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Effect of omega-3 fatty acids supplementation during pregnancy on lung function in preschoolers: a clinical trial

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ABSTRACT

Rationale: Prenatal omega-3 fatty acids improve alveolarization, diminish inflammation, and improve pulmonary growth, but it is unclear whether these outcomes translate into improved postnatal lung function. **Objective:** We assessed the effect of prenatal supplementation with docosahexaenoic acid (DHA) on offspring lung function through 60 months of age. **Methods:** We included a cohort of 772 Mexican preschoolers whose mothers participated in a clinical trial (NCT00646360) of supplementation with DHA or a placebo from week 18–22 of gestation through delivery. **Measurements:** The children were followed after birth and anthropometric measurements and forced oscillation tests were performed at 36, 48, and 60 months of age. The effect of DHA was tested using a longitudinal mixed effect models. **Results:** Overall, mean (Standard Deviation) of the measurements of respiratory system resistance and respiratory system reactance at 6, 8, and 10 Hz during follow up period were 11.3 (2.4), 11.1 (2.4), 10.3 (2.2) and –5.2 (1.6), –4.8 (1.7), –4.6 (1.6), respectively. There were no significant differences in pulmonary function by treatment group. DHA did not affect the average lung function or the trajectories through 60 months. **Conclusions:** Prenatal DHA supplementation did not influence pulmonary function in this cohort of Mexican preschoolers.

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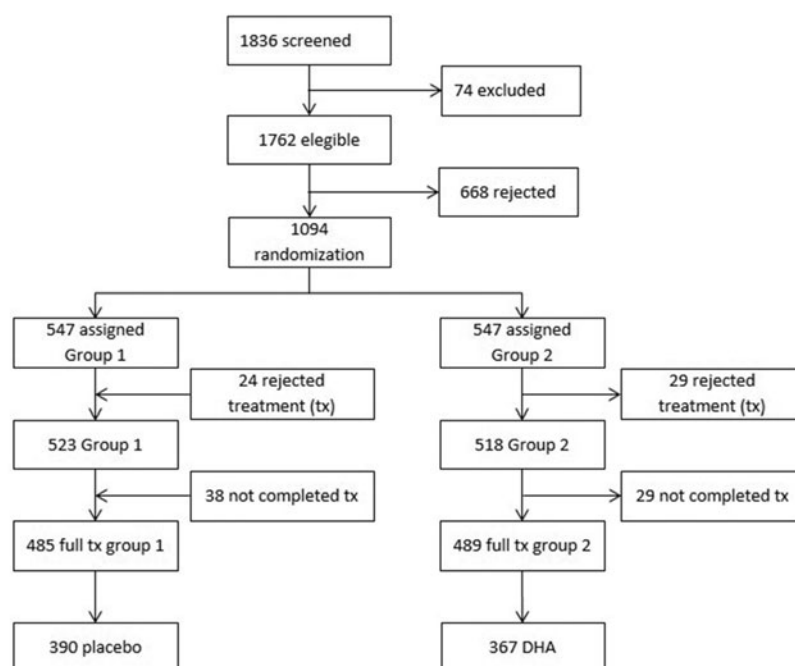
Introduction

Development of the lung begins in utero, with cellular differentiation and formation of the primary structures occurring in stages during fetal development. Substantial growth and maturation of the lung parenchyma occurs postnatally (1–5). The lung function a child is born with determines, to a large extent, the lung function for the rest of his/her life (6–8). Respiratory diseases are amongst the primary causes of morbidity and mortality in infants and respiratory events contribute to a reduction in quality of life, especially in children aged <5 years (9–12). Low pulmonary function is a life-long risk factor for respiratory disease, thus, the study of the pulmonary function in early life is important and can provide an early warning of impending disease. Likewise, normal lung growth and development during infancy is essential for achieving maximal pulmonary-function levels at an adult age (8).

Several factors determine the normal development of the lung (Fetal Respiratory Movements (FRM), adequate intrathoracic space, sufficient volume of intra- and extra pulmonary fluid, and adequate pulmonary blood volume)

(7,13) that are influenced by maternal lifestyle, pregnancy risk factors (diet, hormonal, endocrinal factors, maternal diseases), and environmental exposure (consumption of tobacco, alcohol, drugs, ambient and household air pollution and household chemicals) (5,14). Similarly, some reports indicate that diet plays a very important role in the process of pulmonary development and is central to the pathogenesis of respiratory disease (15,16).

Current reports indicate that adequate omega-3 (n-3) polyunsaturated fatty acids (PUFAs) levels are necessary for normal growth and development. Consumption of n-3 PUFAs during pregnancy or in early postnatal life can modulate immune responses, play a preventive role in the development of allergic disorders, improve alveolarization, diminish inflammation, and improve pulmonary growth (17–20). Tenorio-Lopes et al. (21) reported that n-3 PUFA supplementation via maternal diet improved postnatal respiratory function in rat pups. They suggested that the fatty acids improved pulmonary architecture and improved the response to postnatal respiratory challenges, concluding that prenatal treatment



*The main factors for the women were non included in the present report were: lack of family support, moving away from the study area and for not have the complete information on oscillation test and sociodemographics characteristics during the follow up period.

Figure 1. Consort diagram showing how subjects progressed through the study and how the final study sample was obtained.

was beneficial to the offspring. However, the evidence of the effect of n-3 PUFA during pregnancy on pulmonary function in humans in early postnatal life is limited and the results are inconclusive.

To our knowledge, no randomized clinical trial has evaluated the effects of prenatal supplementation with DHA on pulmonary function at preschool age. For this reason we conducted the present study to evaluate the effect of prenatal supplementation with DHA on lung function in children. We hypothesized that supplementation with DHA during pregnancy would improve lung function in preschoolers. We took advantage of a double blinded controlled clinical trial conducted among pregnant women supplemented with either DHA or placebo and followed their children to five years of age.

Material and methods

Study design and study population

The present study (NCT00646360) used data from the POSGRAD (Prenatal Omega-3 fatty acid Supplements, Growth, And Development) study, a double blind, randomized, placebo controlled trial designed to assess the effect of prenatal supplementation with DHA on offspring growth and development. The study design has been described in detail elsewhere (22); briefly, women were recruited at the Mexican Social Security Institute

General Hospital No. 1 in Cuernavaca, Mexico. Eligible women were 18–35 years of age and in week 18–22 of gestation. All participant had normal risk pregnancies, and none had pregnancy-related diseases or pregnancy complications. One thousand and forty women started treatment, of whom 968 completed the study and delivered 973 live infants. Women provided informed consent for themselves and permission for their children. For the present report, we include 772 mother–child pairs (Figure 1). The Ethics and Investigation Committees of the Mexican Institute of Public Health (INSP) and Emory University Ethics Committee approved the investigation protocol, as well as the letters of informed consent.

Randomization and intervention

All eligible women were randomized to either active treatment or the placebo group using a computer-generated list (22). After signing the letter of informed consent, women were randomly assigned to receive 400 mg/d daily of algal DHA or a soy and corn oil–based placebo from the 18th to 22nd week of pregnancy through to delivery.

Evaluation of treatment adherence

Compliance, defined as the percentage of capsules consumed during the intervention, was 88.9% in the two groups. DHA concentrations in maternal plasma at birth were significantly higher than in the placebo group (22).

Respiratory function by forced oscillation test

Pulmonary function was measured using the Forced Oscillation Technique (FOT) (23,24) at 36, 48, and 60 months of age. Respiratory system resistance (Rrs) and respiratory system reactance (Xrs) were measured with commercially available equipment (COSMED12; Italy) according to European Respiratory Society (ERS) recommendations (23). The commercial FOT equipment is based on the equipment prototype described by Landser et al. (25,26). Pulmonary test methods and validation processes have been described in detail in our previous report (27). Trained personnel conducted all tests and the three best tests selected for averaging. In the final analysis, we included the averages of Rrs and Xrs at 6, 8, and 10 Hz. It is also important to note that all the children were healthy and did not have any respiratory symptoms for 15 days prior to lung function testing.

Information on other variables

At baseline, the participating women responded to a general health questionnaire that included information on weight and height prior to delivery, sociodemographic characteristics, and diseases and gynecological antecedents. A dietary questionnaire was administered during pregnancy, a respiratory health questionnaire recorded for all children prior to the lung function tests and a food consumption frequency questionnaire completed. Growth (height and weight) was recorded each year during the follow-up.

Statistical analysis

Descriptive analysis of the 772 participating population was performed. Baseline characteristics were compared between the intervention and placebo groups utilizing the Mann-Whitney *U* test. Statistical analysis was carried out on an intention-to-treat basis. To evaluate the effect of the supplementation on pulmonary function, we performed a longitudinal analysis for 36, 48, and 60 follow-up months, using mixed models with random intercept, age as randomized slope, and a covariance matrix without structure. We considered the treatment variable as dichotomous (DHA or placebo). The models were adjusted by height, sex, age, and age of the child squared. Other variables, such as weight, diet during pregnancy and exposure to environmental factors (tobacco exposure, pets in home, etc.) were examined but were not significant confounders and not included in the final models. All models were evaluated by examining residuals and the goodness-of-fit through the Akaike Information Criterion (AIC). Data analysis was performed with STATA software version 12.0 (28).

Table 1. Sociodemographic characteristics of the 772 children who completed the study at 60 months according to treatment group.

Variables	Placebo <i>n</i> = 367 (48.5)	DHA <i>n</i> = 390 (51.5)	<i>p</i> Value*
Girls <i>n</i> (%)	342 (49.85)	344 (50.15)	0.15
Weight kg (mean, SD)	16.09 (2.38)	16.15 (2.64)	0.74
Height cm (mean, SD)	103.15 (5.78)	103.25 (5.80)	0.74
Passive Smoking, Yes <i>n</i> (%)	143 (25.72)	143 (24.66)	0.68
Maternal atopy Yes <i>n</i> (%)	204 (32.38)	226 (33.63)	0.63

**P* value of the Student's *t*-test for the difference of means by treatment group. Chi-squared test for categorical variables.

Results

We included 772 preschool-aged children who had complete information on pulmonary function for the study period, giving a total of 1,464 pulmonary function measurements. Preschool boys and girls who were not included in the analysis did not differ in terms of the demographic characteristics of the participants included in the analysis (data not shown). The characteristics of the preschool boys and girls were similar by intervention group (Table 1).

There were no differences in pulmonary function at any age between the treatment groups (Table 2). DHA supplementation during pregnancy had no significant effect on the pulmonary function trajectory from three to five years of age in the crude model or adjusted models (Table 3).

While height was an important predictor of the participant's pulmonary function, no interaction between supplementation and height was observed (data not shown). For each cm of increase in the child's average height, there was an average decrease of −13.5 hPaSL (95% CI, −17.2, −9.8) in Rrs6 frequency and an increase of 8.7 hPaSL (95% CI, 6.2, 11.2) in Xrs6 frequency. Similar results were observed at 8 and 10 Hz.

The instantaneous growth rate for Rrs6 and Xrs6 at 60 months of age was −0.13 hPaSL and 0.13 hPaSL per year, respectively; and for Rr8 and Rrs10 and their counterparts (Xrs8 and Xrs10), between the 36 and 48 months of age, the average growth was very similar (4%), while between 48 and 60 months of age was 8% (Figure 2).

Discussion

Our findings indicate that DHA supplementation during pregnancy at a dose recommended by the Mexican Academy of Medicine (17) does not provide a significant, additional beneficial effect in terms of improve respiratory function at 60 months of age in this cohort of healthy Mexican preschool-aged children. The hypothetical explanations that support these findings probably are; first, that, the DHA dose provided in the study maybe

Table 2. Cross-sectional assessment of lung function stratified by age and treatment group.

Variables	36 months (<i>n</i> = 153)			48 months (<i>n</i> = 513)			60 months (<i>n</i> = 658)		
	Placebo <i>n</i> = 70	DHA <i>n</i> = 83	<i>p</i> Value*	Placebo <i>n</i> = 260	DHA <i>n</i> = 269	<i>p</i> Value*	Placebo <i>n</i> = 375	DHA <i>n</i> = 407	<i>p</i> Value*
Rr ₆ (hPa.s.L ⁻¹)	11.8(2.2)	11.9(2.1)	0.605	11.8(2.4)	11.6(2.4)	0.605	11.7(2.9)	11.6(2.8)	0.276
Rr ₈ (hPa.s.L ⁻¹)	11.6(2.0)	11.6(1.9)	0.590	11.4(2.3)	11.3(2.4)	0.299	11.3(3.0)	11.1(2.8)	0.234
Rr ₁₀ (hPa.s.L ⁻¹)	10.6(1.9)	10.7(1.7)	0.651	10.7(2.1)	10.6(2.3)	0.250	10.5(2.7)	10.4(2.6)	0.284
Xr ₆ (hPa.s.L ⁻¹)	-5.8(1.5)	-5.7(1.6)	0.700	-5.3(1.5)	-5.3(1.6)	0.568	-5.6(1.9)	-5.3(1.9)	0.950
Xr ₈ (hPa.s.L ⁻¹)	-5.5(1.7)	-5.3(1.6)	0.739	-5.0(1.5)	-5.0(1.7)	0.437	-5.4(2.2)	-5.1(2.1)	0.945
Xr ₁₀ (hPa.s.L ⁻¹)	-5.1(1.5)	-5.0(1.5)	0.717	-4.7(1.5)	-4.7(1.6)	0.493	-5.0(2.2)	-4.8(2.0)	0.937

N = Number of participants by age.

P* value of the Student's *t*-test for the difference of means by treatment group.Table 3.** Effect of DHA supplementation during pregnancy on respiratory system resistance and reactance (Crude and Adjusted Models) in preschool children aged 3–5 years in Morelos state, Mexico.

Variable	Resistance*						Reactance*					
	Rrs ₆		Rrs ₈		Rrs ₁₀		Xrs ₆		Xrs ₈		Xrs ₁₀	
	β	<i>p</i> value	B	<i>p</i> value	B	<i>p</i> value	B	<i>p</i> value	β	<i>p</i> value	β	<i>p</i> value
DHA [±]	-0.090	0.562	-0.069	0.649	-0.099	0.486	0.114	0.300	0.070	0.546	0.073	0.510
DHA [≠]	-0.030	0.842	-0.019	0.899	-0.022	0.874	0.054	0.616	0.010	0.936	0.018	0.873

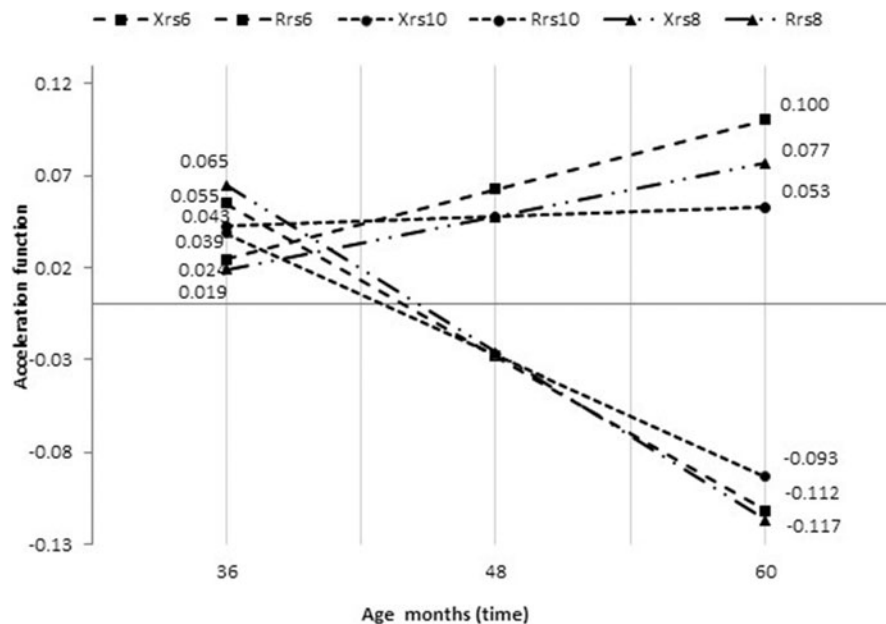
*Mixed models with random intercept, age as random slope and covariance matrix without structure.

[±]Crude model (bivariate). Placebo = reference category.[≠]Adjusted model by age in months, age in months squared, height in cm, gender, and maternal antecedents of atopy. Placebo = reference category.

too small to effectively improve lung development at long term. Second, most of the beneficial effects of supplementation with omega-3 fatty acids have been described for other systems and not directly on pulmonary growth or lung function (29–31) and third, the majority of the beneficial effects have been reported in short-term studies undertaken in animals and at early stages of life

(the perinatal period) and may not be translatable to human children (18–20).

Some experimental studies carried out in animals have demonstrated that maternal omega-3 fatty acid supplementation have beneficial effects. Sharma et al. undertook a study to assess the effects of maternal n-3 PUFA supplementation on lung injuries in newborn



The instantaneous rate was calculated from the coefficients of mixed models.
Lineal Slope = $\beta_{\text{edad}} - 2(\text{Edad}) \beta_{\text{edad}^2}$

Figure 2. Instantaneous longitudinal growth rate of Rrs and Xrs at 6, 8, and 10 Hz by age in months.

rats and reported that supplementation prevented the decrease in alveolarization caused by hyperoxia (32).

In an human study, 83 atopic pregnant women were assigned to receive fish oil supplementation or placebo from 20 weeks of pregnancy until delivery. Results suggested that omega-3 supplementation decreased the levels of interleukin 13 (IL-13) ($p < 0.05$) in umbilical cord and infants in the fish oil group were consistently less likely to develop atopic features including food allergy, recurrent wheeze, persistent cough, diagnosed asthma and anaphylaxis compared with the control group. However, because of the small sample size, the authors could not firmly conclude on the benefit of supplementation (33). In another human study, children who received omega-3 supplementation showed a nonsignificant trend towards less recurrent wheezing, persistent cough, asthma, food allergy, angioedema, or anaphylaxis, compared with the placebo group (34).

Two recent reports in infants suggest that dietary omega-3 supplements in pregnancy or in the early post-natal period could have immune modulatory properties and associated clinical benefits (17, 35). Best et al. (36, 37) examined the role of n-3 rich fish oil during pregnancy in children and found that prenatal n-3 LCPUFA supplementation did not reduce IgE-associated allergic disease at 6 years of age. Secondary outcomes were suggestive of a protective effect of the intervention on the incidence of *D. farinae* sensitization. We previously reported a significant inverse associated between n-3 PUFA supplementation during pregnancy and the risk of children presenting with respiratory symptoms up to 18 months of age, especially in children whose mothers were atopic (38).

The strengths of the present study include: a randomized control trial of DHA supplementation in a population with a documented deficiency that was improved by supplementation; a large sample size; a longitudinal follow-up until 5 years of age; and measurements of lung function and growth in lung function from 3 to 5 years of age. The randomization resulted in an equilibrium of maternal and infant socio-demographic characteristics between the supplementation and placebo groups.

Conclusion

In summary, the results of the present study do not support a beneficial impact of pre-natal DHA supplementation during pregnancy at a dose of 400 mg/day on lung function or lung function growth to 5 years of age. However, this does not preclude other long-term benefits of pre-natal DHA supplementation and continued follow-up of the present cohort is recommended.

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Competing interests statement

The authors declare have no competing of interest.

Role of sponsors

The sponsor had no role in the design of the study, the collection and analysis of the data, or the preparation of the manuscript. The manuscript contents are solely the responsibility of the authors and do not necessarily represent the official view of the Eunice Kennedy Shriver National Institute of Child Health and Human Development or the National Institute of Health.

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