifiers will not be stored with biospecimens.

The study will maintain confidentiality of the data in its databases. Data will be summarized in aggregate for reporting purposes. Data submitted to the study does include participant identifiers, as defined by the U.S. Health Insurance Portability and Accountability Act of 1996 (HIPAA). The study will follow all necessary measures to assure the participant data stored in the database are protected and secure from unauthorized access.

There are no known risks of DHA or AA supplementation at the 200mg dose specified for this study. DHA and AA are naturally occurring nutrients contained in many foods. Three ounces of Atlantic salmon contains 1238 mg DHA, three ounces of canned light tuna contains 190 mg DHA, and three ounces of fish sticks (about 5 fish sticks) contain 216 mg DHA.[23] AA is commonly found in meat and dairy foods. The amount of AA required in this protocol is about the same as three eggs or three sausage links.

Based on information contained in the Food and Drug Administration Agency Response Letter GRAS Notice No GRN 000041 (May 17, 2001), intake of DHASCO and ARASCO in term infant formulas up to 75 mg/kg bw/day are GRAS (see Appendix). The dose for this study is approximately 20mg/kg/day. There are no known risks of consuming either the DHA+AA or the placebo powder at the dose specified for this study. Because participants in some studies have noted stomach discomfort with consumption of study oils of various types, the consent form for this study will note the possibility of these symptoms; however, these symptoms are not expected at the doses used in this study. Allergic reaction is always a possibility and will be noted in the consent form, along with possible associated symptoms.

The knowledge to be gained from this investigation outweighs these minimal risks. This study has the potential to inform future dietary recommendations for toddlers, especially those who were born preterm.

2.3.2 Known Potential Benefits

DHA and AA supplementation may benefit participating children in their cognitive, language, and motor development, behavior, and/or growth. It is not known whether these benefits exist. Participation in this study will aid in acquiring new knowledge that might help other children who are born prematurely and their families in the future. Additionally, parents are provided with some information from the study including their child's height and weight upon request. Some parents may find this information useful. There are no other known benefits expected from participation in this study.

3 OBJECTIVES

3.1 Study Objectives

We will enroll 448 children 10-16 months of age (adjusted for prematurity) into a randomized, double-blind, placebo-controlled trial of supplementation with 200mg DHA + 200mg AA per day for 180 days. The aims of the study are to:

1. *Aim 1:* Compare cognitive ability between the DHA+AA arm and placebo arm after 180 days of intervention.
   Exploratory sub-aim 1a: Explore sub-group differences by child sex,birth weight, and socioeconomic status.
   Hypothesis 1: Children randomized to the DHA+AA arm will demonstrate at least a mean 5-point advantage in Bayley Scales of Infant and Toddler Development-III cognitive composite scores after 180 days of supplementation compared to those randomized to placebo.

2. *Aim 2:* Compare measures of effortful control and activity level between the DHA+AA arm and placebo arm after 180 days of intervention.
   Hypothesis 2: Children randomized to the DHA+AA arm will demonstrate at least a 0.33-standard deviation advantage in effortful control and activity level scores from the Early Childhood Behavior Questionnaire after 180 days of supplementation compared to those randomized to placebo.

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measure

Change in cognitive ability from baseline to visit 3.

Change in effortful control and activity level from baseline to visit 3.

3.2.2 Secondary Outcome Measures

Bayley Scales of Infant and Toddler Development – 3rd edition (language and motor sub-scale scores) – at visit 3.

3.2.3 Other Outcome Measures

Development and behavior: Brief Infant Toddler Social Emotional Assessment, Brief Infant Sleep Questionnaire.

Growth and body composition: Weight, recumbent length, head circumference, mid-upper arm circumference, triceps and subscapular skinfolds.

4 STUDY DESIGN

This single-site study is a randomized, placebo-controlled trial of DHA+AA supplementation for 180 days involving children 10-16 months of age (adjusted for prematurity) at enrollment. The principal investigator, Study Staff, clinic staff, parents, and children will be blind to the child's treatment assignment.

There are two study treatment arms. The DHA+AA supplementation arm is provided with 200 mg DHA (DHASCO) + 200mg AA (ARASCO) to be taken orally daily in the form of a powder for 180 days. The placebo group is provided with a powder form of corn oil (400mg) to be taken daily for 180 days. The DHA + AA supplement and corn oil powder are provided by DSM, the largest manufacturer of algal DHA oils in the U.S. Both products are similar in terms of appearance and taste based on information provided by DSM and also an empirical evaluation conducted by the research team for purposes of this study. Products are sold over-the-counter and are dietary supplements. DHASCO and ARASCO are the supplement products added to most U.S. infant formula products. However, the FDA has been consulted regarding the necessity of an IND for this study. Through these conversations it was determined that an IND was necessary for this product and the application was filed on July 20, 2011. On October 4, 2011 the FDA approved the protocol. The IND Number for this study is 112885.

448 children will be enrolled in this study over a 6 year period to evaluate study aims. If funding is available, the children who participate in the study will continue to be followed to age 5.

This study is conducted at Nationwide Children's Hospital. Participants are children who were born at less than 35 weeks' gestation and who were admitted to any NCH managed NICU (DHW, DMH, GMC, MAIN, RMH, STAN, OSU), or Mt. Carmel East or West NICU (Columbus, OH), or children who have ever had a Neonatalology Clinic (e.g., Developmental Clinic or Bronchopulmonary Dysplasia (BPD) Clinic) follow up visit scheduled at NCH, regardless of attendance. The Neonatal Developmental Clinic and BPD Clinic conduct approximately 3,000 patient visits per year, providing care for children who required neonatal intensive care and infants discharged with special needs. The Clinics also provide developmental milestone testing for any child who was born preterm or otherwise at risk for developmental delay. The interdisciplinary Clinic teams address the medical, nutritional, neurological, developmental and social needs of patients in a single location and consist of neonatologists, neurologists, developmental/behavioral pediatricians, pediatricians, Certified Pediatric Nurse Practitioners, physical therapists, occupational therapists, nurses, social workers, Registered Dietitians, and lactation consultants.

Some data collection elements for the proposed study are collected as part of standard clinical care (see Appendix A) in the Neonatal Developmental and Bronchopulmonary Dysplasia (BPD) Clinics. Staff for the proposed study are not involved in those data collections unless clinic staff cannot complete a data collection item in a manner concordant with study procedures or participants are not attendees of the follow up clinics. Data for items collected as part of clinical care will be abstracted from the medical record. This is indicated on the consent forms for this study.

Some children who attend the Neonatal Developmental or BPD Clinics are participants in the Ohio Perinatal Research Network Perinatal Research Repository (OPRN PRR; IRB10-00035). The OPRN is a collaborative among clinicians and scientists from Central Ohio medical and research institutions with the goal of examining risk factors, associated complications and ultimately the prevention of preterm births. The central PRR of clinical data and specimens from parents and their children supports OPRN and is used in research about how to prevent, diagnose and treat preterm birth and the diseases and complications that may result from preterm birth. Some data collection elements of interest for the present study are also collected as part of participation in OPRN PRR. Staff for the Omega Tots study are not involved in data collections for OPRN PRR participants unless OPRN PRR staff cannot complete a data collection item. Data for these items will be extracted from the OPRN PRR ePerinatal information management system and subsequently merged with data from the present study.

The aims of the study will be addressed at visit 3, after 180 days of follow-up of all study participants. We will explore the effect of DHA+AA supplementation on cognitive development (using the Bayley-3), effortful control (using Infant Behavior Questionnaire and Early Childhood Behavior Questionnaire), and activity level (using Infant Behavior Questionnaire and Early Childhood Behavior Questionnaire) at 180 days post-randomization.

Safety oversight will be under the direction of the Principal Investigator and Study Doctor. All adverse events will be reviewed to rule out the study intervention as a potential cause. Because the intervention has been shown to be very low risk in previous studies, no DSMB will be convened.

5 STUDY ENROLLMENT AND WITHDRAWAL

The total target sample size for enrollment is 448 children. If funding is available, the children who participate will continue to be followed in the full study to age 5.

Participants are children who were born at less than 35 weeks' gestation and who were admitted to any NCH managed NICU (DHW, DMH, GMC, MAIN, RMH, STAN, OSU), Mt. Carmel East or West NICU (Columbus, OH), or children who have ever had a Neonatology (e.g. Developmental Clinic or BPD Clinic) follow up visit scheduled at NCH, regardless of attendance, and their parent(s). The Clinic has a diverse patient population. Where possible, Study Staff will coordinate with Clinic staff and OPRN PRR staff and use EPIC to identify children who have been followed by the clinics for follow-up care or who are enrolled in OPRN and who are age eligible for the study. The EDW and EPIC also will be used to identify children who meet eligibility criteria. Additional eligibility criteria assessed through a prescreening process using EPIC. Abstracted information includes information regarding an indication of any major malformation, that the child is between the 5th and 95th percentiles for weight at his/her most recent visit, and that the child does not currently have a feeding problem that may inhibit full participation. We will attempt to locate OSU NICU admissions in EPIC, however, it is possible that all children may not be in EPIC. All inclusion/exclusion criteria not documented in EPIC will be assessed via phone with the legal guardian.

Families identified as potentially eligible in the EPIC prescreening process are mailed an introductory letter and study brochure 2-6 weeks ahead of a scheduled clinic visit when possible or during their eligibility window when no clinic visit coincides with the window of eligibility. If a clinic visit has not been scheduled the introductory letter and study brochure will be mailed no earlier than 1 month before the child becomes age eligible for participation in this study. Contact information may be verified and/or attempts to locate unreachable families (e.g., those with outdated contact information on file) will be made using a web-based online search service (e.g., http://www.peoplesmart.com/) that gathers information through public records, similar to a Google search. The search service provides a fast and effective way to look-up or verify contact information about a person, phone number of address of interest. Contact information will be verified using names, addresses, and phone numbers.

Starting 4/1/15, a group of 202 children who will not participate in the blood draw component of the study will be recruited (Omega Tots Jr.). Specifically, the 202 participants recruited using HRSA funds will not be asked to complete the blood component of the study. Prior to participant pre-

screening in EPIC, participants will be randomly selected to be recruited for the biospecimen (i.e., blood) or non-biospecimen (i.e., no blood) protocol. Participants in the blood and no-blood groups will be screened, recruited, and followed in the same way, however, recruitment materials and conversations, as well as the informed consent process reflect the appropriate procedures for the respective group. The difference in recruitment information will be if information about the blood draw is included in recruitment materials. Whether a family is being recruited for the biospecimen or non-biospecimen group will be randomly determined prior to sending any recruitment materials. If a family invited to the biospecimen group does not want to participate due to the blood draw, they may be invited to participate in the non-biospecimen group. It is anticipated that 246 children will participate in the biospecimen group and 202 will participate in the non-biospecimen group. *Randomization to the DHA+AA or placebo group is not affected by this.* Participants will continue to be randomized according to the current protocol and randomization scheme.

The introductory letter and study brochure will briefly describe the purpose of the study and will provide contact information for Study Staff. Study Staff phone the family approximately one week after mailing the introductory letter and study brochure to further assess eligibility and gauge general interest in participating. Study Staff use the phone script devised for this study as a conversation guide. During this phone call, Study Staff provide a description of the study and its requirements. Study Staff also assess child eligibility based on inclusion and exclusionary criteria during this call.

To be eligible for this study, children must have discontinued regular breast milk and/or formula consumption (no more than two times per week) because both contain DHA (breast milk varies widely in DHA content, while most formula products contain similar amounts of DHA), not currently be consuming Pediasure more than two times per week, use English in the home (demonstrate ability to communicate in English well-enough to understand study, informed consent, and study questionnaires), have no plans to move out of the area in the next six months, not already consume DHA or Omega-3 supplements more than two times per week, not have a corn allergy, soy, or fish allergy.

If the child is eligible, Study Staff describe the study to the parent and ask if he/she is interested in coming into Nationwide Children's Hospital to learn more about the study. If a family is potentially interested in participating, Study Staff schedule them for an appointment to discuss the study and potentially enroll and carry out informed consent and the baseline visit. If possible, this appointment is arranged to coincide with an already scheduled clinic visit (if that is the family preference). Gauging participant interest in advance will alleviate, as much as possible, Study Staff causing delays in the clinic by approaching families for the first time while at the clinic. Although the primary recruitment process commences with a phone call prior to a clinic visit, it is possible that Study Staff

may approach potentially eligible families in clinics if they were not reachable by phone in advance. Additionally, Study Staff may approach some families in the clinic waiting room if for some reason there was no outreach via letter previously (e.g., new patient who moved to Columbus), study staff unable to contact family via phone etc. Some children are eligible for clinic follow-up but never attend the clinic or stop attending before age 2. Study Staff will reach out to these families as a supplemental recruitment method.

Families will be reminded of scheduled/upcoming appointments via phone, email and/or text (based on family preference). Attempts will be made via phone, email, text, and/or mail to contact families who do not show for their scheduled visits.

Based on trends in clinic attendance and experience with the pilot study, it is anticipated that recruitment of the full 448 participants will take approximately 6 years (end 2018).

During the first scheduled study appointment, Study Staff meet with the parent to provide more detailed information about the study and answer any questions the parent may have. If the parent is interested in enrolling his/her child and the child meets all eligibility criteria and is not excluded based on the exclusion criteria, the Study Staff guide the parent through the informed consent process and obtain written informed consent. Participation or refusal to participate in the study will not affect participation in the OPRN PRR or the child's entitlement to clinical care. Upon enrollment, children are randomized to receive either a daily DHA+AA supplement or the placebo. All Study Investigators, Study Staff, clinical staff, parents, and children are blind to the treatment assignment.

Upon enrollment, Study Staff verify the contact information on file for the family and work to determine the preferred method (e.g., mail or email) of sending the T2 e-visit approximately 60 days later and to schedule the T3 study visit 180 days later. If the family has a clinic appointment in this window, Study Staff will try to coordinate where possible, if that is the family's preference. Study Staff contact families with visit reminders via mailed postcards and phone, email, and/or text message (based on family preference).

Study Staff call, email, and/or text participating families at the following (approximate) intervals after randomization to answer questions about the assigned treatment, identify and resolve acceptability and compliance issues, assess and gather information about possible adverse events, and remind families to complete the study diary: 3, 14, 30, 45, 90, 120, 150 days and as needed based on family and study needs. Regular check-in emails are sent to families. Additionally, families will be contacted around 60 days post randomization regarding the T2 e-visit and necessary follow-up to facilitate survey return. During these communications parents may be asked to estimate what proportion of doses they administered out of the desired number and what proportion of the powder they estimate their child usually consumed. Study staff will work with the family to increase

adherence where possible. Children who do not complete the e-visit and/or do not return for visit 3 will be considered lost to follow-up, but attempts will be made to contact them via phone at these pre-prescribed intervals. Although the T2 data collection instruments are ideally administered 60 (+/- 14) days post randomization, Study Staff will attempt to collect the information outside this window (e.g., at the T3 visit) if necessary. To accommodate families, Study Staff may offer an NCH facility (e.g., Close to Home) for data collection as well as taxi/bus fare for families to travel to the data collection site.

This study intends to enroll children, a special population, and will accordingly adhere to additional protections specified under 45 CFR Part 46 Subpart D – Additional Protections for Children Involved as Subjects in Research (45 CFR Part 46.401-409).

Parents have the right to withdraw themselves and their child from the study at any time. Withdrawal from the study will not affect participation in the OPRN (if applicable) or the receipt of clinical care. If a participant develops a condition that precludes continued participation in the study, he/she will be withdrawn. If the study is halted for any reason, participants will be instructed to discontinue taking the assigned treatment and return the unused portion to Study Staff. Participants who choose to discontinue taking the assigned treatment during the study will continue to be followed for assessment of the outcomes, and their discontinuation will be recorded in the study database. Participants who continue to participate but are found to have low adherence to the assigned supplement or placebo will not be withdrawn and will be continue to be followed. Adherence to study protocol will be assessed throughout participation.

5.1 Participant Inclusion Criteria

Participants must meet all of the inclusion criteria in order to be eligible to participate in the study. These criteria include:

1) Age 10-16 months (corrected age) at baseline
2) Discontinued regular breastfeeding and formula feeding at the time of randomization (no more than two times per week)
3) Gestational age <35 completed weeks at birth
4) English in the home, demonstrate ability to communicate in English well-enough to understand study, informed consent, and study questionnaires
5) Informed consent obtained and signed

6) Child admitted to any NCH managed NICU (DHW, DMH, GMC, MAIN, RMH, STAN, OSU), Mt. Carmel East or West NICU (Columbus, OH; confirmed via EPIC or verbally from guardian), or children who have ever had a Neonatology (e.g., Developmental Clinic or BPD Clinic) follow up visit scheduled at NCH, regardless of attendance.

Additionally and as part of the informed consent process, Study Staff will ensure that parents have a good understanding of study procedures and are able to comply with study procedures for the entire length of the study.

5.2 Participant Exclusion Criteria

All participants meeting any of the exclusion criteria at baseline will be excluded from study participation. These criteria include:

1) Feeding problems
2) Major malformation, metabolic, or digestive disorder that would preclude participation and/or optimal absorption of the supplement.
3) Weight <5th or >95th percentile for age, per WHO growth charts
4) Consume DHA supplement drops, chews, or powders or Pediasure, or fatty fish more than 2x per week
5) Plans to move out of the area within the next 6 months
6) Known corn allergy
7) Known soy allergy
8) Known fish allergy

5.3 Treatment Assignment Procedures

5.3.1 Randomization and Masking Procedures

Treatment randomization involves a block design of randomly varying block size of 2 and 4. 112 participants are randomized to each of four groups, identified by color – 2 groups will be assigned

the DHA+AA supplement powder and two groups will be assigned the placebo powder. A SAS pseudo-random number generator programmed by Dr. Norris-Turner, Assistant Professor in the Department of Internal Medicine, Division of Infectious Disease; and the College of Public Health, Division of Epidemiology at The Ohio State University, is used to develop the randomization scheme. Dr. Norris-Turner has experience in Randomized Controlled Trials and producing complex randomization schemes. Having an experienced researcher not otherwise involved in study operations produce the randomization scheme will allow Study Staff to maintain blinding. Other than providing and overseeing the randomization scheme, Dr. Norris-Turner is not involved in the study. This randomization scheme assigns available participant study ID numbers to one of four groups identified by color: two colors correspond to the DHA+AA supplement and two colors correspond to placebo powder. All Study Staff, OPRN staff who have direct contact with participants, clinic staff, parents, and children will be unaware of which color corresponds to which treatment group. Initially, only Dr. Norris-Turner will know the color assignment and exact block sizes used. The randomization scheme will be balanced with an equal number of study ID numbers assigned to each of four groups. If one participant must be unblinded during the study due to unforeseen circumstances or if individuals involved with the study somehow deduce one participant's assignment, this 4 group scheme will allow a participant to be unblinded without compromising the entire randomization of the study.

All supplement materials are precounted by DSM. Cases will be shipped directly to IDS. Each case contains 12 individually sealed boxes. Each individually sealed box contains 40 supplement sachets. The lot number is printed on the outside of each case, box, and sachet. A packing slip will identify the lot number and the contents of the case.

The PI will request an initial supply of to be delivered to IDS prior to the study commencing. Additional cases will be requested as needed. A supply of at least 1 case per letter group (i.e., at least 4 cases) will be maintained in IDS throughout the study.

Prior to receiving materials from DSM, Dr. Norris-Turner will generate the randomization scheme, consisting of 4 groups, with two groups corresponding to the DHA+AA supplement powder and two groups corresponding to the placebo powder. Once the randomization scheme is derived, Dr. Norris-Turner will randomly assign each of the four groups a different color, which will identify group membership to treatment blinded study staff.

Study Staff will create color coded labels to affix to the supplement upon arrival from DSM. These labels will **not** identify if the product as active or placebo. Rather, they only will identify the group by color.

Upon receiving materials from DSM, one case will be opened at a time by IDS. IDS will label each of the boxes (containing 40 sachets each) within that case appropriately. Upon completion of one case, a second case is opened and the process repeated until all boxes are labelled. The PI may choose to have a witness from outside the study team present for this process for quality assurance.

Marked boxes will be stored in separate cabinets (one cabinet for DHA+AA and one cabinet for placebo) in IDS.

The Study Staff developed a detailed Standard Operating Procedure (SOP) for Randomization and Blinding, and Emergency Unblinding Procedures. This SOP names the people who will generate the randomization scheme, and the scheme will be stored in a locked cabinet. It will also list the people who will have access to a sealed envelope containing the randomization scheme should emergency unblinding be necessary. Only the IRB or PI are allowed to authorize early unmasking in the event of emergency. Otherwise, the randomization assignments will be kept secure until closure of the study.

5.3.2 Reasons for Withdrawal

Study participation will be discontinued under the following circumstances:

- Clinically significant abnormal laboratory results or AEs, which rule out continuation of the study intervention, as determined by the Principal Investigator and the Study Doctor.
- The Principal Investigator believes it is in the best interest of the participant.
- The participant’s legal guardian requests withdrawal from the study.
- Any clinical adverse event (AE), laboratory abnormality, intercurrent illness, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the child.
- Development of any exclusion criteria may be cause for discontinuation.

Participants are free to withdraw from participating in the study at any time upon request. If at any time the Principal Investigator and/or Study Doctor believes participating in this study is not the best choice of care, the study may be stopped and other care prescribed. If the study instructions are not followed, participation in the study may also be stopped. If unexpected medical problems come up, the Principal Investigator or Study Doctor may decide to stop the child’s participation in the study.

5.3.3 Handling of Withdrawals

The study will continue to collect safety data on any participant discontinued because of an AE or SAE. If voluntary withdrawal of adherence to the nutritional supplement/placebo powder occurs, the participant will be asked to continue scheduled evaluations, complete an end-of-study evaluation, and be given appropriate care under medical supervision until the symptoms of any AE resolve or the participant's condition becomes stable.

5.3.4 Termination of Study

Because the intervention has not been found in numerous previous trials to pose harm to young children, it is not anticipated that the study would need to be terminated due to development of toxicities or adverse events due to the intervention. However, the study will be terminated if the PI or IRB conclude based on their findings that termination is in the best interests of the participants.

6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1 Study Product Description

6.1.1 Acquisition

Bulk DHASCO and ARASCO powder-form oil and placebo powder-form oil will be obtained from DSM.

6.1.2 Formulation, Packaging, and Labeling

The DHASCO + ARASCO powder packets each contain approximately 100mg DHA and 100mg AA. The placebo corn oil packets contain 200mg of corn oil powder. Two packets per day will provide the desired amount for the study. Supplements will be dispensed to participants at one time point at the initiation of study participation (unless supplement needs to be replaced at an unexpected interval, if this is done, participants may receive up to 20 boxes of supplement, all dispensions will be recorded).

1st Dispension: Upon enrolment (T1), 10 boxes (400 sachets) are distributed to participants. Participants will be given a bag for ease of carrying,

This amount is enough to cover study duration, however, additional boxes will be distributed if needed (e.g., if family runs out/loses some).

After consent, a prescription is delivered to IDS. The prescription covers the amount of powder needed for the duration of the study (up to 20 boxes). IDS selects the appropriate product and removes any identifying information (DHA+AA/placebo) on the box. An indicator of the group color will remain on the box. A second indicator of group color is removed from the box and then placed by IDS on back of the prescription as proof of what product was dispensed and a label conducive to Ohio BOP and FDA regulations is placed on each of the 10 boxes before being dispensed to study staff. Additionally, the amount of product dispensed to a participant is recorded in an overall master log, maintained by IDS. No information on the boxes of packets or the packets themselves indicates which product is contained in them upon dispension from IDS. Each box will be labeled according to the randomization scheme, according to color, to designate which group the participant was randomized to blinded study staff.

When the product is counted by study staff, the non-identifying group color portion of the label is placed in the appropriate location in the participant's study file to provide a further indication and record of what product group color was dispensed to each participant.

6.1.3 Product Storage and Stability

The supplies of packets shall be kept in a dry, secured environment at controlled and monitored room temperature. The expiration dates labeled on the products will be monitored by IDS to ensure product freshness.

6.2 Dosage, Preparation and Administration of Study Intervention/Investigational Product

DHA+AA and placebo powder will be administered by mouth daily. The products require no special mixing or preparation on the part of the parent other than to place the powder into a serving of food or drink (preferably milk) that the parent feels fairly confident that the child will consume the entirety. Families are asked to avoid mixing in acidic drinks such as apple juice and this can break down the supplement.

6.3 Modification of Study Intervention/Investigational Product for a Participant

No circumstances are known for which the dose should be modified.

6.4 Accountability Procedures for the Study Intervention/ Investigational Product(s)

After the labeling is complete, the Pharmacy maintains the investigational product and will dispense them to the study families at baseline and as needed to maintain supplies if the family loses some or all packets. Families are asked to bring their used and unused packets to each study visit so staff and IDS can assess the amount remaining.

6.5 Assessment of Subject Compliance with Study Intervention/ Investigational Product

Parents are provided with diaries (paper or electronic) and instructions to record whether the child consumed the assigned supplement or placebo powder and what proportion he or she consumed each day as well as any acceptability issues encountered. 12 diaries that each cover 2-week increments of the trial will be provided to families. The parent is asked to record acceptability and compliance (i.e., how many packets consumed each day) and to bring the booklet with them to the third visit (if completed via paper). Study Staff discuss and remind families about the study diaries during routine follow up communications. Additionally, Study Staff probe for any problems encountered that reflect acceptability or compliance problems. At the third study visit, Study Staff review the completed diaries with the parent and probe for any problems encountered that reflect acceptability or compliance. For each diary returned, the participant receives $2 to thank them for completing the diary.

6.6 Concomitant Medications/Treatments

There are no restrictions on what drugs or treatments may be concomitant with the study intervention.

7 STUDY SCHEDULE, STUDY PROCEDURES/ EVALUATIONS

7.1 Screening

An initial prescreen is completed using EPIC. Study Staff administer a brief eligibility screener to assess inclusion/exclusion criteria documented in EPIC (e.g., verify the age of the child, assess whether the child has transitioned off regular infant formula and breast milk consumption (no more than two times per week)). When information is unable to be verified in EPIC, inclusion/exclusion criteria is assessed via the phone during the recruitment phase. Participation in an early intervention/home visiting program will also be noted on the prescreen form if documented in EPIC.

When a child is deemed and/or confirmed eligible during the recruitment phone call, Study Staff further describe the study to the parent and ask if he/she is interested in coming into Nationwide Children's Hospital (NCH) to learn more about the study and its participation. During this appointment, Study Staff will review the written informed consent document, answer any questions, obtain written consent for participation, randomize the family into a treatment group (DHA+AA or placebo), and conduct the baseline visit.

7.2 Parent/Legal Guardian Completed Questionnaires

For purposes of this study, two questionnaires were created for the baseline study visit (i.e., T1). The first questionnaire is a standard maternal questionnaire. As several questions are related to pregnancy and breastfeeding behaviors of the mother, all attempts will be made to assess this information directly from the mother. During recruitment, all attempts will be made to have the mother bring the child in for study visits and thus be available to complete study related questionnaires. However, the Study Staff understand that this is not always possible and have adapted study protocol accordingly. Specifically, a second non-maternal caregiver questionnaire is administered at the baseline study visit in the case that a non-maternal caregiver brings the child in for a study visit. This questionnaire contains the same information as in the maternal questionnaire, but wording is adapted to be completed by a non-maternal caregiver. In cases where a mother is not able to attend study visits and/or a non-maternal caregiver completes the questionnaire, a maternal questionnaire will be sent to the mother (if applicable) along with a postage paid, self-addressed return envelope. Alternatively, a link to the online version of the survey may be emailed

to the mother. Additionally, the non-maternal questionnaire is distributed to those interested in completing it, regardless of study visit attendance. As no pregnancy related information is collected at the second and third data collection intervals, only one caregiver questionnaire was designed for these visits. If available and interested, both caregivers will be asked to complete the survey. Questionnaires are mailed (via post or email) to parents for the T2 data collection interval and in advance of the third data collection interval. Parents are encouraged to complete the questionnaire at home (for T2) and prior to coming to NCH for the study visit (T3); however, Study Staff are available to assist parents in completing the questionnaire over the phone or during the study visit. All questionnaires are available for families in both paper and electronic forms.

The family's intentions to complete the study, attend the next study visit, and give their child the powder on a daily basis throughout study duration; as well as any anticipated barriers to study participation are assessed during each contact with the family.

7.3 Enrollment/Baseline – Visit 1 (T1)

Upon completion of the written informed consent, families are randomized to receive either a DHA+AA supplement powder (200 mg DHA + 200mg AA per day) or placebo powder (400mg corn oil per day) according to the randomization scheme endorsed in this study. At the baseline visit, Study Staff provide instructions about how to administer the powder, frequency of administration, possible foods to mix it in, and anticipatory guidance about what to do when the child is picky about eating. Families are provided with several boxes of packets to take home with them along with a phone number and email address for the study to be able to ask questions. If the participant loses or discards some or all packets during the study, the family is instructed to inform Study Staff and a new partial or full supply will be provided via Fedex shipping to the home.

This study visit occurs at a NCH facility. The primary purpose of the first study visit is to obtain baseline data and basic demographic information for participating children and families. It is estimated that this visit will take approximately 3 to 3.5 hours. Data collection activities at T1 include:

Diet (child): Study Staff administer a questionnaire to parents to capture a breastfeeding and formula feeding history from birth to the present time and instruct parents on how to complete the Harvard Service Food Frequency Questionnaire and a fatty acid mini-FFQ to estimate the child's typical daily dietary intake of n-3 (including DHA) and n-6 fatty acids. Obtaining baseline information related to fatty acid levels and typical daily consumption will allow study staff to monitor changes in DHA and AA levels throughout the course of the study and determine if there is a significant

increase in DHA and AA levels for children in both treatment arms. Additionally, baseline information related to the child's intake of DHA rich foods will allow study staff to examine any changes in the child's consumption of DHA over the course of the study.

Cognition/Behavior (child): Baseline developmental status is directly assessed with children using the Bayley Scales of Infant Development-3. Parents complete the Brief Infant Sleep questionnaire and Infant Behavior Questionnaire-very short form.

Medical Home (child): The presence of a medical home for the child is assessed with caregiver reported questions from the National Survey of Children's Health, 2011/2012. These questions assess usual place for care, utilization of services, referrals, care coordination, provider communication, and compassionate, culturally effective, family-centered care.

Early Intervention: Caregivers report on any early intervention/home visiting programs the child is or has participated in, when the child started, the frequency, and duration of the intervention.

Anthropometrics (child): To assess baseline body composition, length/height, weight, and head circumference plus skinfold measurements (tricep, subscapular) and a mid-arm circumference measurement are taken.

Biospecimens (246 children in the biospecimen group only): To measure baseline fatty acid levels, an experienced nurse or phlebotomist draws 2 ml whole blood into a purple top tube. In the rare case where a child will be having blood drawn for a clinical purpose that day, Study Staff will coordinate with the clinic to collect the study blood sample at the same time with no additional stick required. Study Staff centrifuge and separate the blood and place the sample in a secured -80 degrees Celsius freezer at NCH until it can be analyzed by Dr. Lynette Rogers lab in the Center for Perinatal Research.

Demographics: A basic demographic profile is collected including education, income, household composition, employment, marital status, gender, race, ethnicity, and age.

Mental Health (parent): Study Staff instruct the parent on the completion of a brief, self-administered mental health screener (Spielberger State-Trait Anxiety Inventory (trait scale only) and Centers for Epidemiologic Studies-Depression Scale).

Health History (parent): The parent is asked to complete a brief health history including current height and weight, smoking, alcohol use.

Adherence Diary: The purpose and protocol for completion of adherence diaries are explained to participants. Participants are asked to record whether the child takes the supplement/placebo each day and to record any issues encountered with the powder. This can be done via pen and paper, online, or over the phone. The time period covered by each diary is 2 weeks.

Compensation: At the conclusion of the visit, participants receive $50 in compensation for their time and a Children's Hospital Parking Garage Pass ($2). Children are given a developmentally-appropriate children's book or small toy (value =$5).

Contact and Scheduling: Before the end of the visit, Study Staff confirm the contact information on file for the family, work to determine the preferred method of sending the T2 e-visit approximately 60 days later, and works to schedule the T3 study visit 180 days later. In the circumstance where the first study visit coincides with a scheduled clinic visit, Study Staff will coordinate with clinical staff to find the best opportunity for the collection of data and specimens that are not already collected as part of clinical care. In general, participants who are coming to NCH for a clinic visit at this time may receive the Bayley-3 as part of standard clinical care. All other measures will be completed by Study Staff. If the child does not complete a developmental assessment using the Bayley-3 during the clinic appointment, Study Staff will conduct one.

7.4 Follow-up e-visit 2 (T2)

The T2 e-Visit takes place 60 days (+/-14 days) post-randomization. The primary purpose of this data collection timepoint is to complete a dietary assessment, and obtain additional family, household, and neighborhood information. This visit is done remotely, through a postal or online questionnaire. It is estimated that this visit will take approximately 45 to 60 minutes. The data collection activities at T2 include:

Diet (child): Parents complete a fatty acid mini-FFQ to estimate the child's typical daily dietary intake of n-3 (including DHA) and n-6 fatty acids and the Child Feeding Questionnaire.

Behavior (child): Parents are asked to answer one question regarding the child's television viewing habits and one question regarding family mealtime routines.

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Caregiver Perception of Treatment Group and Adherence: Caregivers are asked what treatment group they perceive their child to be in and to estimate what proportion of doses they administered out of the desired number and what proportion of the powder they estimate their child usually consumed.

Physical Activity (parent): Parents complete the 2-item Stanford Brief Activity Screener.

Social Exposures: Parents complete a brief assessment of the family and neighborhood food and physical activity environment, including an assessment of collective efficacy, cohesion and social control.

Compensation: At the conclusion of the e-visit, participants receive $25 (e.g., gift card/check) in compensation for their time.

Contact and Scheduling: During the follow up communications regarding the e-visit, Study Staff confirm the contact information on file for the family and confirm/schedule (if needed) the final study visit for approximately 120 days later. Parents will be reminded of upcoming appointments via phone, text, and/or email. They may be mailedreminder card about their next scheduled study visit. Ideally, the last study visit will coincide with a previously scheduled clinic visit to try to boost attendance at both visits. Study Staff will work closely the clinic staff and/or schedules on these scheduling issues where possible.

7.5 Follow-up and completion of intervention – Visit 3 (T3)

Visit 3 takes place 180 days (+/-20 days) post-randomization. The primary purpose of this visit is to assess child's cognitive, language, and motor skills and their behavior. Study Staff coordinate with Neonatal Developmental or BPD Clinic staff (where possible) to schedule study activities to coincide with the scheduled clinical visit at 16-22 months of age, if one exists. In general, participants who are coming to NCH for a clinic visit may receive the Bayley-3 as part of standard clinical care. All other measures will be completed by Study Staff. If the child does not receive a Bayley-3, the Study Staff will conduct one. Study Staff coordinate with clinical staff to find the best opportunity to complete the additional data collections. Families will come to a NCH facility for Visit 3. It is estimated this this visit will take approximately 2 hours and 20 minutes to 3 hours. The data collection activities at T3 include:

Diet (child): Study Staff instruct parents on how to complete the Harvard Service Food Frequency Questionnaire and a fatty acid mini-FFQ to estimate the child's typical daily dietary intake of n-3 (including DHA) and n-6 fatty acids.

Cognition/Behavior (child): Cognitive, language, and motor development are directly assessed using the Bayley-3 which is routinely administered by Clinic staff for all patients at the clinic visit. The parent completed Early Childhood Behavior Questionnaire (ECBQ) is used to assess temperament, the parent completed Brief Infant Toddler Social Emotional Assessment (BITSEA) is used to assess social-emotional development, the parent completed Brief Infant Sleep Questionnaire characterizes sleeping patterns of the participating children, the parent completed Pervasive Developmental Disorders Screening Test – II (PDDST-II) is used to assess possible autistic like symptoms in the children, and one question will assess the child's response to joint attention.

Anthropometrics (child): To assess body composition, length/height, weight, and head circumference plus skinfold measurements (tricep, subscapular) and a mid-arm circumference measurement are taken.

Biospecimens (246 children in the biospecimen arm only): To measure fatty acid levels, an experienced nurse or phlebotomist draws 2 ml whole blood into a purple top tube. In the rare case where a child will be having blood drawn for a clinical purpose that day, Study Staff will coordinate with the clinic to collect the study blood sample at the same time with no additional stick required. Study Staff will centrifuge and separate the blood and place the sample in a secured -80 degrees Celsius freezer at NCH until it can be analyzed by Dr. Lynette Rogers lab in the Center for Perinatal Research.

Caregiver Perception of Treatment Group and Adherence: Caregivers are asked what treatment group they perceive their child to be in and to estimate what proportion of doses they administered out of the desired number and what proportion of the powder they estimate their child usually consumed.

Adherence diary: Study Staff review the completed diaries with the parent and will probe for any problems encountered that reflect acceptability or compliance.

Childcare: Parents are asked about their use of child care services.

Mental Health (parent): Parents complete the Parenting Stress Index – Short Form to assess parenting stress, a brief ADHD screener, and parents complete the Autism Spectrum Quotient Questionnaire (AQ) to assess characteristics associated with Broad Autism Phenotype.

Compensation: At the conclusion of the visit, participants in the blood draw group receive $75 in compensation for their time and participants in the no blood draw group receive $50 in compensation for their time. All participants receive $2 per returned diary (up to $24 in total if all diaries are returned), and a Children's Hospital Parking Garage Token ($2). Children are given a developmentally-appropriate children's book or toy (value =$5).

Contact and Scheduling: Before the end of the visit, Study Staff confirm the contact information on file for the family.

Outcomes Only Assessment: If all attempts to schedule a family to come into the hospital for the final study visit are exhausted and the family has not completed the final study visit within the scheduled window, additional strategies will be employed to obtain outcomes data for the child. The outcomes only assessment includes the Bayley-3 developmental assessment and parent completed Early Childhood Behavior Questionnaire. Examples of additional strategies to re-engage families include offering a home visit option to complete the outcomes only assessment. Families who do not attend their final study visit, but re-engage to complete the outcomes only assessment will receive $100 in compensation for their time. Children will receive a book or toy (value=$5).

7.6 Additional Incentive

In addition to the compensation described in sections 7.3, 7.4, and 7.5, we will offer an additional incentive for families. The additional incentive will be in the form of a quarterly $100 drawing. Enrolled participants have the opportunity to receive drawing entries for completing study activities. Participants can receive up to 6 entries into the drawing per child. Participants receive one entry per child for completing the following activities: 1) attend the T1 visit; 2) return the T2 questionnaires; 3) attend the T3 visit; 4) returned used and unused study packets at the end of the study; 5) return study diaries at the end of the study; and 6) an additional entry is given if the participant completes items 1-5.

7.7 Follow-up visits & data analysis post-intervention

The intervention of this study concludes at the third study visit. The feasibility data collected from the Bayley-3 and the child behavior questionnaires at T1 and T3 will form the preliminary data to

support a grant application for continued follow-up. If continued follow-up is pursued at the conclusion of this study, separate informed consent will be obtained for the follow-up study. This possibility will be noted in the consent form.

Data collected on family education, income, stressors, health history and mental health of parents are used to:

1. Assess demographic variables will allow for a check of the randomization procedure, providing investigators with necessary information to assess the demographic composition of treatment groups.

2. Inform the primary outcome measures. Specifically, this information will be used to examine the potential mediating role of family demographics, stress, and health on child developmental outcomes.

3. Conduct secondary data analyses related to child nutrition, development, and growth.

4. Allow for additional subgroup analyses by socio-economic status.

Baseline fatty acid levels will be measured using the biospecimen obtained at the first study visit (i.e., baseline) for 246 of the 448 participants. This will allow study staff to monitor changes in DHA levels throughout the course of the study and determine if there is a significant increase in DHA levels for children in both treatment arms. Additionally, parents of all 448 participants will provide information related to the child's intake of DHA rich foods. These questionnaires are completed at all three data collection time points and will allow study staff to examine any changes in the child's consumption of DHA over the course of the study.

This full-scale study involves 448 total children (224 in each arm; including those in the pilot study). Depending on funding, follow-up will continue beyond 16-22 months of age primarily for the purposes of assessing aspects of cognitive development, and secondarily to assess aspects of growth and body composition. In general, children may be invited to return for study visits annually to age 5. Following the participants for several years is important because previous trials of LCPUFA supplementation in infants have found that effects differed depending on the length of follow-up and developmental stage of the participants at follow-up. Some effects may not become apparent for several years. These visits will include age-appropriate measures of cognitive development as well as related measures of social-emotional development and behavior. Anthropometrics will also be gathered to track growth and body composition. Halfway between annual in-person visits, the study will engage participants in brief remote data collections via phone or internet. The purpose of these data collections is to collect contextual data that may modify the

effect of the treatment on cognitive ability or body size and also, secondarily, to maintain ongoing contact with families to reduce loss to follow-up. If funding is secured, a separate IRB application will be submitted.

For participants who are recruited into the biospecimen group (i.e., they participate in the blood draw), it is possible that a small amount of blood may be leftover from the study and banked in the Nationwide Children's Hospital Biopathology Center to be combined with biospecimens from the full study for research at a later time, perhaps even years from now. This future research may include research on child nutrition, development, and growth and it may include DNA (gene) testing. For example, polymorphism of fatty acid genes may be analyzed. The usual privacy protections will be applied where we label the blood samples with a code number and not your child's name or other information about them or the family. The informed consent process will involve a section specifically related to the banking of leftover biospecimens obtained as part of this study. This choice allowing researchers to bank leftover biospecimens is separate from the choice to participate in the Omega Tots study.

7.8 Laboratory Evaluations

7.8.1 Specimen Preparation, Handling, and Shipping

Laboratory analyses of biospecimens will be conducted by the Dr. Lynette Rogers lab in the Center for Perinatal Research. Fatty acids will be extracted from plasma and RBC samples. One hundred µL aliquots of each sample will be spiked with C23:0 (50 uL, 30 mg/mL) as an internal standard and butylated hydroxytoluene (50 uL, 0.1%) added as an anti-oxidant. The lipids will be immediately separated from the sample using a Bligh-Dyer extraction (Chloroform: Methanol (2:1)). Briefly, a total of 8 volumes of organic solvent will be added to the sample. After thorough mixing, the samples will be centrifuged (2,000 rpm, 2 min, 4oC) and the organic phases removed and dried under N2 in a water bath at 37oC. Care will be taken to keep the samples saturated with dry N2 to minimize artifactual oxidation. The samples will be acidified to pH 4-4.5 with HCl containing 5% NaCl, followed by an additional Bligh-Dyer extraction and drying under N2. The lipids will be dissolved in benzene (500 uL) and methylated using 10% methanolic sulfuric acid (65oC, 1 hr). The samples will be neutralized with saturated NaHCO3, extracted with hexane (2 aliquots, 500 uL), dried under N2, and reconstituted in analytical solvent (100 uL, undecane). Fatty acid methyl esters will be measured by gas chromatography with flame ionization detection using a linear temperature ramp of 80 to 260 degrees C in 64 min (80 degrees hold 2 min, 5 degrees/min ramp to 174, 2 degrees/min ramp to 212, 3 degrees/min ramp to 260, 260 hold 8 min). Fatty acids from C8:0 to C22:6 will be quantified using experimentally derived standard curves.

7.8.1.1 Instructions for Specimen Preparation, Handling, and Storage

Instructions for the preparation, handling, and storage of specimens are explained clearly in the study's MOP, including required temperatures, aliquots of specimens, if samples are frozen, where they will be stored, and how they will be labeled.

Any remaining specimens will be maintained frozen at -80 degrees Celsius in a secured freezer in the Biopathology Core for future research use. Only the investigators for this study will have access to these specimens and permission for future use. The consent form for the study will state that leftover specimens may be used for studies of prematurity, child development or nutrition conducted only by the investigators for the present study.

8 ASSESSMENT OF SAFETY

8.1 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

8.1.1 Adverse Events

Adverse Event: ICH E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for “serious adverse events” should be captured on the appropriate CRF. Information to be collected includes event description, time of onset, study doctor’s assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis, which would include MD, PA, Nurse Practitioner, DO, or DDS), and time of resolution/stabilization of the event. All AEs occurring during study participation must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened should be considered as baseline and not reported as an AE. However, if the medical condition deteriorates at any time during the study, it should be recorded as an AE.

All AEs must be graded for severity and relationship to study product.

Severity of Event: All AEs will be assessed by the study doctor using a protocol defined grading system. For events not included in the protocol defined grading system, the following guidelines will be used to quantify intensity.

- Mild: events require minimal or no treatment and do not interfere with the participant’s daily activities.

- Moderate: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe: events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
- Life threatening: any adverse drug experience that places the participant, in the view of the Principal Investigator, at immediate risk of death from the reaction as it occurred (i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death).

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

Relationship to Study Products: The study doctor's assessment of an AE's relationship to test article (vaccine or study drug) is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study product assessed using the terms: related or unrelated. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

- Associated – The event is temporally related to the administration of the study product and no other etiology explains the event.
- Not Associated – The event is temporally independent of study product and/or the event appears to be explained by another etiology.

8.1.2 Serious Adverse Events

Serious Adverse Event (SAE): An SAE is defined as an AE that meets one of the following conditions:

- Death during the period of protocol defined surveillance
- Life-threatening event (defined as a subject at immediate risk of death at the time of the event)
- An event requiring inpatient hospitalization or prolongation of existing hospitalization during the period of protocol defined surveillance
- Results in congenital anomaly or birth defect
- Results in a persistent or significant disability/incapacity

- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

All SAEs will be:

- recorded on the appropriate SAE CRF
- followed through resolution by a study doctor
- reviewed and evaluated by a study doctor

8.1.3 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

Collection of laboratory data should be limited to those laboratory parameters that are relevant to safety, study outcome measures, and/or clinical outcome. No known fatty acid value is considered to be abnormal or to present a safety concern. As a result, it is not anticipated that any abnormal laboratory values will be found.

Any abnormal clinical findings will be reported to the Neonatal or BPD clinic physicians for children who are patients there. For children who are under the care of other community physicians, reports of abnormal clinical findings will be shared with the appropriate physician.

8.2 Reporting Procedures

8.2.1 Serious Adverse Events

Any AE considered serious by the PI or Study Doctor or which meets the aforementioned criteria must be submitted on an SAE form to the NCH IRB within 3 business days of discovery. All SAEs will be followed until satisfactory resolution or until the Study Doctor deems the event to be chronic or the patient to be stable.

8.2.2 Regulatory Reporting for Studies Conducted Under IND

N/A

8.3 Type and Duration of Follow-up of Subjects after Adverse Events

All AE's will be followed up on at each study visit until resolved

8.4 Halting Rules

If it is found that the study intervention is related to any serious adverse events, enrollment would be temporarily suspended until a safety review is convened, the objective of which is a decision as to whether the study (or intervention for an individual or study cohort) should continue per protocol, proceed with caution, be further investigated, be discontinued, or be modified and then proceed. Suspension of enrollment (for a particular group or for the entire study) is another potential outcome of a safety review.

Subsequent review of serious, unexpected, and related AEs by the Principal Investigator, Study Doctor, the IRB, or the FDA or relevant local regulatory authorities may also result in suspension of further trial interventions/administration of study product. The FDA retains the authority to suspend additional enrollment and study interventions/administration of study product for the entire study, as applicable.

8.5 Safety Oversight

Safety oversight will be under the direction of the Principal Investigator and Study Doctor. All adverse events will be reviewed to rule out the study intervention as a potential cause.

9 STATISTICAL CONSIDERATIONS

9.1 Study Hypotheses

Hypothesis 1: Children randomized to the DHA+AA arm will demonstrate at least a mean 5-point advantage in Bayley Scales of Infant and Toddler Development-III cognitive composite scores after 180 days of supplementation compared to those randomized to placebo.

Hypothesis 2: Children randomized to the DHA+AA arm will demonstrate at least a 0.33-standard deviation advantage in effortful control and activity level scores from the Early Childhood Behavior Questionnaire after 180 days of supplementation compared to those randomized to placebo.

9.2 Sample Size Considerations

448 children will be randomized with equal allocation to either the treatment group (DHA+AA supplementation, *n*=224) or the placebo group (corn oil powder, *n*=224) for this randomized-controlled trial. The pilot trial produced the preliminary data necessary to support a full-scale trial of the effect of DHA supplementation on cognitive development and behavior assessed at intervals starting at 180 days post randomization. The first 24 children enrolled in the pilot trial were part of an evaluation of feasibility (recruitment, retention, acceptability of the intervention) and also biological response to the intervention. To determine the effect of the treatment on plasma and RBC levels of DHA, 12 children in each arm of the trial will provide 83% power to detect a difference in RBC fatty acid levels of 1% (standard deviation=0.8%). A total of 102 children (51 in each arm) will provide moderate statistical power (β=0.64) to explore a difference in mean Bayley-3 scores of 7 points (SD=15) between the DHA and placebo groups for the purposes of the study. To compensate for a projected 10% loss to follow-up or withdrawals between baseline and Visit 3, we plan to enroll 448 children in the study to fully evaluate aims related to the efficacy of DHA as a low-cost early intervention to improve cognitive and behavioral outcomes in children born preterm and to conduct relevant subgroup analyses.

A total of 448 children (224 in each arm)are needed to detect a 5-point difference in Bayley-3 scores with 80% power (alpha=0.05, SD=15). Previous clinical trials of infants randomized to a DHA-containing formula or placebo found 0.9-7.9 point difference using the BSID-II.[15] The most recent published trial in this area used a high dose of DHA and detected a 4.5-point difference.[14] In terms of secondary outcomes, none are expected to require more participants than the Bayley-3

would require. For instance, a sample size of 74 will be required to detect a difference of 10% in the mean proportion of children scoring in the clinical range on a given sub-scale (t-score) of the BRIEF-P, with 80% power and alpha=0.05. (Power calculated using 2 software packages.[24,25])

Participants subject to protocol violations will be included under intent to treat. If a participant withdraws but their outcome data are available, we will include them. If a participant withdraws or is otherwise lost to follow-up such that their outcome data are missing then they will not be included.

9.3 Planned Interim Analyses (if applicable)

No interim analysis will be planned to evaluate the possibility of termination.

9.3.1 Safety Review

Based on the large number of previous studies that have utilized the same intervention in similar populations and the widespread use of the study products in the U.S. consumer marketplace, there are no expectations for halting the study enrollment or intervention due to safety concerns. However, it will be left to the judgment of the Principal Investigator with the advice of the Study Doctor whether a pattern of adverse events or other circumstances warrant halting. See the section “Assessment of Safety” for details about safety monitoring.

9.3.2 Immunogenicity or Efficacy Review

After the second study visit, a biostatistician or epidemiologist not otherwise affiliated with the study will examine the RBC and plasma laboratory values in relation to treatment assignment to evaluate whether there is a statistically significant difference (alpha=0.05) between the groups in the concentration of individual fatty acids, ratios of individual fatty acids to each other, and total omega-3 and omega-6 fatty acid levels.

9.4 Final Analysis Plan

Summary statistics will be calculated and qualitative methods used to evaluate the feasibility of recruiting, enrolling, and following children. We will use two-sample t-tests to determine whether outcomes varied by treatment group.

We also will conduct two-sample t-tests and ANCOVA to explore the effect of DHA versus placebo on Bayley-3 cognitive development scores and behavioral outcomes of effortful control and activity

level. These analyses will be conducted per Intent to Treat. Children who are missing outcome data will not be part of the analysis. If the child has withdrawn or stopped taking the assigned supplement or placebo but outcome data are available, the associated data will be included in the analysis.

10 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The study will maintain appropriate medical and research records for this trial, in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. Only authorized Study Staff and NCH IRB staff will have access to the documents.

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subjects' memory aid or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial. It is acceptable to use CRFs as source documents.

11 QUALITY CONTROL AND QUALITY ASSURANCE

The quality management program for this study will consist of a system of quality checks on all data collection procedures in addition to a system of training.

All Study Staff that will have contact with study participants will be trained in accordance with the gold standard for each data collection method. Experienced NCH staff from the Behavioral Core or the OT/PT clinic staff will train Study Staff to conduct Bayley-3 assessments. Only Study Staff that can conduct reliable and consistent assessments will be permitted to do the Bayley-3 assessments for this study. OSU CCTS bionutrition staff will train Study Staff in anthropometric measures. At periodic intervals the quality of the data collections being performed will be evaluated by direct observations by the PI or a designated subject matter expert. All paper-based data collection will be evaluated for protocol compliance, completeness, and accuracy by the Research Associate via direct observation of Study Staff and checks of the completed forms. Data entry quality will be assessed by a random check of 10% of the data entries. Laboratory quality control and assurance procedures will follow SOP's already in place in the laboratory and used during previous studies.

12 ETHICS/PROTECTION OF HUMAN SUBJECTS

12.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997).

12.2 Institutional Review Board

The Nationwide Children's Hospital IRB will review and approve the protocol for this study including the associated informed consent documents and recruitment material. Any amendments to the protocol or consent materials will also be approved before they are placed into use.

12.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Discussion of risks and possible benefits is conducted with the participants and their families. Consent forms describing in detail the study interventions/products, study procedures, and risks are given to the family and written documentation of informed consent is required prior to starting intervention/administering study product. Consent forms are IRB-approved and the parent is asked to read and review the document. Upon reviewing the document, the Study Staff member explains the research study and answers any questions that may arise. The parent signs the informed consent document prior to randomization and any procedures being done specifically for the study. The parent has the opportunity to discuss the study with his/her family or think about it prior to agreeing to participate. The participant may withdraw consent at any time. A copy of the informed consent document is given to the family for their records. The rights and welfare of the participants is protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

12.3.1 Informed Consent/Assent Process (in Case of a Minor)

Children may participate in this study under the written informed consent provided by a parent or legal guardian.

Children of a parent who is cognitively impaired or mentally ill are not eligible if the parent is not able to understand fully the research project and to grant informed consent.

12.4 Exclusion of Women, Minorities, and Children (Special Populations)

The study will not exclude any special populations.

12.5 Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party.

A study monitor or other authorized representatives may inspect all documents and records required to be maintained, including but not limited to, data collection records and pharmacy records for the subjects in this study. Records will be retained indefinitely.

12.6 Study Discontinuation

In the event that the study is discontinued, participants may discontinue use of the supplement and placebo safely. There is no known risk to discontinuation.

12.7 Future Use of Stored Specimens

Any residual specimens will be maintained after the study is complete and will be available for future analysis and use, per the participant consent document. Specimens may be analyzed for future studies of nutritional and genetic factors in relation to child growth and development.

Specimens will be maintained in a secure location in the NCH Biopathology Code and will be labeled with a numeric code and no identifying information. This will be noted on the consent form and additional consent to bank leftover biospecimens in this manner will be obtained from participating families.

13 DATA HANDLING AND RECORD KEEPING

The Principal Investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Dark ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change.

Copies of the CRF will be used as source documents and maintained for recording data for each participant enrolled in the study. Data reported in the CRF derived from source documents should be consistent with the source documents or the discrepancies should be explained

13.1 Data Management Responsibilities

All source documents and laboratory reports will be reviewed by the investigator team and data entry staff, who will ensure that they are accurate and complete. Adverse events must be graded, assessed for severity and causality, and reviewed by the PI or designee.

Data collection is the responsibility of the Study Staff under the supervision of the PI. During the study, the investigator must maintain complete and accurate documentation for the study.

The Study Staff is also responsible for data management, quality review, analysis, and reporting of the study data under the direction of the investigator team.

13.2 Data Capture Methods

For the purposes of this study, data capture will be via paper and/or electronic methods. Surveys will be computerized surveys using RedCap. The data entry system will include password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

13.3 Types of Data

Data for this study will include questionnaire/interview (pen and paper and/or electronic), medical record, direct developmental assessment, laboratory, and outcome measures.

13.4 Study Records Retention

Study records will be kept in a secure, locked location in Biobehavioral Health and kept per procedures laid out in federal regulations.

13.5 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), or Manual of Procedures requirements. The noncompliance may be either on the part of the subject, the investigator, or the Study Staff. As a result of deviations, corrective actions are to be implemented promptly.

These practices are consistent with ICH E6:

4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, section 5.1.1

5.20 Noncompliance, sections 5.20.1, and 5.20.2.

All deviations from the protocol must be addressed in study subject source documents and must be maintained in the regulatory file, as well as in the subject's source document. Protocol deviations must be sent to the local IRB per their guidelines.

14 PUBLICATION POLICY

Following completion of the study, the Principal Investigator is expected to publish the results of this research in a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov*, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. The Principal Investigator will register this trial in an acceptable registry.

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16 APPENDIX A: SCHEDULE OF EVENTS

Visit 1 – Baseline, Child age: 10-16 months corrected age
e-Visit 2 – Baseline + 60 days, Child age: 12-18 months corrected age
Visit 3 – Baseline + 180 days, Child age: 16-22 months corrected age

		Visit			Administration	
Domain	**Protocol**	1	2	3	Parent	Inter.
Cognition/Behavior	**Bayley Scales of Infant and Toddler Development-3*	☒	☐	☒	☐	☒
	Brief Infant Sleep Questionnaire	☒	☐	☒	☒	☐
	*Early Childhood Behavior Questionnaire-very short form	☐	☐	☒	☒	☐
	Infant Behavior Questionnaire-Revised very short form	☒	☐	☐	☒	☐
	*Brief Infant Toddler Social Emotional Assessment	☐	☐	☒	☒	☐
	Television Viewing	☐	☒	☐	☒	☐
	Pervasive Developmental Disorders Screening Test – II (PDDST-II); Stage 2: Developmental Clinic Screener	☐	☐	☒	☒	☐
	Response to Joint Attention	☐	☐	☒	☒	☐
Diet	Willett Food Frequency Questionnaire	☒	☐	☒	☒	☐
	Food frequency questionnaire of high DHA food items	☒	☒	☒	☐	☒
	Breast/formula feeding history	☒	☐	☐	☐	☒
	Acceptability/compliance diary	☐	☒	☒	☒	☐
	Child Feeding Questionnaire	☐	☒	☐	☒	☐
	Mealtime Routine/Behavior	☐	☒	☐	☒	☐
	Parental Perception of Treatment Group, % consumed	☐	☒	☒	☒	☐
Anthropometrics	Weight	☒	☒	☒	☐	☐
	Recumbent length	☒	☒	☒	☐	☐
	Head Circumference	☒	☒	☒	☐	☐
	Mid-upper arm circumference	☒	☒	☒	☐	☐
	Upper arm length	☒	☒	☒	☐	☐
	Triceps and subscapular skinfolds	☒	☒	☒	☐	☐
Biospecimens	Blood (2 ml) (Omega Tots only)	☒	☐	☒	☐	☐
Mental Health (parent)	Centers for Epidemiologic Studies-Depression Scale	☒	☐	☐	☐	☐
	State Trait Anxiety Inventory (Trait only)	☒	☐	☐	☐	☐
	*Parenting Stress Index	☐	☐	☒	☐	☐

	ADHD Screener	☐	☐	☒	☒	☐
	Autism Spectrum Quotient Questionnaire (AQ)	☐	☐	☒	☒	☐
Health History (parent)	Self-reported height/weight	☒	☐	☐	☒	☐
	Smoking Exposure					
	- During pregnancy	☒	☐	☐	☒	☐
	- Currently	☒	☐	☐	☒	☐
	- In the home	☒	☐	☐	☒	☐
	Alcohol					
	- During pregnancy	☒	☐	☐	☒	☐
	- 30-day quantity and frequency	☒	☐	☐	☒	☐
	Stanford Brief Activity Screener	☐	☒	☐	☒	☐
Social/ Neighborhood Environment	Physical Activity, Neighborhood Environment	☐	☒	☐	☒	☐
	Neighborhood Collective Efficacy – Community Cohesion and Informal Social Control	☐	☒	☐	☒	☐
	Healthy Food Environment	☐	☒	☐	☒	☐
Demographics	Current Age	☒	☐	☐	☒	☐
	*Ethnicity	☒	☐	☐	☒	☐
	*Race	☒	☐	☐	☒	☐
	Gender	☒	☐	☐	☒	☐
	Contact Information	☒	☒	☒	☒	☐
	Current Address	☒	☒	☒	☒	☐
	Current Marital Status / Partner / Father of child	☒	☐	☐	☒	☐
	*Education (both parents)	☒	☐	☐	☒	☐
	*Income	☒	☐	☐	☒	☐
	Health Insurance	☒	☐	☐	☒	☐
	Employment	☒	☐	☐	☒	☐
	Household Roster-Relationships	☒	☐	☐	☒	☐
	Childcare	☐	☐	☒	☒	☐
Health Services (Child)	Participation in home visiting/early intervention services	☒	☐	☐	☒	☐
	Presence of a Medical Home	☒	☐	☐	☒	☐
Participation Intentions	Complete the study, Attend the next study visit, Give supplement daily be introduced for all families/contacts	☒	☒	☒	☐	☒
	Barriers to participation	☒	☒	☒	☐	☒

Italic items – collected as part of Neonatal Developmental Clinic or BPD Clinic follow-up

* Collected as part of OPRN follow-up

Phone calls/emails/text messages – Study Staff call, email, and/or text participating families at the following (approximate) intervals after randomization to answer questions about the assigned treatment, identify and resolve acceptability and compliance issues, assess and gather information about possible adverse events, and remind families to complete the study diary: 3, 14, 30, 45, 90, 120, 150 days and as needed based on family and study needs. Regular check-in emails will be sent to families. Additionally, families will be contacted around 60 days post randomization regarding the T2 e-visit and necessary follow-up to facilitate survey return.

Data in the OPRN Perinatal Research Repository or medical record to be extracted – date of birth, gestational age, Apgar scores, birth weight, birth length, birth head circumference, sex, race/ethnicity, maternal age, maternal education, smoking, NICU feeding history, contact information

Blood will be processed and stored for analysis of fatty acid concentrations (plasma, red blood cells). Any remaining volumes will be stored for future analysis including for possible genetic analysis. Blood will only be collected for 246 out of the 448 participants.

Children will be mailed a greeting card annually on their birthday to check for return address changes and also boost retention. Children will receive a seasonally-appropriate holiday greeting card annually for the same purpose. Families will receive study newsletters annually to keep them informed of study progress. Additionally, thank you cards/letters will be sent to families after all study visits.