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Chantalle Okondo Date

# Long Term Effects of Prenatal Docosahexaenoic Acid Supplementation on Early Childhood Growth in Mexico

By

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Master of Science in Public Heath

Hubert Department of Global Health

_____

Usha Ramakrishnan, PhD
Committee Chair

# Long Term Effects of Prenatal Docosahexaenoic Acid Supplementation on Early Childhood Growth in Mexico

By

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B.S.
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An abstract of
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2012

# Abstract

## Long Term Effects of Prenatal Docosahexaenoic Acid Supplementation on Early Childhood Growth in Mexico

By Chantalle Okondo

**Background:** Long chain polyunsaturated fatty acids such as docosahexaenoic acid (DHA) have been associated with improved outcomes during early infancy. Few studies have examined the longer lasting effects of prenatal DHA supplementation on growth and development in early childhood. A randomized double blind placebo controlled trial was conducted in Mexico to ascertain the effects of prenatal DHA supplementation on childhood outcomes. At the end of the initial study offspring of supplemented primagravid women were reported to have larger birth weights and larger head circumference at birth than offspring of women who received the placebo.

**Objective:** A further follow up study was conducted to examine the longer lasting effects of prenatal supplementation on growth measures of offspring from 18 through 48 months of age.

**Methods:** Pregnant women in Mexico were randomly assigned to receive either 400mg of algal DHA or a placebo during week 18 to 20 of gestation through delivery. The children were followed from 18 months to 48 months and anthropometric data (height and weight) were collected and analyzed. The main outcomes in the study included weight (kg), height (cm), body mass index (BMI), height for age z-scores and weight for height z-scores.

**Results:** Anthropometric measurements were obtained at 18, 24, 36 and 48 months for 732, 675, 351 and 711 of the 973 children respectively from the original study. The overall results indicated that both the intervention and control groups were similar from 18 to 48 months. The results from intention to treat analysis showed no differences between DHA and placebo. Comparison of average weight gain per month between 18-48 months revealed that offspring born to women who received prenatal DHA gained more weight compared to those born to women who received the placebo, however these differences were not statistically significant ($p>0.05$). Mixed effect regression estimates for the effect of the intervention and the interaction between treatment and maternal gravidity were not significant.

**Conclusion:** There were no long term significant effects of prenatal DHA supplementation on child growth between 18 months to 4 years of age.

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## Table of Contents

## List of Tables



## List of Figures

**INTRODUCTION:**

Deficient childhood growth and development are significant public health problems; currently 171 million children are stunted worldwide, 167 million (95%) of whom reside in developing countries (1), with an estimated 103 million children (18%) under five years of age being underweight (2). In Mexico, 16 % of children under five years are stunted (1), while only 3.4 % of children under five are underweight (2). While these numbers continue to decrease, it is important to maintain the downward trend by examining all possible factors related to early childhood growth. Research has shown that inadequate childhood growth during the first few years of life are associated with poor outcomes later in life such as poor educational performance, lost productivity, low adult wages etc. (3). Numerous studies have shown that interventions during the earliest stages of life are likely to have significant positive effects on child nutrition and consequently growth and development (4, 5). Some of the interventions conducted in Mexico have included: micronutrient supplementation during early childhood, that has resulted in increasing child size at 2 years of age for those who are highly compliant (6); conditional cash transfer programs such as Oportunidades, which have reported higher birth weights and improvements in linear growth of children in poor urban households (7, 8).

Recently, essential fatty acids known as long-chain poly-unsaturated fatty acids (LCPUFAs) have come to the forefront of pregnancy, lactation and infancy research (9). The two main classes of LCPUFAs consist of omega-3 and omega-6 fatty acids, which contribute primarily to the normal functioning of cell membranes and to growth and development in infants. Docosahexaenoic acid (DHA) is a long chain omega-3 fatty acid,

found in higher concentrations in the retina and brain; it is necessary for membrane functioning and the development of vision and cognitive functions in early childhood. DHA can be found preformed in breast milk, oil-rich fish and algae. The need for LCPUFAs increases during pregnancy due to fetal growth and maternal tissue expansion. LCPUFAs are transported through the placenta during pregnancy with the fetus retaining a high amount (50-60 mg/day) during the last trimester (10, 11). However, the amount of n-3 LCPUFAs—especially DHA—supplementation during pregnancy required to improve childhood growth and development remains unclear.

This particular study was a follow up to a randomized double blind placebo controlled trial conducted in Cuernavaca Mexico from February 2005 to February 2007 that examined childhood growth and development outcomes following prenatal supplementation of DHA (12). Expectant mothers received either 400 mg of algal DHA or the placebo. The pregnancies resulted in 968 live births and 5 stillbirths. At the end of the initial study there were no significant differences between intent to treat on weight, length and head circumference but the offspring of primagravid women who received DHA supplementation were heavier and had larger head circumferences at birth. Women classified as primagravid were younger, taller, had more schooling and had smaller infants than did multigravidae, therefor it was important to separate the two groups but despite this DHA status did not differ by gravidity. Growth to age 18 months was examined. It was found that offspring of supplemented primagravid women had an increased length at 18 months by 0.72cm (13).

The objectives of the follow-up study were to determine:

- How DHA supplementation during pregnancy influenced child growth during 18, 24, 36 and 48 months of age
- If the positive effects seen at birth and at length during 18 months among primagravid women remained after the intervention was discontinued

## LITERATURE REVIEW:

Maternal and child malnutrition is highly prevalent among children in developing countries; it influences poor growth and development outcomes in children and is the most important risk factor contributing to the burden of disease. Lipids are a major source of energy and can provide some of the essential nutrients needed to support appropriate growth and development. Omega-6 and omega-3 fatty acids are composed of linoleic acid (LA) and alpha - linolenic acid (ALA) respectively; they cannot be synthesized by the body and must therefore be consumed in the diet. The most common dietary sources for the fatty acids are vegetables and marine meat. It is important to note that omega-3 fatty acids compete with omega-6 fatty acids for the enzymes responsible for desaturation and elongation to form products such as DHA. Omega 3 fatty acids help reduce inflammation whereas most omega-6 fatty acids tend to promote inflammation. Imbalance in intake has been associated with numerous behavioral abnormalities as well as neurological and psychiatric disorders in children and adults (14, 15, and 16). The ratio of omega-6 to omega-3 in westernized diets is known to be as high as 15:1 (17). The ideal ratio is yet to be determined but the French Food Safety Agency recommends a 5:1 intake of omega-6: omega-3.

Arachidonic acid (AA) found in higher concentrations in erythrocytes, plasma and other tissues, is formed from LA and is an integral component of membranes and used as a substrate for eicosanoid synthesis. Similarly DHA and eicosapentaenoic acid (EPA) are formed from ALA; the predominant LCPUFAs in human tissues and human milk are AA and DHA (18). The main sources of DHA include animal products, algae and fatty fish such as tuna, salmon and fish oils. Human milk or infant formulas enriched with

LCPUFAs are the only sources of DHA for infants. Deficiencies in essential fatty acids result in poor growth and neurological abnormalities (19). During the weaning process, children no longer receive breast milk or formula exclusively. If complimentary foods do not provide adequate levels of DHA, the weaning child may be at increased risk for development deficiencies (20). The purpose of this literature review is to explore DHA intake among pregnant and lactating women and to examine the association between DHA supplementation during and after pregnancy, and childhood growth and development.

DHA intakes during pregnancy and lactation:

Several studies have examined dietary intake of long chain poly unsaturated fatty acids (LCPUFAs) during pregnancy and lactation. A review of the major findings from studies that examined DHA intakes and pregnancy outcomes are presented in the following section:

A cross sectional survey conducted in Cuernavaca Mexico by Parra-Cabrera et al ascertained LCPUFAs intakes in pregnant women at the Mexican Social Security Institute (IMSS) hospital using a food frequency questionnaire developed for our study population. They reported that among 1,364 pregnant women between 18-22 weeks of gestation, daily intakes of DHA were 55 (37; 99) mg which are much lower than the recommended values, with the median ratio of n-6 to n-3 PUFA as 11.8:1. The study reported the main dietary contribution to DHA intake were eggs, chicken and fresh canned fish. Intakes were higher among women who completed high school (21). The researchers concluded that DHA intakes among pregnant women in Cuernavaca, Mexico

were very low with very few food sources of DHA being consumed. Despite the large sample size, the data could not be generalized to the whole population mainly because the services offered to pregnant women at this particular hospital were not available to the rural poor or the self-employed and the wealthy generally chose private medical care. In different observational study also conducted in Mexico, the researchers ascertained that maternal intake of DHA was only 0.11mg/day for women seeking prenatal care at the hospital. However, the estimate was thought to be conservative considering the small sample size and suspected misclassification of LCPUFAs intake. (22). More research needs to be conducted to ascertain the correct intakes among pregnant women in developing countries.

Not all women consume low amounts of LCPUFAs. A prospective cohort study conducted in 3 European countries (Spain, Germany and Hungary) determined levels of DHA and folic acid intakes among 270 healthy women aged 18-41 years who were between weeks 12 and 20 of gestation. The study used a food frequency questionnaire that was specifically focused on sources of DHA and folic acid. The results indicated that Spanish participants had significantly higher intakes of DHA than Hungarian and German participants. However, despite the significant differences, nearly 90% of all women reached the DHA recommended intake of 200mg/d (24).

Despite lack of concrete evidence to confirm intakes of DHA, most pregnant women both in developing and developed countries are encouraged to supplement their diets to increase gestational period and therefore reduce the risk of premature birth. The current recommendation by the European Consensus Group is to consume at least 200 mg of

DHA daily (9) while the Food and Agriculture Organization (FAO) recommends 300 mg/day of EPA and DHA, of which 200mg must come from DHA (23).

Maternal DHA levels are dependent on pre-pregnancy status and dietary intakes. It may be essential to increase DHA intakes during pregnancy because LCPUFAs are transported through the placenta to the fetus and fetal accretion is relatively high during the last trimester (50-60 mg) (10, 11). Having multiple or closely-spaced pregnancies can create an even higher demand for maternal LCPUFAs (25, 26).

Women who suffer from postpartum depression are less likely to care for their children adequately in order to maintain appropriate growth and development. Research has been conducted on the relationship between DHA levels and postpartum depression. Results have been incongruous and indicate that DHA may or may not be associated with reduced prevalence of postpartum depression. A cross national ecological study conducted by Hibbeln et al found that higher concentrations of DHA in human milk and increased seafood intake predicted a lower prevalence of postpartum depression (27). On the other hand, recent intervention studies conducted in Mexico by Ramakrishnan et al and in Australia by Makrides et al did not show any relationship between DHA supplementation during pregnancy and reduced postpartum depression (12, 28).

During lactation, maternal DHA levels are highly variable (29, 30) and dependent on dietary intakes and fatty acid stores (31, 32). Women who consume low amounts of or no animal or seafood products and / or have a low energy diet are at risk for deficiencies. This is especially true in developing countries where low socio-economic status of households doesn't allow for adequate consumption of DHA in the diet (29, 30, and 33).

Mothers with low intakes depend on conversion of omega-3 fatty acids to the long chain derivatives of eicosapentaenoic acid (EPA) and DHA; research however has shown that conversion rates are relatively low and may not be sufficient to adequately meet the needs of the mother and infant. (34). Some studies have shown that lactating women supplemented with DHA see an increase in DHA levels in their breast milk (35). For example, a study conducted in the USA by Jensen et al supplemented 227 women with either 200 mg of algal DHA or vegetable oil with no DHA for 4 months after delivery. Their main outcome variables were maternal plasma phospholipid, milk lipids 4 months postpartum and the fatty acid pattern of plasma phospholipids of their infants. The study reported that the DHA content of milk lipid and infant plasma phospholipid of the supplemented and control groups were 75% and 35% higher respectively than baseline levels, 4 months postpartum (35).

More information is needed on DHA intake. The research shows that dietary intakes of DHA among pregnant and lactating women in several developing countries is highly variable and sometimes dependent on location; for example, women living near coastal areas have been reported to have higher DHA levels in their plasma and breast milk (36). Excluding those from coastal areas, most women in developing countries are less likely to consume a diet rich in DHA due to the low intake of animal and marine sources, putting them at increased risk for poor status and deficiencies during pregnancy and lactation. A direct relationship has been established with DHA intake and status both in mothers and infants shortly after supplementation but additional research is necessary in order to ascertain the long term effects of DHA intake during pregnancy and/or lactation on later stage growth and development.

Dietary DHA intakes of young children:

Infants and young children need DHA for normal growth and development (37) yet there is very little research regarding DHA intake among infants and children especially those at the preschool age. There are varying recommendations for DHA intake among young children; the FAO recommends 100 mg/d of DHA for those between the ages of 2 and 4 years (23). While the Institute of Medicine (IOM) recommends 63mg/d of omega-3 for infants, they do not have a recommendation for DHA alone (19).

For infants, especially those living in developing countries, breast milk is an important source of fat intake (38). Infants in rural Gambia were followed in an observational study from birth to 2 years of age with complimentary foods being introduced at 3 months. Consumption of fish featured regularly in the diet of mothers in small quantities while meat and milk were scarce. Complimentary foods that included thin gruels made from cereal and water, or on occasion, cow's milk were generally low in energy and fat. The results showed that the fat intake from breast milk was highest in the first 3 mo. As children got older, complimentary foods containing little fat replaced breast milk consumption. The percentage of energy from fat was initially >50%, but declined to 30% by 17 months. Once the infants were fully weaned—at ≈2 y of age—both fat intake and fat as a percentage of energy decreased substantially (38).

Low intakes of DHA aren't just observed in early childhood but in older children as well (8-10 years). In Guatemala the researchers found intakes to be very low in DHA and EPA regardless of socio-economic status, primarily due to low consumption of fish and seafood (39). These studies emphasized the importance of maintaining exclusive

breastfeeding up to one year as well as providing complimentary foods and main meals that are nutritionally adequate to promote growth and development. Children at the preschool age who are no longer dependent on breast milk, are at a higher risk for deficiencies in essential fatty acids especially DHA, which is known to deplete rapidly after weaning. It is important to examine whether supplementation in utero would have beneficial effects later in life when dietary intakes are inadequate.

Prenatal DHA supplementation on childhood outcomes:

During the last trimester of pregnancy and in early infancy, the human brain and eye undergo growth spurts that require increased amounts of DHA from the mother. Inadequate intake during pregnancy has been linked to impaired cognitive systems and impaired visual development (40). Approximately 400mg of omega-6 and 50 mg of omega-3 per kg of body weight are deposited daily in the infant during the last trimester (41). Newborns have been shown to have better DHA status than their mothers, indicating preference of DHA to the infants. For women with low intakes it is important to determine whether providing omega-3 or fish oil supplements versus preformed DHA can improve DHA status in both mothers and infants.

One study conducted by van Houwelingen et al in the Netherlands randomly supplemented healthy pregnant women with fish-oil capsules (n=23) containing omega-3 during week 30 of gestation until delivery while the control group received either no supplementation (n=10) or olive oil capsules (n=16). The study found that children born to mothers supplemented with fish oil capsules during their last trimester were born with better DHA status (42). Whereas another study in the Netherlands that randomly assigned

women to receive either margarine enriched with alpha-linolenic acid (ALA) or margarine without ALA from week 14 of gestation until delivery did not increase either maternal or infant plasma phospholipid DHA status (43). The conflicting results correspond to previous knowledge that conversion of DHA from parent omega-3 ALA is low and may not be adequate to influence child outcomes (11, 37). These results support the idea that interventions should focus on increasing preformed DHA intake rather than increasing omega-3 fatty acid intake.

Several studies both cross sectional and randomized controlled trials have looked at the relationship between fatty acid intake during pregnancy and growth and developmental outcomes in offspring. The majority have resulted in mixed findings (44, 45, 12), due to the type and amount of treatment provided, sample sizes and duration of treatment. Observational studies conducted in higher income countries show that increased consumption of fish during pregnancy increases birth weight (46) and visual and cognitive development in full term infants (47, 48). While a randomized control trial in higher income countries reported that increased DHA and EPA intake during pregnancy resulted in small increases in head circumference (49) there was no increase in birth weight or length (50), and it provided no effect on visual and cognitive development in infancy or later in life. Ramakrishnan et al did not report any effects on growth but did observe a positive interaction between maternal DHA supplementation and birth size among primagravid women (12). More research focusing on the association between birth outcomes and DHA alone is required.

Majority of the studies mentioned above suggest that increased DHA intake during pregnancy may result in improved gestational age, birth weight and birth length.

Developmental outcomes such as visual and cognitive measures have a strong association with prenatal DHA intake in developed countries but research has revealed mixed results in developing countries. With beneficial effects in infancy being observed, it is important to determine whether the effects continue later in life. There are very few studies that look at the long term effects of prenatal DHA supplementation on growth in preschool children, and according to our search, only three are being conducted in developed countries (51-53). Helland et al conducted a study in Norway and reported that pregnant and lactating women supplemented with omega-3 LCPUFAs promoted higher IQ scores at 4 years as compared with maternal supplementation with omega-6. They followed the children at 7 years of age and performed the same cognitive test performed at 4 years and examined the relationship between plasma fatty acids pattern and BMI in the children. The study did not observe any significant effects on IQ and BMI at 7 years of age but suggested that maternal concentration of omega-3 during pregnancy might be of important for later cognitive functions (51). Similar results were observed in the Campoy et al and van Goor et al studies where maternal supplementation during pregnancy did not influence neurodevelopment at 18 months (53) or cognitive function at 6.5 years of age (52). These results could be due to the duration or type of treatment and to small sample sizes.

Postnatal DHA supplementation on childhood outcomes:

As was mentioned earlier, breast milk is an important source for DHA in infants. DHA concentrations in breast milk have been positively associated with infant size at 5 months in Congo (54) perhaps due to high amounts of DHA in the diet. An observational study in Brazil found that increased intake of omega-3 LCPUFAs during lactation improved

growth in pre-term infants (55). In Norway the content of ALA in breast milk 4 weeks and 3 months after birth correlated positively with BMI. However, the benefits of DHA supplementation during pregnancy did not continue beyond infancy as no correlation was found with BMI at 7 years (51). Supplementation during lactation may improve infant growth, but more information is needed to determine intake guidelines for adequate childhood growth.

There have also been numerous studies that have examined formula fortified with DHA and its effects on child growth. In China Ben et al compared infants from four feeding groups: (1) AA plus DHA supplemented formula; (2) standard formula; (3) breast milk; and (4) breast milk plus supplemented formula. The study showed that there were no significant differences found in growth and development for the infants in any of the four feeding groups (56). A meta-analyses of well conducted RCT's from developing countries also showed that there were no beneficial effects of DHA plus AA supplementation of formula-milk on the anthropometric measurements (weight, length and head circumference) of infants born at term (57, 58 and 59). In contrast a study conducted by Udell et al found that children supplemented with alpha linolenic acid (ALA) enriched formula were heavier and longer compared to infants in the control group (60). There is very limited data from developing countries that shows a link between DHA supplementation and child growth especially during the preschool stage.

<u>DHA Supplementation of children on growth and development:</u>

Mixed results have been reported among studies that examine the effects of LCPUFAs supplementation in children. A study in Ghana aimed to test the hypothesis that multiple

micronutrients added to home-prepared complementary foods would increase growth and that the effect would be greatest in the presence of added energy from fat. Infants were randomly assigned to either receive sprinkles powder, crushable nutritabs tablets, or energy dense, fat based nutributter. The results indicated that increased consumption of the 3 supplements were associated with motor milestone acquisition at 12 months compared to those who received no intervention. Nutributter was the only supplement that was positively associated with growth (56). These outcomes could have been due to the fact that nutributter not only contains LCPUFAs but other essential vitamins and minerals that are positively associated with growth as well.

Three randomized controlled trials conducted in Dallas, Texas in the United States recruited a total of 229 infants to receive either formula supplemented with DHA and arachidonic acid (AA) or a control formula beginning at 1–5 days (for a 12-month feeding study), or following 6 weeks (for a 6-week-weaning study) or 4–6 months of breastfeeding (for a 4-to 6-month weaning study). The researchers used a 2-step problem solving task to ascertain cognitive development in the infants. They reported that in the 12-month feeding and 6-week weaning studies, supplemented children had more intentional solutions (successful task completions) and higher intention scores (goal-directed behaviors) than controls indicating positive effects of supplementation on cognitive development in children at 9 months. (62)

The same results were not observed in older children who are supplemented with LCPUFAs, regardless of study location, socio-economic status, and type and duration of supplementation provided. All the studies reviewed indicate that increasing LCPUFAs

intake of children older than 2 years of age has no effect on improvement of growth and development (63, 64, and 65).

In summary, DHA is most important during pregnancy, lactation and early childhood growth. DHA status in women is dependent on pre-pregnancy status and dietary intakes. Increased supplementation in mothers and infants has been linked to improved growth and development outcomes. However, more research is needed to ascertain long term effects of prenatal supplementation on growth in later childhood, especially for children at the preschool age who no longer depend on breast milk or fortified formulas to provide DHA.

# MANUSCRIPT

## Long Term Effects of Prenatal Docosahexaenoic Acid Supplementation on Early Childhood Growth in Mexico

**Okondo C A,**

## Contribution of Student

All secondary data was collected by the study team in Cuernavaca Mexico. I merged all the datasets from the different time points that they were collected, then cleaned and organized the data sets required for analysis. I generated all the tables and figures using SAS version 9.3 (Cary NC) with guidance from my thesis advisor. I obtained majority of the content in the methods section from previous studies that the project has already published with the main contribution coming from Ramakrishnan U, Stein A.D, Parra-Cabrera S, Wang M, Imhoff-Kunsch B, Juarez-Marquez S, Rivera J, and Martorell R, 2010. Effects of docosahexaenoic acid supplementation during pregnancy on gestational age and size at birth: Randomized, double-blind, placebo-controlled trial in Mexico. Food Nutr. Bull.2010;31:S108-16. I wrote the rest of the thesis content and manuscript.

## Abstract

### Long Term Effects of Prenatal Docosahexaenoic Acid Supplementation on Early Childhood Growth in Mexico

By Chantalle Okondo

**Background:** Long chain polyunsaturated fatty acids such as docosahexaenoic acid (DHA) have been associated with improved outcomes during early infancy. Few studies have examined the longer lasting effects of prenatal DHA supplementation on growth and development in early childhood. A randomized double blind placebo controlled trial was conducted in Mexico to ascertain the effects of prenatal DHA supplementation on childhood outcomes. At the end of the initial study offspring of supplemented primagravid women were reported to have larger birth weights and larger head circumference at birth than offspring of women who received the placebo.

**Objective:** A further follow up study was conducted to examine the longer lasting effects of prenatal supplementation on growth measures of offspring from 18 through 48 months of age.

**Methods:** Pregnant women in Mexico were randomly assigned to receive either 400mg of algal DHA or a placebo during week 18 to 20 of gestation through delivery. The children were followed from 18 months to 48 months and anthropometric data (height and weight) were collected and analyzed. The main outcomes in the study included weight (kg), height (cm), body mass index (BMI), height for age z-scores and weight for height z-scores.

**Results:** Anthropometric measurements were obtained at 18, 24, 36 and 48 months for 732, 675, 351 and 711 of the 973 children respectively from the original study. The overall results indicated that both the intervention and control groups were similar from 18 to 48 months. The results from intention to treat analysis showed no differences between DHA and placebo. Comparison of average weight gain per month between 18-48 months revealed that offspring born to women who received prenatal DHA gained more weight compared to those born to women who received the placebo, however these differences were not statistically significant ($p>0.05$). Mixed effect regression estimates for the effect of the intervention and the interaction between treatment and maternal gravidity were not significant.

**Conclusion:** There were no long term significant effects of prenatal DHA supplementation on child growth between 18 months to 4 years of age.

**INTRODUCTION:**

Essential fatty acids such as alpha linolenic acid (ALA) and linoleic acid (LA) cannot be synthesized in the body and have to be consumed in the diet; they are used to form omega-3 and omega-6 fatty acids respectively. Docosahexaenoic acid (DHA) is then derived from omega-3 fatty acids and belongs to the long chain polyunsaturated fatty acids (LCPUFA) family. LCPUFAs such as DHA have received a lot of attention in the last decade for their roles during pregnancy, lactation and infancy (1). The nutritional demands during pregnancy for DHA are increased mainly due to high accretion by the fetus in the third trimester, (2, 3) and their accumulation in the brain and retina membranes of infants that aid in visual and neurological functions (4, 5). The demands are solely met by maternal stores or maternal dietary intake during pregnancy (6). A cross sectional survey conducted in Mexico determined intakes among pregnant women to be much lower than the recommended amounts mainly because of low consumption of foods rich in DHA such as animal sources and marine life (7).

Both observational and randomized controlled trial studies have shown that prenatal DHA supplementation is associated with cognitive functions early in life (8 -12) but the link between DHA supplementation and growth in early childhood have produced mixed results (10, 13, 14). One study in particular reported that increased intakes of DHA can be limiting to the growth of infants and young children (15). The intervention supplement examined in that study included not only DHA, but other constituents of fish oil as well. It is unclear as to whether DHA was indeed the cause of the limited growth, or if other factors were responsible. In spite of the benefits of prenatal DHA supplementation on birth outcomes and early infancy, there have been very few studies that have examined

long term effects of prenatal DHA supplementation on the growth and development of young children, specifically in developing countries where maternal and child malnutrition is highly prevalent.

### Study Background

The study is a follow-up to a randomized double blind placebo controlled trial in Cuernavaca, Mexico. A total of 1,094 women were randomly assigned to two groups and 1,040 began treatment, expectant mothers received either a daily supplement of 400 mg of algal DHA or a placebo during the second half of their pregnancy to delivery. Birth data were available for 973 pregnancies; the results reported no difference in intent to treat between the two groups but that DHA may have increased birth size among supplemented primagravid women (16). They also examined growth to age 18 months of the offspring and found children born to primagravid women who received prenatal DHA supplements were longer compared to the control group (17).

The main objective of this follow up study was to determine whether the benefits of prenatal DHA supplementation in improving size of supplemented primagravid women had any effect on growth of young children between 18 through 48 months after the intervention was discontinued.

## METHODS:

### Study site and experimental design

The study was conducted in collaboration with the Instituto Nacional de Salud Publica (INSP) in Cuernavaca and the Mexican Institute of Social Security (IMSS) General

Hospital 1 and the Hubert Department of Global Health, Rollins School of Public Health, Emory University Atlanta Georgia, USA. The study protocol was approved by the Emory University Institutional Review Board and by the Instituto Nacional de Salud Publica Research, Biosafety and Ethics Commission. Written consent was obtained from all the women for themselves and their infants before enrollment into the study.

**Study Setting:**

Study participants were recruited at the Mexican Institute of Social Security (IMSS) General Hospital 1, a large hospital which is located in Cuernavaca, Mexico and 3 small health clinics within the IMSS system in Cuernavaca during routine prenatal care visits between February 2005 and February 2007. Generally the women who use the hospital are of medium to low socio-economic status and either they and/or their husbands are employed. In majority of the cases, a patient at IMSS pays one third of their healthcare cost while their employer and the federal government pays the remaining two thirds of the cost.

**Sample size:**

The study had previously estimated that a final sample of 338 infants per group would have at least 90% power to detect an effect size of 0.25 SD or greater for the major outcomes at the end of the study assuming a significance level of alpha = 0.05 for a two-tailed test. This sample size would allow us to detect differences in weight of 0.43kg (0.2 SD), length of 0.87cm (0.2 SD) and BMI (kg/m2) of 0.29 (0.2SD) with at least 80% power.

**Eligibility Criteria:**

Eligible women were between 18-35 years, in gestation week 18-22 and planned to deliver at the IMSS General Hospital in Cuernavaca, exclusively or predominantly breast-feeding for at least 3 months and planned to live in the area for at least 2 years after delivery. Women were excluded if any of the following criteria were present: high risk pregnancy [history and prevalence of pregnancy complications, including placental abruption (separation of the placenta from its attachment to the uterus wall before the baby is delivered) , preeclampsia, pregnancy induced hypertension, any serious bleeding episode in the current pregnancy, and /or physician referral]; lipid metabolism or absorption disorders; regular intake of fish oil or DHA supplements; or chronic use of certain medication (e.g. medications for epilepsy). For the sub sample of this follow up study any child that had a measurement for weight and/or height at 18, 24, 36 and 48 months of age was included in the analytical sample.

**Prenatal Supplementation:**

Women were randomized to receive either 400mg of algal DHA daily or placebo until delivery. Study participants and members of the study team remained unaware of the treatment scheme throughout the intervention period and follow-up period of the study. The supplements (Martek Biosciences) were in color coded bottles (2 colors/treatment arm) and were distributed by trained field workers during weekly visits at the participant's homes and/or work place. The DHA capsules contained 200 mg DHA each derived from algal source. The placebo capsules contained olive oil and were similar in appearance and taste to the DHA capsules. Women were instructed to take 2 capsules

daily, together at the same time each day. During each weekly home visit, participants received 14 capsules in a precoded container; the capsules remaining from the prior visit were counted. Supplements were provided for more than 1 week in cases where the participants planned to travel. Women ceased supplement ingestion at delivery.

**Compliance:**

Compliance was calculated as the total number of capsules actually consumed during pregnancy expressed as a percentage of the total number expected to be consumed. The 858 women whose children were followed to 48 months consumed 228 capsules, on average, representing 95% compliance in both intervention and control groups. DHA concentrations in maternal plasma at delivery and cord blood were higher ($p<0.05$) in the intervention group compared with the control group in a random subsample of the study participants (16). Additional details regarding the original study are described elsewhere (16).

**Data Collection:**

All anthropometric measurements were obtained by trained study personnel at the study headquarters located at IMSS General Hospital 1 using standardized equipment and techniques (16). Birth weight was measured using a pediatric scale to the nearest 10g. Birth length and head circumference were measured to the nearest 1 mm using a portable anthropometer with a fixed head piece and a flexible tape, respectively. We obtained weight and length / height at 18, 24, 36 and 48 months. . Children that were measured a few weeks before their second birthday were measured with a standing height and not recumbent length. We measured length and height (each to 1mm) using a calibrated

length and height board and weight (to 10g) using pediatric scales (18). We calculated age at measurement from the date of birth and the date of measurement and computed BMI (kg/m2).

**Data Analysis:**

Anthropometric data was measured and available at 18, 24, 36 and 48 months of age for the children that were retained at follow up from the original study. All analyses were implemented in SAS version 9.3 (Cary NC). Length measurements for 18 months were converted to height measurements by subtracting 0.7 cm (19). The anthropometric measurements for 24, 36 and 48 months were converted to Z-scores using the 2006 WHO reference standards (20). The follow-up data were merged with the data from the original study. We first compared intervention and control groups on maternal characteristics at randomization and infant characteristics at birth. To identify potential sources of selection bias, we compared the final analytic sample to those infants lost to follow-up on several baseline characteristics. We used Student's t-test for normally distributed continuous variables and chi-square tests for categorical variables.

Our main outcome measures included height (cm), height for age z-scores (HAZ), weight (kilograms), weight for height z-scores (WHZ), BMI (kg/m2) and BMI for age z-scores (BMIZ) among singleton births. All analyses were done following the intention to treat design. We controlled for child sex, age at measurement and maternal height (given the importance of maternal size as a predictor of child growth) to estimate adjusted differences between groups and their 95% confidence intervals using general linear models. We calculated change in weight and height measures to detect differences

between the intervention and control group, this was done by dividing the difference of any two measurements by time in months. The average weight gain per month was obtained for children with any two measurements at (18,24,36 or 48 months) and for those with measurements at (18, 24 or 48 months) we excluded the sample size at 36 months due to the low rate of follow up at that time period.

For analysis of repeated measures of height (cm), weight (kg), HAZ, WAZ, WHZ and BMIZ, the mixed-effects regression model was used to evaluate the effects of the intervention, controlling for child age and sex at measurement, birth weight and maternal height. This method allowed for repeated measures on each child while accounting for the considerable variation across children in overall maternal and infant characteristics. As previously reported, maternal gravidity modified the effect of DHA supplementation on weight and head circumference (16), and in the follow- up study the interaction was significant for weight ($p<0.08$), length ($p<0.02$), HAZ ($p<0.02$) and head circumference ($p<0.05$) (17). To examine whether this interaction persisted through 48 months, we examined the estimates of differences between intervention and control groups on our main outcome indicators. A p-value of less than 0.05 was considered statistically significant for all tests.

## RESULTS:

The original study reported that of the 1,094 women randomized, 1040 started treatment and 973 completed the study. Five had stillbirths and 968 delivered 973 live born infants: 963 singletons and 5 pairs of twins (16). Anthropometric measurements were obtained for 858 children out of 973 (88.1%) from the original birth cohort, the 858 children were

those who had at least one measurement between 18-48 months of age. The children who were lost to follow up didn't differ by intervention or by maternal or infant characteristics. The comparison of several maternal characteristics at randomization and infant characteristics at birth also didn't differ between the two groups and are presented in Tables 1 and 2. The mean values for weight, height and BMI at 18, 24, 36 and 48 months of age are shown in Table 3. At 48 months children whose mothers had received the placebo had an unadjusted weight of 16.18kg (+/-) 2.25kg, whereas children whose mothers received DHA supplementation had an unadjusted weight of 16.40kg (+/-) 2.55kg. There were no statistically significant differences between the groups ($p>0.05$). When evaluating the trend in average unadjusted HAZ from 18 to 48 months, we found no difference in rate of growth or initial status at 18 months between DHA and placebo (Figure 1). The trend for BMIZ also illustrated that both groups were similar. Initial status was the same at 18 months with the intervention group dropping slightly below the placebo around 24 months, but eventually increasing and surpassing the placebo group at 48 months (Figure 2).

The results of the intent to treat are shown in Table 4; there were no differences between the two groups (DHA – placebo) on weight 0.22kg (-0.12, 0.56) and height 0.230cm (-0.31, 0.77) at 48 months after adjusting for maternal height, child sex and age at measurement. When examining the average weight gain per month, it appears that children in the intervention group with measurements at 18, 24, 36 or 48 months had gained more weight per month 189.5g (+/-) 63.6g as compared to the control group 183.5g (+/-) 52.9g ($p>0.05$). Taking into account the smaller sample size of children measured at 36 months, we calculated the average weight gain per month for children

with anthropometric measurements at 18, 24 and 48 months. It appears that those in the intervention group gained 195.2kg (+/-) 70.3 kg per month compared to control group who gained 188.6kg (+/-) 58.3 kg per month; however, this difference was not statistically significant. The average height gain per month for both sample groups was similar (Tables 5 and 6). The results of the mixed effects regression analysis for various growth outcomes are presented in Table 7. The regression coefficients for supplementation were not significant for any of the growth outcomes after adjusting for maternal height, child's age and sex at measurement and birth size ($p > 0.05$). Because previous results indicated significant interaction with maternal gravidity on length at 18 months, mixed effects regression analysis was conducted with the interaction term of treatment by gravidity (Table 8). No effect of maternal DHA supplementation was observed for children born to primagravid and multigravid women. Estimates for all the main outcomes except BMIZ, were in the same direction for both sub groups though they were not significantly different.

## DISCUSSION:

The study was conducted in Cuernavaca, Mexico and previous research indicated that dietary intakes of DHA were particularly lower at 55mg/d (14), than the recommended amount of 200 mg/d (1). The results show the compliance rate of the study intervention for women whose children were followed to 48 months was very high and was similar between treatment groups.

The intervention resulted in increased maternal plasma concentrations of DHA in cord blood at delivery (16) and significantly higher concentrations of DHA and ALA in breast milk at 1 month postpartum following prenatal supplementation (21). Breast milk

concentration is particularly important to this specific study population because in the overall study, over 95% of the mothers breast-fed with a mean duration of 10 months. Like in the original study, maternal characteristics at randomization and infant characteristics at birth were similar for both intervention and control groups. However, the offspring of primagravid women who received DHA were heavier and had a larger head circumference. When investigating postnatal growth at 18 months, the interaction with gravidity did not hold for birth weight and head circumference but a significant interaction on length was reported (17).

We reported that anthropometric measures at 18, 24, 36 and 48 months were similar across the board, but that children in the intervention group were slightly heavier and gained more weight per month than those in the placebo group. The difference however, was not significant.

To compare our results we reviewed other studies that were conducted in developed countries; we found just 3 main studies that reported on growth measurements later on in childhood after prenatal supplementation was discontinued. They included one study in the Netherlands where they conducted a double blind placebo controlled trial and randomly assigned 183 women between their $14^{th}$ to $20^{th}$ week of gestation to receive DHA 220 mg/day, DHA+ Arachidonic Acids (AA) 220 mg/day or placebo. They followed 114 children up till 18 months and didn't observe any difference in weight and height measures (22). Another study conducted by Helland et al in Norway randomly assigned 590 pregnant women during week 18 of gestation to receive either 10 ml of cod liver oil (which contained 1183 mg/10 mL DHA, 803 mg/10 mL EPA (20:5 n-3), and a total of 2494 mg/10 mL $\sum$ n-3 PUFAs) or corn oil until 3 months after delivery (33, 34).

Three hundred and forty one mothers in total took part in the study until giving birth, with 143 children being followed up to 7 years of age, the researchers in this study did not observe any differences between the groups on weight and height measurements (23). Lastly a study conducted in 3 European centers in Spain, Germany and Norway had 270 pregnant women in their 20$^{th}$ week of gestation, randomly assigned to receive a daily supplement of 500 mg (DHA) + 150 mg EPA [fish oil (FO)], 400 μg 5-methyltetrahydrofolate (5-MTHF), or both or a placebo. (24) They obtained data from 154 children at 4 years of age at follow up and also observed no significant differences in height and weight.

In comparison to all the above RCT's our study had a much larger sample size with over 800 children followed between the ages of 18 through 48 months, we supplemented women solely with preformed DHA from algal sources as compared to the other studies that used supplements from fish oil which contained other constituents. Our intervention period was conducted during the gestation period alone and was carried out in Mexico, a developing country undergoing an epidemiologic transition where obesity rates among children are increasing (25). In spite of the major differences between the studies, we arrive at the same results, that prenatal supplementation with DHA does not influence child growth beyond infancy.

One of the main limitations in the study was the unbalanced data or the lack of data during certain time points, especially at 36 months. The inconsistency in follow up could have reduced our power and effect size and comprised our ability to detect a significant difference. Data on dietary intake of children was collected and could have contributed to the understanding of the outcomes but has yet to be analyzed. However because both

groups are similar in socio economic status and because randomization in the study was considered to be successful, the likelihood of a significant difference in dietary DHA intake between the two groups is minimal.

The lack of long-term effects on growth could be attributed to several factors. The original study only observed heterogeneity among offspring of primagravid women, and the difference in birth weight was determined to be about 100-200g and there was no observed difference between groups on birth weight and length. The differences observed early on could have subsided with maturation of children in the study. Due to the imbalance of omega-6 and omega-3 fatty acids in the diet of the pregnant women (7), maternal stores of DHA would have been significantly lower because the enzymes required for DHA conversion would not have been present at sufficient amounts. The children may have had underlying risk factors not addressed by the study that could have influenced growth—such as micronutrient malnutrition. Improvements in early childhood growth may be most easily achieved by implementing small changes in the daily maternal and child dietary intakes of DHA versus supplementing with moderate doses later in pregnancy.

Our study allowed us to examine prenatal DHA supplementation without having to consider EPA or other constituents in fish oils (that are commonly used to increase intake of LCPUFAs) that may be limiting to growth. We also identified no harmful or beneficial long term effects from increasing DHA in the maternal diet. Because the main effects were observed during gestation and directly after birth, optimal doses for improving growth among children during the preschool age are still unknown and require further research. In conclusion a double blind randomized controlled trial that supplemented

women with 400 mg/d of algal DHA from 18-22 weeks of gestation through delivery had no significant effects on growth of their offspring after the intervention was discontinued.

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**Table 1: Maternal Characteristics at randomization among 858 children with measurements at 18, 24, 36 or 48 months at follow-up by treatment group[12]**

| Variables | n | Placebo | n | DHA | P value |
|---|---|---|---|---|---|
| Age, y | 430 | 26.3 (+/-) 4.6 | 428 | 26.4 (+/-) 4.9 | 0.56 |
| Weight, kg | 376 | 63.5 (+/-) 11.1 | 396 | 62.2 (+/-) 11.6 | 0.12 |
| Height, cm | 376 | 155.6 (+/-) 5.6 | 396 | 154.9 (+/-) 5.8 | 0.06 |
| BMI, kg/m2 | 376 | 26.2 (+/-) 4.3 | 396 | 25.9 (+/-) 4.2 | 0.32 |
| Gestational age, wk. | 430 | 20.6 (+/-) 2.1 | 428 | 20.6 (+/-) 1.9 | 0.92 |
| Socio-economic status | 430 | 0.0 (+/-) 1.0 | 428 | 0.0 (+/-) 1.0 | 0.78 |
| Ravens score | 430 | 41.0 (+/-) 9.3 | 428 | 41.0 (+/-) 9.0 | 0.67 |
| High school or more % | 430 | 60.8 | 428 | 55.8 | 0.14 |
| Primagravid % | 430 | 38.1 | 428 | 35.1 | 0.35 |

[1] Values are mean (+/-) SD or percent.
[2] t test for comparison of means and chi-square test for comparison of proportions

**Table 2: Characteristics at birth among 858 children with measurements at 18, 24, 36 or 48 months at follow-up by treatment group[12]**

| Variables | n | Placebo | n | DHA | P-value |
|---|---|---|---|---|---|
| Weight gm. | 376 | 3239 (+/-) 450 | 396 | 3243 (+/-) 436 | 0.90 |
| Length cm | 375 | 50.6 (+/-)2.1 | 396 | 50.4 (+/-) 2.2 | 0.22 |
| Head circumference, cm | 324 | 34.3 (+/-)1.7 | 338 | 34.5 (+/-) 1.5 | 0.32 |
| Low birth weight % | 376 | 3.5 | 396 | 4.0 | 0.67 |
| Gestational age wk. | 428 | 39.2 (+/-) 1.6 | 427 | 39.1 (+/-) 1.8 | 0.62 |
| Preterm % | 428 | 7.5 | 427 | 9.4 | 0.32 |
| Sex % | 430 | 53.3 | 428 | 53.7 | 0.89 |

[1] Values are mean (+/-) SD or percent.
[2] t test for comparison of means and chi-square test for comparison of proportions

**Table 3: Anthropometric measures among 858 children at follow-up by treatment group[12]**

| Outcome Variable | n | Placebo | n | DHA | P-value |
|---|---|---|---|---|---|
| Weight, kg at 18 months | 368 | 10.41 (+/-) 1.19 | 364 | 10.41 (+/-) 1.14 | 0.92 |
| Weight, kg at 24 months | 335 | 12.64 (+/-) 1.99 | 340 | 12.56 (+/-) 1.84 | 0.61 |
| Weight, kg at 36 months | 172 | 14.06 (+/-) 1.72 | 179 | 14.25 (+/-) 1.93 | 0.32 |
| Weight, kg at 48 months | 342 | 16.18 (+/-) 2.25 | 369 | 16.40 (+/-) 2.55 | 0.23 |
| | | | | | |
| Height, cm – 18 months | 368 | 78.84 (+/-) 2.81 | 364 | 78.91 (+/-) 2.77 | 0.74 |
| Height, cm – 24 months | 335 | 88.66 (+/-) 5.28 | 339 | 88.63 (+/-) 5.19 | 0.94 |
| Height, cm – 36 months | 171 | 94.79(+/-) 3.75 | 179 | 95.19 (+/-) 4.01 | 0.3 |
| Height, cm – 48 months | 342 | 102.1(+/-) 4.21 | 371 | 102.3 (+/-) 4.45 | 0.48 |
| | | | | | |
| BMI at 18 months | 368 | 16.42 (+/-) 1.23 | 364 | 16.38 (+/-) 1.13 | 0.66 |
| BMI at 24 months | 335 | 16.00(+/-) 1.34 | 339 | 15.92 (+/-) 1.27 | 0.43 |
| BMI at 36 months | 171 | 15.62 (+/-) 1.17 | 179 | 15.68 (+/-) 1.31 | 0.66 |
| BMI at 48 months | 342 | 15.48 (+/-) 1.40 | 369 | 15.60 (+/-) 1.55 | 0.29 |

[1] Values are mean (+/-) SD.
[2] t test for comparison of means - unadjusted model

**Table 4: The between group intent to treat differences (DHA-Placebo) among 858 children during follow up[1]**

| Outcome Variables | Adjusted Difference (95% CI) |
|---|---|
| Weight, kg at 18 months | -0.02 (-0.18, 0.15) |
| Weight, kg at 24 months | -0.07 (-0.30, 0.15) |
| Weight, kg at 36 months | 0.19 (-0.18, 0.56) |
| Weight, kg at 48 months | 0.22 (-0.12, 0.56) |
| | |
| Height, cm – 18 months | 0.002 (-0.39, 0.40) |
| Height, cm – 24 months | -0.034 ( -0.42,0.49) |
| Height, cm – 36 months | 0.402 (-0.31, 1.11) |
| Height, cm – 48 months | 0.230 (-0.31, 0.77) |
| | |
| WAZ at 18 months | -0.04 (-0.17, 0.10) |
| WAZ at 24 months | -0.05 (-0.19, 0.10) |
| WAZ at 36 months | 0.06 (-0.15, 0.26) |
| WAZ at 48 months | 0.07 (-0.08, 0.21) |

| | |
|---|---|
| HAZ at 18 months | -0.03 (-0.17, 0.11) |
| HAZ at 24 months | -0.02 (-0.16, 0.11) |
| HAZ at 36 months | 0.04 (-0.15, 0.22) |
| HAZ at 48 months | 0.03 (-0.09, 0.16) |
| | |
| WHZ at 18 months | -0.03 (-0.16, 0.10) |
| WHZ at 24 months | -0.06 (-0.20, 0.09) |
| WHZ at 36 months | 0.04 (-0.16, 0.24) |
| WHZ at 48 months | 0.07 (-0.08, 0.22) |
| | |
| BMIZ at 18 months | -0.02 (-0.15, 0.11) |
| BMIZ at 24 months | -0.06 (-0.20, 0.09) |
| BMIZ at 36 months | 0.04 (-0.16, 0.24) |
| BMIZ at 48 months | 0.08 (-0.07, 0.23) |

[1] Model adjusted for maternal height, child sex and age at measurement.

**Table 5: Average weight and height gain per month for children with measurements at 18, 24, 36 or 48 months by treatment group[1, 2]**

| Variables | n | Placebo | n | DHA | P-value |
|---|---|---|---|---|---|
| Weight gm. | 358 | 183.5 (+/-) 52.9 | 371 | 189.5 (+/-) 63.6 | 0.17 |
| Height cm. | 358 | 0.74 (+/-) 0.10 | 371 | 0.74 (+/-) 0.09 | 0.77 |

[1] Values are the difference between any two observations for weight and height divided by amount of time passed in months between the two observations.
[2] Values are mean (+/-) SD, t test for comparison of means

**Table 6: Average weight and height gain per month for children with measurements at 18 24 or 48 months of age by treatment group[1, 2]**

| Variables | n | Placebo | n | DHA | P-value |
|---|---|---|---|---|---|
| Weight gm. | 329 | 188.6 (+/-) 58.3 | 328 | 195.2 (+/-) 70.3 | 0.19 |
| Height cm. | 329 | 0.79 (+/-) 0.13 | 327 | 0.79 (+/-) 0.12 | 0.79 |

[1] Values are the difference between any two observations for weight and height divided by amount of time passed in months between the two observations.
[2] Values are mean (+/-) SD, t test for comparison of means

**Table 7: Effects of DHA on weight, height, height for age, weight for age and weight for height z-scores among 858 children during 18 to 48 months of age**

| | **Estimate** | **95% CI** | **P-value** |
|---|---|---|---|
| Weight, kg | 0.06 | -0.17, 0.30 | 0.60 |
| Height, cm | 0.11 | -0.38, 0.62 | 0.64 |
| HAZ | 0.02 | -0.09, 0.14 | 0.69 |
| WAZ | 0.02 | -0.10, 0.14 | 0.76 |
| WHZ | 0.01 | -0.12. 0.14 | 0.88 |
| BMIZ | 0.01 | -0.11, 0.13 | 0.87 |

[1] Estimates are differences between DHA and Placebo groups and are derived from mixed effects regression models. Controlling for maternal height, child's age and sex at measurement and birth weight 95% CI are presented

**Table 8: Effects of DHA on anthropometric measurements among 858 children during 18 to 48 months of age stratified by parity**

| | Primagravid | | | Multigravid | | |
|---|---|---|---|---|---|---|
| | **Estimate** | **95% CI** | **P-value** | **Estimate** | **95%CI** | **P-value** |
| Weight, kg | 0.32 | -0.07, 0.71 | 0.11 | -0.06 | -0.35, 0.23 | 0.69 |
| Height, cm | 0.64 | -0.20, 1.47 | 0.14 | -0.10 | -0.73, 0.53 | 0.75 |
| HAZ | 0.09 | -0.10, 0.29 | 0.34 | -0.01 | -0.15, 0.14 | 0.94 |
| WAZ | 0.11 | -0.09, 0.31 | 0.23 | -0.02 | -0.17, 0.13 | 0.77 |
| WHZ | 0.11 | -0.11, 0.33 | 0.32 | -0.04 | -0.21, 0.12 | 0.62 |
| BMIZ | 0.09 | -0.12, 0.29 | 0.40 | -0.03 | -0.18, 0.12 | 0.71 |

[1] Estimates are differences between DHA and Placebo groups and are derived from mixed effects regression models. Controlling for maternal height, child's age and sex at measurement and birth weight 95% CI are presented

**Figure 1:**

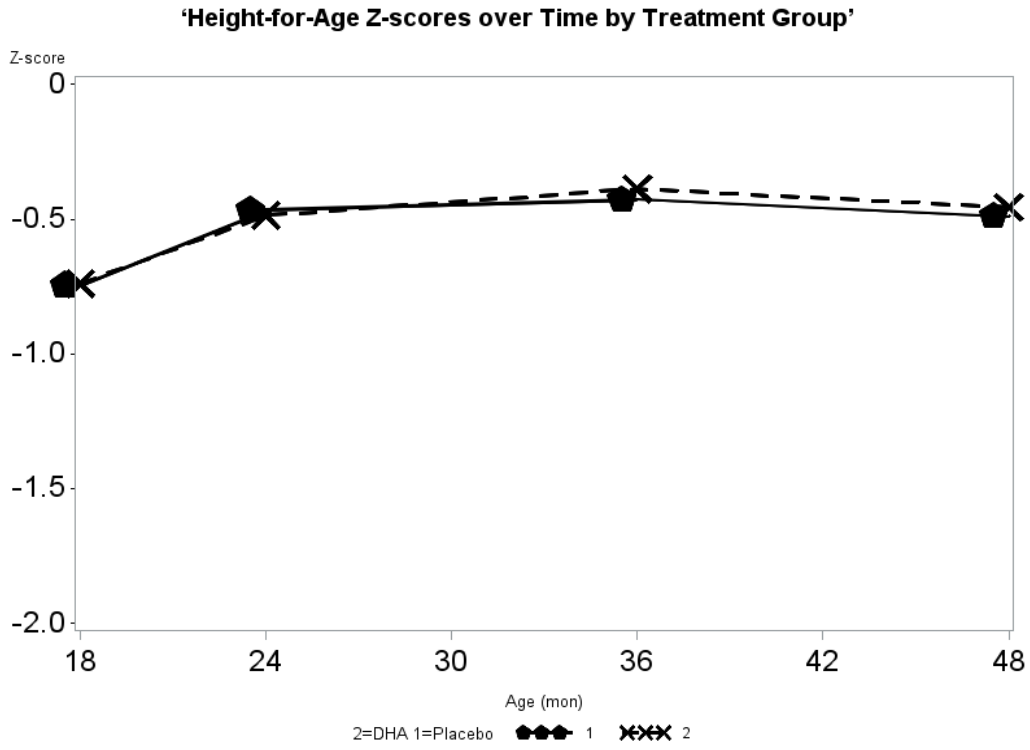
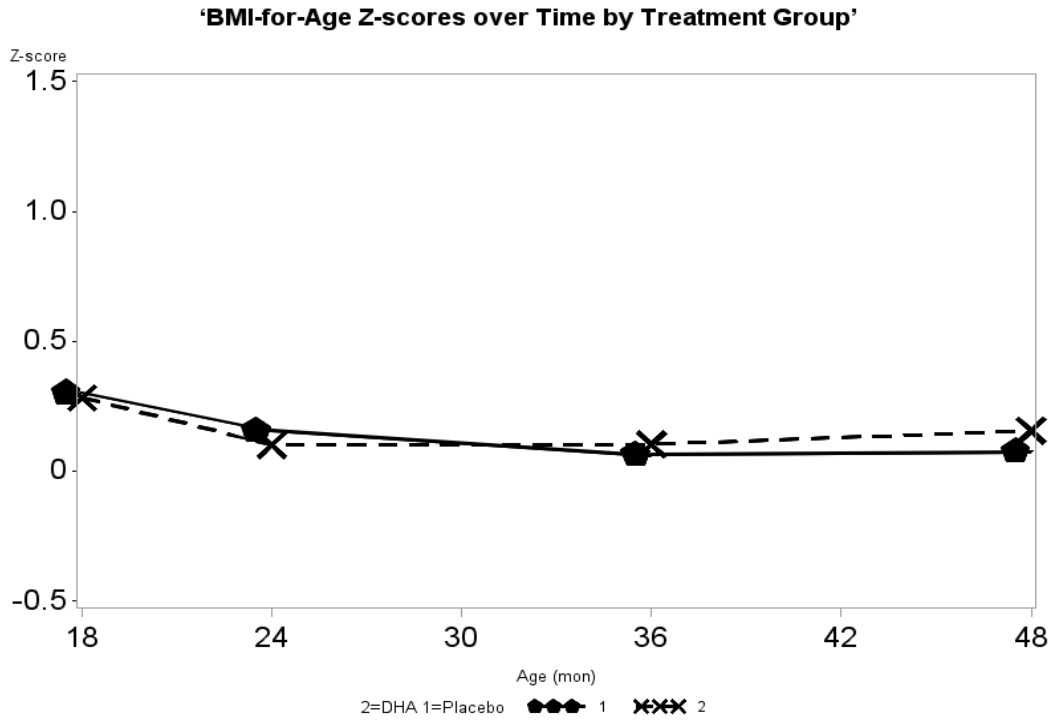



**Figure 2:**



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