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Jill Shah Date

Evaluating the effect of prenatal Docasahexaenoic Acid (DHA) supplementation on cognitive development at 7 years of age in Mexico

By

Jill Shah

Master in Public Health

Epidemiology

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Aryeh D. Stein

Committee Chair

_____

Usha Ramakrishnan

Committee Member

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By

Jill Shah

B.S.

University of Maryland

2011

Thesis Committee Chair: Aryeh D. Stein, Ph.D.

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Abstract

Evaluating the effect of prenatal Docasahexaenoic Acid (DHA) supplementation on cognitive development at 7 years of age in Mexico

By Jill Shah

The essential n-3 long-chain polyunsaturated fatty acid, docosahexaenoic acid (DHA) is important in fetal brain and retina development. Most of the amounts of DHA in the brain are deposited towards the second half of pregnancy and into early infancy, and is abundant in the nonmyelin membranes of the brain and retina. The high concentrations of DHA in these neural membranes suggests that providing additional preformed DHA during pregnancy to mothers may improve the structural and functional development of cognitive systems of their infants. Nutrition and early childhood intellectual achievement are important factors for intellectual functioning in adulthood.

A large double-blind, randomized, placebo-controlled trial of DHA supplementation was conducted starting in 2007 and followed a cohort of 1,094 women and their offspring (n=973) in Cuernavaca, Mexico. Women received daily supplementation with 400 mg DHA or a placebo from 18-22 weeks of gestation through delivery. A follow-up study was conducted to evaluate the effects of prenatal DHA supplementation on child cognitive development at 7 years of age (n=679), using the Wechsler Abbreviated Scale of Intelligence (WASI). The outcome measure included the Full Scale IQ, Performance IQ and Verbal IQ tests and the subset tests, which included Matrix Reasoning, Similarities, Vocabulary and Block Design.

The intent to treat analysis showed that DHA supplementation during the second half of pregnancy until delivery did not significantly affect cognitive development at 7 years of age as measured by WASI ($p>0.10$). A priori tests for heterogeneity showed significant effect modification by HOME score at 60 months and by gender. Full Scale IQ and Verbal IQ scores were higher among children from poorer home environments at 60 months of age who were exposed to prenatal DHA supplementation compared to those that received a placebo and came from similar home environments ($p < 0.10$). This could demonstrate that children from poorer home environment benefit from prenatal DHA supplementation compared to their unexposed peers.

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Chapter 1

LITERATURE REVIEW

This literature review introduces early childhood cognition and gives an overview of neurodevelopment *in utero* and during the early years of a child's life. It then discusses key factors that affect childhood cognition, and then addresses the importance of maternal nutrition and childhood cognition. Next, it specifically discusses DHA intake and child cognitive development, along with discussing literature related to prenatal DHA supplementation and cognitive development.

Introduction to childhood cognition

Cognition is broad and spans various processes such as learning, language, memory and attention. Cognitive development refers to the changes in these processes observed over a longer period of time, and is usually measured in children by performance tests that assess specific cognitive abilities (1). Cognitive development is multidimensional and a result of various factors such as physical growth, interactions with the environment, and neurological maturation (1). It is characterized by plasticity, which is the mechanism for development and learning that refers to the ability of the human brain to adapt to environmental pressures, experiences and physiologic changes (2). Cognitive performance is important in all stages of life and early childhood intellectual achievement is an important factor for intellectual functioning in adulthood (3).

Neurodevelopment in utero and early years

The primary growth phase for the human brain is between birth and two years, with the brain reaching a significant amount of its growth prenatally, and reaching 60% of its total mass by 2 years of age (4). The areas of the brain that are not fully developed continue to grow throughout childhood and adolescence. During the early life stages (i.e., embryonic, fetal and early postnatal life) genetic and environmental determinants play an important role in the development of brain regions and shaping the neural configuration of the brain (5). Genetic determinants also regulate synaptic signal transmission and are crucial in the establishment and maintenance of the central nervous system (5). Important controllable factors that can impact brain development are maternal nutrition, exposures and behaviors. Nutrition is one of the most influential environmental factors on the fetus and is important for the maturation and development of the central nervous system (6). It can also directly modify gene expression and structure, provide specific molecules that can affect brain growth and development, and also act as growth factors (5).

Prenatal development has well defined milestones, referred to as critical periods, where a specific experience must occur during a relatively narrow time frame for the development of certain brain regions. The essentiality of a nutrient depends on when it is delivered in relation to these critical periods during brain development (5). Nutrient deficiencies that occur during the prenatal months usually cause irreversible effects because of the time-sensitive critical periods (5). However, postnatal development timeframes are less defined, and are broader and more flexible in time (5).

The brain is a specialized tissue that depends on the generation of electric potentials for its functionality, therefore requiring certain nutrients such as folic acid, iron, zinc and

essential fatty acids (5). Long-chain polyunsaturated fatty acids (LCPUFA) are necessary for structural integrity and function throughout the body (7). In utero, the accumulation of LCPUFAs occurs mainly in the last trimester of pregnancy (7) and brain development is rapid during this time suggesting LCPUFA may be important in brain development.

Infants are born with an intrinsic capacity to learn, but the environment can regulate what and how the infant learns (5). Between the ages of 1 and 5, there is rapid and dramatic postnatal brain development, characterized by neural plasticity, and also in the acquisition of fundamental cognitive development skills such as working memory and attention. During this age range, the child's spoken vocabulary significantly increases; they gain greater motor coordination and have longer attention spans when focusing on tasks (5).

Factors that affect cognitive development

Cognitive development depends on numerous biological, environmental, and sociological factors, therefore it important to consider these factors when assessing cognitive development (8).

Mineral Deficiencies

Iodine and iron deficiencies are two important minerals in a child's development. Iodine is an integral part of the thyroid hormones, which affects the development of the central nervous system. Iodine is the most common preventable cause of mental retardation, as a deficiency can lead to irreversible mental retardation. A meta-analysis of 18 studies concluded that children and adolescents with iodine deficiency averages 13.5 IQ points lower (9).

Iron deficiency anemia is prevalent in about 25-30% of children younger than 4 years of age in developing countries. Animal models show that early iron deficiency anemia alters brain metabolism and neurotransmission. Numerous studies have shown that infants with iron deficiency anemia had poorer mental, motor, and neurophysiologic functioning (10, 11).

Zinc deficiency affects about one of third of world's population, but research has provided inconsistent findings on the effects of zinc alone. In Bangladesh, zinc with iron improved motor development and behavior. However, these results were not seen in India or Indonesia (12-14).

Environmental exposures (metals and infectious agents)

Environmental exposures to metals such as lead, arsenic and manganese can lead to chronic diseases and developmental delays in children. Children in developing countries are at greater risk for being exposed to lead, such as through contaminated drinking water. Studies from both developing and developed countries show that after the adjustment for social confounders, lead exposure is associated with a small decrease in IQ level (15, 16). Additionally, other studies show that modest impairments in intellectual, motor and behavioral development occurred between ages 2-10 (15). Arsenic is another metal often found in water from wells, and children who drank water contaminated with arsenic had dose response decrements in IQ. This was seen in Bangladeshi, Mexican and Taiwanese adolescents (17, 18) . Similar findings were also seen after exposure to manganese in children (19).

Infectious diseases globally affect children under 5 years and can affect development. Infections from intestinal helminthes and HIV/AIDS affect cognitive development in millions of children. For example, infants with HIV/AIDs infection are at a risk for delays in language acquisition and in some cases, infection can lead to severe encephalopathy (20). The lack of clean water or proper sanitation also put children at a higher risk for diarrheal diseases, especially between birth and the first 2 years of life which may impair cognitive performance (21).

Breastfeeding

Breastfeeding could be a protective factor on child development due to nutrients found in breast milk, such as essential fatty acids. A meta-analysis that included 11 studies in developing countries showed that breastfeeding lead to small cognitive benefits of about 2-5 IQ points (22, 23). Additionally, three reports from developing countries concluded small improvements in motor development with greater duration of breastfeeding (24).

Maternal Education and Intelligence

Maternal education and intelligence can be important factors in cognitive development both directly and indirectly. The indirect influence is mediated by home environment and family income (25). A study by Anderrson et al. observed maternal IQ to be the strongest predictor of a child's cognitive abilities at 5 years of age (26). Also, a study by Bakker et al., found no main effects of DHA and cognitive function at 7 years of age, but reported significant relationships between maternal education and intelligence and cognitive outcome measures (27).

Parental and home factors

Many children from developing countries are exposed to violence such as war and community violence. A study of South African children exposed to community violence had higher levels of post-traumatic stress disorders and attention problems (28). The negative effect of the exposure to violence is likely to be greater when family stability or mental health of primary caregiver is disrupted. There are consistent findings from developing countries that show reduced levels of cognitive function in young children of depressed mothers. It is likely that maternal depression can affect child rearing behaviors (29, 30).

Cognitive stimulation or child learning opportunities have also shown to increase cognitive performance. Multiple studies have shown significantly higher cognitive functioning in young children who were given additional cognitive stimulation or learning opportunities, compared to non-stimulated children. Some follow-up studies consistently report long lasting effects of early cognitive stimulation, with some benefits lasting as long as 17 years (31).

Socio-economic status

Socio-economic status (SES) is an important environmental factor that can affect cognitive development. SES is a measure that encompasses education, social status and wealth that affects the ability for families to purchase the goods and services they need (5). It is likely that low SES leads to inadequate dietary intakes and nutrient deficiencies which can cause complications later in life (32). SES also affects access to healthcare and housing which in turn affect nutrition. High-risk pregnancies are more likely in low SES families

and children are more likely to have experienced adverse cognitive and behavioral events (5).

Importance of maternal prenatal nutrition

Maternal and child undernutrition is the underlying cause of over a third of the disease burden in children younger than five years (33). Poor maternal and child nutrition negatively impacts social, economic, and human capital development. A mother's nutritional status before and during pregnancy is important for a healthy pregnancy outcome, and prenatal maternal nutrition can impact fetal growth development as well as neurological outcomes later in life (33). Malnutrition adversely affects numerous developmental aspects such as brain growth, neurogenesis and neural processes. Additionally, malnutrition during certain critical periods can create permanent effects on brain development which can affect cognitive development in the future (6).

Intrauterine growth restriction largely indicates a deficiency in fetal nutrition during a critical period for brain development. In developing countries, intrauterine growth restriction is mainly due to infections and poor maternal nutrition. Numerous studies have shown that term low-birth weight infants with intrauterine growth restriction had lower developmental levels and cognitive levels and poorer problem solving ability (8). Additionally, stunting is a measure of chronic under nutrition that is caused by the combination of poor nutrition and infectious diseases. Multiple prospective cohort studies show associations between stunting by ages 2 and 3 and cognitive deficits later in life (8).

Therefore, investing in maternal nutrition is a cost effective approach in the prevention of various complications (34). There is evidence that adequate nutrition *in utero* and continuing on to the first two years of life is essential for strong human capital.

Undernutrition can be associated with lower adult intellectual functioning and lower economic status in adulthood. (35).

Introduction to fatty acids

Over the past decades, there has been a focus on the role of essential lipids in the central nervous system. Dietary lipids provide essential fatty acids and further the absorption of lipid-soluble vitamins (36). Lipids are structural components of all tissues and the brain, retina and other neural tissues are especially rich in LCPUFAs such as omega-3 (n-3) and omega-6 (n-6) fatty acids.

Essential LCPUFAs are important for neurodevelopment and optimal brain function as they are crucial components of the phospholipid bilayer, and contribute to the functional and structural integrity of the body. In the brain and retina, they are important for signal transduction, neurotransmission and neurogenesis (7). A deficiency in LCPUFAs can alter synaptogenesis and membrane function (37). The n-3 and n-6 polyunsaturated fatty acids comprise 14% and 17% respectively of the total fatty acids in the brain. The body cannot readily produce these fatty acids therefore, they must be acquired through the diet and the fetus must receive these nutrients by placental transfer (38-40).

LCPUFAs are transported across the placenta, and present in human milk, and accumulate in the brain and retina during fetal and infant development (38). Placental transfer is both active and passive. Active transport occurs through fatty acid transport proteins and passive transport depends on maternal blood levels (7). N-6 fatty acids are abundant in the diet and are commonly found in vegetables oils, seeds and nuts. However, n-3 fatty acids are less abundant in the diet, and found in marine animals and water plants such as algae (7, 41). It is important to note that both n-6 and n-3 fatty acids share and

compete for the same enzymes during the conversion process (42), which may decrease the availability of n-3 fatty acids. Therefore, it is not clear whether the maternal needs of n-3 LCPUFAs during pregnancy can be met through these means.

Docasahexaenoic acid (DHA) is an essential n-3 fatty acid that plays an important role in the development of the fetal brain and retina (43, 44), and is the main n-3 fatty acid in brain gray matter. DHA and arachidonic acid (AA), another important LCPUFA, rapidly accumulate in neural tissues during gestation and the first year of life (43, 44). DHA has shown to accumulate in areas of the brain associated with learning and memory, and AA is important for normal cognitive growth (45, 46).

Docasahexaenoic acid

DHA is an important component in the myelination of brain frontal lobes that continuous throughout childhood and adolescence, and may be especially important during periods of brain growth spurts which occur between the last trimester of pregnancy and up to two years of age (46). Additionally, DHA is an important structural component of the highly specialized membrane lipids of the central nervous system (38). DHA rich frontal lobes are thought to be an important factor in executive and higher order cognitive activities such as planning and problem solving (47). Furthermore, intakes during pregnancy and infant years have shown to affect growth and cognitive performance later in childhood (48). The high concentrations of DHA in the retina and brain suggest that these fatty acids may have an important role in retinal and neuronal function (38).

Preformed DHA is found in cold water fish, such as salmon and tuna, breast milk, and algae but DHA can also be synthesized in the liver from its precursor, a-linolenic acid (ALA) (42). Breast milk is one the best sources of ALA and DHA, however, the efficiency

of conversion of ALA to DHA may be low (48). Since DHA is an essential fatty acid and cannot be synthesized by the body, the fetus depends on maternal sources for its intake.

Evidence shows that conversation rates are lower in infants than adults, and that precursors are not efficiently converted to DHA to allow for biochemical and functional normality to occur (36, 49, 50). When there is not enough DHA, the specialized phospholipid membranes in the retina and brain may be substituted with alternate fatty acids which can alter cognitive functions such as memory, attention and visual processing (7).

Since the efficiency of conversion of DHA may be low, it is not clear whether maternal needs of n-3 LCPUFAs during pregnancy can be met. It may be more efficient to increase DHA status through increasing fish consumption or DHA supplementations (48). However, some oil-rich fish are contaminated with high levels of mercury which raises the concern about pregnant women being exposed to mercury, since the placenta does not protect the fetus from this neurotoxin (51). Additionally, some pregnant women avoid fish because of concerns regarding polychlorinated biphenyls contamination. Therefore, providing preformed DHA supplementation during pregnancy may reduce the risk of mercury and polychlorinated biphenyl exposure.

Previous studies examining prenatal DHA supplementation and cognitive development

Numerous studies have explored the relationship between prenatal DHA supplementation on cognitive development but there have been mixed conclusions (52). Different outcome measures, differences in study design and differences in the dose and duration of supplementation are all factors that impact study results (1, 52).

A systematic review by Campoy et al. (52) highlights the varied results of studies that aimed to determine the effect of prenatal DHA supplementation on infant and child cognitive development. Two RCTs reported no differences between DHA supplementation and placebo groups on Bayley Scales of Infant Development at 10 and 18 months (53, 54). Another study assessing cognitive development at 6.5 years, randomly assigned 154 mothers to receive one of four interventions. Starting at the 20th week of pregnancy until delivery, mothers received a daily supplement of Fish oil composed of 500mg DHA+150mg EPA, 400ug 5-MHTF, or both, or a placebo. There were not any significant differences across the intervention groups for Kaufman Assessment Battery for Children scores for children at 6.5 years (55).

However, an RCT in Australia reported better eye and hand coordination in the LCPUFA supplemented compared to the placebo group at 2.5 years (56). Ninety-eight pregnant women received Fish oil that contained 2.2g DHA + 1.1g EPA or a placebo made of olive oil from 20 weeks gestation until delivery. Results showed that children in the fish oil-supplemented group (n=22) had higher scores for eye and hand coordination compared to children in the placebo group (n=39) (56).

The lack of conclusive information and mixed findings exploring the associations and effects of prenatal DHA supplementation and child cognitive development, especially in later years, indicates the gap in research on this topic. Additionally, most of the studies conducted have been observational and are not randomized in design and also few studies look at the effects of DHA alone, and usually examine DHA in combination with other supplementations such as AA and EPA.

Chapter 2

INTRODUCTION

Undernutrition continues to be a major public health problem, and the negative consequences of maternal undernutrition on both the mother and the child are particularly higher in low and middle income countries. The women in these settings are often undernourished before pregnancy and their nutritional deficiencies intensify throughout pregnancy (57). Maternal nutrition preconception and during pregnancy is a crucial determinant of the proper growth and development of newborns. Moreover, there is evidence that adequate nutrition *in utero* and during the first two years of life is essential for strong human capital, and undernutrition may be associated with lower economic status in adulthood (35). Inadequate nutrition and lack of essential of micronutrients and fatty acids has shown to lead to poorer birth and child development outcomes (37).

Essential fatty acids are critical in central nervous development since they are important structural elements of cell membranes and for the formation of new tissues, both processes that occur at a higher rate during pregnancy and fetal development (58, 59). Brain development and optimal brain function are dependent on the intake of essential fatty acids such as n-3 and n-6 fatty acids, which are polyunsaturated fatty acids (PUFAs) that cannot be readily produced by the body (39, 60). Since these essential fatty acids cannot be produced by the body, the fetus must receive these nutrients by placental transfer (38).

The n-3 fatty acid DHA is involved in the development of the fetal brain and retina, and is the main n-3 fatty acid in brain gray matter (43, 44). The high DHA content supports an essential role for this fatty acid in brain and visual function (36). Inadequate DHA intake in early life may be associated with later complications in structural and functional development of visual-sensory and cognitive systems (61). Dietary intake of DHA is

limited in many parts of the world, especially in the Mexican diet (42). Adult DHA recommendations range from 200-900mg per day, however most Western diets contain 60-80mg per day (62).

Preformed DHA can be found in cold water fish, such as salmon and tuna, and algae but DHA can also be synthesized in the liver from its precursor, ALA (42). Along with the low intake of DHA in the Mexican diet, the efficiency of conversion to DHA from ALA may be low, because both n-6 and n-3 fatty acids share and compete for the same enzymes during the conversion process (42). The nutrition transition has been accompanied by increases in n-6 fatty acids, such as those widely found in vegetable oils, seeds, nuts, eggs and some meats, that may interfere with the conversion of ALA to DHA, and the intakes of n-6 fatty acids have increased over time (42). Furthermore, previous studies have shown that the rate of conversion from ALA to DHA is insufficient to meet the needs of pregnancy and infancy. Conversion rates are lower in infants than adults because precursors may not be efficiently converted to DHA to allow for biochemical and functional normality to occur (36, 49, 50).

Most of the amounts of DHA in the brain are deposited towards the second half of pregnancy and into early infancy, and is abundant in the nonmyelin membranes of the brain and retina (44, 63). Since DHA is concentrated in these neural membranes, it is likely that providing additional preformed DHA during pregnancy to mothers may improve the structural and functional development of cognitive systems of their infants. The importance and essentiality for DHA in human brain development is known, however the need for DHA in later cognitive development is still unclear. Few studies examine the long-term benefits of increasing prenatal DHA supplementation (52), and it would be beneficial to

better understand the role of prenatal DHA supplementation in long term cognitive development. Nutrition and early childhood intellectual achievement are important factors in intellectual functioning in adulthood (3).

Research has shown that DHA is essential for growth and development of the brain, but the current evidence examining DHA's effects on birth outcomes and cognitive development in the later years is limited and inconclusive, indicating a need for more research regarding prenatal DHA supplementation. This study used data from a large, placebo-controlled trial conducted in Cuernavaca, Mexico to evaluate the benefits of Prenatal DHA (Omega-3 fatty acid) Supplements on infant GRowth And Development (POSGRAD). The objective of this study was to examine the effects of prenatal DHA supplementation on cognitive outcomes, measured by the Wechsler Abbreviated Scale of Intelligence (WASI) test, at 7 years of age. It is hypothesized that children whose mothers received prenatal DHA supplementation will display better cognitive scores on one or more of the WASI scales (Verbal IQ, Performance IQ or Full Scale IQ) compared to those children born to women who received a placebo.

Chapter 3

METHODS

Overview

This study is a follow-up of the offspring of women who participated in the POSGRAD study, a double-blind, randomized, placebo-controlled trial designed to assess the effect of prenatal DHA supplementation on child growth and development. 1,094 pregnant women between 18 to 22 weeks of pregnancy were randomized to receive a daily supplement of DHA (400mg) or placebo of similar taste and appearance. Offspring born

to these women have been followed up to 7 years and repeated measures of growth and development have been collected since birth. At 7 years, cognitive development was assessed using the Spanish language version of the WASI (n=679). The objective of this study was to examine the effects of prenatal DHA supplementation and cognitive outcomes at 7 years. This research was supported by NIH HD-043099.

Study setting and population

This study is a collaboration between Emory University's Hubert Department of Global Health in Atlanta, Georgia, the Instituto Nacional de Salud Pública (INSP) and Instituto Mexicano del Seguro Social, (IMSS) General Hospital I in Cuernavaca, Mexico. In general, the women who use this hospital are of medium to low socioeconomic status and either they or their husbands, or both, are employed. IMSS healthcare system provided employed persons access to medical care, and IMSS usually pays one-third of the healthcare costs and the employer and federal government pay the remaining costs. Study recruitment began in February 2005 and the intervention was completed in July 2007.

Women were considered for inclusion in the study if they were between 18-35 years of age, 18-22 weeks of gestation, planned to breastfeed predominantly for at least 3 months and planned to deliver at the IMSS General Hospital in Cuernavaca and reside in the area for 2 years after delivery. Women were excluded from enrollment in the study if they had: high risk pregnancy, regular intake of DHA supplements or fish oil during pregnancy, chronic use of medication for chronic illnesses or lipid metabolism or absorption disorders. Following recruitment, 1,094 eligible women were randomized to receive a 400mg/day of algal DHA (two 200 mg capsules), or a soy and corn oil-based placebo of similar taste and

appearance. A total of 1,040 women started treatment and 968 women completed the study and delivered 973 live infants (including 5 pairs of twins). Subjects were followed through 7 years of age, and cognitive measurements at 7 years were collected and assessed using the Spanish language version of the WASI (Figure 1).

Randomization, Intervention and Compliance

Block randomization was used to create balanced replication of four treatments (two colors for DHA and two for control) using a block size of eight. All study participants and members of the study team remained blinded to the treatment scheme throughout the intervention period. Since the study is ongoing for follow-up of child development, the participants and fieldworkers in Mexico remain blinded to treatment scheme. The analytical study team was unblinded after the youngest baby born turned 6 months of age. At this time all participants had stopped taking supplements.

The supplements were produced by Martek Biosciences and distributed by Mead Johnson. The DHA capsules contained 200mg DHA derived from an algal source. The placebo capsules contained olive oil and were similar in taste and appearance to the DHA supplements. During the weekly home visits, mothers received 14 capsules in a precoded container and were instructed to take two capsules daily together, at the same time each day. The remaining capsules from the previous week were counted each week. Compliance was calculated as a percentage, by dividing the total number of capsules actually consumed over the number of capsules expected to be consumed. Capsule count, side effects and illnesses were recorded during the weekly home visits.

Measurement of maternal and child characteristics

Maternal Baseline characteristics

Trained nurses obtained baseline measurements of weight, height, skin folds, and obstetric history at the first scheduled visit when participants received the first week's supply of supplements.

Maternal Schooling

Maternal schooling was defined as the highest level of school completed by mother. For this study, schooling was categorized as greater than or less than a high school education.

Dietary Intake

Trained fieldworkers using pretested questionnaires from previous studies in Mexican populations measured dietary intake and socioeconomic status. Dietary intakes of fatty acids were measured using a previously validated food frequency questionnaire that was adapted for pregnant women. This questionnaire asked to recall 110 food items in the past 3 months.

Socioeconomic Status

A continuous SES variable was used in this analysis that was creating using principle component analysis (PCA). This factored in occupation, housing and personal assets to determine an SES score.

Child characteristics at the time of birth

Study personnel obtained data on birth outcomes from hospital records within 24 hours after delivery. Birth outcome data included, live birth, occurrence of multiple births, sex of baby, type of delivery, and anthropometric measurements obtained within 1 hour of birth.

- Low birth weight was defined as less than 2,500g
- Birth length and head circumference were measured by trained hospital staff to the nearest 1mm.
- Gestational age at birth was calculated based on the date of the last menstrual period reported of the mother during recruitment
- Preterm birth was defined as delivery before 37 weeks.

Home environment

Home environment is an important factor in the development and fostering of child cognitive intelligence. The Home Observation for Measurement of the Environment (HOME) Inventory is designed to measure the quantity and quality of stimulation and support available to a child in the home environment. It can be conducted from 0-14 years with different versions available for different time intervals.

The Infant/Toddler HOME Inventory is designed to use during birth to 3 years of age. It includes 45 items that are clustered into six subscales: Parental responsivity, acceptance of child, organization of environment, learning materials, parental involvement and variety in experience. The Infant/Toddler HOME inventory is out of a total of 55 points (64). The Early Childhood HOME Inventory is designed for use during 3 to 6 years of age. This inventory contains 55 items clustered into eight subscales: learning materials,

language stimulation, physical environment, parental responsivity, learning stimulation, modeling of social maturity, variety of experience and acceptance of child. The Early Childhood HOME inventory is measured out of a total of 545 points (64).

Measurement of Outcomes

Cognitive functioning was assessed using the WASI, the abbreviated version of the *Wechsler Intelligence Scale for Children®—Fourth Edition* (WISC–IV®). This assessment can be administered across a broad range of ages spanning from 6 to 90 years. The WASI assessment provides composite scores that estimate Verbal Comprehension and Perceptual Reasoning abilities and includes four subsets: Block Design, Matrix Reasoning, Similarities and Vocabulary. These subsets are scaled to a T-score metric and yield Verbal IQ, Performance IQ and Full-Scale IQ scores.

The Verbal IQ score is a measure of crystallized abilities and consists of two measures:

- Vocabulary subset: measuring word knowledge and verbal concept formation
    - includes 31 items
        - 3 picture items, where the examinee names the objects presented
        - 28 verbal items, where examinee defines words visually and orally presented
- Similarities subset: measuring verbal reasoning and concept formation
    - includes 24 items

- 3 picture items: examinee selects an option that shares common characteristic to the target
- 21 verbal items: examinee describes how two words are similar

The Performance IQ score is composed from the two performance measures:

- Matrix Reasoning subset: for measuring visual information processing and abstract reasoning skills
  - includes 30 items
  - examinee views an incomplete matrix or series and chooses the option that completes the series
- Block Design subset for measuring the ability to analyze and synthesize abstract visual stimuli.
  - includes 13 items
  - examinee views a constructed model or picture and used red and white blocks to recreate the design within a specified time limit

The 4 subsets were administered in the Spanish version and it has been pretested, standardized and applied by members of our study team in previous studies.

Statistical Analysis

In this analysis, we retained 679 individuals who had plausible WASI cognitive scores at 7 years of age. Comparison of several characteristics among the DHA supplementation intervention group and the placebo group were examined for effectiveness of randomization. These characteristics were assessed using the Student T-test for

continuous variables or Chi-Square Tests (X^2) for categorical variables. A p-value of less than 0.05 was considered as significant for baseline comparisons between treatment groups. All analyses were done following the intention to treat design.

Distributions of the variables were examined for normality, and they were assessed for outliers and implausible values. Observations with implausible values were removed. A two sample pooled T-test was used to assess the differences in child cognitive development between the DHA intervention group and placebo group. Group means were analyzed and compared for the main outcome measures (Verbal IQ, Performance IQ and Full Scale IQ) and the subset measures (Block design, Matrix Reasoning, Similarities and Vocabulary).

Each main WASI test outcome was examined individually, and two models were used for each assessment. The first model adjusted for age at time of test administration, and another model further adjusted for age at time of test administration, gender, maternal height and whether or not the child was breastfeed for the first 3 months. Covariates were selected *a priori,* and maternal height was included in the adjusted model due to being close to significantly different at baseline between the treatment groups (p=0.08).

Effect modification was assessed by individually examining the interaction between treatment and the following covariates: public school (public school*treatment), HOME score at 12 months, HOME score at 60 months, maternal schooling, maternal intelligence, gender of child, offspring breastfed for at least 3 months, and primigravida. Interactions were considered significant at $p < 0.10$. HOME score at 12 months, HOME score at 60 months and maternal Raven score were continuous variables, and all other covariates were examined as categorical variables. All analyses were completed in SAS

9.4. The analysis for this study was approved by the Emory University Institutional Review Board.

Chapter 4

RESULTS

Primary Analysis

A total of 973 infants born to the 1,094 pregnant women who originally enrolled in the POSGRAD trial were eligible for the 7-year follow-up visit. Figure 1 describes recruitment and follow-up of pregnant women and their children until 7 years of age. A total of 681 children were measured for WASI cognitive tests, but 2 of them were dropped due to implausible values. This resulted in a sample size of 679 children born to 675 women, including four pairs of twins. The placebo group had 333 participants and the DHA supplementation group had 346 participants (Figure 1). Comparison of several baseline maternal characteristics and child characteristics for this final sample revealed no significant differences between the intervention and control group ($p<0.05$) (Table 1).

At baseline, the mean maternal age was 26.4 ± 4.8 years and the mean BMI for the mothers was 26.2 ± 4.3. Dietary intake of preformed DHA was very low, with a median intake of 56.5g/day (IQR: 38.6-99.5g/day) combined with a high ratio of *n*-6 to *n*-3 fatty acids (12:3). (Table 1). Fifty-five percent of the offspring were male. Mean birth weight and gestational age were 3227.3 ± 450.2g and 38.9 ± 1.8 weeks, respectively, and the prevalence of low birth weight and preterm delivery were only 5% and 9%, respectively (Table 2). The prevalence of small for gestational age was 10% (Table 2).

Loss to follow-up did not differ by treatment group, however some characteristics differed among the children who had 7 yr WASI data and those who did not ($p<0.05$). The

children with 7-year WASI data were 70g heavier (p=0.03) and 0.37cm taller, at birth (p=0.04). Head circumferences differed by 0.34cm in the children who had 7 year WASI data compared to children who were not included in the study due to missing 7 year data (p=0.01). Additionally, 72.2% of the children who were included in the study were breastfed for 3 months or more, compared to 40.2% for those who were not included in this study (Supplementary Table S1).

Intent to treat analysis revealed no significant differences by treatment group in the mean scores of the main outcome measures (Verbal IQ, Performance IQ and Full Scale IQ) or the subset measures (Block design, Matrix Reasoning, Similarities and Vocabulary) (p>0.10) (Table 3). However, we found evidence of heterogeneity for the effect of the treatment by gender and home environment at 60 months of age. There was however no evidence of effect modification by either HOME score at 12 months, maternal schooling, maternal intelligence, child breastfed for at least 3 months, or primigravida. In general, all covariates examined appeared to have a significant relationship with one or more of the main WASI cognitive measures (Verbal IQ, Performance IQ and Full Scale IQ). For example, correlation between HOME score at 60 months and the WASI outcomes was significant (p<0.001) and had correlation coefficients ranging from r=0.35 to r=0.43.

When examining the effect modification by gender on treatment through stratified analysis, we found that males who were exposed to prenatal DHA performed 1.7 (-0.48, 3.89; p=0.07) points better on Full Scale IQ compared to their unexposed peers, compared to no differences among females. We also found these interactions were stronger after adjusting for age of child during WASI test, sex of child, breastfeeding and maternal height, for the Full Scale IQ as well as Verbal IQ (p=0.07 and p=0.08, respectively) (Table 4). The

adjusted differences between DHA and placebo groups for the Full Scale IQ was 2.30 (0.15, 4.46) for males compared to -0.74 (-2.79, 1.31) for females.

After stratifying HOME score into tertiles, the interaction by HOME score at 60 month on treatment was significant for Verbal IQ (p=0.05) and Full Scale IQ (p=0.04) and Performance IQ was borderline significant at p=0.10. Among those children from a Low HOME environment (score <39), children exposed to prenatal DHA scored 3.73 (0.69, 6.77) points higher for Verbal IQ and 3.40 (1.07, 5.74) points higher for Full Scale IQ. Among children from a Medium or High HOME environment (score ≥ 39), no effect of exposure to prenatal DHA was observed (Table 5).

We further explored the interaction of home environment by stratifying into categories for gender. No significant interaction by HOME score at 60 months on treatment was seen for males (p>0.10) (Table 6), although males from Low HOME score environments who were exposed to prenatal DHA performed better on Verbal IQ and Full Scale IQ. For females, all three of the WASI outcome measures had significant interaction terms (p <0.10), but the HOME specific estimates were not significant, except for Verbal IQ and Full Scale IQ in the Medium HOME score tertile (Table 6).

Sub- Analyses

Sub Analyses were carried out in two restricted datasets: singleton births (n=671) and those who had HOME score data at 60 months (n=575). There were no significant differences in baseline characteristics in the singleton births sample (data not shown) or the HOME score only sample (Supplementary Table S2). The results for the main effect of intervention in the restricted datasets were similar to that of the primary analysis.

Additionally, examining the effect modification between HOME score environment and treatment and effect modification between gender and treatment yielded similar results as the primary analysis (results not reported).

Chapter 5

DISCUSSION

In this follow-up study of a large double blinded randomized controlled trial, daily supplementation of 400mg DHA during the second half of pregnancy did not have a significant effect on Full Scale IQ, Performance IQ or Verbal IQ as measured by the WASI test, at 7 years of age. The two groups were similar at baseline, compliance was high (65), and loss to follow up did not differ by treatment group.

A much larger DOMInO trial in Australia showed similar findings. They did not report any differences in the overall mean scores of Cognitive, Language, Motor or Behavior Rating Scale of the BSID-III between the DHA intervention and placebo group at 18 months of age. However they saw differences by gender. Unlike the males, females exposed to prenatal DHA had poorer mean adaptive behavior and language scores (53). Our results did not report significant interaction between gender and treatment on WASI outcomes when only adjusting for age at time of WASI test. Males who were exposed to prenatal DHA seemed to score higher on the Full Scale IQ compared to the males in the placebo group. Interaction was significant after adjusting for age of child during WASI test, sex of child, breastfeeding and maternal height. More research of cognitive scores at later ages could be important to observe if any positive or negative effects of DHA supplementation arise.

The quality of home environment, which includes factors such as caregiving competence, parental responsivity, and the quality of home learning environment are important for a child's development (25, 66). There was significant effect modification between the treatment group and Low HOME score at 60 months suggesting that children who are exposed to DHA prenatally and come from poor home environments may receive important cognitive benefits. Children who experience unfavorable home environments such as lack of parental responsivity or cognitive stimulation, may benefit from exposure to DHA *in utero*.

These results are consistent with previously reported findings from the POSGRAD cohort. Ramakrishnan et al., found significant effect modification by HOME environment at 12 months and DHA treatment on infant motor development as measured by the Psychomotor Scale in the Bayley Scales of Infant Development (67). Additionally, significant effect modification by HOME environment at 12 months was observed at 5 years on cognitive development as measured by the McCarthy Scales of Children's Abilities for Global Development (68). In our study, there was no evidence of significant effect modification by HOME environment at 12 months and treatment group, but it was seen at 60 months. This may suggest that the HOME measure closest to our outcome of 7 years is a more relevant factor in cognitive development.

Additionally, when examining the effect of treatment by gender, HOME environment was a source of heterogeneity, only among the females and not in males. Among the females from Low HOME score environment, those who were exposed to prenatal DHA performed better on the WASI cognitive tests compared to those unexposed, as represented by the positive HOME specific estimates. For males, almost all of the

HOME specific estimates were in the same direction, although they were not significant. Perhaps suggesting that all males benefitted from prenatal DHA supplementation, so the effect modification by HOME environment may be lost.

As mentioned before, studies have explored the impact of prenatal DHA supplementation on cognitive development but there have been mixed conclusions (52). Different outcome measures, differences in study design and differences in the dose and duration of supplementation are all factors that impact study results (1). Our research adds evidence to the relatively unexplored field of prenatal DHA supplementation and cognitive development past infancy and early childhood. Additionally, it addresses the limitation of other studies, most of which have been observational, and there are a limited number of randomized controlled trials that explore prenatal DHA supplementation and cognitive outcomes in children in early childhood (1).

Our results are consistent with other studies that show no main effect of prenatal DHA supplementation on the cognitive function of children (55) (69). Our results also strengthen the argument that home environment is an important factor in child cognitive development (25), as the effect of poor home environment was mediated in children who received prenatal DHA supplementation. More research needs to be conducted to establish a definitive link between prenatal DHA supplementation and cognitive function at 7 years of age, and more evidence is needed to explain the role of environmental factors in cognitive development.

In conclusion, daily supplementation of 400mg DHA during the second half of pregnancy did not have a significant effect on Full Scale IQ, Performance IQ or Verbal IQ or the subset tests as measured by the WASI test, at 7 years of age. Children from a Low

HOME score environment may benefit from prenatal DHA supplementation compared to the children who do not receive prenatal DHA supplementation. Additionally, our results show that HOME score was a source of heterogeneity among females, but not among males.

Strengths

This study was a large randomized controlled trial which is considered the "gold standard" of study designs. The randomization was successful, as reflected by the well-balanced maternal and infant characterizations by treatment at baseline. Additionally, the study's large sample size, high intervention compliance, and dedicated study and field team in Cuernavaca, Mexico have contributed to the success of the study. The study included a large sample size of 675 mothers, compared to other studies that had fewer participants (55, 70). Furthermore, information on growth and development outcomes of the children continues to be collected, which will add to the field of prenatal DHA impact on cognitive growth and development after infancy and early childhood.

Limitations

This study is not without limitations. For this study, 69% of the original birth cohort completed the WASI cognitive tests, due to children being lost to follow up and missing WASI scores. This was especially a concern when examining the significant interaction with HOME environment at 60 months since the sample dropped from n=675 to n=575. However, the baseline maternal and child characteristics were similar in a restricted dataset with only those participants with HOME environment data (Supplementary Table S2).

In our sample, 80% of the children had been breastfed for at least the first three months. However, this was significantly different than the children who were lost to follow up and did not have 7-year WASI data, where only 40% had been breastfed for the first three months. This could indicate that women who breastfed were more likely to stay enrolled and participate in the study. Additionally, previous studies have reported an association between breastfeeding and better cognitive outcomes (71), suggesting that this cohort may report better cognitive outcomes than the original sample. Since breastfeeding status did not significantly differ by treatment group, cognitive differences due to breastfeeding are unlikely in our sample.

Another limitation is the lack of dietary data during early childhood. Previous research shows that deficiencies in iodine, iron and zinc could be associated with child cognition (72) and although we do not expect to find differences by treatment group due to the study design, unmeasured differences in dietary intakes during early childhood could be an important confounder of cognitive outcomes for this population.

Future Directions and Public Health Implications

The current evidence examining DHA's effect on birth outcomes and cognitive development is limited and the results are inconsistent, indicating a need for more research regarding prenatal DHA supplementation and child cognition, especially into the school years. Therefore, it would be beneficial to conduct more studies to definitively determine the effects of prenatal DHA supplementation on childhood cognitive development in later years, as well as examining the effect of home environment, and other socioeconomic factors that may contribute to the heterogeneity in study results.

More research in this area would help in determining the need for DHA recommendations for pregnant women. Studies have examined prenatal DHA in relation to infant birth and developmental outcomes. However DHA intake levels, time and duration of supplementation vary, making the establishment of guidelines difficult (73). Although specific DHA recommendations are not yet established, it is likely that pregnant women need higher intakes of DHA and EPA than nonpregnant women. Several expert groups recommend that between 200-300mg DHA+EPA daily during pregnancy and breastfeeding, but this may be a conservative estimate since this is also the minimum recommendation for the general adult population (62). Additionally, examining cognitive outcomes at later ages would be helpful in determining if positive or negative effects of DHA persist later on in a child's life.

This analysis contributes to the growing field of research regarding prenatal DHA supplementation and infant and child outcomes. Although no main effect of prenatal DHA supplementation was seen, our research suggests that children from poorer home environments significantly better from DHA supplementation compared to their unexposed peers. More research on prenatal DHA supplementation could be beneficial in establishing DHA recommendations for pregnant women.

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TABLES

Table 1: Selected maternal baseline characteristics of women (n=675) who participated in a trial of 400mg/d DHA supplementation during pregnancy and had children with measures of infant cognitive development at 7 years of age using the WASI assessment, by treatment group [1]

	n	Placebo	n	DHA	P-value[3]
Maternal characteristics at baseline					
Maternal age, yr	332	26.5 ± 4.7	343	26.4 ± 4.9	0.70
Gestational age, weeks	332	20.5 ± 2.1	343	20.5 ± 1.9	0.94
Socioeconomic status, z score	332	0.03 ± 1.0	343	0.08 ± 1.0	0.55
Highschool education or above, %	331	60.4%	343	56.0%	0.24
First pregnancy, %	332	37.1%	343	35.0%	0.58
Maternal BMI	332	26.3 ± 4.3	343	26.1± 4.4	0.69
Maternal height, cm	332	155.5 ± 5.52	343	154.7 ± 5.7	0.08
Maternal weight, kg	332	63.5 ± 10.9	343	62.7 ± 11.8	0.34
Smoking, %	301	3.3%	304	4.6%	0.42
Raven Score	332	40.8 ± 9.5	343	40.8 ± 9.0	0.96
DHA, g/day [2]	332	0.05 (0.04, 0.09)	343	0.06 (0.04, 0.10)	0.98
AA, g/day [2]	332	0.14 (0.10, 0.18)	343	0.14 (0.10, 0.18)	0.68
EPA, g/day [2]	332	0.02 (0.01, 0.04)	343	0.02 (0.01, 0.04)	0.86
LA, g/day [2]	332	18.1 (14.3, 21.9)	343	18.2 (13.7, 23.1)	0.61
ALA, g/day [2]	332	1.54 (1.06, 2.07)	343	1.42 (1.04, 2.12)	0.78
(n-3):(n-6) Dietary fatty acid ratio	332	12.0 ± 5.0	343	12.6 ± 6.0	0.17

[1] Values are (mean ± SD) unless otherwise indicated

[2] median (Q1, Q3)

[3] T-test for comparison of means and chi-square test for comparison of proportion

Table 2: Selected offspring characteristics among children (n=679) born to women (n=675) who participated in a trial of 400mg/d DHA supplementation during pregnancy and had measures of infant cognitive development at 7 years of age using the WASI assessment, by treatment group[1]

	n	Placebo	n	DHA	P-value[2]
Child Characteristics at birth					
Male	332	53.3%	343	55.7%	0.54
Weight, g	332	3233.3 ± 458.7	343	3221.5 ± 442.5	0.73
Length, cm	331	50.5 ± 2.5	343	50.4 ± 2.2	0.48
Head circumference, cm	287	34.4 ± 1.6	292	34.4 ± 1.4	0.91
Gestational age, wk	330	39.0 ± 1.7	342	38.9 ± 1.8	0.41
Preterm birth <37 weeks, %	330	8.2%	342	10.2%	0.36
Intrauterine growth restriction, %	330	8.5%	342	11.7%	0.17
Child Characteristics after birth					
Breastfed for first 3 months, %	328	75.9%	344	70.9%	0.14
Public school attendance (vs private)[3]	326	80.7%	342	79.8%	0.78
HOME total score (12 months)	215	37.0 ± 4.4	246	36.7 ± 4.4	0.48
Home total score (60 months)	279	41.2 ± 7.5	296	41.7 ± 6.8	0.36
Age at time of WASI test	332	7.15 ± 0.19	346	7.17 ± 0.19	0.38

[1] Values are (mean ± SD) unless otherwise indicated

[2] T-test for comparison of means and chi-square test for comparison of proportions

[3]at 7 years of age

Table 3: Comparison of mean raw scores on the WASI tests for the DHA and placebo treated groups for children at 7 years of age, by treatment group[1]

Test	Placebo n=333	DHA n=346	T-statistic	P-value[2]
Full Scale IQ	88.5 ± 9.7	89.2 ± 10.2	-0.84	0.40
Performance IQ	91.6 ± 9.6	91.5 ± 9.2	0.13	0.90
Verbal IQ	88.2 ± 11.4	89.2 ± 12.3	-1.05	0.30
Vocabulary	39.5 ± 8.3	40.6 ± 9.4	-1.52	0.13
Similarities	44.4 ± 10.0	44.7 ± 9.9	-0.38	0.70
Matrix Reasoning	42.8 ± 7.8	42.6 ± 7.1	0.40	0.69
Block Design	46.3 ± 7.0	46.5 ± 6.9	-0.35	0.73

[1] Values are (mean ± SD)
[2] T-test for comparison of means

Table 4: Examining effect modification of gender on treatment: mean WASI cognitive scores stratified by gender

Test	Male			Female			
	Placebo[1]	**DHA[1]**	**Difference[2] (95% CI)**	**Placebo[1]**	**DHA[1]**	**Difference[2] (95% CI)**	**p-value[3]**
	n=177	**n=191**		**n=155**	**n=152**		
Full Scale IQ*	88.6 ± 10.2	90.3 ± 10.9	1.70 (-0.48, 3.89)	88.5 ± 9.2	87.8 ± 9.0	-0.74 (-3.28, 1.80)	0.13
Performance IQ	92.1 ± 9.9	92.5 ± 10.3	0.38 (-1.71, 2.47)	91.0 ± 9.2	90.3 ± 7.3	-0.69 (-2.56, 1.17)	0.46
Verbal IQ **	87.8 ± 11.7	90.1 ± 12.7	2.32 (-0.20, 4.84)	88.8 ± 11.0	88.1 ± 11.7	-0.69 (-2.72, 1.35)	0.10

[1] Values are (mean ± SD)

[2] Estimates are β coefficients showing differences between DHA and placebo, derived from regression models adjusted for age during WASI test

*p=0.07 for interaction, in a model adjusted for age of child during WASI test, sex of child, breastfeeding and maternal height using test for heterogeneity

** p=0.08 for interaction, in a model adjusted for age of child during WASI test, sex of child, breastfeeding and maternal height using test for heterogeneity

[3] test for heterogeneity

Table 5. Effect modification by HOME environment, stratified by tertiles, on treatment

	Low HOME score <39 n=184	**Medium HOME score 39-46 n=195**	**High HOME score >46 n=196**	**p-value[2]**
	Estimate[1] (95% CI)	**Estimate (95% CI)**	**Estimate (95% CI)**	
Verbal IQ	3.73 (0.69, 6.77)	-2.26 (-5.27,0.75)	1.30 (-2.10, 4.69)	0.05
Performance IQ	1.94 (-0.24, 4.14)	-0.50 (-3.00, 1.99)	-2.12 (-4.93, 0.68)	0.10
Full Scale IQ	3.40 (1.07, 5.74)	-1.50 (-4.00, 1.01)	-0.28 (-3.15, 2.59)	0.04

[1] β-Estimates are differences between the DHA and placebo groups, adjusted for age during WASI test
[2] test for heterogeneity

Table 6. Effect modification by HOME score and treatment, stratified by gender

Males				
	Low HOME score <39 n=106	**Medium HOME score 39-46 n=102**	**High HOME score >46 N=102**	**p-value[2]**
	Estimate[1] (95% CI)	**Estimate (95% CI)**	**Estimate (95% CI)**	
Verbal IQ	5.13 (0.89, 9.38)	0.37 (-3.77, 4.52)	1.85 (-3.09, 6.80)	0.47
Performance IQ	1.75 (-1.08, 4.59)	1.15 (-2.78, 5.08)	-1.35 (-5.57, 2.88)	0.54
Full Scale IQ	4.20 (1.12, 7.28)	0.95 (-2.67, 4.57)	0.36 (-3.95, 4.67)	0.40

Females				
	Low HOME score <39 n=78	**Medium HOME score 39-46 n=92**	**High HOME score >46 n=93**	**p-value[2]**
	Estimate (95% CI)	**Estimate (95% CI)**	**Estimate (95% CI)**	
Verbal IQ	1.88 (-2.44, 6.20)	-5.46 (-9.93, -1.00)	0.93 (-3.71, 5.59)	0.07
Performance IQ	2.26 (-1.18, 5.71)	-2.34 (-5.39, 0.70)	-2.82 (-6.58, 0.91)	0.07
Full Scale IQ	2.38 (-1.20, 5.96)	-4.37 (-7.86, -0.87)	-0.77 (-4.53, 3.00)	0.04

[1]β-Estimates are differences between the DHA and placebo groups, adjusted for age during WASI test

[2] test for heterogeneity

Supplementary Tables

S.1a Analysis for Selection Bias Characteristics for mothers who were lost to follow up/did not have 7-yr WASI data[1]

	n	Non-missing	n	Missing	p-value[3]
Maternal characteristics at baseline					
DHA treatment, %	681	50.8%	418	49.0%	0.57
Maternal age, yr	677	26.4 ± 4.8	417	25.9 ± 4.5	0.07
Gestational age, weeks	677	20.5 ± 2.0	417	20.7 ± 2.2	0.06
Socioeconomic status, z score	677	0.05 ± 0.9	405	-0.06 ± 1.0	0.08
High school education or above, %	676	58.1%	404	57.9%	0.94
First pregnancy, %	677	36.0%	364	41.5%	0.08
Maternal BMI	677	26.2 ± 4.3	364	25.7 ± 4.0	0.07
Maternal height, cm	677	155.0 ± 5.6	364	155.3 ± 5.9	0.44
Maternal weight, kg	677	63.0 ± 11.4	364	62.1 ± 10.9	0.20
Raven Score	677	40.8 ± 9.2	362	40.9 ± 9.0	0.91
DHA, g/day[2]	677	0.06 (0.04, 0.09)	405	0.06 (0.04,0.09)	0.34
AA, g/day[2]	677	0.14 (0.10, 0.18)	405	0.13(0.10, 0.18)	0.59
EPA, g/day[2]	677	0.02 (0.01, 0.04)	405	0.02(0.01, 0.03)	0.20
LA, g/day[2]	677	18.1 (14.0, 22.4)	405	17.4 (13.6, 22.4)	0.70
ALA, g/day[2]	677	1.5 (1.1, 2.1)	405	1.5 (1.0, 2.0)	0.47
(n-3):(n-6) Dietary fatty acid ratio	677	12.3 ± 5.5	405	12.1 ± 5.1	0.59

[1] Values are (mean ± SD) unless otherwise indicated

[2] median (Q1, Q3)

[3] T-test for comparison of means and chi-square test for comparison of proportion

S.1b Characteristics for children who were lost to follow up/did not have 7-yr WASI data [1]

	n	Non-missing	n	Missing	p-value[2]
Child Characteristics at birth					
Male	677	54.4%	296	49.0%	0.12
Weight, g	677	3227.0 ± 449.9	294	3156.9 ± 486.9	0.03
Length, cm	676	50.4 ± 2.4	293	50.1 ± 2.7	0.04
Head circumference, cm	581	34.4 ± 1.5	249	34.1 ± 2.1	0.01
Gestational age, wk	674	39.0 ± 1.8	297	39.3 ± 2.1	0.05
Preterm birth <37 weeks, %	674	9.2%	297	9.1%	0.96
Breastfed for ≥ 3 months	674	72.2%	259	40.2%	<0.01
HOME score at 12 months	461	36.9 ± 4.4	137	36.1 ± 4.7	0.07

[1] Values are (mean ± SD) unless otherwise indicated

[2]T-test for comparison of means and chi-square test for comparison of proportion

Table S.2a Limiting dataset to those with only HOME scores at 60 months and comparing maternal baseline characteristics[1]

	n	Placebo	n	DHA	P-value[3]
Maternal characteristics at baseline					
Maternal age, yr	278	26.5 ± 4.7	295	26.3 ± 5.0	0.58
Gestational age, weeks	278	20.4 ± 2.0	295	20.4 ± 2.0	0.83
Socioeconomic status, z score	278	0.05 ± 1.0	295	0.05 ± 1.0	0.93
Highschool education or above, %	277	58.5	295	56.3	0.60
First pregnancy, %	278	35.3	295	33.9	0.74
Maternal BMI	278	26.4 ± 4.4	295	26.2 ± 4.4	0.60
Maternal height, cm	278	155.6 ± 5.4	295	154.5 ± 5.8	0.02
Maternal weight, kg	278	63.9 ± 11.1	295	62.6 ± 12.0	0.20
Smoking, %	252	4.0	261	4.2	0.89
Raven Score	278	40.8 ± 8.8	295	40.8 ± 8.8	0.68
DHA, g/day [2]	278	0.05 (0.04, 0.09)	295	0.06 (0.04, 0.10)	0.95
AA, g/day [2]	278	0.14 (0.10, 0.17)	295	0.14 (0.10, 0.18)	0.62
EPA, g/day [2]	278	0.012 (0.01, 0.04)	295	0.02 (0.01, 0.04)	0.87
LA, g/day)	278	17.6 (14.2, 21.6)	295	17.9 (13.6, 22.3)	0.38
ALA, g/day [2]	278	1.47 (1.02, 2.05)	295	1.40 (1.02, 2.09)	0.95
(n-3):(n-6) Dietary fatty acid ratio	278	12.1 ± 5.1	295	12.7 ± 6.1	0.20
Fat g/day [2]	278	93.0 (72.9, 112.0)	295	92.9 (74.4, 121.6)	0.42

[1] Values are (mean ± SD) unless otherwise indicated

[2] median (Q1, Q3)

[3] T-test for comparison of means and chi-square test for comparison of proportion

Table S.2b: Selected offspring characteristics restricted to those with HOME data[1]

	n	Placebo	n	DHA	P-value[2]
Child Characteristics at birth					
Male, %	278	54.0	295	54.2	0.95
Weight, g	278	3246.4 ± 457.2	295	3242.1 ± 430.8	0.91
Length, cm	277	50.5 ± 2.6	295	50.5 ± 2.2	0.84
Head circumference, cm	242	34.5 ± 1.6	253	34.4 ± 1.4	0.53
Gestational age, wk	277	39.2 ± 1.6	294	39.0 ± 1.8	0.57
Preterm birth <37 weeks, %	277	7.2	294	10.2	0.21
Intrauterine growth restriction, %	277	7.6	294	10.5	0.22
Child Characteristics after birth					
Breastfed, %	275	76.4	294	71.2	0.21
Public school attendance (vs private)[3], %	273	79.5	294	80.6	0.74
HOME total score (12 months)	182	36.8 ± 4.6	215	36.5 ± 4.5	0.63
Home total score (60 months)	279	41.2 ± 7.5	296	41.7 ± 6.8	0.36
Age at time of WASI test	278	7.2 ± 0.2	296	7.2 ± 0.2	0.54

[1] Values are (mean ± SD)
[2] T-test for comparison of means and chi-square test for comparison of proportions
[3]at 7 years of age

FIGURE

Figure 1. Flow chart describing recruitment and follow-up of pregnant women and offspring for WASI cognitive data at 7 years.