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Evaluating the Effect of Docosahexaenoic Acid (DHA) Supplementation during Pregnancy on Child Cognitive Development at 5 Years of Age in Morelos, Mexico.

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By

Beth Catherine Pallo

B.A., Ithaca College, 2006

Thesis Committee Chair: Usha Ramakrishnan, Ph.D.

An abstract of
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Abstract

Evaluating the Effect of Docosahexaenoic Acid (DHA) Supplementation during Pregnancy on Child Cognitive Development at 5 Years of Age in Morelos, Mexico.

By Beth Catherine Pallo

Maternal nutritional status is an important determinant of child growth and cognitive development outcomes. Recently there has been increased attention on maternal nutrition requirements of dietary lipids, especially essential fatty acids (EFAs).

Docosahexaenoic acid (DHA), a long-chain polyunsaturated fatty acid (LCPUFA) derived from the n-3 EFA family, is found predominately in the metabolically active neural membranes of the brain and retina and is known to influence cognitive ability and visual acuity. The significance of maternal n-3 LCPUFAs status is of interest to researchers since fetal accretion is highest during the last trimester of pregnancy.

Since 2007, a large double-blind, randomized placebo-controlled trial of DHA supplementation has followed a cohort of Mexican women (n=1,094) and the offspring (n=978) born to them from birth to early childhood. Secondary data analysis was carried out to evaluate the impact of the intervention on child cognitive development for 802 children (88% of the birth cohort) as measured by the Spanish version of the McCarthy Scales of Children's Abilities (MSCA) for Global Development at 5 years of age. Outcome measures calculated were the raw and standardized scores of the MSCA six scales: verbal, perceptual-performance, quantitative, memory, motor and general cognitive.

Intent-to-treat analysis determined that DHA supplementation (400mg/day) mid-pregnancy until delivery did not significantly improve child cognitive development at 5 years of age ($p>0.05$). A significant treatment by home environment at 12 months of age interaction was detected for verbal, perceptual-performance, memory, and general cognitive raw and standardized scores ($p\leq0.10$), indicating that offspring from poor home environments benefited from prenatal DHA supplementation.

There were no main effects of prenatal DHA supplementation on offspring development at 5 years of age. However, verbal, perceptual-performance, memory, and general cognitive raw and standardized scores were higher among children from poor home environments at 12 months of age who were exposed to DHA in utero compared to those from similar home environments that received the placebo.

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List of Acronyms

AA- arachidonic acid
ALA- alpha-linolenic acid
BRS- Behavioral Rating Scale
BSID-II- Bayley Scale of Infant Development-II
DHA- docosahexaenoic acid
EFA- essential fatty acid
GCI- General Cognitive Index
HOME- Home Observation for Measurement of the Environment
IQ- Intelligence Quotient
K-ABC- Kaufman Assessment Battery for Children
LA- linoleic acid
LCPUFA- long chain polyunsaturated fatty acid
LMIC- low and middle income country
MDI - Mental Development Index
MLR – multiple linear regression
MSCA- McCarthy Scales of Children's Abilities
PERILIP- Perinatal Lipid Intake Working Group
PDI- Psychomotor Development Index
SES- socioeconomic status
SLR- simple linear regression
RCT- randomized control trial
VIF- variance inflation factor
X^2 - Chi-squared
WHO- World Health Organization

Chapter 1: INTRODUCTION

Despite recent progress and improvements in overall nutritional status among children under 5 years of age worldwide, undernutrition continues to be a major public health problem, particularly in poorer rural regions of the world [1]. At the same time, obesity due to overnutrition is also becoming a growing concern in low and middle-income countries (LMICs). The coexistence of undernutrition and obesity is common in countries experiencing a nutrition transition [2].

There are many non-biological factors that can impede a child's developmental potential, such as demographics, socioeconomic status, inequality, and education. Additionally, nutrition and maternal nutritional status are recognized as important determinants of child health and development outcomes [3]. Nutrient deficiencies in pregnant women and children under the age of 5 lead to health disparities that have long-term economic and social consequences [4]. Inadequate nutrition and the absence of essential micronutrients and fatty acids among infants and children under 5 years of age have been shown to lead to poorer birth and child development outcomes [3, 5].

Research has established the need for both n-6 and n-3 fatty acids for the development and health of the brain and vascular system [5]. Specifically, the n-3 fatty acid docosahexaenoic acid (DHA) can be obtained directly from dietary sources (e.g. oil-rich fish, breast milk, and algae) or synthesized from the precursors α-linolenic acid (ALA) and linolenic acid (LA). However, the efficiency of conversion of DHA from ALA or LA may be low and it remains unclear whether maternal needs for n-3 long-chain polyunsaturated fatty acids (LCPUFAs) during pregnancy can be met in most women by

synthesis from precursor essential fatty acid (EFA) stores [2]. DHA availability is important because DHA is essential for membrane function and is known to play a key role in the development of the brain and retina [6]. Fetal accretion of LCPUFAs is rapidly incorporated in the nervous tissue of the retina and brain during the last trimester of pregnancy up until two years of age; LCPUFA intake during pregnancy influences both maternal and infant fatty acid status at birth [2, 6].

As such, DHA supplementation during pregnancy is of interest to researchers because the current body of evidence examining DHA's effects on birth outcomes is limited and inconclusive [6]. Maternal preconception, prenatal, and postnatal dietary balance and composition of LCPUFAs influence the quantity of DHA available to the fetus and breast milk-fed infant [7]. Diets low in DHA and LCPUFAs during pregnancy are associated with poorer DHA status and slower reestablishment of maternal stores [8]. Furthermore, the LCPUFA status of neonates is strongly correlated with maternal stores indicating that DHA status is typically higher among infants born to women with higher maternal DHA stores [9].

Although there are still knowledge gaps in regard to vulnerable groups (e.g. pregnant and lactating women, children under 5 years of age) meeting nutritional requirements, evidence is emerging that EFAs play an important role in neurological function. Specifically, there is evidence that dietary DHA can improve birth outcomes and visual and cognitive development [6]. It is recommended that the fetus and neonate receive LCPUFAs in amounts sufficient to support optimal visual and cognitive development, however there is currently no scientific consensus as to what level of DHA intake is optimal to lead to a saturation of neural membranes [6, 7, 10]. Women in LMICs are

reported to have inadequate intakes of EFAs. In high-income countries where intakes are higher, infants born to supplemented mothers have demonstrated improvements in visual acuity, attention, and aspects of cognitive performance in some studies [11].

While some studies have reported a positive association between LCPUFA status and/or dietary intake of LCPUFAs during pregnancy and child neurodevelopment, these findings are mixed [12-14]. Prior research suggests that prenatal DHA levels are predictive of better cognitive performance, yet these associations are limited to the first months of life [10, 15]. The long term benefits of improving DHA status during pregnancy, especially in regard to older children, are still uncertain [16]. Differences in the study population, type of supplement, duration of exposure, and the variety of cognitive assessment tools used are some limitations that make comparison difficult.

Although we know that DHA is essential for human brain growth and development and that available evidence suggests maternal n-3 LCPUFA supplementation during pregnancy benefits the cognitive development of infants, evidence on the impact of prenatal LCPUFA supply and later cognitive development is not as convincing [6]. There is a strong interest for researchers to explore the long-term benefits of DHA status to fill in the knowledge gaps for this essential fatty acid, especially after infancy. To summarize these relationships, **Figure A**. provides a conceptual framework that explains the connection between in-utero exposure to LCPUFAs and child development.

This analysis aims to determine if maternal DHA supplementation during pregnancy improves cognitive outcomes of offspring, measured by the Spanish version of the McCarthy Scales of Children's Abilities (MSCA) at 5 years of age among study participants in Morelos, Mexico. It is hypothesized that children whose mothers were prenatally supplemented with DHA will exhibit better performance scores on one or more of the MSCA six scales (verbal, perceptual-performance, quantitative, memory, motor, and general cognitive) when compared to children born to women who received the placebo.

Chapter 2: REVIEW OF THE LITERATURE

This literature review first explains neurodevelopment during and after pregnancy into the early years of a child's life. It then provides a general overview on the key risk and protective factors identified to impact child cognitive development, followed by a discussion of the role nutrition plays in child cognitive development. Finally, the literature that specifically addresses DHA intake and child cognitive development is examined and reviewed.

Neurodevelopment

During Pregnancy

The rate of human brain growth is greatest in utero and during the early years of life. Prenatal maternal nutrition, exposures and behaviors are important controllable factors that may impact fetal brain development and neurological outcomes later in life. Prenatal development has well defined milestones, often called critical periods, which are specific to the development of a particular brain region over a specific period of time. Neural proliferation peaks in the third and fourth month of gestation and after this period migration activity declines. During the fifth month of gestation neuronal cells mature, attain proper alignment and orientation, and differentiate. Fetal brain development is a complex and highly metabolic process dependent on adequate oxygen, protein, energy and micronutrients. Nutrient deficiencies during the prenatal months usually cause irreversible effects on neurological development because these processes only occur during a specific programmed time [17]. Eliminating important maternal

nutritional deficiencies and toxic exposures can optimize fetal brain development and potential development during the early years of life [18]. While genetics are a main determinant of neuronal progenitors and their migration to brain regions during embryonic, fetal and early postnatal life, environmental factors also play a critical role in shaping neural configuration and gene expression modification [17].

First 12 Months of Life

The time period following fetal development and birth is one of accelerated postnatal brain development, characterized by neural plasticity. Neural plasticity refers to the ability of the human brain to adapt to environmental influences can lead to system reorganization at behavioral, anatomical, physiological, cellular and molecular levels [19]. During the first year of life, an infant's capacity to discriminate between sounds, colors, objects and characteristics of persons develops rapidly In the first 2-3 months of life the infant has two main developmental tasks: to develop a basic capacity for self-regulation and to become oriented to the external world. Increasing maturation of the central nervous system during the first month of life facilitates the infant's capacity for self-regulation by making their reactions to stimuli more predictable and organized. During the first year of life, an infant starts to develop meaningful relationships, cognitive abilities and gain a greater understanding of the external world. During the second half of the first year, infants advance rapidly in cognitive, motor and social development [20].

Early Life (1-5 years of age)

Early childhood, the 1-5 year age range, is a time of rapid and dramatic growth, serving as the foundation of development for cognitive and interpersonal skills. The timing of the relationship between nutrient availability and brain development is not only relevant to prenatal development, but also postnatal. There are few published studies specifically addressing the role of nutrition in cognitive development among preschool aged children (n=125) compared to those of infants (n=232) or school-aged children (n=303). Rosales and colleagues suggest that assessing preschool aged children for neural and cognitive development may be more difficult due to age-related variability, individual differences in temperament, linguistic ability, and patterns of neural activity. There is a suggested "window of sensitivity" during which specific nutrients may affect postnatal neural development, yet it is important to recognize that other factors (i.e. environmental and sociological) may exacerbate, confound or compensate for the effects of nutrients on the developing nervous system [17].

Risks and Protective Factors Influencing Cognitive Development

Brain development is dependent on the overall health of the mother during pregnancy, adequate prenatal and postnatal nutrition, and the length of gestation. After birth, brain growth is enhanced by secure and stimulating relationships. Alternatively, a host of biological and environmental factors can compromise brain development [20].

Risk Factors of Early Child Development

The Lancet's 2011 Child Development Series identifies the following as the key risks that prevent children from attaining their development potential: inadequate cognitive stimulation, stunting, iodine deficiency, and iron-deficiency anemia [21, 22]

Inadequate Cognitive Stimulation

In a review of existing studies on cognitive stimulation for children in developing countries, only 10-41% of parents provide cognitively stimulating material to their child and only 11-33% of parents actively involve their children in cognitively stimulation activities. Consistent evidence from intervention studies indicates that providing increased stimulation or learning opportunities to young children significantly increases both cognitive and social-emotional competence [22].

Stunting

Stunting is a commonly used as an indicator of undernutrition and is defined as a low height for age (> -2 z score), indicating chronic restriction of a child's potential growth [1]. Growth stunting affects about a third of children in less wealthy regions of the world and is more common in areas with limited diet variability and poor quality of food. In children, zinc deficiency can lead to stunting. The effect of maternal zinc status

on pregnancy outcomes is unclear at this time [4]. In Mexico, the most recent data from the 2006 Mexican National Nutrition Survey found that 15.5% of children under the age of five were stunted based on 2006 WHO standards. While this is a marked decrease from 26.9% in 1988, stunting remains the main malnutrition problem in Mexico. Although gaps among ethnic and socioeconomic groups have decreased over time, the highest rates of stunting were found in the more economically deprived regions of Mexico [23].

Iodine Deficiency

An estimated 2 billion people have inadequate iodine nutrition, which places them at risk of iodine deficiency disorders [21]. Complications from iodine deficiency include birth defects, increased infant mortality, cognitive and neurological impairment (cretinism in its most severe form), and an increased resistance to infectious disease [4].

Maternal iodine deficiency can lead to hypothyroidism, cretinism, and impaired brain function. For severe deficiency, maternal iodine supplementation completed by the second trimester can improve neurological and cognitive development of the infant [1]. Sources of dietary iodine include marine food sources, iodized salt, and processed foods that use iodized salt. While there have been major improvements worldwide to address health problems related to iodine deficiency, areas with insufficiencies still exist [18].

Iron-deficiency Anemia

Iron is an essential micronutrient for hemoglobin synthesis and is also important for the synthesis of DNA and proteins. Dietary sources include fruits, vegetables, fortified grain products, meat and poultry. Pediatricians recommend iron supplementation for mothers during pregnancy and for children during infancy [20]. Iron deficiency has been associated with alterations of excitorary and inhibitory neurotransmitter receptors in the fetal brain, supporting the notion that early iron deficiency in the developing brain can have significant long term and irreversible developmental implications [18].

Worldwide, there are an estimated 2 billion cases of anemia, with the highest prevalence in developing countries among pregnant women and infants under two years of age. Iron deficiency is responsible for approximately 50% of all anemia cases. The health consequences of iron deficiency include reduced cognitive performance, work performance and endurance [4]. For example, long-term studies of children who were iron deficient during infancy show continuing lower scores on tests that measure cognitive and motor functioning [20]. In Mexico, the most prominent nutrition problem identified from the most recent Mexican National Nutrition Surveys was iron deficiency among children under 5 years of age and pregnant women who reside in rural areas [23].

Other Factors

Environmental risk factors such as dietary and environmental exposures to environmental toxins can also impair fetal neurological growth. The fetal brain is susceptible to these toxins as they easily pass through the placenta and can expose the developing brain, which has no defense against these harmful toxins. Lead is a long recognized neurotoxin that can lead to impairments in cognition, fine motor skills, and language processing skills. Mercury exposure can lead to neurological deficits in motor development, auditory and visual evoked potentials, and attention and memory abilities [18].

Finally, there is evidence that intrauterine growth restriction, malaria, lead exposure, HIV infection, maternal depression, institutionalism and exposure to violence also pose risks to the developmental potential of children [11]. While evidence of the role these risk factors play is still emerging, it is important that they are considered when planning interventions aimed at promoting cognitive development.

Protective Factors of Early Child Development

The Lancet's 2011 Child Development Series identifies breastfeeding and maternal education as protective factors that encourage children to attain their development potential.

Breastfeeding

The World Health Organization (WHO) recommends exclusive breastfeeding for the first 6 months of life in order to achieve optimal growth, development and health. An estimated 39% of infants in LMICs are exclusively breastfed until 6 months of age, but exclusive breastfeeding varies widely by country *[21]*. In particular, Mexico has low prevalence of exclusive breastfeeding, only 22.3% of mothers reported exclusive breastfeeding <6 month old infants on the country's Health and Nutrition survey [24].

Breastfeeding provides nutrients necessary for adequate brain growth. Breast milk has a high concentration of essential fatty acids, which are required during the first two years of life to promote myelination [20]. Studies have noted that it is difficult to discern if the advantage of breastfeeding resides in the nutrient composition of breast milk or maternal variables (e.g. IQ, educational attainment, SES, social interaction with child) [15, 25]. While it is difficult to measure the relationship between breastfeeding and cognitive growth due to potential confounders, it is undisputed that breast milk provides all the essential nutrients for infants and may help reduce their susceptibility to disease.

Observational studies have yielded different conclusions on breastfeeding and cognitive development. Holme and colleagues found no measurable IQ advantage among breast-fed infants, yet Quigley and colleagues concluded that breastfeeding was associated with improved cognitive development at 5 years of age, most notably among those born preterm [25]. Another cohort study in Australia demonstrated a significant association between the duration of breastfeeding and cognitive development as measured by the Peabody Picture Vocabulary Test Revised even after confounding social

and parenting factors were taken into account [26]. Further strengthening evidence that breastfeeding positively impacts development and educational attainment, a Randomized Controlled Trial (RCT) assessing development potential and exclusive breastfeeding promotion in Belarus found that intervention children had significantly higher IQ and verbal scores [21].

Maternal Education and Intelligence

Maternal education and intelligence are recognized to be important factors that influence child cognitive outcomes. Anderrson and colleagues found maternal IQ to be the strongest predictor of children's cognitive abilities at 5 years of age in a longitudinal study of Scandinavian small for gestational age birth data [27]. A study by Bakker et al. that investigated associations between DHA and cognitive function at 7 years of age found no main effects but reported significant relationships between cognitive outcome measures and maternal education and intelligence [14].

Maternal Nutrition

The nutritional status of a woman before and during pregnancy is important for a healthy pregnancy outcome, but there is a paucity of peer-reviewed research reporting associations between maternal nutritional status and child development [11]. Some observational studies have found that maternal undernutrition is associated with smaller head circumference and lower brain weight. Maternal nutrient restrictions may

have devastating effects on fetal brain development, but nutritional deficiencies may be equally damaging [18]. While maternal undernutrition does not severely impact the volume or composition of breast milk in the absence of severe malnutrition, the concentration of certain micronutrients and fatty acids is dependent on maternal status and intake, so maternal deficiency can impeded the nutritional status of an infant [1]. Prenatal nutritional deficits may have long term effects on adult mental health, research is needed to determine the effect of food supplementation before and during pregnancy on child development [11].

Cognitive Development

Nutrition and Child Cognitive Development

Poor nutritional status of mothers and children remain a public health problem that negatively impact social, economic, and human capital development, especially in LIMCs [1]. More research is warranted in regard to prenatal nutritional supplementation programs that have the potential to alleviate the burden of inequality and benefit the cognitive development of the world's poorest children [21].

Cognitive development refers to changes of the cognitive process observed over periods of time and is usually assessed in children by batteries of performance tests that evaluate specific abilities [6]. Some examples of these abilities are fine motor, verbal communication and memory skills. Cognitive development is a multidimensional and non-liner process characterized by neural plasticity [6, 17]. Neural plasticity is a mechanism for development and learning; it refers to the ability of the human brain to

adapt to environmental pressures, physiologic changes, and experiences [19]. The human brain has a great deal of plasticity in the first two years of life and a strong ability to recover from deprivation and neglect during this time period [20]. Biological, environmental and sociological exposures are a few of many factors that influence cognitive development and should be considered when assessing cognitive development [6, 28].

Davies' book, *Child Development: A Practitioner's Guide*, notes that during the second half of pregnancy through 2 years of age the developing human brain is critically dependent on adequate nutrition [20]. Nutrition is an environmental factor that can directly modify gene structure and expression [17]. Two basic types of cells constitute brain composition: neurons and glia. Neurons send and receive messages and store information. Glial cells nourish and provide supportive tissues for the neurons. Specialized glia, called myelin cells, provides insulation for brain circuits. Malnutrition can result in the underproduction of neuronal and glial cells, slower myelination, and poor overall brain growth. Consequently, malnutrition can impede IQ potential and lead to other cognitive deficits that can have a lasting impact; yet these effects can be reversed if proper nutrition is provided during early childhood [20].

Nutrients provide specific molecules that enable genes to exert their potential or intended effects of brain growth and development. Unique functions of the brain are reflected in its requirement of certain nutrients and special fats such as DHA. Evidence continues to accumulate that DHA is important for synaptogenesis during the third trimester of gestation [17]. Nutrition plays a critical role in mediating brain development

and demonstrates the interplay of biological and nurturing factors on cognitive growth and development [28].

Effects and outcomes of nutrition are almost always correlated to broader influences from environmental factors such as socioeconomic status (SES) and social interaction [6]. The causal relationship between nutrition and brain development is complex, therefore it is important to delineate measured outcomes and specific mechanisms that link nutrition interventions to these outcomes when determining the success of a nutrition intervention [17].

DHA and Cognitive Development

DHA is an essential constituent of neural membranes and found in relatively high concentrations in the brain and retina. DHA influences retinal membrane dynamics and nervous system function by altering neural membrane physical properties, enzymatic activities, and receptor structure [7]. Infants acquire LCPUFAs from their mothers either prenatally via the placenta or postnatally in breast milk [29]. The developing brain and nervous system have an essential requirement for DHA, placental transfer is crucial as neither the fetal brain nor retina initially synthesize DHA. The postnatal period is also very crucial for accumulation of LCPUFA in infants. There are still many unanswered questions about what influences maternal to fetal DHA accretion and how much dietary DHA is incorporated into neural membranes at the postnatal times [7].

As stated previously, maternal PUFA status varies according to diet and/or n-3 consumption during pregnancy and dietary sources of preformed DHA are oil rich fish,

algae, and breast milk. However, some species of oil-rich fish are known to be contaminated with high levels of mercury and there is concern about mercury exposure from seafood ingestion, particularly for pregnant women and women of child bearing age, as the placenta does not protect the fetus from this neurotoxin [18]. Providing preformed DHA supplementation during pregnancy may reduce the risk of mercury exposure from cold-water fish. Studies have indicated that regular consumption of oily fish or supplementation with n-3 LCPUFA results in increased maternal DHA circulation during pregnancy and at term [9, 30].

Accumulation of LCPUFA by the fetus is elevated to levels higher than those in the mother during gestation. Babies that are inadequately supplied with DHA in utero or postnatally accumulate lower amounts in the blood and tissue and may be at a neurodevelopmental disadvantage [29]. Furthermore, circulating DHA status in mothers was found to increase between 15 and 28 weeks, followed by a decline after 28 weeks until birth. Previous studies have reported elevated DHA and LCPUFA in the early and mid-trimesters of pregnancy. The decline after 28 weeks until birth indicates that elevated maternal levels are unsustainable due to accretion of DHA by the fetus at this time. The biomagnification of DHA from the mother to the fetus appears to be physiologically predetermined [29].

Studies Examining Prenatal DHA and Child Cognitive Development

Various outcome measures across studies that examine the relationship between prenatal maternal DHA status and cognitive development make comparison difficult. Factors that impact outcomes and interpretation of research studies include differences in study design, dose and duration of supplementation, age at cognitive assessment, and methodology used for cognitive assessment [6]. The following examples demonstrate the varied results of studies that aim to determine the impacts of LCPUFA status and later child cognitive development.

A randomized, double blinded study in Norway investigated the impact of supplementing mothers with cod liver oil (1.2g DHA/day) or corn oil (4.7g LA/day) from 17-19 weeks of pregnancy until delivery and for three months during lactation on cognitive development using the Kaufman Assessment Battery for Children (K-ABC) at 4 years of age. Researchers found that children's mental processing score on the K-ABC at 4 years of age correlated significantly with maternal intake of DHA during pregnancy. They also reported the K-ABC composite score was significantly correlated with the child's head circumference, which is used as an indicator of a young child's brain development. In a multiple regression model, maternal intake of DHA was the only variable of statistical significance for the children's mental processing score at 4 years of age, suggesting that maternal intake n-3 LCPUFAs during pregnancy and lactation may be favorable for mental development of children in later years [12]. It is unclear whether this effect is due to DHA supplementation during pregnancy, during lactation, or both. However, the study suggests that the effects of DHA supplementation during pregnancy may appear later in life, when cognitive function is more mature [6].

An observational study in Spain by Mendez et al. analyzed the relationship between maternal intake of fish and other seafood and child neurodevelopment at 4 years of age. Results reported that among children breastfed for more than 6 months, high maternal fish intake (>2-3 servings/week) was associated with significantly higher children's cognitive performance scores on the McCarthy Scales of Children's Abilities compared to low maternal fish intakes (less than or equal to 1 serving). There was no association among children breastfed for longer periods. Regardless of breastfeeding duration, maternal intakes of other seafood were inversely associated with scores on several subscales, which could be due to low n-3 LCPUFA levels in smaller fish species such as shellfish and squid [13].

Alternatively, another prospective cohort study investigated the relationship between DHA and AA status at birth and cognitive function at 7 years of age and found no significant association between fatty acid status at birth and cognitive performance using the Kaufman Assessment Battery for Children (K-ABC) [14].

Mixed findings and the lack of conclusive evidence when assessing the relationship between prenatal DHA status and later child cognitive development, especially from studies that are randomized in design, indicate the need for more research on this topic.

Chapter 3: METHODS

Overview

A secondary analysis was carried out from data collected in a randomized control trial examining the effect of prenatal DHA supplementation on infant growth and development. This study was carried out through collaboration between Instituto Nacional de Salud Publica (INSP), Instituto Mexicano del Seguro Social (IMSS) in Cuernavaca, Mexico and Emory University Rollins School of Public Health in Atlanta, Georgia. Study recruitment began in February 2005 and the intervention was completed in July 2007. Pregnant women (n=1,094) were randomized to receive a daily supplement of DHA (400mg) and a placebo from 18 to 22 weeks of pregnancy until delivery. Infants born to women enrolled in the trial have been followed at regular intervals through 5 years of age and repeated measures of growth and development have been administered since birth. At 5 years of age, child development was assessed using the Spanish language version of the McCarthy Scales of Children's Abilities (n=803). The goal of this secondary data analysis is to assess the relationship between DHA supplementation and child cognitive development at 5 years of age as measured by the McCarthy Scales of Children's Abilities.

Study Population, Setting, and Eligibility Criteria

Recruitment of pregnant women took place during prenatal care visits between February 2005 and February 2007 at the Instituto Mexicano Seguro Social (IMSS) General Hospital I and three other health clinics located in Cuernavaca, Mexico. The city

of Cuernavaca is located approximately 50 miles from Mexico City; the study population can be characterized as living in mostly urban and peri-urban settings and generally of low-medium socioeconomic status. At the time of recruitment, the women and/or their husbands were employed, thus they qualified for medical care and insurance coverage from IMSS. In most cases, IMSS hospital patients pay one-third of the healthcare costs and the employer or federal government pays the remaining two thirds of the costs.

Women eligible to be included in the study were aged 18-35 years with gestation between 18-22 weeks, planned to give birth at IMSS general hospital, would permanently reside in the Cuernavaca area for the next 2 years, intended to predominantly breastfeed for at least the first 3 months of the infant's life and agreed to participate with informed consent. Participants were excluded from enrollment in the study if any of the following criteria applied: high risk pregnancy as documented by clinical records, hyperlipidemia and/or absorption disorders, regular intake of DHA supplements or fish oil during pregnancy and chronic use of medication for chronic illness such as epilepsy. Following recruitment, 1,094 eligible women were enrolled in the DHA study.

The Emory University Institutional Review Board (IRB) and the biosafety and ethics committee of the Instituto Nacional de Salud Publica (INSP) approved this study. Informed consent was obtained from all subjects prior to enrollment in the study and again from the parent and/or caretaker at the time of the infant's birth. The consent process explained the details of the study procedures, respondent burden, potential risks and benefits, provided contact information of individuals so that participants are able to obtain more information, and clearly stated participants are free to withdraw

from the study at any time. In addition, a Data Safety and Monitoring Committee monitored the safety of the study and ensured human subject protection during the data collection process.

Intervention

From 18-22 weeks gestation until delivery, women were randomly assigned to receive two capsules of 200mg of DHA or a placebo daily. The DHA supplements were derived from an algal source and supplied from Martek Biosciences Corporation based in Columbia, MD. The placebo capsules were similar in appearance and taste to the DHA supplements; they contained a mix of corn and soy oils with no additional antioxidants. Each week, field workers delivered 14 capsules to the home or workplace of study participants. Women were instructed to take two pills at the same time each day. Field workers monitored participant compliance by counting remaining pills and conducting regular interviews. Compliance was calculated as the total number of capsules actually consumed, expressed as a percentage of the total number expected to be consumed. Consumption of the capsules was discontinued at the time of infant delivery.

Randomization and Blinding

Randomization of all eligible study participants to either intervention or control group was conducted using block randomization. A computer-generated list created by the study biostatistician at Emory University randomly created balanced replication of four treatments (two colors for DHA and two for control) using a block size of eight. All members of the DHA study team as well as the study participants were blinded to the treatment assignment throughout the intervention. The treatment code was maintained in sealed envelopes at INSP and Emory University, only to be made available when the study is complete, for data analysis purposes, or if requested by the external Data Safety and Monitoring Committee.

Outcome Measures

McCarthy Scales of Children's Abilities

Child cognitive development was measured at 5 years of age using the Spanish language version of the McCarthy Scales of Children's Abilities (MSCA). The MSCA is a comprehensive test battery that measures a variety of cognitive and motor behaviors in children aged 2 ½ through 8 ½ years. The test uses game-like tasks that are suitable for children of both genders as well as various ethnic, regional, and socioeconomic backgrounds. The test is designed to facilitate the measurement of children's general intellectual level, as well as their strengths and weaknesses in particular abilities [31]. Six scales were chosen for measurement: verbal, perceptual-performance, quantitative, memory, motor, and general cognitive. Predictive validity of the McCarthy Scales of

Children's Abilities has been examined using a variety of measures. Good predictive validity of the general cognitive scale index has been demonstrated through significant correlation with achievement tests such as the Peabody Individual Achievement Test and the Stanford Achievement Test [32]. Additionally, previous studies have successfully used the Spanish version of MSCA in Mexico [33].

The MSCA contains 18 separate tests that assess a child's cognitive and motor abilities. During validation of the MSCA, weights were assigned to each test by evaluating the largest standard deviation obtained for that test across the entire age range. To obtain raw scores for the six scales, the total weighted raw score for each of the 18 individual tests is first computed. Then the weighted composite raw scores for each of the six scales are computed. The score for each scale is based on a linear sum of the weighted raw score for that scale's component tests. Raw scores of each scale are converted into scale indices using the Scale Index Equivalents of Composite Raw Scores Table, which is standardized for the child's age at the time of test administration. Each distribution is normalized and converted to a scaled score distribution with a fixed mean and standard deviation. The verbal, perceptual-performance, quantitative, memory, and motor scale indices have a mean of 50 and a standard deviation of 10. The general cognitive scale index has a mean of 100 and a standard deviation of 16. After the six scale indices are computed and recorded, the child's MSCA profile is plotted and interpreted per manual instruction.

The administration of the McCarthy test took place at IMSS hospital, either in a windowless room located on a busy floor of the hospital or in a room with one window located on a quiet floor of the hospital. A team of psychologists (n=3) were trained and

supervised by the lead study psychologist. The administration was supervised through direct random observations and the assessment of completed McCarthy forms to ensure proper scoring techniques.

Children received full credit for questions they answered correctly on each test. Partial credit was also given for questions; details for scoring instructions are provided in the MSCA manual. The psychologist noted on the test form when the child answered "I don't know" or refused to answer the question and these were treated as missing values when totaling the scores. When a child answered a question incorrectly, they did not receive any points for that question. The psychologist administering the test recorded the points the children received for each question in the test booklet immediately following their response. These results were then entered into the computer system by a designated data collection and entry team at IMSS.

Description of the Six MSCA Scales

1. Verbal Scale

The verbal scale is designed to test the child's ability to express oneself verbally and also considers the child's maturity of verbal concepts. The child provides one-word answers, phrases, sentences to answer questions that yield mental processes such as short and long term memory, divergent thinking and deductive reasoning. Verbal ability has also been shown to be an excellent predictor of school achievement. The tests of the verbal scale include:

- Pictorial Memory (3*)
- Word Knowledge (4)
- Verbal Memory (7)
- Verbal Fluency (15)
- Opposite Analogies (17)

**Tests are numbered 1 through 18 to indicate the order of their administration*

2. Perceptual-Performance Scale

The perceptual-performance scale consists of "game-like" tasks that do not require the child to speak. The child demonstrates the skills of imitation, logical classification, and visual organization through a variety of spatial, visual-perceptual and conceptual tasks. The tests of the perceptual-performance scale include:

- Block Building (1)
- Puzzle Solving (2)
- Tapping Sequence (6)
- Right-Left Orientation (8)
- Draw-a-Design (12)
- Draw-a-Child (13)
- Conceptual Grouping (18)

3. Quantitative Scale

The quantitative scale measures a child's ability with numbers and understanding of quantitative words. This scale aims to assess the child's number aptitude rather than computational skills.

- Number Questions (5)
- Numerical Memory (14)
- Counting and Sorting (16)

4. Memory Scale

Each test comprising the memory scale assesses the child's short-term memory. There are auditory and visual stimuli simultaneously and separately. These tests assess the child's ability to memorize specific content.

- Pictorial Memory (3)
- Tapping Sequence (6)
- Verbal Memory (7)
- Numerical Memory (14)

5. Motor Scale

The motor scale assesses the child's coordination as a variety of gross and fine motor tasks are performed. The motor index reflects the child's developmental level and is a vital adjunct to the picture of the child as revealed other scores specific to cognitive development.

- Leg Coordination (9)
- Arm Coordination (10)
- Imitative Action (11)
- Draw-a-Design (12)
- Draw-a-Child (13)

6. General Cognitive Scale

The general cognitive scale is composed of all the tests in the verbal, perceptual-performance, and quantitative scales. It provides a measure of the child's overall cognitive function. The general cognitive scale, sometimes referred to as the general cognitive index (GCI), measures the child's cognitive ability in relation to other children the same age and is presented as an index of the child's ability at a given point in time. It represents the child's ability to integrate accumulated learning and adapt to the tasks of MSCA. The individual general cognitive scale is of most use when viewed in the context of indices on the other five scales. The overall MSCA score profile indicates behavioral

and developmental maturity, as well as strengths and weaknesses, in the cognitive and motor domains.

Other Variables of Interest

As previously noted, certain maternal characteristics have been identified as determinants of child cognitive development. Data collection of maternal characteristics took place during study recruitment. A social worker administered a sociodemographic and obstetric history questionnaire. The National Institute of Perinatology in Mexico validates the questionnaire for use in pregnant women from low to medium socioeconomic status. The assessment inquired about marital status, place of birth, years of school, occupation, and household income and composition. Additionally, a study psychologist administered The Raven's Progressive Matrices test, a non-verbal assessment of cognitive ability.

1. Socioeconomic Status: Socioeconomic status (SES) is a proxy for a broad array of human activities (e.g. education, social status, and wealth) that affect the ability of a family to purchases goods and services essential for well-being [17]. Two SES variables, one continuous and the other categorical, were created from the data collected. The continuous SES variable was used in this analysis. It was created using principle component analysis (PCA) to determine an SES score based on occupation, housing and personal assets.

2. Educational Attainment: Maternal educational attainment was defined as the highest level of school completed by the mother.

3. Maternal Intelligence: Maternal intelligence was assessed using the computed Raven's Progressive Matrices score. The Raven exam is a 60-question test that consists of five parts with 12 questions each (out of 60 possible points). The test assesses non-verbal intellectual function through the completion of abstract patterns. The Standard Progressive Matrices form was used for scoring; a point was given for each matrix the participant answered correctly [34].

In addition to maternal characteristics, infant characteristics and measurements of development collected at the time of birth and during the study follow up period were also used in this analysis.1. Preterm birth: A preterm birth was defined as gestational age <37 weeks in this study. Gestational age at birth in days was determined based on the date of the last menstrual period reported at recruitment. If the woman had delivered in the previous 6 months, dating ultrasound was used.

2. Congenital Diseases: Congenital anomalies at the time of birth such as trisomy, hydrocephalus, spinal bifida, enzyme abnormalities and "other anomaly" were recorded at IMSS hospital.

3. Bayley Scales of Infant Development: The Spanish version of the Bayley Scales of Infant Development-II (BSID-II) was administered to the cohort of children at 18 months of age. The two outcome measures calculated from the BSID-II of interest in this analysis were the Mental Development Index (MDI) and Psychomotor Development Index (PDI) scores.

The BSID-II yields three scales: the Mental Scale, the Psychomotor Scale, and the Behavior Rating Scale. The Mental Scale evaluates memory, learning and problem solving, verbal communication, mental mapping, sensory perception and early mathematical abilities. The Psychomotor Scale evaluates body control, coordination, and fine motor skills. The Behavior Rating Scale measures attention, orientation, emotional arousal and motor quality throughout the administration of the test [35].

Following training by the lead study psychologist, the BSID-II was administered by a team of psychologists (n=5). The test was administered in a quiet setting at IMSS hospital. Periodic direct observation and examination of completed forms by the team supervisor ensured proper test administration and data collection procedures. Children received credit for items within the Mental and Psychomotor Scales. The scores of these scales were calculated by adding the total number of items for which the child receives credit and then converting the summary scores to scale indices (MDI and PDI) per manual instructions. Standardization relative to age of the MDI and PDI yields a mean of 100 with a standard deviation of 15, with a range between 50-150; an index score between 85 and 114 is considered “within normal limits” for both. The Behavior Rating Scale (BRS) is translated into a percentile rank; scores greater or equal to the 26th percentile are classified as “within normal limits”. The BRS was not assessed in this analysis.

The BSID-II has been validated and correlates well with developmental tests indicative of later academic achievements, such as the McCarthy Scales of Children’s Abilities and the Wechsler Preschool and Primary Scale of Intelligence-Revised [36].

5. Home Environment: Housing, parenting, social experiences and cognitively-stimulating play materials are recognized as mechanisms that can affect the brain and behavioral development of children [17, 37, 38]. The Home Observation for Measurement of the Environment (HOME) inventory is a widely used measure of the quality of the home environment. The Spanish version of the HOME exam was administered to the cohort at 12, and 60 months of age.

The infant version, administered at 12months of age consists of 45 questions divided into 6 subscales: parental responsivity, acceptance of child, organization of the environment, provision of appropriate materials, parental involvement and variety of stimulation. The preschool version, administered at 60 months, consists of 45 questions divided into 8 subscales: stimulation through toys and learning materials, language stimulation, physical environment, pride, affection and warmth, stimulation of academic behavior, social maturity, variety of stimulation and physical punishment. The HOME exam is scored out of total of 45 possible points [39].

The HOME exam interview was conducted by a trained study psychologist at the residence in which the child spent the majority of their time outside of school. Each question is worth one point, zero points are given in the event a requirement was not met and no partial credit was given for any questions. During the interview, the study psychologist determined whether or not the family received a point for each question. At each visit, the location was noted on the HOME exam form and the child was required to be present with the caregiver at the time of the interview.

Statistical Analysis

Statistical analysis was carried out to determine maternal and infant baseline characteristics and child characteristics at 5 years of age among study participants who completed the McCarthy Scales of Children's Abilities cognitive assessment. Comparison of characteristics among the intervention and control group was assessed using the Student T-test for comparison of means of continuous variables or Chi-squared (X^2) tests for comparison of proportions of categorical variables.

Distributions and frequencies of variables in the McCarthy test data were examined to ensure completion of data entry and normality. Variables were assessed for outliers and implausible values to ensure that each MSCA test score fell within its specified point range (Table 4). Scores for each of the 18 tests that comprise the MSCA test battery, as well as the computed MSCA raw scores and scale indices, were examined for extreme values. If any of the 18 separate test scores were missing for a child, a corresponding scale that constituted that test was also missing for that child and these observations were excluded from the analysis. Additionally, distribution of each of the six MSCA raw scores and scale indices that comprise the McCarthy test were examined to ensure completion of data entry and normality. To assess the validity of the computed MSCA raw scores and scale indices scores, a one sample T-test compared the results of the DHA study sample to those of the MSCA standardization sample at 5 years of age. Inter-interviewer variability for the MSCA was examined using paired T-tests to compare the mean and variance of the scores given by the three study psychologists who administered the McCarthy test.

Bivariate associations assessed the effect of DHA supplementation on child cognitive development at 5 years of age. A two sample pooled T-test was used to determine differences in child cognitive development between the intervention and control group. Group means across the main outcome measures (verbal, quantitative, perceptual-performance, memory, motor and general cognitive raw scores and scale indices) were analyzed and compared.

Simple linear regression (SLR) models were formulated between the primary independent variable (treatment) and each main MSCA outcome variable. SLR analysis was also carried out to determine covariates associated with the main MSCA outcome variables. The covariates assessed were age at the time of test administration, gender, socioeconomic status, maternal educational attainment, maternal IQ, the HOME environment score at 12 and 60 months of age, and the BSID-II MDI and PDI scores at 18 months of age. Pair-wise correlations between independent covariates of interest were examined using a correlation matrix. If there was a high degree of correlation between two covariates only one was considered in the multiple regression model and a high degree of correlation was defined at r=0.8.

Multiple linear regression (MLR) analysis was carried out to determine the effect of maternal DHA supplementation during pregnancy on child cognitive development. The regression models were not assessed for potential confounding as the DHA study is randomized by design and the treatment groups were well balanced at baseline. After determining important covariates to be included in the final model, regression diagnostics were performed in order to ensure accurate analytical results. A variation inflation factor (VIF) > 10 indicated a problem with multicollinearity. If collinearity

existed between 2 or more variables, each variable was individually dropped from the model one at a time and the variable that explained the most variance was no longer considered in the final model.

Effect modification was tested by individually examining the interaction between treatment and the following covariates: socioeconomic status (i.e. treatment*SES), mother's educational attainment, maternal IQ, HOME score at 12 and 60 months of age, and BSID-II MDI and PDI scores at 18 months of age. All covariates were selected *a priori* based on previous findings. Quality of the home environment was examined since previous analyses of this study data suggest the intervention's effect on cognitive development outcomes varies in regard to the quality of the home environment [40]. Effect modification of the BSID-II MDI and PDI scores 18 months of age were examined to determine if the intervention's effect on cognitive performance at 5 years of age varies as a function of cognitive performance in early life. Additionally, the above analyses were also performed after restricting the sample to term births, singleton births, and subjects born without congenital abnormalities. Statistical significance was defined as $\alpha = 0.01$ when assessing for interaction; for all other analyses statistical significance was defined as $\alpha = 0.05$. All analyses were performed using SAS 9.3 (SAS Institute, Cary, NC).

Chapter 4: RESULTS

Primary Analysis

I. Description of Study Sample

A total of 973 infants born to 1,094 women enrolled in the trial were followed beginning at birth until 5 years of age. Figure B. describes the study recruitment and follow-up of pregnant women and their offspring until 5 years of age. A sample of 802 children with a mean age of 5.10 ± 0.17 years who completed the McCarthy Scales of Children's Abilities was analyzed to determine the effect of DHA supplementation beginning at 18-22 weeks of pregnancy until birth on child cognitive development. The maternal characteristics at study enrollment, child characteristics at birth, and child characteristics at the time of test administration were assessed. There were no significant differences detected for these characteristics among the intervention and control group (Tables 1 and 2).

At baseline, the mean maternal age was 26 years old and socioeconomic status was similar for both groups. Overall, 58% of mothers completed high school or more schooling and the mean score on the Raven's test was 40.8 out of a total of 60.

The mean birth weight of infants was 3,214.0 grams and only 5.0% of infants in the study were born with low birth weights. As previously reported in earlier publications, anthropometric indicators at birth and at 5 years of age did not differ between groups and there were no differences between groups in infant feeding practices [30, 41].

Additionally, HOME summary scores at 12 and 60 months of age and BSID-II outcome scores at 18 months were similar for both groups (Table 2). Table 3 provides the average age of children overall and stratified by treatment group at the time of test administration for all tests included in this analysis. There was a significant age difference among groups for the BSID-II exam at 18 months, as children assigned to the placebo were slightly older at the time of test administration ($p=0.05$)

II. Bivariate Analysis

Analysis of the six MSCA scales (both the raw and scale indices) indicated that one study psychologist consistently scored higher on a majority of the measures in comparison to the other two psychologists. However, there were no differences between mean scores and treatment group when stratified by study psychologist, indicating that although these higher scores may have increased the overall mean of the six MSCA scales raw and standardized scores, the score distribution was not impacted by treatment group ($p>0.05$).

All scores for the eighteen tests that comprise the MSCA test battery fell within their specified point range, as did the composite raw scores and composite scale indices. The majority of the 18 MSCA tests were normally distributed, yet some were heavily skewed in one direction. This is expected as the MSCA tests have varying degrees of level of difficulty. Higher scores were more common for easy tests and were skewed right. On the other hand, tests that were more difficult to complete were skewed left as lower scores were more common. Heavily skewed component test scores were not

transformed because the six MSCA composite scales (both the raw scores and scale indices) were normally distributed.

The mean raw scores of the study population and the standardization sample for each test at 5 years of age were similar for MSCA tests 8, 11, 12, 14 (Part 2), 15, 16, 17, and 18. The mean raw scores of all other tests were significantly lower than the standardization sample, with the exception of test 5, which was higher ($p \leq 0.05$) (Table 7). The computed verbal scale index scores of the study population and the standardization sample were similar ($p > 0.05$), but all other scale indices scores were significantly lower for the study sample ($p \leq 0.05$) (Table 5).

To determine main effects of the intervention, group means of the MSCA outcome measures were compared; no significant differences were found between children in the intervention and control group ($p > 0.05$) (Table 6).

III. Linear Regression Models

In general, all covariates of interest (e.g. SES, maternal IQ, and HOME score) appeared to have a significant relationship with one or more of the MSCA outcome measures. For example, correlation between the general cognitive standardized score and the HOME score at 60 months of age was significant ($p < 0.01$) and had a correlation coefficient of medium strength ($r = 0.41$). Confounding was not an issue in this analysis; there were no significant differences between the primary independent variable (treatment group) and the independent covariates of interest. Although age at the time

of test administration and gender were well balanced at baseline, they remained in the models as the literature has historically adjusted for these variables [40].

We found significant interactions between treatment group and HOME score at 12 months of age for the MSCA verbal, perceptual-performance, memory and general cognitive raw scores and scale indices ($p<0.10$) as shown in Table 7. We found that the slope estimate of the effect of home environment on cognition was reduced by half among those who were exposed to DHA in utero compared to the placebo as shown in Figures 1 – 8. The effect was in the same direction for all interactions.

In contrast, there was no evidence of significant interactions between treatment group and the HOME exam at 60 months of age for any of the MSCA outcome measures (Table 8). Similarly, there were no significant differences in MSCA outcome measures when assessing interaction between treatment group and BSID-II MDI and PDI scores at 18 months of age (Tables 9 and 10, respectively).

Sub Analyses

Sub analyses were carried out in order to determine if the main effects of the intervention on child cognitive outcomes held true for three different restricted datasets: term births (n= 725, 90.4% of MSCA sample), infants born without congenital disease (n=779 97.0% of MSCA sample), and singleton births (n=794, 99.0% of MSCA sample). There were no observable significant differences in baseline characteristics in the

restricted samples. The main effects of the intervention on child cognitive outcomes in the restricted analysis were comparable to the primary analysis (*results not reported*). Additionally, assessing the effect modification between treatment and quality of home environment in the restricted datasets revealed results similar to the main analysis, as significant interaction was still observed for the verbal, perceptual-performance, memory and general cognitive raw scores and scale indices ($p<0.10$).

Chapter 5: DISCUSSION

In a large, randomized controlled trial, supplementation of 400mg/day DHA mid-pregnancy until delivery did not have a significant effect on child's cognitive development at 5 years of age. The two groups were well matched at baseline, intervention compliance was high at 88%, and loss to follow up at the time of cognitive assessment was low with 82% of the original birth cohort completing the McCarthy Scale of Children's Abilities cognitive assessment test. Furthermore, compliance and loss to follow up did not differ by treatment group.

The significant effect modification between the treatment group and HOME score at 12 months of age suggests that children who come from poor home environments and are exposed to DHA supplementation prenatally may be at a developmental advantage over their equal, unexposed peers. These results are similar to previous analysis of the DHA study data that reported significant interaction by treatment and home environment for the BSID-II MDI and PDI scores at 18 months of age. However, the

significance of this interaction disappeared when the children's home environment was assessed during study follow up at 60 months of age.

These results strengthen the argument that quality of the home environment is an important determinant of child development in the earliest years of life. Despite unfavorable conditions such as lack of parental involvement at home, children may benefit the most developmentally if they are exposed to higher levels of DHA in utero. Furthermore, this analysis adds evidence to a topic that has not been well researched, as studies that determine the effect of prenatal DHA supplementation on cognitive performance in healthy children older than two years of age is limited at this time. The total number of randomized controlled trials examining prenatal DHA exposure is limited, especially in regard to the cognitive outcomes of children after infancy and into early childhood [6]. Exposure to higher levels of DHA in utero, infancy, and early childhood may lead to better cognitive outcomes for at risk children, such as those of poor nutritional status. However, a definitive link between prenatal DHA supplementation and cognitive function at 5 years of age is yet to be established and more evidence explaining the role environmental and social factors play in the cognitive development process is needed.

Bias

Although the randomization of this RCT controlled for selection bias successfully, bias due to measurement error could potentially be a problem. It is difficult to pinpoint the reason for the observed differences of the mean MSCA raw and scale indices scores

between the children in this study sample and those in the standardization sample at 5 years of age. One explanation could be that more children in our study population simply refused to cooperate during the administration of the test. This would result in lower scores on the 18 component tests, as oftentimes points are accumulated after the previous question is answered correctly. Additionally, the psychologists were expected to keep a record of children that were especially uncooperative, yet these notes were general to the overall exam and not specific to each component test. However, since there were not obvious differences in test score performance between the groups, it can be assumed measurement error was not a significant source of bias in this analysis.

Another explanation and possible source of bias is that missing data for the McCarthy test scores was handled incorrectly. If children were erroneously not assigned the correct points for the different scales, this would result in lower overall test scores observed in our population. The complex skip patterns involved when scores the MSCA test make this a reasonable argument to be a source of bias in regard to impact of missing test values. There are complex skip patterns in the MSCA test, which can result in incorrect scores, especially when there are many missing values. This is not likely as all the MSCA scores were calculated using a dataset based on the original observations.

Finally, an additional source of bias could be related to the location of the McCarthy test administration. The McCarthy test was administered in two different rooms at the IMSS hospital but the room location was not recorded on the test form. The fact that the rooms were quite different (one windowless on a louder floor and the other on a quiet floor with a window) may have impacted the child's test performance. Unfortunately, we

cannot determine if the room location impacted test performance or control for this variable if it were found to be a confounder.

A large portion of the original birth cohort (88%) completed the McCarthy Scores of Children's Abilities, yet some children were lost to follow up in this study and have missed previous follow up appointments. This is particularly of concern when considering the significant interaction with the home environment at 12 months of age since the total sample size dropped from n=802 to n=533 in this analysis. Even though baseline maternal and infant characteristics were well balanced in the smaller sample ($p>0.05$ for all characteristics), it is interesting that the significant interaction disappeared when assessing the home environment at 60 months of age (n=799). Future analyses of the DHA study follow up data should always ensure that maternal and infant characteristics at baseline do not differ when the sample size meaningfully changes, as significance differences could introduce bias.

Strengths

The DHA study is a large randomized controlled trial, the "gold standard" of study designs. The randomization was highly successful, as indicated by the well-balanced maternal and infant characteristics by treatment allocation at baseline. The study's large sample size, high intervention compliance, excellent follow up since it began in 2007, and dedicated study team in Cuernavaca have contributed to the continuation of findings and the study's ongoing success. Additionally, there is potential to continue following the growth and developmental outcomes of the children enrolled in the study,

which will add to the growing body of research investigating the impact of fatty acids on brain growth and development, especially for later child development.

Limitations

There are some limitations to this study. In regard to interviewers, one psychologist in particular administered the majority of the MSCA tests for this sample. Furthermore, another psychologist consistently gave children significantly higher scores on the MSCA test, but fortunately this did not impact the homogeneity of test scores between treatment groups. The DHA study could have benefitted from having more trained psychologists (e.g. n=5, as did the BSID-II exam) administering the MSCA test as well as more frequent and routine test standardization procedures to ensure the quality of scoring techniques.

While it has been established that infant feeding practices were similar for both treatment groups during the first months of life, the duration the infant was breastfed would be a more useful measure to assess in our study. Although participants agreed to breastfeed their child exclusively for three months upon entering the study, the duration of breastfeeding and breastfeeding practices may vary between the treatment groups. This is especially important because breastfeeding is associated with better cognitive development outcomes.

Specifically, findings from a prospective study by Gustafsson et al. that measured duration of breastfeeding in relation to child IQ ay 6.5 years of age support that high levels of LCPUFAs are important for cognitive development [42]. However, results from

this study should be interpreted with caution due to its small sample size. Another cohort study, this one larger, found a significant positive association between duration of breastfeeding and cognitive performance in children at 5 years of age even after controlling for important confounders such as the home environment and parenting style [26]. Because breast milk is one of the few dietary sources rich in DHA, determining breastfeeding duration and practices would enable researchers to assess and control for DHA exposure after the prenatal period, especially if it were found to be an important confounder of cognitive outcomes for this study population.

Future Directions

Inconsistencies in the available evidence indicate it would be beneficial to conduct more studies that assess the relationship between prenatal DHA supplementation, cognitive development, and the home environment in early and later childhood. Both infant and maternal DHA status should be reported during multiple time points in future studies, as this will provide more information about the effects of prenatal DHA status and/or supplementation on child cognitive development later in life. Collection of maternal blood prior to and following birth, along with infant cord blood samples should be a priority so that researchers can better establish the direct relationship between maternal and infant LCPUFA status.

Following birth of the infant, detailed reports on breastfeeding duration and practices are necessary since postnatal nutrition plays an important role in child development. Previous analyses of this study data have determined that mothers who

were supplemented with DHA had higher levels of DHA in breast milk than mothers who received the placebo [30]. Future analyses should examine possible differential effects by breastfeeding duration and practices, as this may impact the effect of prenatal DHA supplementation when assessing child cognitive development outcomes of the study cohort.

As identified by the Lancet Series on Child Development, there are key micronutrients that play a protective role in child cognitive development. Future analyses of the DHA study data should assess the micronutrient status of the children to control for other factors that may have a significant effect on cognitive development. For example, does iron or iodine status differ by treatment group and do these factors explain cognitive development outcomes in early or later childhood? Iron and anemia status of DHA study participants is of particular importance for this study population since the most prominent nutrition problem identified from the most recent Mexican National Nutrition Surveys was iron deficiency among children under 5 years of age and pregnant women [23].

There is a need to determine global recommendations for fatty acid intakes, especially during pregnancy as currently there is no scientific consensus as to what level of DHA intake is optimal to lead to a saturation of neural membranes [6, 7, 10]. Yet studies that have examined prenatal DHA in relation to infant birth, growth, and developmental outcomes vary by DHA intake levels as well as the time and duration of supplementation which makes the formulation of guidelines difficult [43].

Although the evidence is still growing and more research is needed, it is still important to provide prenatal DHA supplementation guidelines for pregnant women and women of child bearing age. A targeted approach would focus on vulnerable populations and take into consideration that some countries generally have lower fatty acid dietary intakes than others. In the United States, it is recommended that pregnant and lactating women intake a minimum of 300mg/day DHA. Yet pregnant and lactating women are advised by the European based Perinatal Lipid Intake Working Group (PERILIP) to intake 200mg/day DHA [8]. Since the populations these recommendations target most likely have higher fatty acid intakes than those in LMICs, it might be suggested that pregnant women and women of child bearing age in LMICs intake at least 400mg/day DHA. However, it is unlikely there will be increased efforts to spread educational awareness and key messages about DHA requirements to targeted populations (i.e. women in LMICs) until there is a better understanding of the role that DHA plays in birth outcomes, neurodevelopment and cognitive function.

Public Health Implications

Many people are unaware that essential fatty acids, especially DHA, play an important role in neural and retinal growth and function. The available evidence demonstrates that DHA is an important dietary requirement, especially for pregnant women, women of childbearing age, and the developing fetus. Prenatal DHA supplementation is an important nutrition intervention that warrants more pronounced public health consideration because there are only a few natural dietary sources by

which humans can meet their fatty acid requirements. Low reports of adverse effects from DHA supplementation trials indicate that DHA supplementation is safe [44]. It also may be an environmentally sound and safer alternative to dietary sources such as tuna and other large oil-rich fish, which are known to be contaminated with high levels of mercury [13, 18].

Furthermore, a recent systematic review on the effect of LCPUFA intake during pregnancy concluded that prenatal DHA status can reduce preterm deliveries in high risk populations; these findings might enhance governmental and public support for recommending targeted prenatal DHA supplementation [43]. Prenatal DHA supplementation could serve as an important nutrition intervention that improves birth outcomes, future growth, and cognitive development of children, especially those who are already born at a disadvantage into poverty or poor physical, social, and built environments.

This analysis adds to the growing body of research exploring the importance of maternal and infant DHA status. This study did not find any main effects of prenatal DHA supplementation on cognitive development, but the findings suggest that children exposed to DHA in utero from low quality home environments may have a cognitive advantage over placebo children from similar home environments. Continuing research on prenatal DHA supplementation will ensure that better recommendations and interventions are carried out moving forward. Establishment of the timing, duration, and adequate intake level of supplementation based on sound evidence will hopefully improve the birth, growth, and development outcomes of children in LIMCs moving forward.

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Table 1: Selected maternal characteristics at randomization and child characteristics at birth among children (n=802) born to women (n=798) who participated in a trial of 400 mg/d docosahexaenoic acid (DHA) during pregnancy and had measures of infant cognitive development at 5 years of age using the McCarthy Scales of Children's Abilities, by intervention group[1]

	n	Placebo	n	DHA	P value [2]
Maternal characteristics at randomization					
Age in years	398	26.3 ± 4.7	400	26.4 ± 4.9	0.93
Gestational age in weeks	398	20.5 ± 2.1	400	20.5 ± 2.0	0.86
Socio-economic status	398	0.05 ± 1.0	400	0.02 ± 1.0	0.72
Schooling (high school or more) (%)	398	60.7	400	55.8	0.16
School (highest level completed)	398	12.1 ± 3.6	403	11.8 ± 3.5	0.31
Ravens score	398	41.1 ± 9.3	400	40.4 ± 9.1	0.32
Primigravida (%)	398	38.7	400	34.8	0.25
Weight (kg)	398	63.5 ± 11.0	400	62.4 ± 11.6	0.15
Height (cm)	398	155.4 ± 5.6	400	154.8 ± 5.7	0.13
Body mass index (kg/m^2)	398	26.3 ± 4.3	400	26.0 ± 4.3	0.32
Child characteristics at birth					
Weight (g)	399	3,212.5 ± 466	403	3,215 ± 451	0.93
Length (cm)	398	50.4 ± 2.6	403	50.3 ± 2.3	0.82
Head Circumference (cm)	342	34.3 ± 1.8	346	34.4 ± 1.6	0.33
Low birth weight (<2500 g) (%)	399	4.5	403	5.5	0.54
Gestational age in weeks	397	39.1 ± 1.7	402	39.0 ± 1.9	0.63
Preterm birth[3] (%)	397	8.1	402	10.5	0.24
Sex (male) (%)	397	54.1	402	53.9	0.93
Intrauterine growth restriction (%)	397	10.6	402	11.0	0.87

[1] Values are (mean, ± SD) unless otherwise indicated
[2] T-test for comparison of means and chi-square test for comparison of proportions
[3] Defined as <37 weeks of gestation

Table 2: Selected child characteristics at 5 years of age among children (n=802) born to women (n=798) who participated in a trial of 400 mg/d docosahexaenoic acid (DHA) during pregnancy and had measures of infant cognitive development at 5 years of age using the McCarthy Scales of Children's Abilities, by intervention group[1]

	n	Placebo	n	DHA	P value[2]
Child characteristics					
Weight (kg)	399	18.4 ± 3.0	403	18.3 ± 3.0	0.89
Height(cm)	399	108.4 ± 4.5	403	108.3 ± 4.4	0.91
Arm circumference (cm)	399	17.4 ± 1.8	403	17.4 ± 1.7	0.84
Triceps Skinfold	399	8.9 ± 1.8	403	9.0 ± 2.6	0.81
Subscapular Skinfold	399	6.6± 2.4	403	6.6± 2.6	0.91
Abdominal Circumference	399	54.8 ± 4.9	403	54.8 ± 4.9	0.95
HOME Score[3]	250	36.8 ± 4.4	283	36.6 ± 4.4	0.54
Home Score[4]	387	41.0 ± 7.4	391	41.6 ± 6.2	0.42
BSID-II MDI[5]	323	95.1 ± 9.3	334	94.4 ± 10.9	0.35
BSID-II PDI[6]	323	93.1 ± 9.6	334	92.9 ± 9.0	0.72

[1] Values are (mean, ± SD) unless otherwise indicated
[2] T-test for comparison of means and chi-square test for comparison of proportions
[3] Home Measurement of the Environment Exam at 12 months of age
[4] Home Measurement of the Environment Exam at 60 months of age
[5] BSID-II Mental Development Index Score at 18 months of age
[6] BSID-II Psychomotor Index Score at 18 months of age

Table 3: Average age of children at time of study test administration born to women who participated in a trial of 400 mg/d docosahexaenoic acid (DHA) during pregnancy [1]

Exam	Target Age (yrs)	Average Age (yrs) (Overall)	Age Range, Minimum-Maximum (Overall)	n	Placebo	n	DHA	P value[2]
MSCA	5.0	5.10 ± 0.17	5.0-6.1	399	5.1 ± 0.2	403	5.1 ± 0.2	0.20
HOME	5.0	5.35 ± 0.22	5.0-6.3	398	5.4 ± 0.2	401	5.3 ± 0.2	0.30
BSID-II	1.5	1.51 ± 0.03	1.5-1.7	365	1.5 ± 0.0	365	1.5 ± 0.0	0.05
HOME	1.0	1.01 ± 0.03	1.0-1.2	250	1.0 ± 0.0	283	1.0 ± 0.0	0.57

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

Table 4: Comparison of mean raw scores on the 18 tests that comprise the McCarthy Scales of Children's Abilities for the docosahexaenoic acid (DHA) study sample (n=802) and the MSCA standardization sample (n=102) for children at 5 years of age[1]

	Possible Score, Minimum-Maximum	Study Sample (n=802)	Standardization Sample (n=102)	P value[2]
Test				
1. Block Building	0-10	9.9 ± 0.4	9.3 ± 1.2	< 0.01
2. Puzzle Solving	0-27	5.4 ± 2.9	10.7 ± 6.8	< 0.01
3. Pictorial Memory	0-6	3.2 ± 1.2	3.6 ± 1.2	< 0.01
4. Word Knowledge: *Parts I & II*	0-29	14.2 ± 3.7	16.1 ± 2.0	< 0.01
5. Number Questions	0-12	7.7 ± 2.2	4.4 ± 1.4	< 0.01
6. Tapping Sequence	0-9	1.5 ± 1.5	3.5 ± 1.6	< 0.01
7. Verbal Memory: *Part I* *Part II*	 0-30 0-11	 9.9 ± 2.5 4.6 ± 2.7	 22.0 ± 6.5 5.2 ± 3.0	 < 0.01 0.04
8. Right-Left Orientation	0-12	5.9 ± 2.5	6.1 ± 3.2	0.46
9. Leg Coordination	0-13	10.5 ± 1.9	11.1 ± 1.8	< 0.01
10. Arm Coordination Parts I, II, & III	0-28	6.8 ± 3.8	8.3 ± 4.0	< 0.01
11. Imitative Action	0-4	3.7 ± 0.5	3.8 ± 0.4	0.05
12. Draw-A-Design	0-19	6.6 ± 2.3	6.7 ± 3.0	0.69
13. Draw-A-Child	0-20	10.3 ± 3.1	9.1 ± 3.7	< 0.01
14. Numerical Memory: *Part I* *Part II*	 0-12 0-10	 4.8 ± 1.7 1.2 ± 2.4	 5.9 ± 1.9 1.1 ± 1.6	 < 0.01 0.68
15. Verbal Fluency	0-36	14.0 ± 4.4	12.9 ± 4.8	0.02
16. Counting and Sorting	0-9	6.3 ± 2.2	6.3 ± 1.9	1.00
17. Opposite Analogies	0-9	4.7 ± 2.3	4.6 ± 1.5	0.67
18. Conceptual Grouping	0-12	7.2 ± 2.4	7.6 ± 2.4	0.11

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

Table 5: Comparison of mean scores for the six MSCA scale indices scores that comprise the McCarthy Scales of Children's Abilities for the docosahexaenoic acid (DHA) study sample (n=802) and the MSCA standardization sample (n=102) for children at 5 years of age[1]

	Possible Score, Minimum-Maximum	Study Sample (n=802)	Standardization Sample (n=102)	P value[2]
MSCA Scale Index Score				
Verbal	22-78	43.4 ± 8.6	50.0 ± 9.9	< 0.01
Perceptual-Performance	22-78	48.9± 8.3	50.5± 10.3	0.08
Quantitative	22-78	45.2 ± 9.7	50.4 ± 10.1	<0.01
Memory	22-78	42.2 ± 8.9	50.3 ± 10.4	<0.01
Motor	22-78	48.1 ± 8.9	50.1 ± 10.3	<0.04
General Cognitive	50-150	90.6 ± 13.3	100.3 ± 16.4	<0.01

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

Table 6: Unadjusted comparison of measures of cognitive development using McCarthy Scales of Children's Abilities at 5 years of age among children (n=802) born to women who participated in a trial of 400 mg/d docosahexaenoic acid (DHA) during pregnancy, by intervention group[1]

	n	Placebo	n	DHA	P value[2]
Raw McCarthy Score					
Verbal	392	50.9 ± 11.5	398	50.6 ± 11.1	0.67
Perceptual Performance	389	46.7 ± 9.3	397	46.9 ± 9.3	0.73
Quantitative	394	20.1 ± 6.4	401	19.9 ± 6.3	0.60
Memory	392	25.3 ± 7.6	399	25.3 ± 7.6	0.98
Motor	388	37.8 ± 7.0	396	38.1 ± 6.8	0.53
General Cognitive	387	117.9 ± 23.3	392	117.7 ± 22.0	0.94
Scale Index McCarthy Score					
Verbal	392	43.5 ± 8.7	398	43.3 ± 8.5	0.69
Perceptual Performance	389	48.7 ± 8.3	397	49.0 ± 8.3	0.60
Quantitative	394	45.3 ± 9.7	401	45.1 ± 9.6	0.69
Memory	392	42.1 ± 8.7	399	42.2 ± 9.0	0.92
Motor	388	47.9 ± 8.8	396	48.3 ± 8.9	0.54
General Cognitive	382	92.9 ± 13.3	391	92.4 ± 13.4	0.64

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

Table 7: Comparison of measures of cognitive development using McCarthy Scales of Children's Abilities at 5 years of age among children (n=802) born to women who participated in a trial of 400 mg/d docosahexaenoic acid (DHA) during pregnancy, by intervention group[1]

	n	Placebo	n	DHA	P value[2,3]
Raw McCarthy Score		β		β	
Verbal	246	0.79	279	0.40	0.08
Perceptual Performance	245	0.55	279	0.22	0.06
Quantitative	248	0.30	282	0.19	0.37
Memory	246	0.41	280	0.15	0.07
Motor	244	0.17	278	0.09	0.53
General Cognitive	243	1.68	274	0.76	0.03
Scale Index McCarthy Score					
Verbal	246	0.58	279	0.30	0.10
Perceptual Performance	245	0.50	279	0.21	0.06
Quantitative	248	0.43	282	0.29	0.45
Memory	246	0.47	280	0.19	0.09
Motor	244	0.22	278	0.12	0.57
General Cognitive	240	0.89	273	0.44	0.08

[1] Values are β coefficients
[2] p value from interaction term between group and HOME score at 12 months of age
[3] Model adjusted for child gender and child age at measurement

Table 8: Comparison of measures of cognitive development using McCarthy Scales of Children's Abilities at 5 years of age among children (n=802) born to women who participated in a trial of 400 mg/d docosahexaenoic acid (DHA) during pregnancy, by intervention group[1]

	n	Placebo	n	DHA	P value[2,3]
Raw McCarthy Score		β		β	
Verbal	381	0.63	386	0.51	0.27
Perceptual Performance	378	0.50	385	0.50	1.00
Quantitative	383	0.32	389	0.25	0.19
Memory	381	0.37	387	0.26	0.12
Motor	377	0.26	384	0.30	0.54
General Cognitive	376	1.45	380	1.24	0.30
Scale Index McCarthy Score					
Verbal	381	0.46	386	0.39	0.41
Perceptual Performance	378	0.45	385	0.45	0.98
Quantitative	383	0.49	389	0.39	0.26
Memory	381	0.43	387	0.30	0.11
Motor	377	0.33	384	0.38	0.52
General Cognitive	371	0.78	379	0.73	0.71

[1] Values are β coefficients
[2] p value from interaction term between group and HOME score at 60 months of age
[3] Model adjusted for child gender and child age at measurement

Table 9: Comparison of measures of cognitive development using McCarthy Scales of Children's Abilities at 5 years of age among children (n=802) born to women who participated in a trial of 400 mg/d docosahexaenoic acid (DHA) during pregnancy, by intervention group[1]

	n	Placebo	n	DHA	P value[2,3]
Raw McCarthy Score		β		β	
Verbal	320	0.40	329	0.35	0.61
Perceptual Performance	318	0.21	329	0.22	0.89
Quantitative	321	0.23	332	0.18	0.30
Memory	320	0.25	330	0.25	0.91
Motor	317	0.14	328	0.15	0.83
General Cognitive	317	0.86	324	0.78	0.62
Scale Index McCarthy Score					
Verbal	320	0.29	329	0.27	0.68
Perceptual Performance	318	0.20	329	0.20	0.93
Quantitative	321	0.37	332	0.27	0.18
Memory	320	0.31	330	0.29	0.76
Motor	317	0.18	328	0.19	0.87
General Cognitive	314	0.48	323	0.48	0.99

[1] Values are β coefficients
[2] p value from interaction term between group and BSID-II MDI score at 18 months of age
[3] Model adjusted for child gender and child age at measurement

Table 10: Comparison of measures of cognitive development using McCarthy Scales of Children's Abilities at 5 years of age among children (n=802) born to women who participated in a trial of 400 mg/d docosahexaenoic acid (DHA) during pregnancy, by intervention group[1]

	n	Placebo	n	DHA	P value[2,3]
Raw McCarthy Score		β		β	
Verbal	320	0.30	329	0.21	0.33
Perceptual Performance	318	0.20	329	0.16	0.59
Quantitative	321	0.14	332	0.07	0.18
Memory	320	0.20	330	0.11	0.16
Motor	317	0.18	328	0.21	0.64
General Cognitive	317	0.65	324	0.46	0.29
Scale Index McCarthy Score					
Verbal	320	0.22	329	0.16	0.33
Perceptual Performance	318	0.18	329	0.14	0.55
Quantitative	321	0.21	332	0.11	0.19
Memory	320	0.24	330	0.14	0.13
Motor	317	0.24	328	0.27	0.66
General Cognitive	314	0.35	323	0.26	0.39

[1] Values are β coefficients
[2] p value from interaction term between group and BSID-II PDI at 18 months of age
[3] Model adjusted for child gender and child age at measurement

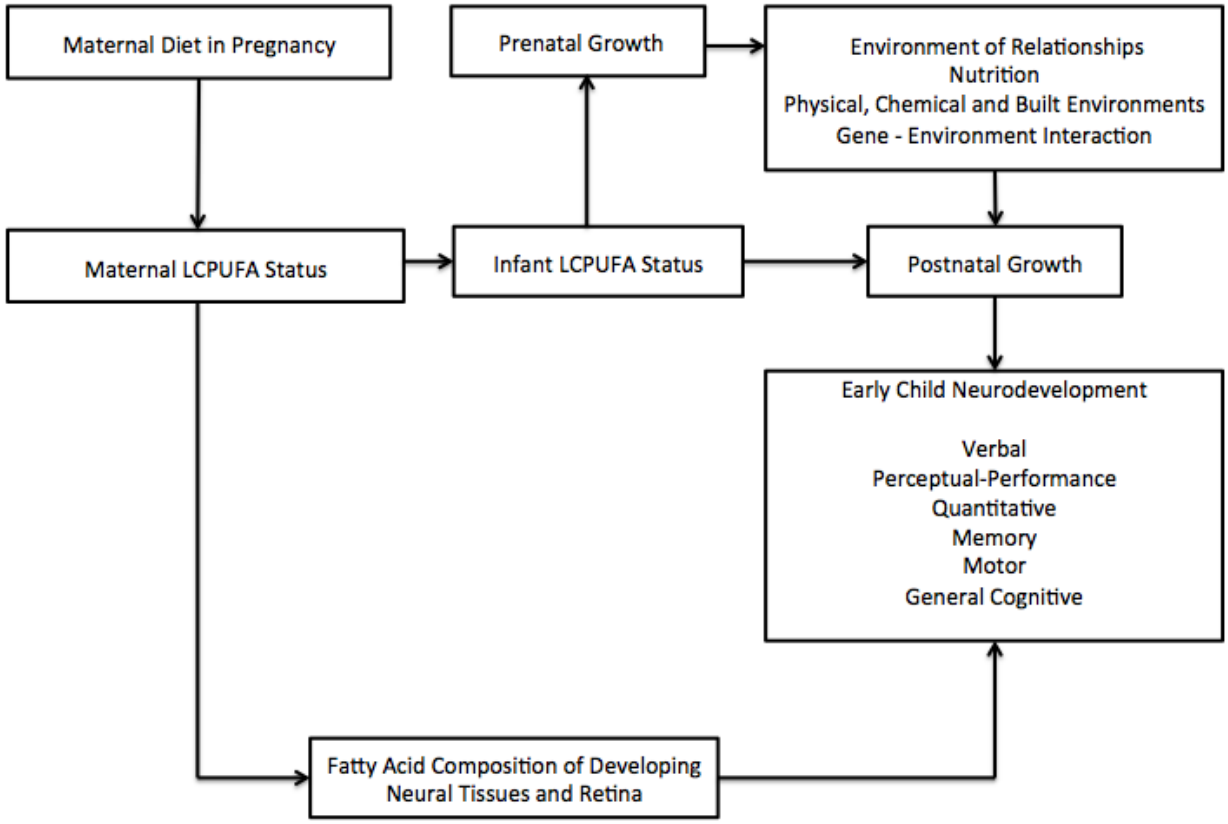


Figure A. Conceptual framework relating to in-utero exposure to long chain polyunsaturated fatty acids (LCPUFAs) and child development.

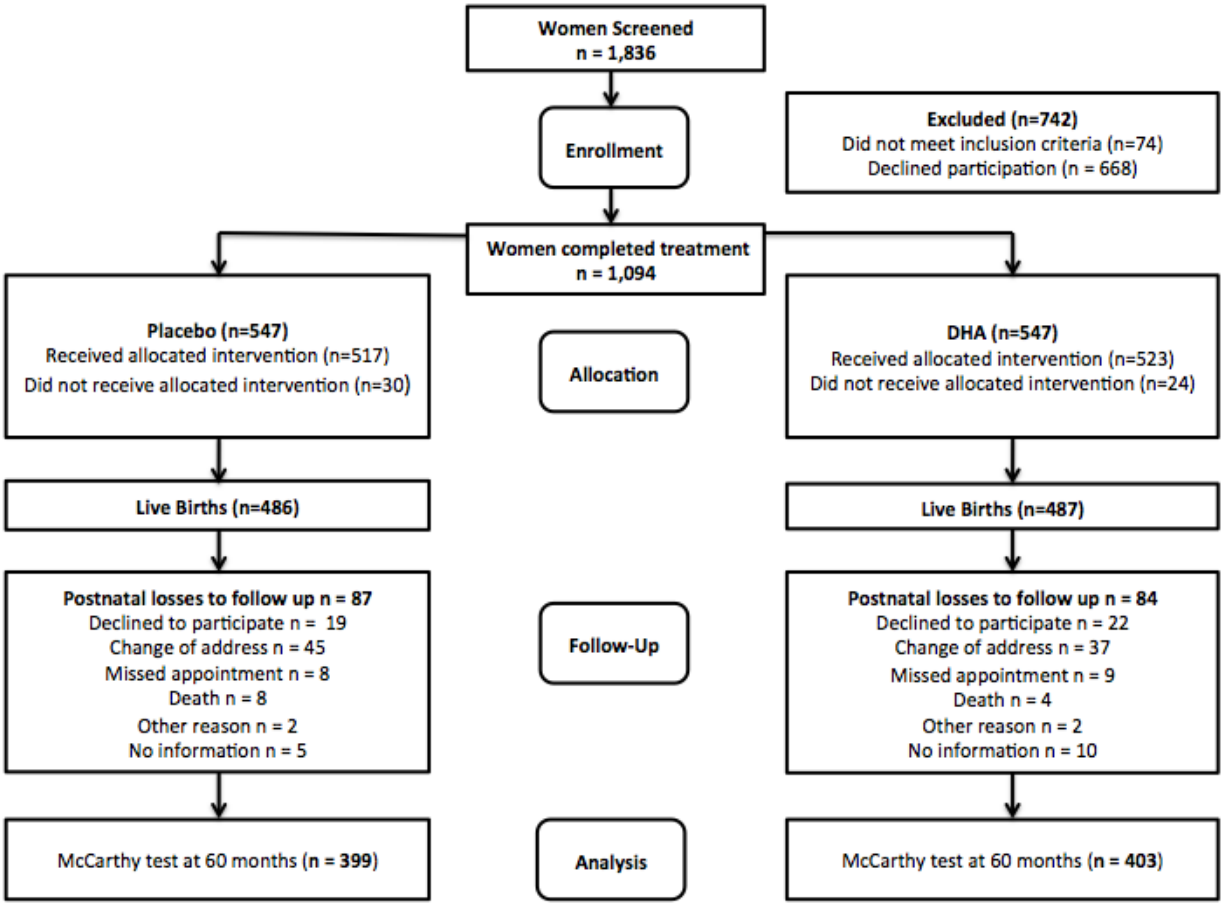
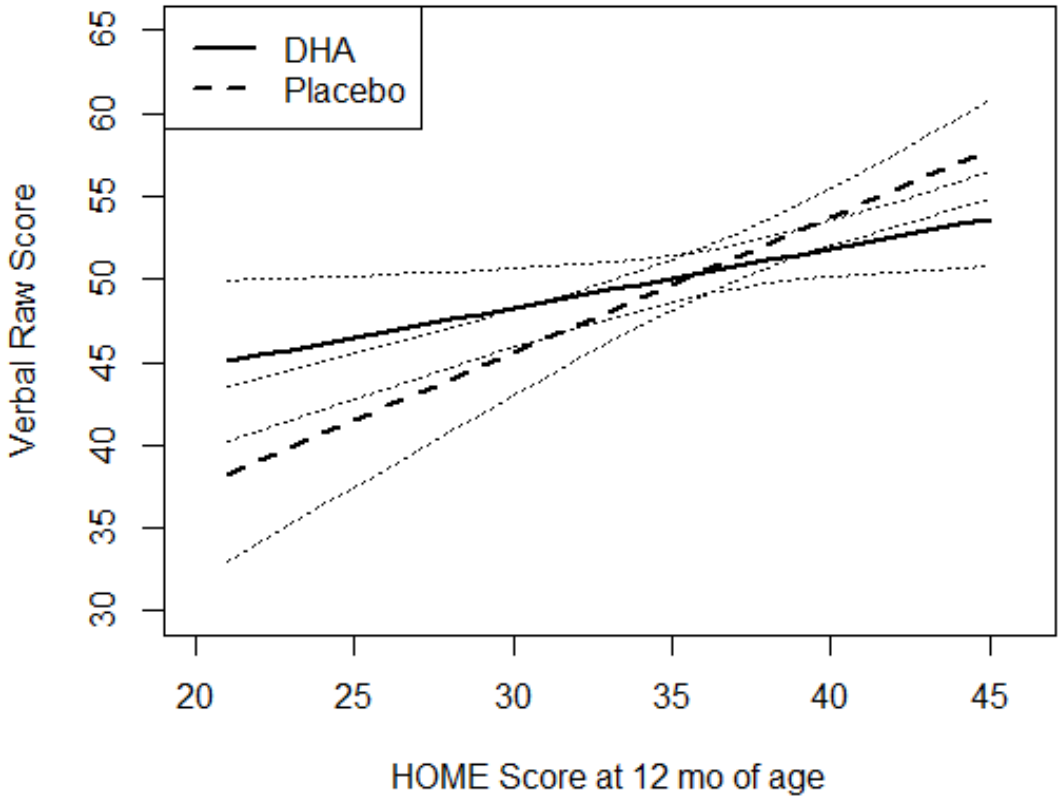


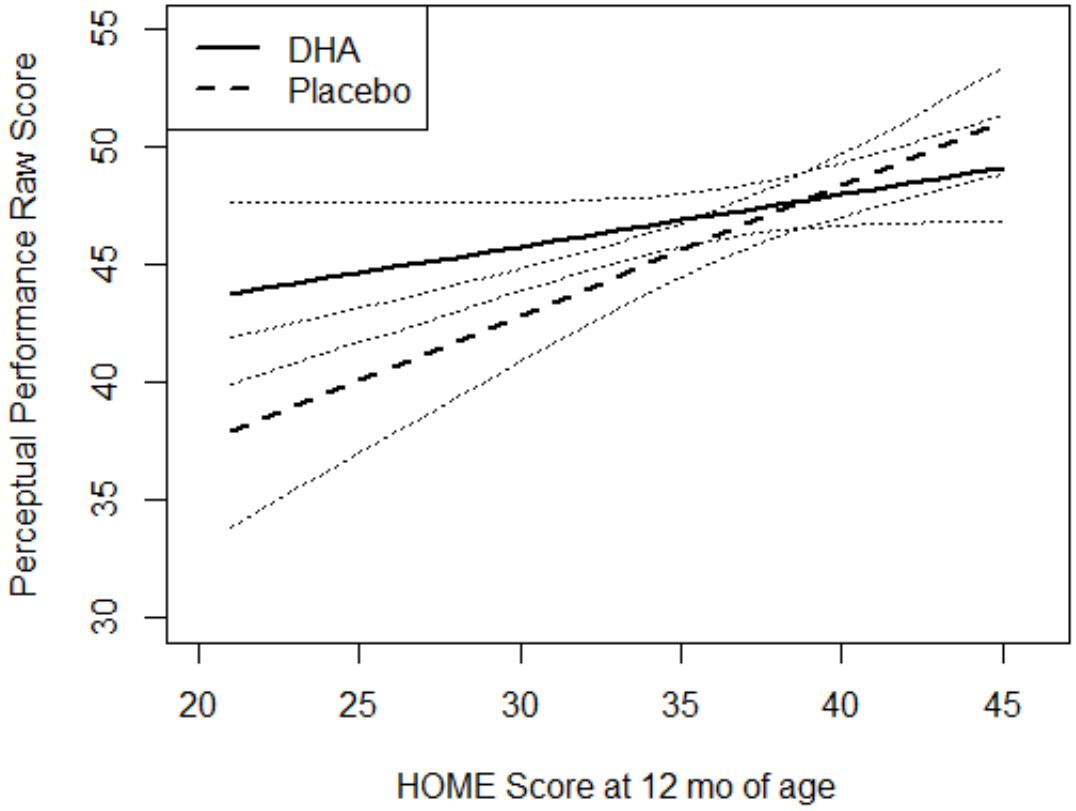
Figure B. Details on the recruitment of mothers and follow-up of infants in a randomized controlled trial of 400 mg/d docosahexaenoic acid (DHA) during pregnancy in Cuernavaca, Mexico.

Figure 1: Relationship between HOME Score and Verbal Raw Score at 5 yr of age, by intervention group*



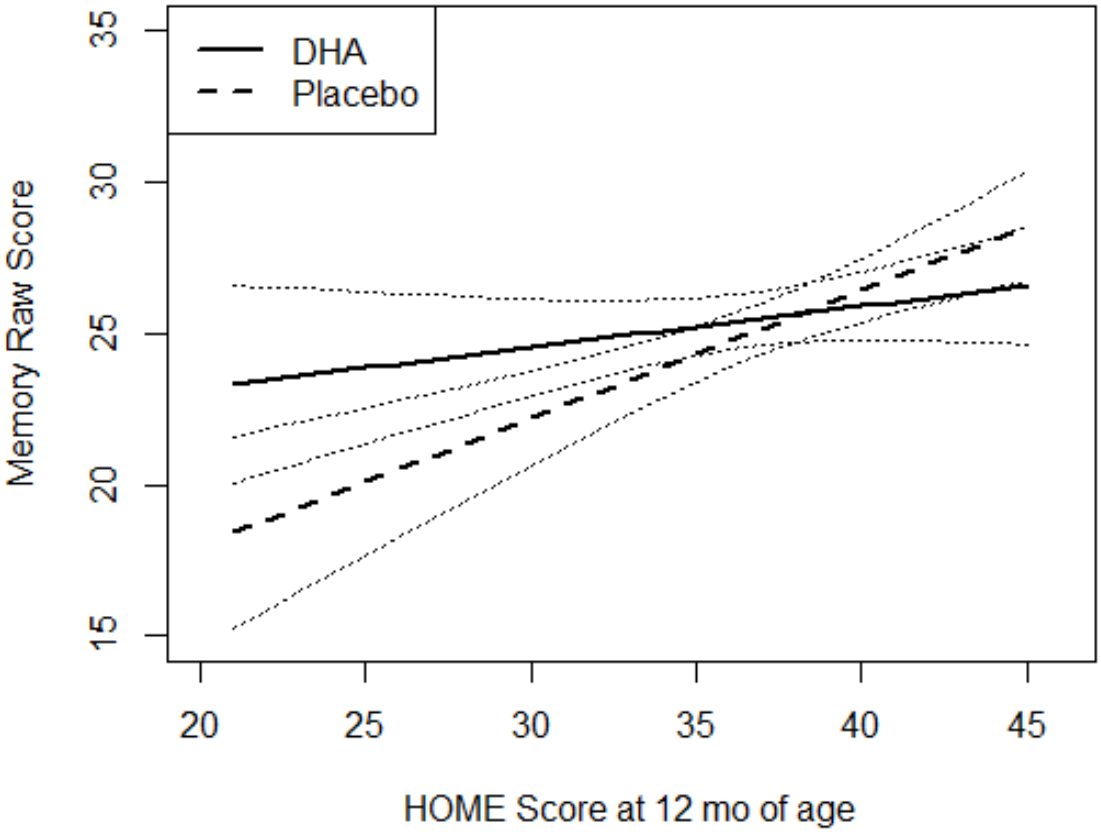
*p-value=0.08 for test of interaction between treatment group and home environment adjusting for gender and age of measurement

Figure 2: Relationship between HOME Score and Perceptual Performance Raw Score at 5 yr of age, by intervention group*



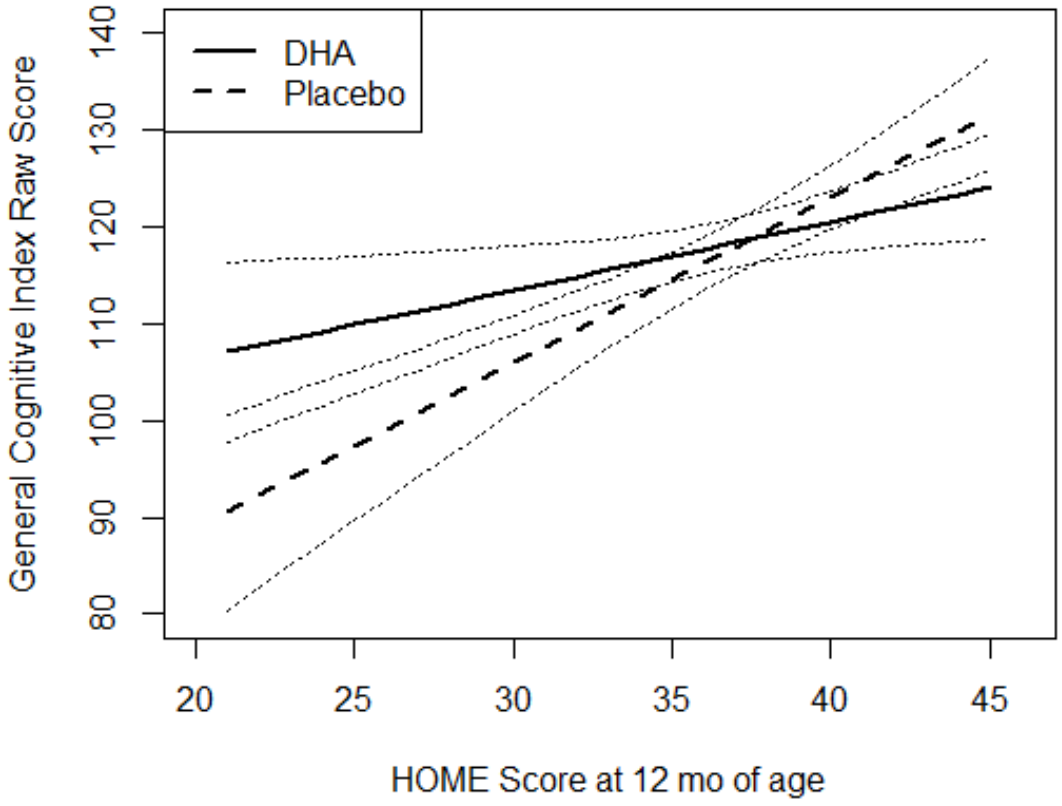
*p-value=0.06 for test of interaction between treatment group and home environment adjusting for gender and age of measurement

Figure 3: Relationship between HOME Score and Memory Raw Score at 5 yr of age, by intervention group*



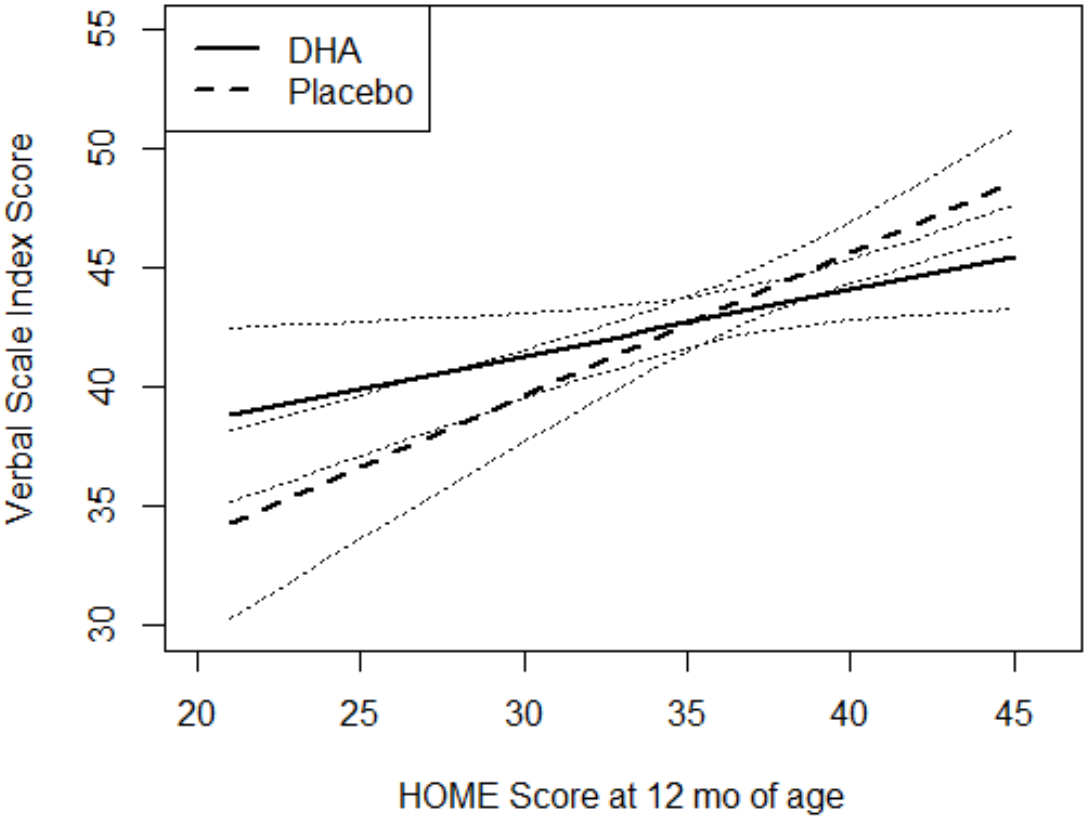
*p-value=0.07 for test of interaction between treatment group and home environment adjusting for gender and age of measurement

Figure 4: Relationship between HOME Score and General Cognitive Index Raw Score at 5 yr of age, by intervention group*



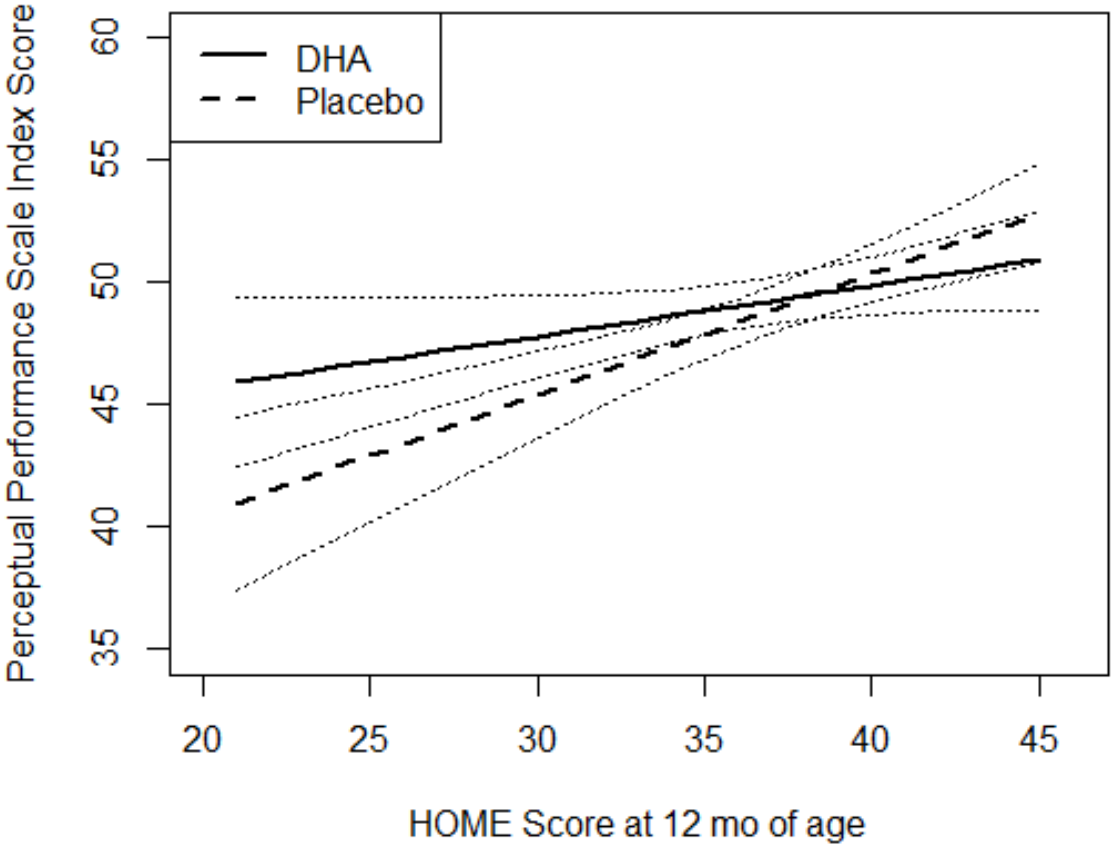
*p-value=0.03 for test of interaction between treatment group and home environment adjusting for gender and age of measurement

Figure 5: Relationship between HOME Score and Verbal Scale Index Score at 5 yr of age, by intervention group*



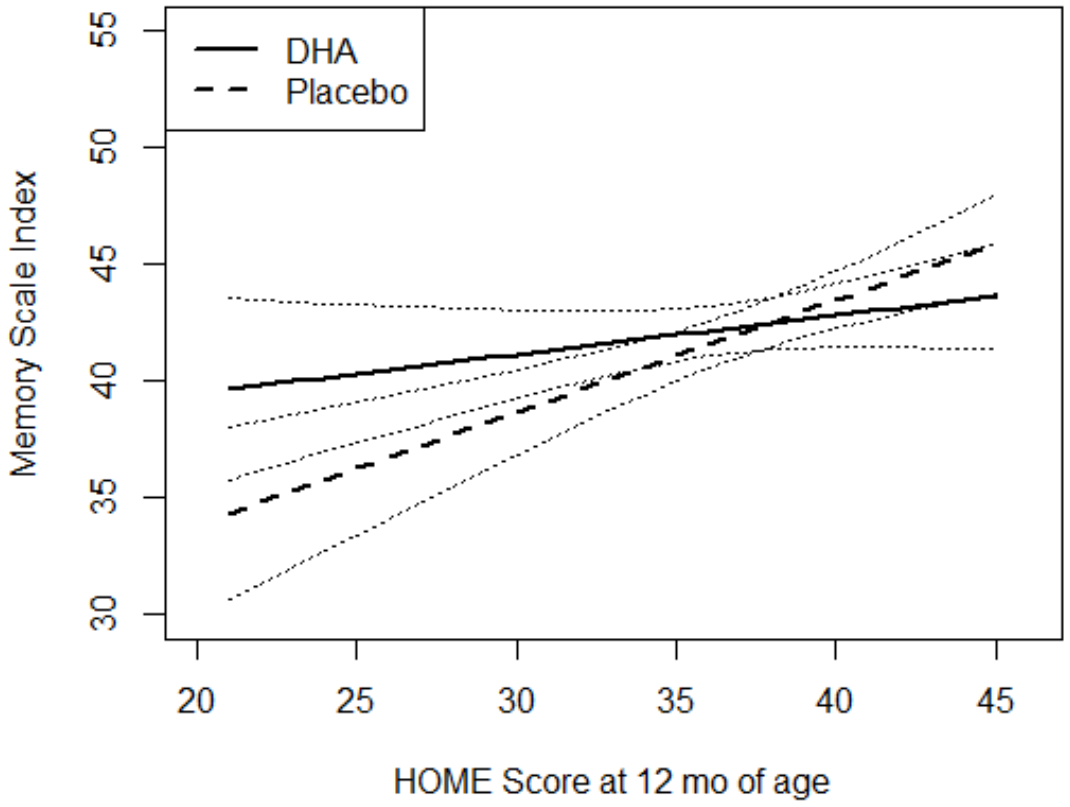
*p-value=0.10 for test of interaction between treatment group and home environment adjusting for gender and age of measurement

Figure 6: Relationship between HOME Score and Perceptual Performance Scale Index Score at 5 yr of age, by intervention group*



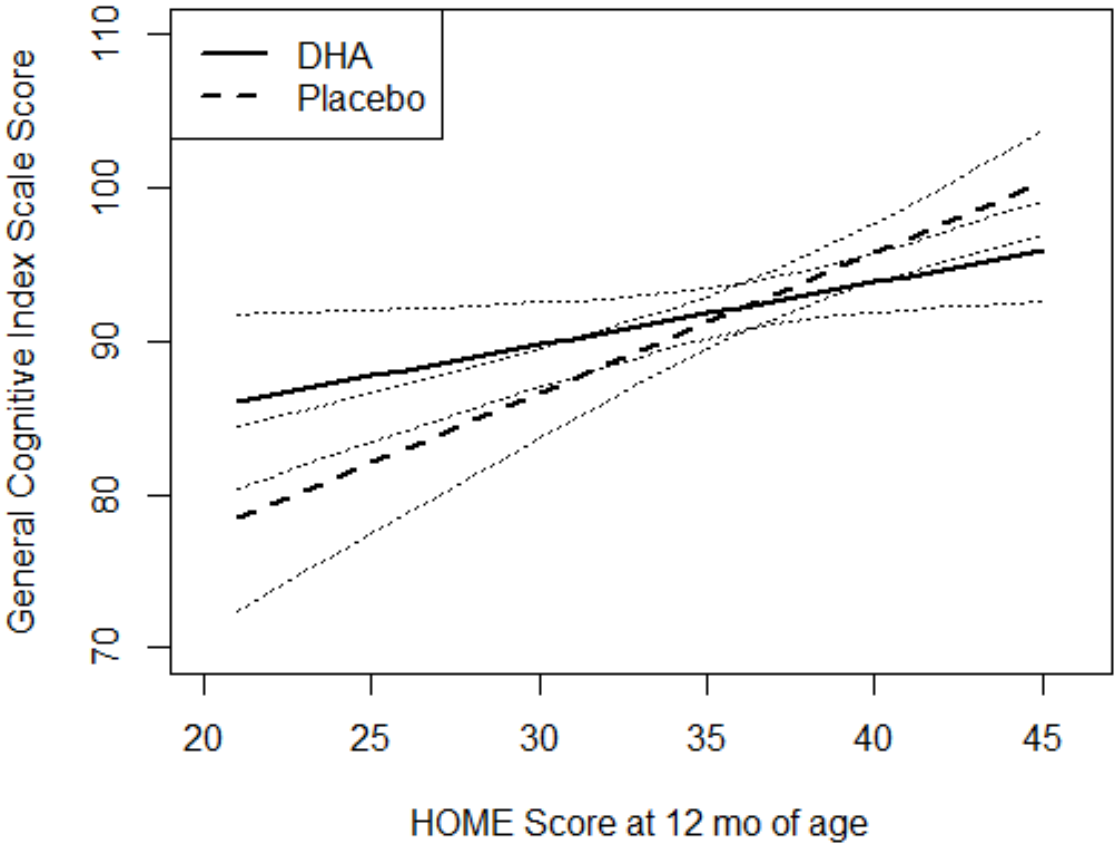
*p-value=0.06 for test of interaction between treatment group and home environment adjusting for gender and age of measurement

Figure 7: Relationship between HOME Score and Memory Scale Index at 5 yr of age, by intervention group*



*p-value=0.09 for test of interaction between treatment group and home environment adjusting for gender and age of measurement

Figure 8: Relationship between HOME Score and General Cognitive Index Scale Score at 5 yr of age, by intervention group*



*p-value=0.08 for test of interaction between treatment group and home environment adjusting for gender and age of measurement