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Original Research Article

Effects of prenatal docosahexaenoic acid supplementation on offspring cardiometabolic health at 11 years differs by maternal single nucleotide polymorphism rs174602: follow-up of a randomized controlled trial in Mexico

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A B S T R A C T

Background: There is limited evidence regarding long-term effects of prenatal docosahexaenoic acid (DHA) supplementation on offspring cardiometabolic health (CMH). Inconsistent results may be attributable to variants of fatty acid desaturase (*FADS*) genes.

Objective: We aimed to evaluate the effect of prenatal DHA supplementation on offspring CMH and investigate effect modification by maternal *FADS2* single nucleotide polymorphism (SNP) rs174602.

Methods: We used follow-up data from a double-blind, randomized controlled trial in Mexico in which pregnant females received 400 mg/d of algal DHA or placebo from midgestation until delivery. The study sample included 314 offspring with data at age 11 y and maternal *FADS* genetic data (DHA: $n = 160$; Placebo: $n = 154$). We derived a Metabolic Syndrome (MetS) score from body mass index, HDL, triglycerides, fasting glucose concentrations, and systolic blood pressure. Generalized linear models were used to evaluate the effect of the intervention on offspring MetS score and test interactions between treatment group and genotype, adjusting for maternal, offspring, and household factors.

Results: Offspring MetS score did not differ significantly by treatment group. We observed evidence of effect modification by maternal SNP rs174602 ($P = 0.001$); offspring of maternal TT genotype who received DHA had lower MetS score relative to the placebo group (DHA (mean ± standard error of the mean (SEM)): -0.21 ± 0.11, $n = 21$; Placebo: 0.05 ± 0.11, $n = 23$; Δ= -0.26 (95% CI: -0.55, 0.04), $P = 0.09$); among CC maternal genotype carriers, offspring of mothers who received DHA had higher MetS score (0.18 ± 0.06, $n = 62$) relative to the placebo group (-0.05 ± 0.06, $n = 65$, Δ=0.24 (0.06, 0.41), $P < 0.01$).

Conclusion: The effect of prenatal DHA supplementation on offspring MetS score differed by maternal *FADS* SNP rs174602. These findings further support incorporating genetic analysis of *FADS* polymorphisms in DHA supplementation trials.

Clinical trial details: This trial was registered at clinicaltrials.gov as NCT00646360.

Keywords: Mexico, DHA, FADS, gene-nutrient interactions, cardiometabolic health, prenatal supplementation

Introduction

Nutritional exposures may have long-term implications for offspring metabolic health, including risk of cardiovascular disease and type II diabetes [1–4]. DHA is an n-3 long-chain PUFA (LC-PUFA) that typically accumulates during the second half of pregnancy to support optimal fetal DHA tissue deposition, development and immune function [5]. Animal models and epidemiological studies suggest that alterations in the prenatal DHA supply may also influence long-term offspring cardiometabolic risk via altered cell and organ

Abbreviations: AA, arachidonic acid; CMH, cardiometabolic health; FADS, fatty acid desaturase; HWE, Hardy-Weinberg equilibrium; IMSS, Mexican Social Security Institute; INSP, Mexican National Public Health Institute; LC-PUFA, long-chain PUFA; LD, linkage disequilibrium; MAF, minor allele frequency; MetS, metabolic syndrome; PC, Principal Component; PCA, Principal Components Analysis; POSGRAD, Prenatal Omega-3 Supplementation and Child Growth and Development; RCT, randomized controlled trial; SBP, Systolic Blood Pressure; SNP, single nucleotide polymorphism; SES, Socioeconomic Status.

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development, gene expression, and development of neuroendocrine signals [1, 4, 6, 7]. For example, studies in rat and mouse models have shown that prenatal n-3 LC-PUFA supplementation results in lower adiposity, insulin resistance, and dyslipidemia among offspring [8–11].

In humans, observational studies report associations of higher maternal n-3 LC-PUFA status during pregnancy with lower adiposity, dyslipidemia, and leptin concentrations among offspring in early and midchildhood [12, 13]. Additional evidence suggests that, especially among females with overweight or obesity, mother-offspring dyads may benefit from prenatal DHA supplementation via improvements in maternal insulin sensitivity, circulating lipids, and placental inflammation, thus reducing fetal overnutrition and adiposity [14]. However, systematic reviews of results from randomized controlled trials (RCT) report inconsistent effects of prenatal DHA supplementation on offspring cardiometabolic health (CMH) outcomes [6, 15–18]. Although these inconsistencies may be attributable to differences in the dose, type, and timing of supplementation during pregnancy [19, 20], variants of fatty acid desaturase (*FADS*) genes that modulate the conversion of n–3 and n–6 fatty acids into LC-PUFAs may also contribute to this heterogeneity [5].

Tissue LC-PUFA concentrations are determined by both dietary intake of n-6 and n-3 LC-PUFAs and endogenous formation from dietary PUFA precursors, which occurs through a series of consecutive desaturation and elongation steps. The rate-limiting desaturase steps are mediated by Δ-6 and Δ-5 desaturase enzymes encoded in the *FADS* gene cluster (*FADS1, FADS2, FADS3*) [5]. Multiple variants in *FADS* genes have been associated with lower LC-PUFA concentrations, indicating reduced conversion of dietary precursors [5, 21]. Although a few observational studies suggest that maternal *FADS* genotype influences offspring LC-PUFA status and lipid profiles, to our knowledge, this association has not been investigated in the context of an intervention trial [22].

To address these research gaps, we leveraged data from a large prenatal DHA supplementation RCT in Mexico, in which pregnant females received either 400 mg algal DHA (treatment) or placebo daily from midpregnancy through delivery. We previously reported that maternal *FADS2* SNP rs174602 modified the effect of prenatal DHA supplementation on offspring birth weight [23], metabolome at age 3 mo [24], and cognition at age 5 y [25]. The objective of this study is to evaluate the effect of prenatal DHA supplementation on offspring CMH at age 11 y and assess whether it differed by variations in maternal *FADS* SNP rs174602.

Methods

Participants and study design

This study included children of pregnant females who participated in the Prenatal Omega-3 fatty acid Supplementation and Child Growth and Development (POSGRAD) trial in Cuernavaca, Mexico (NCT00646360). A detailed description of the trial design and protocol has been published previously [26]. Briefly, pregnant females were recruited at 18 to 22 wk gestation at the Mexican Social Security Institute (IMSS) and were eligible for inclusion if they were 18 to 35 y old, planned to deliver at the IMSS hospital, breastfeed for at least 3 mo, and continue living in the area for ≥ 2 y after delivery. Exclusion criteria included high-risk pregnancies, lipid metabolism or absorption disorders, regular intake of fish oil or DHA supplements, or chronic use of certain medications. Once eligibility was confirmed, participants were contacted and provided with a thorough explanation of the study protocol, and written informed consent was obtained. Of 1762 eligible pregnant females, 1094 were randomly assigned to receive 2 capsules containing either 200 mg algal DHA per capsule (treatment) or a corn/soy oil blend (placebo) daily through delivery; the fatty acid composition of the supplements has been previously published [24]. Block randomization was used to randomly assign study participants into groups of equal sample size using a block size of 8. Assignment codes were placed in sealed envelopes at the beginning of the study and were held in a sealed location by a faculty member at Emory University who was not involved with the study. Enrollment took place from February 2005 to March 2007, and the last child was born in July 2007. All study participants and members of the study team were blinded to treatment allocations throughout the intervention period of the study. Data were unblinded for the analytical study team after the last infant was born and reached 6 mo of age. The participants and fieldworkers remain blinded to the intervention, as the study is still ongoing for follow-up. Due to budgetary limitations, venous blood samples were collected in a subsample of 485 children who were contacted and agreed to participate in the 11-y follow-up study from 2016 to 2018.

The study was conducted according to the guidelines of the Declaration of Helsinki. The Emory University Institutional Review Board and the Mexican National Public Health Institute (INSP) ethics committee approved all procedures involving human subjects. Informed consent was obtained from all pregnant females at study enrollment. At the 11-y follow-up visit, mothers provided written informed consent, and children provided written assent.

Maternal genotype data

Fasting venous blood samples were obtained from all pregnant females at recruitment. Plasma, buffy coat, and red blood cells were separated and stored at INSP laboratories at −80°C until buffy coats were transported to the Helmholtz Center, Munich, Germany. The genetic analysis was carried out during 2012 and 2013 for those who provided consent to genotyping (n = 720), using methods that have been previously described [23]. The resulting data sets containing information on 15 *FADS1, FADS2,* and *FADS3* SNPs, selected based on biological evidence of an effect on LC-PUFA metabolism [27–31], were sent to Emory University via encrypted files. Key SNPs were selected on the basis of previous associations with cardiometabolic health in the literature (rs174548, rs174556, rs174570, rs174575, rs174576, rs174579, rs174602) [27, 28, 30, 31]. Maternal *FADS2* SNP rs174602 was selected as the focus of this study based on previous evidence of effect modification on offspring birth weight, metabolome at 3 mo, and cognition at 5 y within the POSGRAD trial [23–25]. Allele frequencies were calculated, and Hardy Weinberg Equilibrium (HWE) was tested with Fisher's exact test using the R 'genetics' package.

Follow-up study of children at 11 y

At age 11 y, body weight (kg) and height (cm) were collected in triplicate by trained personnel following standard procedures [32]. Children were weighed wearing light clothing with a portable electronic pediatric scale (Tanita model 1582) with a precision of 100 g, which was calibrated daily with a known reference weight. Height was measured using a stadiometer with a precision of 0.1 cm. Average values of all 3 measurements were calculated. We calculated BMI-for-age z-scores according to the 2007 WHO Growth Reference Standards using the 'z scorer' R package [33]. Blood pressure (mmHg)

was measured using a digital device (OMRON model HEM-711ACINT), which has been validated for use in children and adolescents. Blood pressure was taken when the child was at rest (> 5 min after the child arrived at the study visit). In each arm, 4 measurements were made with 2-min intervals; the first measurement was discarded, and the subsequent 3 were averaged [34].

Outcome assessment: cardiometabolic markers

Venous blood samples were obtained from children after a 12-h fasting period (verified by documenting the approximate time that food was last consumed) and centrifuged. Aliquots of serum were frozen in liquid nitrogen and stored at −80°C until further analysis at the National Institute of Medical Sciences and Nutrition Salvador Zubirán in Mexico. HDL cholesterol, triglyceride, and glucose concentrations were assessed using the Beckman Coulter SYNCHRON CX 5 Delta automated kit and expressed in mg/dL. Cardiometabolic risk factor thresholds were defined as follows: triglycerides ≥150 mg/dL, HDL <40 mg/dL, systolic blood pressure (SBP) ≥ 90th percentile for sex and height, and fasting glucose ≥100 mg/dL [35, 36].

Derivation of continuous MetS score

To operationalize cardiometabolic health and reduce multiple testing, we used principal components analysis (PCA) to calculate a continuous MetS score (primary outcome) using SBP, BMI-z, HDL, triglycerides, and glucose concentration values [37]. We used BMI-z instead of waist circumference because it has greater reliability and has been used in other similar studies [38, 39]. We log-transformed triglycerides and glucose concentration values and used the inverse of HDL when standardizing, so a higher factor loading score would have a similar interpretation to other measures in the model. Top principal components (PCs) were identified visually using scree plots and quantitatively using the Kaiser criterion (eigenvalues > 1). Subsequently, the score was calculated as the sum of the top 2 components, weighted by variance explained. The score can be interpreted as a z-score, with higher scores representing increased cardiometabolic risk. PCA was performed using the 'FactoMineR' R package [40]. Given the observed sex differences in cardiometabolic health measures, we performed a sensitivity analysis comparing sex-specific and overall MetS scores; their correlation coefficient was 0.97, so we used the overall score in subsequent analyses.

Covariates

Data on maternal, offspring, and household factors were available to further characterize the study population. Household SES at enrollment was calculated with the use of PCA on a list of assets collected through interviews [26]. Maternal BMI at enrollment was assessed based on weight and height measurements that were obtained using standard procedures. Maternal dietary intake at study enrollment was also assessed using a 110-item food-frequency questionnaire that was specifically designed to include important PUFA sources [41]. Data on infant feeding practices at 3 mo of age were obtained by maternal interview and used to categorize infant feeding practices according to the WHO classification [42, 43]. Dietary intake of children at 11 y was assessed via multiple-pass 24-h dietary recall developed for Mexican populations [44]. Trained personnel administered the diet recall tool to the child's primary caregiver (in presence of the child). Nutrient and energy estimations were obtained using the 2012 and 2016 Mexican Food Database (BAM in Spanish): Compilation of the Frequently Consumed Foods in the Country, which is maintained by INSP [45]. Sedentary time was estimated using a self-reported physical activity and inactivity questionnaire that was developed for and validated among children ages 10 to 14 y in Mexico [46]. Sexual maturation was assessed by proxy using testosterone concentrations for males and age at attainment of menarche for females via a self-reported questionnaire.

Statistical analysis

Normality of data was assessed using histograms and quantile-quantile plots, and residual plots were used to check model assumptions. We calculated means and standard deviations for maternal and offspring characteristics at trial enrollment and birth and assessed differences by treatment group and maternal genotype using t-tests, ANOVA, and Wilcoxon rank-sum tests as appropriate. We compared these characteristics between those included in the analytic sample and the rest of the birth cohort, and variables that differed were considered for inclusion in models as covariates.

We used multivariable generalized linear models (GLMs) to assess the effect of prenatal DHA supplementation on offspring MetS scores and test interactions between maternal treatment group and genotype using 5 different models: 1) unadjusted model; 2) adjusted for household SES score, maternal age (y), parity (number of live births), BMI (kg/m^2), and offspring sex and age at measurement (d); 3) model 2 additionally adjusted for birth weight (g) and gestational age at birth (wk); 4) model 3 additionally adjusted for energy intake (kcal/d) and omega-3 fatty acid intake (g/d); and 5) model 4 additionally adjusted for monounsaturated fat (MUFA) (g/d) and saturated fat intake (g/d). We used multiple imputations to account for missing values of covariates (diet [n = 85, 27.1%], sedentary time [n = 26, 8.3%], sexual maturation [testosterone concentrations in males: n = 101, 58.4%; attainment of menarche in females: n = 1, 0.7%], and infant feeding practices [n = 30, 9.6%] and duration [n = 2, 0.6%]). We used the R 'mice' package to generate 20 imputed datasets using fully conditional specification with 50 iterations, conducted GLMs for each of the 20 models, and pooled the estimates [47]. Inclusion of covariates specified in models 3, 4, and 5 did not alter estimates; therefore, we report all findings adjusted for the covariates specified in model 2. All statistical analyses were performed using R version 4.0.4 (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was held at $P < 0.05$. We tested 6 different outcomes (MetS score and 5 components included in MetS score) and used the Bonferroni correction to adjust for multiple testing ($P < 0.008$).

Results

The analytic sample included all children with complete data on maternal genotype and cardiometabolic risk factors (BMI-z, HDL, triglycerides, glucose, and SBP) at age 11 y (n = 314) (Figure 1). Maternal and offspring characteristics at trial enrollment and birth were balanced by treatment group and maternal genotype (Table 1). Mean maternal age and BMI at enrollment were 26.2 ± 4.7 y and 26.0 ± 4.2 kg/m^2, respectively. Median maternal dietary intake of DHA was very low (median [IQR]: 56 [40–105] mg/d), combined with a dietary n-6:n-3 ratio of 12:1. Mothers of children in the analytic sample tended to be older and have a higher BMI and higher SES score at trial enrollment relative to those lost to follow-up or missing data (Supplemental Table 1). Lifestyle factors of children at age 11 y by treatment group and maternal genotype are presented in Table 2; children whose mothers received prenatal DHA tended to have higher intakes of polyunsaturated fatty acids, including total omega-3 and omega-6 intake, relative to children whose mothers received placebo ($P < 0.05$).

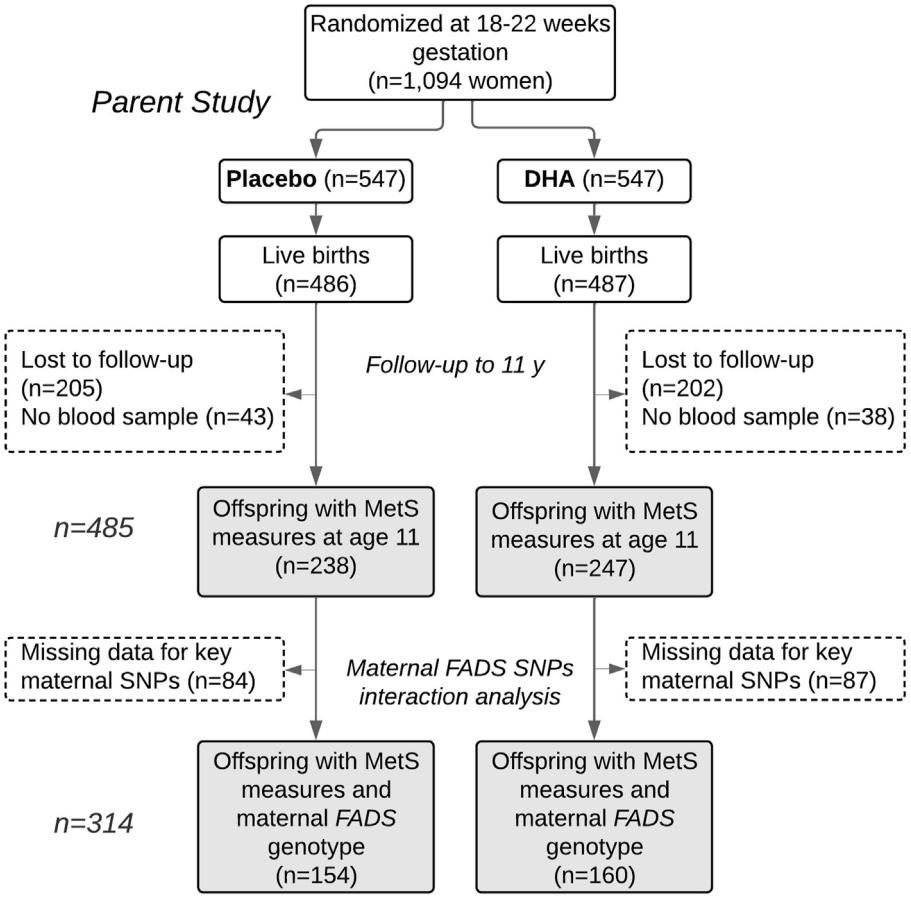

FIGURE 1. Procedures of study sample selection. MetS, metabolic syndrome; FADS, fatty acid desaturase

Maternal and offspring characteristics at study enrollment and birth, and offspring lifestyle factors at age 11 y were similar when stratified by both prenatal treatment group and maternal genotype (Supplemental Table 2).

Genotype distribution of maternal SNP rs174602

Within this sample, the minor allele frequency for maternal SNP rs174602 was 0.37; 44 mothers (14%) were homozygous carriers of the minor T allele, 143 (46%) were heterozygous carriers (TC), and 127 (40%) were homozygous carriers of the major C allele. There were no significant differences in genotype distribution by treatment group, and there were no HWE violations observed ($P = 0.72$).

Cardiometabolic health of children at age 11 y

At age 11 y, 42% of the children had BMI z-score > 1 SD, and 39% had ≥ 1 cardiometabolic risk factor (HDL ≤40 mg/dL, triglycerides ≥150 mg/dl, fasting glucose ≥100 mg/dL, SBP ≥90th percentile). The most frequently observed cardiometabolic risk factor was low HDL (30%), followed by high triglycerides concentration (16%). Derivation of the MetS score via PCA showed that the top 2 PCs collectively explained 56% of the variance in the measured data (Supplemental Figure 1A). Triglycerides, BMI-z, and HDL concentration contributed to the first PC (35.0% variance explained), whereas SBP and fasting glucose concentration contributed to the second PC (20.7% variance explained). The distribution of the MetS score in the study population is shown in Supplemental Figure 1B.

Impact of prenatal DHA supplementation on offspring MetS score

Differences in MetS components and offspring MetS score by prenatal treatment group and maternal genotype are shown in Table 3. Intent to treat analysis showed no differences by treatment group for the MetS components (all $P > 0.05$) or MetS score at 11 y ($\Delta = 0.02$, 95% confidence interval [CI]: -0.09, 0.13). We observed evidence of effect modification by maternal SNP rs174602 ($P = 0.001$) (Figure 2). Offspring of homozygous minor T allele carriers who received prenatal DHA had lower MetS score relative to the placebo group (DHA [mean ± SEM]: -0.21 ± 0.11, $n = 21$; Placebo: 0.05 ± 0.11; Δ= -0.26 [95% CI: -0.55, 0.04], $n = 23$, $P = 0.09$). Among homozygous major C allele carriers, offspring of mothers who received prenatal DHA had higher MetS score (0.18 ± 0.06, $n = 62$) relative to offspring whose mothers received placebo (-0.05 ± 0.06, $n = 65$, Δ=0.24 [0.06, 0.41], $P < 0.01$). Individual cardiometabolic risk factors, stratified by both maternal genotype and treatment group, are shown in Supplemental Table 3. Although we observed similar trends with individual MetS components, the results were not statistically significant after adjustment for multiple testing (Figure 3). Finally, we evaluated 3-way a priori interactions with maternal BMI at study enrollment and offspring

TABLE 1
Maternal baseline characteristics and offspring characteristics at birth, stratified by treatment group and maternal genotype of SNP rs174602.[1]

	Treatment group		Maternal Genotype of SNP rs174602		
	Placebo $N = 154$	DHA $N = 160$	CC $N = 127$	TC $N = 143$	TT $N = 44$
Maternal factors, enrollment					
Age, y	26.6 (4.61)	27.4 (5.18)	27.0 (4.81)	27.2 (5.01)	26.8 (5.02)
BMI, kg/m^2	26.3 (4.10)	26.6 (4.13)	26.3 (4.12)	26.4 (3.92)	27.1 (4.65)
Height, cm	156 (5.54)	155 (5.57)	155 (5.66)	155 (5.46)	157 (5.54)
First pregnancy, %	53 (34.4%)	45 (28.1%)	37 (29.1%)	50 (35.0%)	11 (25.0%)
SES Score[2]	0.13 (1.05)	0.13 (0.89)	0.01 (1.03)	0.13 (0.94)	0.46 (0.81)
Schooling, y	12.3 (3.39)	11.9 (3.45)	11.6 (3.56)	12.3 (3.40)	12.6 (2.96)
Dietary intake at enrollment, g/d					
ALA	1.76 (1.00)	1.79 (1.08)	1.76 (1.04)	1.80 (1.00)	1.73 (1.18)
DHA	0.08 (0.07)	0.08 (0.08)	0.08 (0.08)	0.08 (0.06)	0.10 (0.11)
LA	19.3 (7.76)	19.7 (8.64)	19.4 (7.78)	19.7 (7.79)	19.2 (10.6)
EPA	0.03 (0.04)	0.03 (0.04)	0.03 (0.04)	0.03 (0.03)	0.04 (0.06)
AA	0.15 (0.07)	0.16 (0.09)	0.15 (0.06)	0.16 (0.09)	0.15 (0.06)
Compliance to intervention, %	95.0 (5.00)	95.7 (4.99)	95.1 (5.55)	95.8 (4.53)	94.6 (4.76)
Offspring factors, birth					
Sex					
Male	81 (52.6%)	93 (58.1%)	69 (54.3%)	80 (55.9%)	25 (56.8%)
Female	73 (47.4%)	67 (41.9%)	58 (45.7%)	63 (44.1%)	19 (43.2%)
Birth weight, g	3220 (490)	3230 (449)	3229 (532)	3220 (440)	3231 (367)
Gestational age, wk	39.0 (1.66)	39.1 (1.91)	38.8 (1.97)	39.1 (1.70)	39.4 (1.44)
Infant feeding practices, 3 mo, %					
Exclusively fed human milk	23 (16.2%)	17 (12.0%)	18 (15.8%)	15 (11.6%)	7 (17.1%)
Predominantly fed human milk	20 (14.1%)	15 (10.6%)	11 (9.65%)	17 (13.2%)	7 (17.1%)
Partially fed human milk	75 (52.8%)	80 (56.3%)	59 (51.8%)	78 (60.5%)	18 (43.9%)
Not fed human milk	24 (16.9%)	30 (21.1%)	26 (22.8%)	19 (14.7%)	9 (22.0%)
Duration of human milk feeding, mo	9.71 (7.96)	8.84 (7.65)	8.15 (6.86)	10.3 (8.56)	9.21 (7.53)

SES, socioeconomic status score; ALA, Alpha-Linolenic Acid; LA, Linoleic Acid; AA, Arachidonic Acid.
[1] Chi-square tests, t-tests, and ANOVA were used to test differences between groups. No differences by treatment group were observed. Values presented are mean (SD) unless otherwise stated.
[2] Differs by maternal genotype ($P < 0.05$).

sex but did not find any evidence of further effect modification (G×BMI interaction P value = 0.24, G×sex interaction P value = 0.28).

Discussion

In this follow-up study of mother-offspring dyads from Mexico, we found no main effect of prenatal DHA supplementation on offspring cardiometabolic health at age 11 y, but there were differences by variants of maternal *FADS2* SNP rs174602. Offspring of homozygous minor allele (TT) carriers who received prenatal DHA had lower MetS scores relative to the placebo group, whereas offspring of homozygous major allele (CC) carriers who received DHA had higher MetS scores relative to offspring whose mothers received placebo. These exploratory findings suggest that prenatal DHA supplementation may have differential effects on a child's long-term cardiometabolic risk based on their mother's genotype.

The lack of main effects of the intervention is similar to the findings from the few studies that have evaluated the long-term effect of prenatal n-3 LC-PUFA supplementation on offspring metabolic health; Rytter et al. found no significant differences by treatment group in blood pressure or lipids in a Danish population at age 19 y, but the sample sizes were small ($n = 180$ for blood pressure outcomes, $n = 243$ for adiposity-related outcomes) [48, 49]. When the analysis was restricted to mothers with low fish intake, however, children born to mothers who received fish oil trended toward better lipid profiles. A prenatal DHA RCT that was conducted in the United States among 171 mothers and their offspring also did not find evidence of a main intervention effect but observed a statistically significant interaction between prenatal DHA supplementation and child weight status for SBP and DBP from 4 to 6 y of age and higher fat free mass at age 5 y [50]. A previous study within the POSGRAD cohort also did not find any differences by treatment group in nonfasting serum lipid and glucose concentrations at age 4 y [51]. Some evidence suggests that differences in cardiometabolic risk because of metabolic programming may become more apparent later in childhood, near the onset of puberty. One observational study reported associations of cord blood LC-PUFA composition with child BMI at 2 and 10 y, but not 6 y, highlighting the importance of age of outcome assessment [19]. However, within our study, results were still null at age 11 y.

Our results provide additional support that the differences in the genetic makeup of individuals may partially explain null results observed across prenatal DHA supplementation RCTs. Here, we showed that the effect of the intervention on the offspring MetS score at 11 y differed by maternal *FADS2* SNP rs174602, located at an intron/exon boundary of the *FADS2* gene. *FADS2* encodes for the Δ-5 desaturase enzyme that regulates the conversion of 20:3n-6 to 20:4n-6 (Arachidonic acid [AA]) and 20:4n-3 to 20:5n-3 (Eicosapentaenoic acid). Pregnancy is a complex period characterized by necessary metabolic adaptations, including alterations in lipid and glucose metabolism, to ensure an adequate supply of nutrients, including DHA, to the mother and growing fetus [52]. During this critical period with heightened nutrient requirements, individuals with genotypes associated with lower endogenous conversion to DHA may be at greater risk for DHA deficiency and subsequently benefit more

TABLE 2
Offspring characteristics at 11 y follow-up, stratified by treatment group and maternal genotype of SNP rs174602.[1]

	Prenatal treatment group		Maternal Genotype of SNP rs174602		
	Placebo $N = 154$	DHA $N = 160$	CC $N = 127$	TC $N = 143$	TT $N = 44$
Dietary intake[2]					
Energy intake (kcal/d)	1904 (595)	2064 (746)	1999 (709)	2216 (660)	2216 (660)
Cholesterol (g/d)	246 (183)	300 (219)	276 (201)	328 (235)	328 (235)
Lipids (g/d)	70.2 (33.8)	78.3 (35.7)	75.3 (35.0)	85.2 (42.0)	85.2 (42.0)
Saturated fat (g/d)[3]	27.5 (14.2)	30.6 (17.7)	28.8 (15.3)	35.8 (18.4)	35.8 (18.4)
Monounsaturated fat (g/d)[3]	23.3 (12.8)	26.1 (12.9)	24.8 (12.7)	30.0 (16.9)	30.0 (16.9)
Polyunsaturated fat (g/d)[4]	13.6 (8.21)	16.2 (9.55)	14.9 (9.06)	16.3 (9.03)	16.3 (9.03)
Total omega-3 intake (g/d)[4]	0.71 (0.65)	1.09 (1.39)	0.88 (1.08)	0.80 (0.67)	0.80 (0.67)
Total omega-6 intake (g/d)[4]	5.32 (4.67)	7.34 (7.73)	6.71 (7.49)	6.12 (4.33)	6.12 (4.33)
Total sedentary time, hours/d	4.82 (2.21)	4.75 (2.06)	4.83 (2.05)	5.03 (2.31)	5.03 (2.31)
Maturation					
Females: attained menarche, n (%)	13 (8.5%)	11 (6.9%)	12 (8.39%)	3 (6.82%)	3 (6.82%)
Testosterone concentrations (pg/mL)	1.55 (0.84)	1.55 (0.72)	1.44 (0.77)	1.38 (0.65)	1.38 (0.65)
Age at examination, y	11.12 (0.20)	11.12 (0.17)	11.13 (0.18)	11.14 (0.16)	11.14 (0.16)

[1] Chi-square tests, t-tests, and ANOVA were used to test differences between groups. Values presented are mean (SD) unless otherwise stated.
[2] Dietary data only available for 229 individuals at age 11 y.
[3] Differs by maternal genotype ($P < 0.05$).
[4] Differs by maternal prenatal treatment group ($P < 0.05$).

from supplementation with preformed DHA. One RCT in the United States showed that only among individuals with *FADS* SNPs associated with lower conversion of precursors (i.e., had minor alleles for *FADS* SNPs), prenatal DHA supplementation increased DHA concentrations and reduced AA:DHA ratios at delivery. These findings suggested a selective benefit of supplementation among carriers of variants for some *FADS* SNPs [53]. A recent birth cohort study in China also reported significant interaction between DHA supplementation and maternal SNP rs174602 on DHA concentrations in colostrum [54]. Additionally, previous studies in European populations have shown that the CC genotype for SNP rs174602 is associated with lower Δ-5 desaturase activity [55].

We have also previously reported from the POSGRAD study that children born to TT carriers who received prenatal DHA had higher birthweight relative to those who received placebo, while no differences were observed among CC carriers (23). The fatty acid analysis performed in a subset of the study population ($n = 140$) showed that the minor T allele for SNP rs174602 was inversely associated with maternal plasma DHA concentrations at study enrollment. This suggests that these individuals were at greater risk of DHA deficiency, especially within the context of a diet high in n-6 fatty acids [23]. To add further context to these differences in findings, it is important to consider ancestral variations in the distribution of *FADS* genotypes. Most studies assessing the role of maternal and offspring *FADS* genes in child health have been conducted in European populations [56, 57]. However, Native American and Mexican populations have a greater proportion of carriers of alleles associated with slower conversion of precursors, along with diets high in n-6 PUFAs and low in n-3 LC-PUFAs [5, 58]. Targeting provision of preformed LC-PUFAs to these populations may be particularly important. Overall, our findings reinforce the potential need for targeted interventions and inclusion of genotype information in the design and interpretation of supplementation trials to optimize benefit-risk ratios, particularly in the clinical nutrition setting. However, additional research is needed to reproduce these findings, confirm whether mother-offspring dyads with specific maternal genetic profiles and/or nutritional statuses benefit more from prenatal DHA supplementation, and determine whether it will ever be feasible to utilize genetic information in the design of public health interventions.

Several limitations should be acknowledged when interpreting our findings. First, selection bias may influence the generalizability of our results. The offspring in our study sample (32% of the birth cohort) tended to have mothers who were older and had higher BMI and household SES at study enrollment relative to those lost to follow-up,

TABLE 3
MetS components by treatment allocation and maternal genotype of SNP rs174602 ($n = 314$).

	Treatment group		Maternal genotype		
	Placebo $N = 154$	DHA $N = 160$	CC $N = 127$	TC $N = 143$	TT $N = 44$
Glucose (mg/dL)	88.2 (86.9, 89.5)	87.3 (86.0, 88.6)	86.6 (85.1, 88.1)	88.3 (87.0, 89.6)	89.0 (86.6, 91.4)
HDL (mg/dL)	46.9 (45.1, 48.6)	46.0 (44.3, 47.8)	45.8 (43.8, 47.7)	46.5 (44.7, 48.2)	48.4 (45.2, 51.6)
Triglycerides (mg/dL)	104 (94.3, 113)	111 (101.7, 120)	113 (102.7, 123)	104 (94.2, 113)	103 (85.5, 120)
SBP (mmHg)	103 (101, 104)	102 (101, 104)	103 (101.6, 105)	102 (100.5, 103)	102 (99.1, 104)
BMI-z score	0.64 (0.43, 0.85)	0.69 (0.49, 0.91)	0.88 (0.65, 1.12)	0.55 (0.22, 0.76)	0.48 (0.08, 0.87)
MetS score	-0.01 (-0.10, 0.07)	0.01 (-0.08, 0.09)	0.06 (-0.03, 0.16)	-0.04 (-0.12, 0.05)	-0.07 (-0.22, 0.08)

Values presented are adjusted means (95% CI).
SBP, systolic blood pressure; MetS, metabolic syndrome.

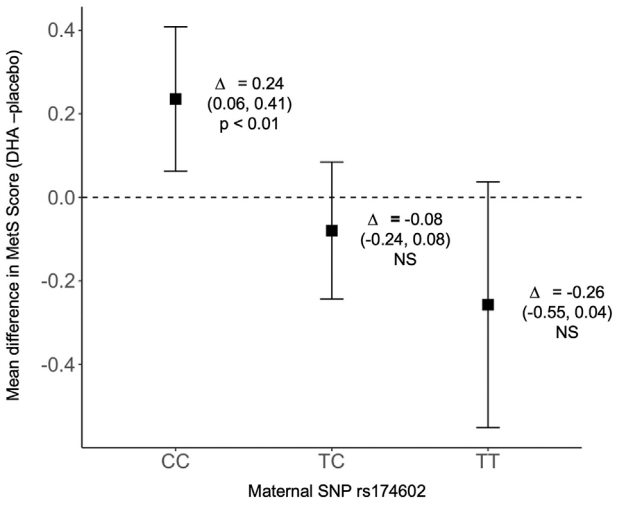

FIGURE 2. Effect modification by maternal SNP rs174602 on offspring MetS Score (*P*-interaction = 0.001). Values are contrast-specific mean differences (95% CI) between DHA and placebo groups from generalized linear models testing the interaction between *FADS2* single nucleotide polymorphism rs174602 and supplementation group on MetS score adjusted for child sex and age at measurement, maternal SES, BMI, parity and age at trial enrollment (n = 314). MetS, metabolic syndrome; NS, not significant; SNP, single nucleotide polymorphism.

but these values did not differ by prenatal treatment group or maternal genotype. Given the high loss to follow-up, sample sizes were limited, which may have contributed to the nonsignificant findings in the TT group. Additionally, cardiometabolic health can be influenced by many factors over the life course, starting with prenatal and early life factors (e.g., maternal BMI during pregnancy, birth weight, infant feeding practices). However, lifestyle factors over childhood, including diet, physical activity, and maturation, may be equally important in determining an individual's cardiometabolic risk [59]. Although there were no differences in maternal characteristics at baseline and offspring characteristics at birth by treatment group or genotype, we did observe differences in diet at age 11 y. Total PUFA intake differed by treatment group, and MUFA and saturated fat intake differed by genotype. However, when interaction between treatment and genotype was considered, no differences in diet were observed. Although these differences may be due to chance, we performed a sensitivity analysis additionally adjusting for PUFAs, MUFAs, and saturated fat, but effect estimates were not attenuated. There is potential bias related to the focus of this analysis on an SNP we have previously shown to be associated with birth weight; however, our findings remained significant even after adjusting for birth weight (i.e., the differences observed were not mediated by the effects of the interaction on birth weight).

Although PCA is a powerful dimensionality-reduction tool that allows us to maximize power by reducing the number of tested outcomes, loading coefficients of individual cardiometabolic factors

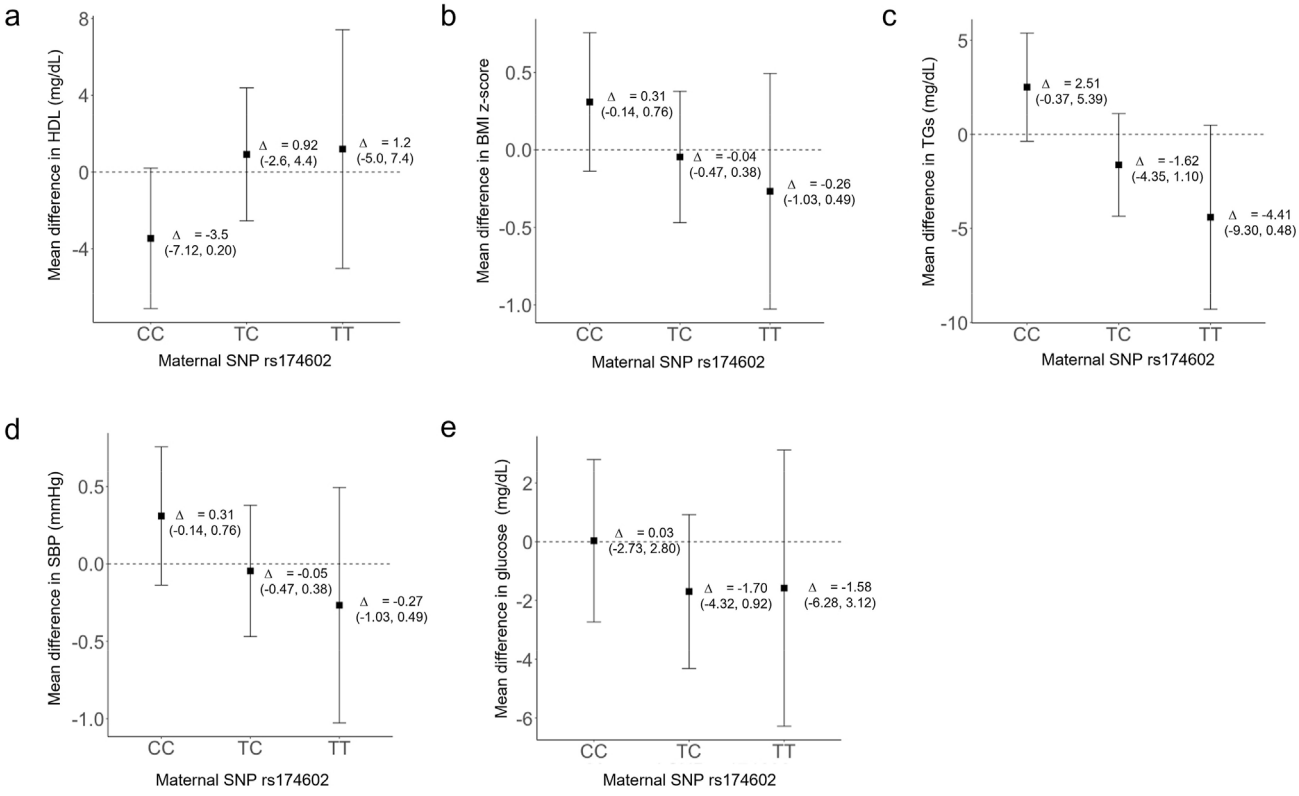

FIGURE 3. Effect modification by maternal SNP rs174602 on the impact of DHA supplementation on offspring MetS components, including A) HDL (*P*-interaction = 0.10); B) BMI z-score (*P*-interaction = 0.14); C) triglycerides (*P*-interaction = 0.03); D) systolic blood pressure (*P*-interaction = 0.01); and E) glucose (*P*-interaction = 0.43). Values are contrast-specific mean differences (95% CI) between DHA and placebo groups from generalized linear models testing the interaction between *FADS2* single nucleotide polymorphism rs174602 and supplementation group on MetS components adjusted for child sex and age at measurement, maternal SES, BMI, parity and age at trial enrollment (n = 314). SNP, single nucleotide polymorphism; TGs, triglycerides; SBP, systolic blood pressure.

from PCA are only applicable to the population from which they are derived. Dietary data were collected via single 24-h recall and may be subject to recall bias. As the trial was not originally designed to assess offspring cardiometabolic health, sample sizes are small, and there may be limited statistical power to detect differences by treatment group or genotype. Additionally, although plasma fatty acid concentrations were available in a small subset of the original birth cohort ($n = 75$), they were not included in the current manuscript due to small sample sizes. Future work should focus on identifying reliable markers of fatty acid status, which can potentially be used as a proxy for genotype. It is unclear whether the strong interaction observed with maternal genotype of SNP rs174602 is due to its high minor allele frequency in this sample (0.37) or because it is a functional SNP. To our knowledge, no biological function has been established for this SNP; it is, therefore, likely just a marker, not the causal variant. Further work is needed across larger, diverse datasets to reproduce these findings and investigate underlying biological mechanisms. Finally, although we show the possible importance of maternal *FADS* genotype in directing supplementation strategies, the role of offspring genotype remains unclear. Future work in adequately powered studies should incorporate offspring genotype information to elucidate this complex relationship.

Strengths of this study include the double-blind RCT design, high compliance to the prenatal intervention, extensive characterization of mothers and children throughout the trial and follow-up period, and availability of genetic information. Furthermore, our study participants were representative of a population with low dietary intakes of preformed DHA, high dietary intakes of n-6 fatty acids, and a high prevalence of alleles associated with lower conversion of precursor PUFAs into LC-PUFAs. Data collection and laboratory assays were standardized, validated, and conducted by trained personnel. The age at follow-up offers a stable time for lipid assessment, as current guidelines promote universal screening of lipids in children aged 9 to 11 y to establish baseline cardiometabolic risk.

In summary, we showed that the effect of prenatal DHA supplementation on offspring MetS score at the age of 11 y differed by maternal *FADS2* SNP rs174602. Population differences in *FADS* genotypes and diet may partially explain mixed results observed across prenatal DHA supplementation trials. However, given the large variation in genotype distributions across populations, this work should be reproduced in larger, independent cohorts. These findings further support the need to incorporate genetic analysis of *FADS* polymorphisms in DHA supplementation trials and may ultimately help guide the development of targeted supplementation recommendations early in the life course to improve cardiometabolic health in the clinical setting.

Author contributions

The authors' responsibilities were as follows—STW, IG-C, JAR, ADS, BK, and UR: designed the research; IG-C, ADS, HD, IRS, and UR: conducted the research; STW: analyzed the data and wrote the paper; STW and UR: had primary responsibility for the final content; and all authors: read and approved the final manuscript.

Data availability

Data described in the manuscript, code book, and analytic code will be made available upon request.

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Conflicts of interest

Usha Ramakrishnan is a member of the Journal's Editorial Board.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2023.10.005.

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