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Dietary and Plasma Polyunsaturated Fatty Acids Are Inversely Associated with Asthma and Atopy in Early Childhood

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Abstract

BACKGROUND: Polyunsaturated fatty acids (PUFAs) influence immune function and risk of allergic disease. Prior evidence of the effect of PUFA intake on childhood asthma and allergy is inconclusive.

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Conflicts of interest: J. Lasky-Su is a consultant to Metabolon Inc. R. S. Zeiger is a consultant for AstraZeneca, DBV Technologies, Genentech, Inc., GlaxoSmithKline, Novartis, Patara Pharma, Regeneron, TEVA Pharmaceuticals, and Theravance Biopharma, and has received research support from Aerocrine, AstraZeneca, Genentech, Inc., GlaxoSmithKline, National Heart, Lung, and Blood Institute (NHLBI), MedImmune, and Merck. G. T. O'Connor is a co-investigator on a grant from Janssen Pharmaceuticals to Boston University that funds a study of the pathogenesis of chronic obstructive pulmonary disease. L. B. Bacharier participates on the Data Safety Monitoring Board of DBV Technologies. A. Beigelman holds stock from DBV Technologies. A. A. Litonjua has received author royalties from UpToDate, Inc. and consultant fees from AstraZeneca, LP. The rest of the authors declare that they have no relevant conflicts of interest.

OBJECTIVES: To investigate associations of PUFA plasma levels and dietary intake with asthma and allergy at age 3 years in this ancillary study of the Vitamin D Antenatal Asthma Reduction Trial.

METHODS: Plasma PUFA levels were reported as relative abundances from mass spectrometry profiling, and dietary PUFA intake was derived from food frequency questionnaire responses. Associations between PUFA and outcomes, including asthma and/or recurrent wheeze, allergic sensitization, and total IgE at age 3 years, were evaluated in adjusted regression models. Additional regression models analyzed the combined effects of antenatal vitamin D and early childhood PUFA on outcomes.

RESULTS: Total, omega-3, and omega-6 plasma PUFA relative abundances were significantly ($P < .05$) inversely associated with both asthma and/or recurrent wheeze and allergic sensitization. Likewise, dietary PUFA intake was inversely associated with asthma and/or recurrent wheeze ($P < .05$ for omega-6 PUFA only). For both dietary and plasma measures of total, omega-3, and omega-6 PUFAs, inverse associations with outcomes were strongest among subjects with both high umbilical cord blood 25-hydroxyvitamin D and high PUFA at age 3 years.

CONCLUSIONS: PUFA dietary intake and plasma levels are inversely associated with asthma and/or recurrent wheeze and atopy at age 3 years. Antenatal vitamin D could modulate the effect of early childhood PUFA on risk of asthma and allergy. © 2018 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2019;7:529–38)

Keywords

Asthma; Allergy; Sensitization; Polyunsaturated fatty acid; Omega-6; Omega-3

Wheezing illnesses in the preschool age population are common and associated with significant morbidity and cost.[1,2] Risk of asthma and wheeze in this age group is multifactorial with genetic and environmental contributions,[3,4] and the presence of allergic sensitization has been linked to asthma that persists later in life.[5] Environmental and behavioral changes, including dietary factors, may be major contributors to childhood asthma risk.

Polyunsaturated fatty acids (PUFAs) including omega-3 and omega-6 fatty acids have dietary and endogenous sources and have long been thought to influence immune function and risk of asthma and other allergic disease.[6] However, reported associations between PUFAs and allergic disease in childhood have been inconsistent in observational analyses,[7–25] and data from randomized controlled trials are limited.[26,27]

To better understand this association, we used both nutrient analysis of food frequency questionnaires (FFQs) and plasma metabolomics to determine the associations of plasma and dietary PUFA at age 3 years and occurrence of asthma and/or recurrent wheeze (a composite outcome) or allergic sensitization by age 3 years in offspring of participants in the Vitamin D Antenatal Asthma Reduction Trial (VDAART). In this cross-sectional analysis, we tested the hypothesis that plasma and dietary PUFA at age 3 years would be inversely associated with the occurrence of asthma and/or recurrent wheeze and/or allergic sensitization. Given evidence suggesting that antenatal vitamin D is associated with reduced

risk of childhood asthma or wheeze,[28] we additionally hypothesized that the risk of asthma and/or recurrent wheeze or sensitization would be lowest among children who had both high antenatal vitamin D, as reflected by umbilical cord blood 25-hydroxyvitamin D (25-OH) concentration, and high early childhood PUFA plasma levels and/or dietary intake.

METHODS

Study design

Subjects were offspring of participants in VDAART, a randomized, double-blind, placebo-controlled trial of vitamin D supplementation during pregnancy to reduce the risk of asthma and/or recurrent wheeze in offspring (NCT00920621).[29,30] Participants were recruited at gestational age 10–18 weeks from study centers in Boston, St. Louis, and San Diego, USA and were randomized to daily 4400 IU (treatment arm) or 400 IU (placebo or usual care arm) of oral cholecalciferol supplementation during the remainder of pregnancy. Inclusion criteria required atopy (asthma, eczema, or allergic rhinitis) in either the biological mother or father. All participants were nonsmokers. The study protocol was approved by the institutional review boards at each participating institution and at Brigham and Women's Hospital. All participants provided written informed consent. A flow diagram for study participants is provided in Figure 1.

Outcome ascertainment

The composite outcome of asthma and/or recurrent wheeze has been described previously[29] and is based on the parental report of physician-diagnosed asthma, recurrent wheeze, and asthma controller medications. Parents were queried about these outcomes by telephone every 3 months and at annual in-person visits for the first 3 years of life, and data throughout follow-up were used to ascertain asthma and/or recurrent wheeze.

All subjects were asked to provide blood samples at age 3 years for the measurement of total IgE and serum specific IgE to common allergens (*Alternaria alternata, Dermatophagoides farinae, Dermatophagoides pteronyssinus*, German cockroach, cat dander, dog dander, grass pollen mix, tree pollen mix, egg white, walnut, milk, peanut, soybean, and wheat). Total IgE and serum specific IgE were available for 534 subjects, including 235 (96%) of the 245 subjects with available plasma metabolomics. All 534 subjects had available FFQ data. Allergic sensitization was determined to be present if specific IgE levels to at least one of the tested foods or environmental allergens were ≥0.35 kU/L.

PUFA ascertainment

Plasma metabolomics.—A subset of 245 subjects who provided plasma samples at age 3 years were selected for untargeted metabolomic profiling using a 1:2 frequency matching of children with (n = 84) and without (n = 161) asthma and/or recurrent wheeze for use in nested case-control analyses with sex, race, and study center as matching factors as previously described.[31] Metabolomic profiling, mass spectrometer platforms, sample extraction and preparation, instrument settings and conditions, and data handling were performed at Metabolon (Research Triangle Park, NC).[32] Results were expressed as relative

abundances, as mass spectrometry yields ion peaks corresponding to metabolites, which are quantified relative to a standard or to the most intense ion peak.

Two steps for additional processing of metabolite data were performed using R version 3.4.0 (R Foundation for Statistical Computing). First, metabolite relative abundance missing values were replaced with half of the minimum relative abundance observed for that metabolite, based on an assumption that missingness is due to low signal intensity.[33] All analyzed metabolites had interquartile ranges greater than zero, and all analyzed metabolites had 1% or fewer missing values except for adrenic acid (17% missing) and docosatrienoic acid (20.8% missing). Second, relative abundances were $\log_{10}$ normalized and Pareto-scaled (mean-centered and divided by the square root of the standard deviation).

Measurements for 13 plasma PUFA metabolites were available, including eicosapentaenoic acid (EPA), docosatrienoic acid, omega-3 docosapentaenoic acid (omega-3 DPA), stearidonic acid, docosahexaenoic acid (DHA), linoleic acid, arachidonic acid, dihomolinoleic acid, docosadienoic acid, adrenic acid, omega-6 DPA, dihomolinolenic acid (omega-3 or omega-6), and linolenic acid (omega-3 or omega-6). A summary variable was created for omega-3 plasma PUFAs by taking the sum of EPA, DHA, omega-3 DPA, docosatrienoic acid, and stearidonic acid relative abundances; and for omega-6 plasma PUFAs by taking the sum of linoleic acid, arachidonic acid, dihomolinoleic acid, docosadienoic acid, adrenic acid, and omega-6 DPA relative abundances.

FFQ nutrient intake.—Child nutrient intake was evaluated at age 3 years, when parents completed a modified version of a semi-quantitative FFQ that was previously validated in preschool-age children.[34] All participants were asked to complete the FFQ, and FFQ data were available for 738 (92%) of 806 subjects. We used FFQ responses to estimate daily calorie and PUFA intake. Nutrient compositions of FFQ items were determined using the Harvard nutrition composition database, which is based on US Department of Agriculture publications supplemented by other publications and information from manufacturers.[35–37] Further details are provided in the Methods section in this article's Online Repository at www.jaciinpractice.org.

Relevant nutrient components included total PUFA, linolenic acid, arachidonic acid, EPA, DPA, and DHA. A summary variable was created for omega-3 PUFA intake by taking the sum of EPA and DHA estimated intakes, and for omega-6 PUFA intake by taking the sum of linoleic and arachidonic fatty acid intakes. To account for total energy intake, nutrient density was calculated for each nutrient by dividing nutrient intake by total calorie intake and all analyses of nutrient densities included estimated calorie intake as a covariate.[38]

Other covariates

Baseline characteristics and other covariates were collected at enrollment, birth, or via questionnaires or annual study visits. These included sex, race/ethnicity, gestational age, VDAART study treatment assignment, cord blood 25-OH, study center, maternal education, household income, and body mass index (BMI) at age 3 years.

Statistics

Statistical analyses were conducted using R version 3.4.0. χ^{22} and *t* tests were used to test for differences in categorical and continuous baseline characteristics, respectively, by phenotype (asthma/recurrent wheeze vs no asthma/recurrent wheeze).

Average daily PUFA dietary intakes corresponding to each FFQ item were calculated. Spearman correlation was used to look for associations between FFQ items and plasma PUFA relative abundances. Pearson correlations were used to evaluate associations between dietary and plasma PUFAs; dietary PUFAs were right-skewed and were log-transformed for these analyses.

Subjects were divided into quartiles by dietary PUFA intake and the 4th quartile was compared with the 1st in analyses of associations with asthma/recurrent wheeze, total IgE, and allergic sensitization. Total IgE was right-skewed and was log-transformed before analysis in linear regression models. Asthma/recurrent wheeze and allergic sensitization were analyzed in logistic regression models or conditional logistic regression models, with the latter used to account for matching on sex, race, and study center in subjects with plasma PUFA measurements. These and all other adjusted models included sex, race/ethnicity (black vs nonblack), gestational age, study center, maternal education (college or higher vs non-college graduate), BMI, and total daily caloric intake on the basis of univariable associations between these variables and asthma/recurrent wheeze (Table I). Maternal education and household income were highly correlated (χ^2P value < .01), and maternal education was included in adjusted models instead of household income, as over 20% of subjects refused to report or did not know their household income (Table I).

To evaluate the joint effects of vitamin D and early childhood PUFA, subjects were categorized as high or low on these measures according to whether they were above or below sample median values on umbilical cord blood 25-OH, 25-OH at age 3 years, and plasma PUFAs, and in the 3rd or 1st tertile of dietary PUFA intake. Subjects were categorized into tertiles instead of quartiles for dietary intake, and at the median for plasma PUFA measures to ensure adequate sample size for adjusted analyses. Subjects were then cross-classified to create 4-level categorical variables with levels “high PUFA/high vitamin D,” “high PUFA/low vitamin D,” “low PUFA/high vitamin D,” and “low PUFA/low vitamin D.” This variable was used as a predictor of outcomes at age 3 in adjusted linear, logistic, and conditional logistic regression models. To generate *P* values for tests of trend, this variable was analyzed as a numeric variable (1 for “low PUFA/low vitamin D,” 2 for “low PUFA/high vitamin D,” or “high PUFA/low vitamin D,” and 3 for “high PUFA/high vitamin D). For this and all other analyses, nominal *P* values are reported.

RESULTS

Subject characteristics

Of 806 offspring of VDAART participants with clinical follow-up to early childhood, FFQs were completed by 738 (92%) and plasma metabolomics were available for 245 (30%) subjects at age 3 years. The distribution of demographic variables was similar between subjects with FFQ data, those with plasma metabolomics data, and the overall cohort (Table

I). Two hundred and eighteen (27%) of 806 total subjects, 207 (28%) of 738 subjects with FFQ data, and 84 (34%) of 245 subjects with plasma metabolomics developed asthma and/or recurrent wheeze by age 3 years. All 541 subjects with serum specific IgE data also had FFQ data, and 253 (47%) of these subjects had allergic sensitization (IgE ≥ 0.35 kU/L to at least 1 allergen) at age 3 years. Of subjects with available serum specific IgE and plasma metabolomics, 112 (48%) of 235 had allergic sensitization.

Children who developed asthma and/or recurrent wheeze by age 3 years, compared with those who did not, differed in several baseline characteristics. They were more likely to be male (62% vs 49%; $P < .01$) and of black rather than white or Hispanic race/ethnicity (50% vs 36% black, 15% vs 22% white, and 34% vs 42% Hispanic; $P < .01$). They were more likely to be born at an earlier gestational age (mean standard deviation [SD] 38.5[2.5] weeks vs 39.2 [1.7] weeks; $P < .01$), to mothers with lower education (26% vs 38% with college or higher education, $P = .01$), and to households with lower incomes (37% vs 27% with household income less than $30,000; $P < .01$). They also differed in frequency by study center ($P < .01$) and had higher BMI at age 3 years (mean [SD] 17.0 [2.0] vs 16.5 [1.6]; $P < .01$) (Table I). Accordingly, subsequent analyses were adjusted for these potential confounders.

Foods contributing to dietary and plasma PUFA

Both dietary PUFA intake and plasma PUFA levels were determined at age 3 years. FFQ items that contributed most to average estimated dietary PUFA intakes included potato, corn, or other chips (median daily PUFA intake 0.86 g and omega-6 PUFA intake 0.80 g), and nuts (median daily PUFA intake0.84 g and omega-6 PUFA intake 0.83 g) (Table E1, available in this article's Online Repository at www.jaci-inpractice.org). Nonfried fish made the largest contribution to omega-3 PUFA intake (estimated daily average intake 0.10 g omega-3 PUFA) (Table E1). In contrast, soup, egg, and cream cheese intakes were positively correlated with plasma total, omega-3, and omega-6 PUFAs (Table E2, available in this article's Online Repository at www.jaci-inpractice.org), and these correlations were weak to modest (maximum Spearman rho = 0.21). This discrepancy suggests that plasma PUFA abundances may depend on factors other than dietary intake such as endogenous production or differences in PUFA metabolism.

Correlations between dietary and plasma PUFAs

Dietary total PUFA was strongly correlated with dietary omega-6 PUFA (Pearson rho = 0.99, $P < .001$), reflecting that omega-6 PUFA made up the vast majority of total dietary PUFA intake. Both total and omega-6 dietary PUFA intakes were moderately associated with dietary omega-3 PUFA intake (Pearson rho = 0.47 and 0.45, respectively; $P < .001$ for both) (Table E3, available in this article's Online Repository at www.jaci-inpractice.org).

Estimated intake of dietary omega-3 PUFA, but not dietary total or omega-6 PUFA, was associated with plasma total, omega-3, and omega-6 PUFA levels (Pearson rho = 0.15, 0.14, and 0.15; $P = .02$, .03, and .02, respectively) (Table E3, available in this article's Online Repository at www.jaci-inpractice.org). In turn, plasma total, omega-3, and omega-6 PUFAs were highly correlated with one another (Pearson rho range 0.85–0.96; all $P < .001$) (Table

E3, Figure 2). These results suggest that dietary omega-3 PUFA intake contributes more to variation in plasma PUFAs than dietary omega-6 PUFA intake.

For 5 individual PUFAs, both dietary intakes and plasma levels were available. Of these, dietary and plasma values were correlated for DHA (Pearson rho = 0.23; $P < .001$) and linoleic acid (Pearson rho = 0.17; $P = .006$). Dietary and plasma values were positively but nonsignificantly correlated for DPA, EPA, and arachidonic acid (Pearson rho = 0.10, 0.09, and 0.07; $P = .13$, .17, and .29, respectively).

Early childhood PUFA and clinical outcomes

Plasma PUFA.—In adjusted analyses, plasma total, omega-3, and omega-6 PUFAs were all statistically significantly inversely associated with asthma and/or recurrent wheeze and with allergic sensitization (Table II). Plasma total, omega-3, and omega-6 PUFAs were also inversely associated with total IgE, though this was statistically significant only for omega-6 PUFA (beta −0.09; 95% confidence interval [CI] −0.17, −0.01; $P = .03$) (Table II). All 13 measured individual PUFAs showed a trend toward inverse associations with all 3 outcomes (Table E4, available in this article's Online Repository at www.jaciinpractice.org), and these associations were significant for 11 of 13 PUFAs for asthma and/or recurrent wheeze, 4 of 13 for allergic sensitization, and 4 of 13 for total IgE (Table E4).

Dietary PUFA.—Subjects were divided into quartiles by dietary total, omega-3, and omega-6 PUFA intakes (Table E5, available in this article's Online Repository at www.jaci-inpractice.org) and subjects in the 4th quartile were compared with those in the 1st quartile. In adjusted analyses, inverse associations were seen between asthma and/or recurrent wheeze and dietary intakes of total PUFA (odds ratio [OR] 0.65; 95% CI 0.38–1.09; $P = .10$), omega-3 (OR 0.61; 95% CI 0.36–1.02; $P = .06$), and omega-6 PUFA (OR 0.53; 95% CI 0.31–0.90; $P = .02$), though this was statistically significant only for omega-6 PUFA intake (Table II). In analyses of individual metabolites, intakes of all PUFAs were inversely associated with asthma/recurrent wheeze, and this was statistically significant for DHA and linoleic acid (Table E6, available in this article's Online Repository at www.jaciinpractice.org). Neither total, omega-3, nor omega-6 dietary PUFA intakes were associated with total IgE or allergic sensitization at age 3 years (Table II).

These findings reveal that PUFA plasma levels and dietary intake are inversely associated with asthma and/or recurrent wheeze in 3-year-old children, with stronger associations seen with plasma PUFAs than dietary PUFA intake. Plasma PUFA was also inversely associated with total IgE and allergic sensitization. The same directions of associations were seen for omega-3 and omega-6 PUFAs. In analyses of independent associations of omega-3 or omega-6 PUFAs with outcomes, adjusting for both in the same model, results were not statistically significant, likely owing to collinearity due to the high correlation between omega-3 and omega-6 PUFAs (data not shown).

Early childhood PUFA, antenatal vitamin D, and clinical outcomes

Given evidence suggesting protective effects of both antenatal vitamin D and early childhood PUFA, we hypothesized that the occurrence of clinical allergic outcomes would

be lowest among children who were exposed to a combination of high antenatal vitamin D and high early childhood PUFA. To test this, we constructed 4-level factor variables cross-classifying subjects based on whether they had high or low antenatal vitamin D (ie, above or below the sample median for umbilical cord blood 25-OH) and whether they had high or low PUFA at age 3 years (ie, above or below the sample median for plasma PUFAs and in the 3rd or 1st tertile for dietary PUFAs). The median umbilical cord blood 25-OH concentration was approximately 35 ng/mL, and offspring of mothers randomized to high-dose vitamin D supplementation were significantly more likely to have umbilical cord blood 25-OH concentration above the median compared with offspring of mothers assigned to low-dose vitamin D supplementation (χ^2test $P < .001$, with 28% of those below the median in the high-dose group and 71% of those above the median in the high-dose group).

In adjusted regression models, we found a consistent pattern whereby high vitamin D and high PUFA were associated with decreased asthma and/or recurrent wheeze and allergic sensitization and lower total IgE in comparison with low vitamin D and low PUFA (Figure 3, Table E7, available in this article's Online Repository at www.jaci-inpractice.org). This pattern held for analyses of both dietary and plasma PUFAs, and for total, omega-3, and omega-6 PUFAs. In contrast, subjects with high PUFA but low vitamin D did not significantly differ from subjects with low PUFA and low vitamin D for any clinical outcome or PUFA type (dietary, plasma, omega-3, omega-6, or total). Subjects with low PUFA but high vitamin D did have lower occurrence of allergic sensitization in comparison with subjects with low PUFA and low vitamin D, though this difference was generally not as large as the difference between subjects with high PUFA and high vitamin D compared with those with low PUFA and low vitamin D.

These findings suggest that the inverse association between early childhood PUFA and allergic outcomes is most robust in those who were exposed to higher *in utero* vitamin D. In the case of allergic sensitization, antenatal vitamin D may be more influential than early childhood PUFA. Of note, no such pattern of association was seen in analyses of the joint associations of 25-OH concentration at age 3 years and PUFA at age 3 years on allergic outcomes (Table E8, available in this article's Online Repository at www.jaci-inpractice.org).

DISCUSSION

In this analysis of a large and diverse cohort of children including PUFA plasma and dietary data, we found that total, omega-3, and omega-6 plasma PUFA relative abundances were inversely associated with asthma and/or recurrent wheeze and allergic sensitization at age 3 years. Likewise, dietary PUFA intake, especially omega-6 PUFA intake, was inversely associated with asthma and/or recurrent wheeze.

Both omega-3 and omega-6 PUFAs were inversely associated with allergic outcomes. Omega-3 PUFA is generally thought to be an anti-inflammatory source of specialized proresolving mediators, whereas omega-6 PUFAs including arachidonic acid are precursors for inflammatory lipid mediators.[6] However, arachidonic acid is also precursor for mediators

associated with protection against allergic inflammation, such as prostaglandin E2,[39,40] and with resolution of inflammation, such as lipoxin specialized proresolving mediators.[41]

Previously reported associations between PUFA intake and allergic disease in childhood have been markedly inconsistent in cross-sectional analyses. Our analysis is in agreement with studies reporting inverse associations between PUFA and allergic disease[7,8] and with consistently observed inverse associations between adherence to fish intake and a Mediterranean diet and asthma symptoms in children in observational studies,[42,43] though the Mediterranean diet is associated with higher omega-3 than omega-6 intake.[44,45] Of note, our findings also agree with a recent longitudinal analysis of PUFAs measured both by blood composition and dietary intake at age 8 years in 940 children in Sweden that found that plasma PUFAs, both omega-3 and omega-6, were inversely associated with allergic disease at age 16 years.[9]

In contrast to our findings, several cross-sectional studies found no association between PUFA and allergic disease in childhood,[10–12] and others found variable effects of different PUFAs. Of the latter, some,[13–16] but not all,[17–20] found that omega-3 fatty acids were inversely associated with allergic disease and omega-6 fatty acids were positively associated with allergic disease. Some cross-sectional studies found a positive association between childhood PUFA and asthma, wheeze, or atopy,[21–25] though most of these studies were limited by small sample size,[24] absent measurement of blood or red blood cell membrane PUFAs,[21–24] and use of questionnaires that asked only about fats in cooking or on bread and/or toast without ascertaining intake of foods high in PUFAs.[22,23]

Randomized controlled trial evidence of postnatal PUFA supplementation to prevent or treat childhood allergic disease is limited. Two recently published meta-analyses on asthma or allergy prevention found no effect of postnatal omega-3 fatty acid supplementation on risk of asthma[27] and no effect of PUFA supplementation in infancy on allergic outcomes.[26] Both concluded that the amount and quality of evidence was low.[26,27] Of 3 trials that suggested a benefit of PUFA supplementation, only 1 was included in the aforementioned meta-analyses —a trial that randomized 89 infants to formula supplementation with DHA and arachidonic acid versus control formula and found lower incidence of allergic disease in the first 3 years of life in the treatment group.[46] A more recent ancillary study of a trial of DHA and arachidonic acid infant formula supplementation (n = 72) versus control formula (n = 19) found that PUFA supplementation delayed the time to first allergic illness.[47] A third study geared toward treatment rather than prevention of asthma found that among 29 children requiring inpatient long-term treatment of asthma in Japan, randomization to fish oil versus olive oil capsules was associated with reduced asthma symptom scores and responsiveness to acetylcholine.[48] Neither these studies nor the present study considers the question of maternal PUFA intake during pregnancy and its relationship to asthma and allergy outcomes in offspring, though there is evidence suggesting a protective effect of antenatal PUFA on asthma and allergic disease in offspring.[49–51]

The inconsistency of findings to date suggests that genetic or other nutritional factors may influence the effect of PUFA supplementation on asthma and allergy risk. Our study design allowed us to investigate the influence of antenatal vitamin D, as subjects were born to

participants in a trial of high-dose antenatal vitamin D supplementation, an intervention that recent clinical trial evidence suggests may reduce risk of childhood asthma.[28] Little is known about whether vitamin D and PUFAs have overlapping, complementary, or even synergistic mechanisms of benefit in prevention of allergic disease. We found higher umbilical cord blood 25-OH in combination with higher dietary or plasma PUFA at age 3 years was associated with lower occurrence of asthma and/or recurrent wheeze, allergic sensitization, and lower total IgE. These findings suggest that the combination of antenatal vitamin D and postnatal PUFA may yield more benefit than either intervention alone, and add to the body of evidence in favor of maintaining vitamin D sufficiency during pregnancy. Of note, the children analyzed in the present study were of high genetic risk of allergic disease, and it is possible that this is another factor that could promote or inhibit a beneficial effect of PUFA.

We found that plasma omega-3 and omega-6 PUFAs were highly positively correlated, whereas dietary omega-3 and omega-6 intakes were modestly positively correlated. This is in keeping with an analysis of a cohort of 8-year-old children that found that plasma phospholipid arachidonic acid was strongly correlated with omega-3 PUFAs (Spearman rho 0.80; $P < .01$), though linoleic acid was not correlated with either omega-3 PUFAs or arachidonic acid in that analysis.[9] This high correlation between omega-3 and omega-6 PUFAs compounds the challenge of discerning which PUFA type is the greatest contributor to the observed associations with asthma/recurrent wheeze and allergic sensitization.

Some study limitations occurred. Like many other observational studies,[52–54] this was an ancillary analysis of data from a randomized clinical trial designed to investigate a different research question, which may limit generalizability of our results. FFQ analysis of nutrient intake is subject to presumably non-differential measurement bias. Although we found that different foods contributed to dietary and plasma PUFAs, the strength of correlations we observed between dietary and plasma PUFAs is similar to that previously reported in pediatric populations,[9,55] and the associations we observed of dietary and plasma PUFAs with asthma and/or recurrent wheeze were consistent with one another. Summing highly correlated PUFAs to create total PUFA, total omega-3, and total omega-6 variables may amplify differences between subjects and could lead to overestimation of associations with outcomes. However, these differences may be biologically relevant. We did not have plasma PUFA data for all subjects, and though there was a large sample of subjects with dietary data, comparison of top with bottom quartile or tertile on dietary variables limited the number of subjects included in these analyses. Subjects with plasma PUFA measurements were selected for a case-control analysis of asthma and/or recurrent wheeze, and this could result in some degree of bias in analyses of other outcomes. Although the consistency of our findings suggests a true biologic effect, multiple tests were performed without P value correction, and P values should accordingly be interpreted with caution. Neither FFQs nor plasma metabolomics likely capture medium- and long-term PUFA intake as well as measurement in erythrocyte membranes or other tissues,[56] which was not available for this study. Dietary data were available at age 3 years only, and findings may not apply to PUFA dietary intake earlier or later in life. As offspring of VDAART participants accrue ongoing clinical follow-up, it will be valuable to determine the effects of antenatal vitamin D and early childhood PUFA on outcomes later in childhood.

In conclusion, plasma PUFA relative abundances and dietary PUFA intake are inversely associated with asthma and/or recurrent wheeze, and plasma PUFA relative abundances are also inversely associated with allergic sensitization at age 3 years. Our findings suggest that antenatal vitamin D could modulate the effect of early childhood PUFA intake on risk of asthma and allergy.

METHODS

Ascertainment of nutrient intake

Child nutrient intake was evaluated at age 3 years, when parents completed a modified version of a semiquantitative food frequency questionnaire (FFQ) that was previously validated in preschool-age children.[E1] Parents were asked: “In the past month, on average, how often did your child eat the following foods?” and for each food or beverage, chose from 6 or 7 response categories ranging from “Never” to “2 or more times/day” or “5 or more times/day.”

We used FFQ responses to estimate caloric intake and intake of polyunsaturated fatty acids. Nutrient compositions of FFQ items were determined using the Harvard nutrition composition database, which is based on US Department of Agriculture publications supplemented by other publications and information from manufacturers.[E2–E4] Of the 87 items on the FFQ, 4 (bacon, margarine tub, margarine stick, and cereal [cold]) were not in the Harvard nutrient composition database and nutrient compositions for these foods were obtained from the US Department of Agriculture Nutrient Database for Standard Reference (Release 28).[E4] Where nutrient values were not available for a food, nutrient contents were assumed to be negligible. Standard portion sizes were obtained using the US Department of Agriculture What’s In the Foods You Eat Search Tool.[E5] The portion size associated with the label “quantity not specified” was selected for each food item except for pizza, for which 119 g, the portion size for “1 piece, not further specified,” was selected instead of the “quantity not specified” portion size of 238 g.

For each of the 87 FFQ items, reported food frequencies were converted to estimated number of daily servings: “Never” was replaced by 0, “<1 time per week” was replaced with 0.5/7, “Once per week” was replaced with 1/7, “2–4 times/week” was replaced with 3/7, “Nearly daily or daily” was replaced with 1, “2–4 times/day” was replaced with 3, “2 or more times/day” was replaced with 3, and “5 or more times/day” was replaced with 5. Daily intake of calories and of each nutrient of interest was calculated by multiplying the estimated number of daily servings for each food item by the nutrient content in a standard portion of that food item, and then summing the result for each nutrient across all food items for each subject.

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Abbreviations used

25-OH	25-Hydroxyvitamin D
BMI	Body mass index
CI	Confidence interval
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
EPA	Eicosapentaenoic acid
FFQ	Food frequency questionnaire
PUFA	Polyunsaturated fatty acid
SD	Standard deviation
VDAART	Vitamin D Antenatal Asthma Reduction Trial

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What is already known about this topic?

Prior evidence of the effect of postnatal polyunsaturated fatty acids (PUFAs) on childhood asthma and allergy is inconclusive.

What does this article add to our knowledge?

Dietary and plasma PUFAs were inversely associated with asthma and/or recurrent wheeze and atopy at age 3 years, and associations were strongest among subjects with high cord blood vitamin D and high PUFA at age 3 years.

How does this study impact current management guidelines?

These findings provide evidence that postnatal PUFA may be protective against childhood asthma and allergy, and add to the evidence supporting maintenance of antenatal vitamin D sufficiency.

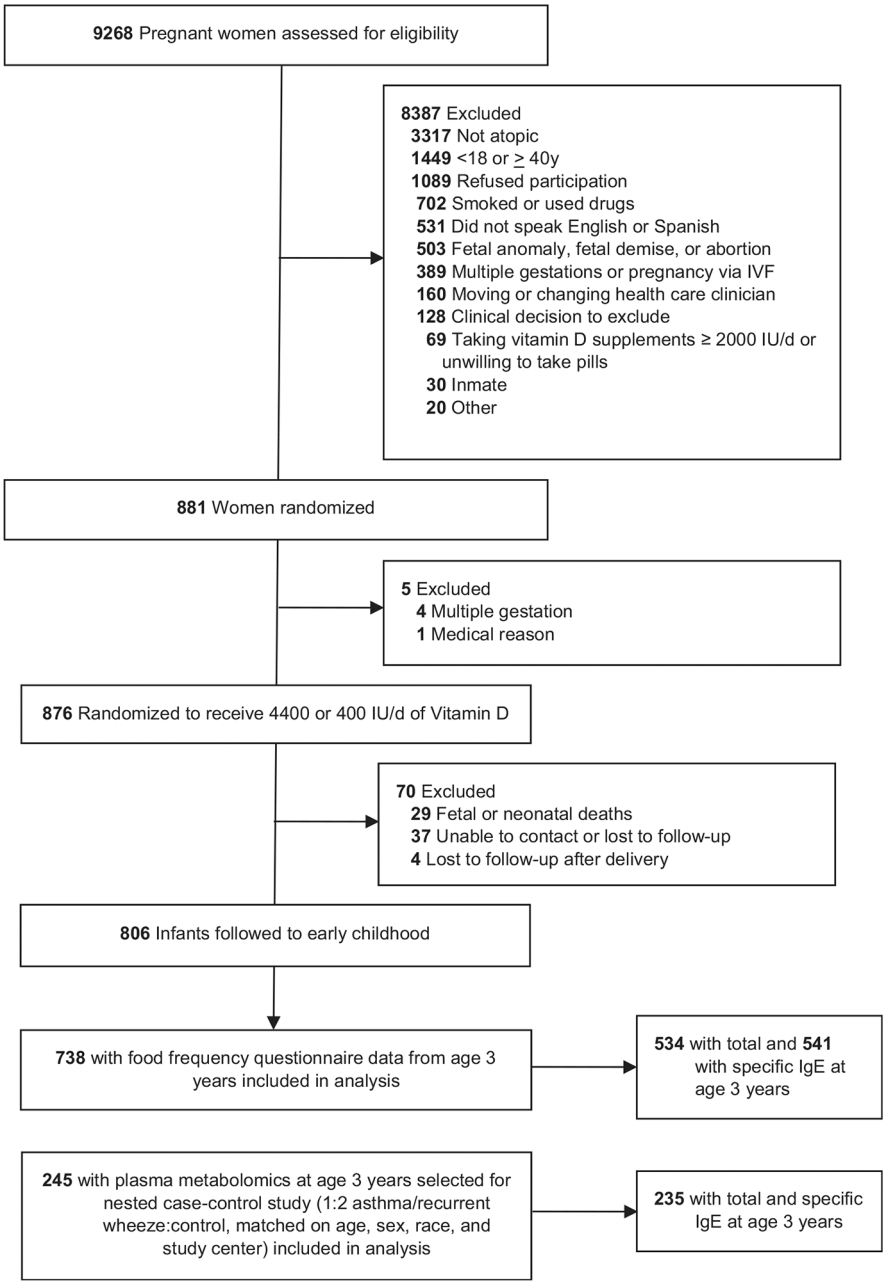

FIGURE 1.

Flow diagram of subjects included in study. A portion of this figure has been published previously.[29] *IVF, in vitro* fertilization.

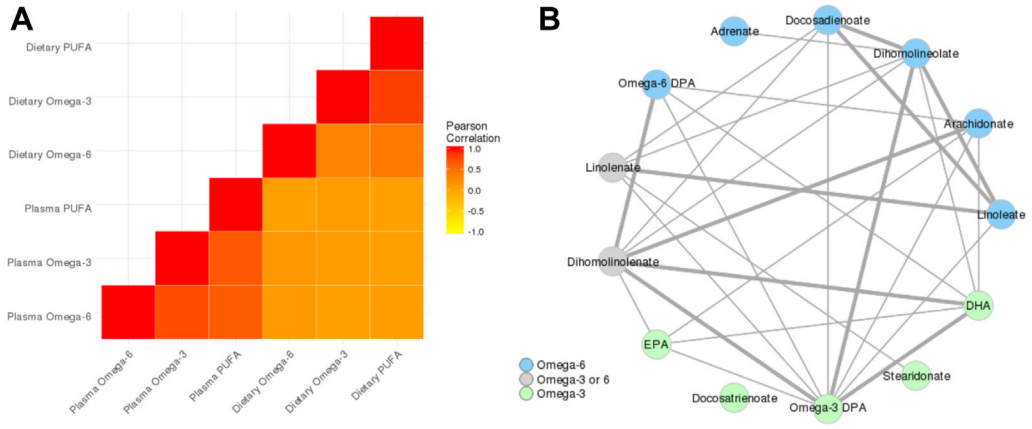

FIGURE 2.

Correlations between dietary and plasma polyunsaturated fatty acids (PUFAs). **A**, Pearson correlation heatmap reveals high (red) correlation between plasma total, omega-3, and omega-6 PUFA, and between dietary total and omega-6 PUFA. Other correlations were weak-to-moderate (orange). Dietary PUFA intake was log-transformed before Pearson correlation analyses. **B**, Spearman correlation network of individual plasma PUFAs shows high correlations within and between omega-3 and omega-6 PUFAs. Thin edges signify Spearman rho >0.7 and thick edges signify Spearman rho >0.8. ***DHA***, Docosahexaenoic acid; ***DPA***, docosapentaenoic acid; ***EPA***, eicosapentaenoic acid.

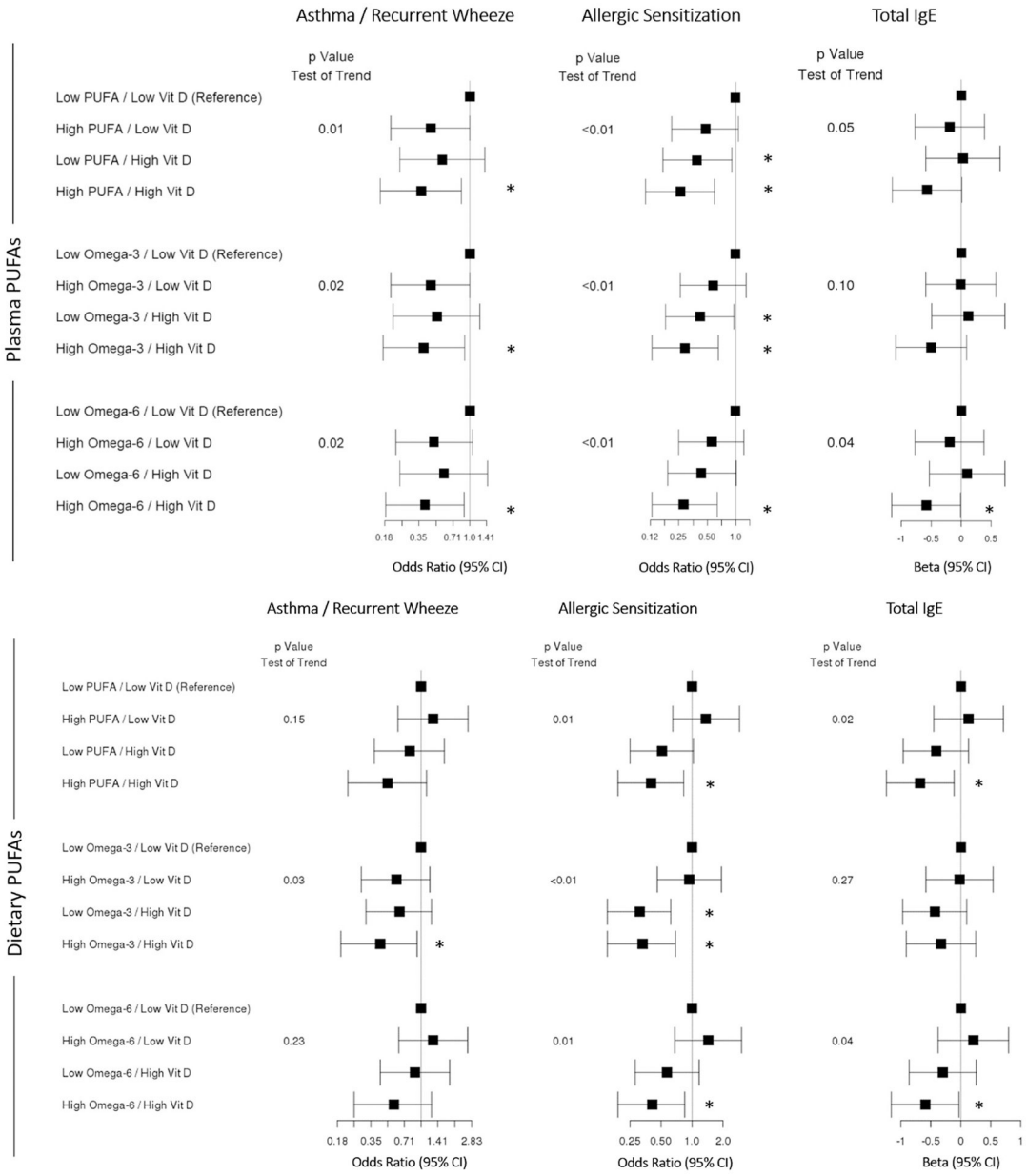

FIGURE 3.

Associations between childhood polyunsaturated fatty acid (PUFA)/antenatal vitamin D and clinical outcomes. Subjects were cross-classified into one of 4 categories based on whether they had high or low antenatal vitamin D (ie, above or below median for umbilical cord blood 25-hydroxyvitamin D) and whether they had high or low PUFA at age 3 years (ie, above or below median for plasma PUFAs or 3rd or 1st tertile for dietary PUFAs). Logistic, conditional logistic, and linear regression models were adjusted for sex, race/ethnicity, gestational age, study center, maternal education, body mass index, and total daily caloric intake. *P* values are for tests of trend. Both vitamin D and dietary PUFA data were available

for 569 subjects, and both vitamin D and plasma PUFA data were available for 242 subjects. *CI*, Confidence interval.

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TABLE I.

Baseline characteristics; tabulated for subjects who provided food frequency questionnaire responses, those who provided plasma samples for metabolomics analysis, and for the entire VDAART offspring cohort

	Food frequency questionnaire data available				Plasma metabolomics available				Entire VDAART cohort			
Variable	**All children (n = 738)**	**Asthma/wheeze (n = 207)**	**No asthma/wheeze (n = 530)**	***P* value**	**All children (n = 245)**	**Asthma/wheeze (n = 84)**	**No asthma/wheeze (n = 161)**	***P* value**	**All children (n = 806)**	**Asthma/wheeze No (n = 218)**	**asthma/wheeze (n = 530)**	***P* value**
Sex, number (%)				**.002**				**.19**				**.001**
Male	391 (53)	129 (62)	261 (49)		133 (54)	51 (61)	82 (51)		421 (52)	136 (62)	261 (49)	
Female	347 (47)	78 (38)	269 (51)		112 (46)	33 (39)	79 (49)		385 (48)	82 (38)	269 (51)	
Race/ethnicity, number (%)				**.0004**				**.002**				**.001**
Black, non-Hispanic	301 (41)	108 (52)	192 (36)		93 (38)	42 (50)	51 (32)		317 (39)	110 (50)	192 (36)	
White, non-Hispanic	146 (20)	32 (15)	114 (22)		44 (18)	18 (21)	26 (16)		161 (20)	33 (15)	114 (22)	
Hispanic or other	291 (39)	67 (32)	224 (42)		108 (44)	24 (29)	84 (52)		328 (41)	75 (34)	224 (42)	
Gestational age (wk), mean (SD)	39.0 (2.0)	38.4 (2.5)	39.2 (1.7)	**.0001**	39.2 (1.6)	38.8 (1.8)	39.4 (1.4)	**.01**	39.0 (2.0)	38.5 (2.5)	39.2 (1.7)	**.0003**
VDAART treatment group, number (%)				.06				.37				.07
4,400 IU/d vitamin D	370 (50)	92 (44)	278 (52)		119 (49)	37 (44)	82 (51)		405 (50)	98 (45)	278 (52)	
400 IU/d vitamin D	368 (50)	115 (56)	252 (48)		126 (51)	47 (56)	79 (49)		401 (50)	120 (55)	252 (48)	
Cord blood 25 OH (ng/mL), mean (SD)	37.3 (18.3)	33.6 (17.1)	38.6 (18.6)	**.003**	36.3 (17.2)	33.6 (18.6)	37.6 (16.3)	.09	37.0 (18.3)	33.2 (16.9)	38.6 (18.6)	**.001**
Study center, number (%)				**.001**				**.001**				**.001**
Boston	207 (28)	69 (33)	138 (26)		54 (22)	22 (26)	32 (20)		240 (30)	76 (35)	138 (26)	
St. Louis	289 (39)	92 (44)	197 (37)		101 (41)	45 (54)	56 (35)		292 (36)	92 (42)	197 (37)	
San Diego	242 (33)	46 (22)	195 (37)		90 (37)	17 (20)	73 (45)		274 (34)	50 (23)	195 (37)	
Maternal education, number (%)				**.02**				.07				**.01**
<High school	94 (13)	33 (16)	61 (12)		31 (13)	16 (19)	15 (9)		100 (12)	35 (16)	61 (12)	
High school or technical School	217 (29)	68 (33)	149 (28)		63 (26)	24 (29)	39 (24)		241 (30)	72 (33)	149 (28)	
Some college	170 (23)	51 (25)	118 (22)		64 (26)	21 (25)	43 (27)		192 (24)	54 (25)	118 (22)	
College graduate or higher	257 (35)	55 (27)	202 (38)		87 (36)	23 (27)	64 (40)		273 (34)	57 (26)	202 (38)	
Household income, number (%)				**.001**				.24				**.001**
<$30,000	221 (30)	78 (38)	142 (27)		69 (28)	29 (35)	40 (25)		236 (29)	81 (37)	142 (27)	
$30,000-$49,999	94 (13)	32 (15)	62 (12)		33 (13)	10 (12)	23 (14)		105 (13)	33 (15)	62 (12)	
$50,000-$74,999	88 (12)	18 (9)	70 (13)		22 (9)	7 (8)	15 (9)		97 (12)	19 (9)	70 (13)	

	Food frequency questionnaire data available				Plasma metabolomics available				Entire VDAART cohort			
Variable	All children (n = 738)	Asthma/wheeze (n = 207)	No asthma/wheeze (n = 530)	*P* value	All children (n = 245)	Asthma/wheeze (n = 84)	No asthma/wheeze (n = 161)	*P* value	All children (n = 806)	Asthma/wheeze No (n = 218)	asthma/wheeze (n = 530)	*P* value
$75,000-$99,999	71 (10)	8 (4)	63 (12)		29 (12)	5 (6)	24 (15)		79 (10)	10 (5)	63 (12)	
$100,000-$149,999	59 (8)	12 (6)	47 (9)		23 (9)	7 (8)	16 (10)		62 (8)	12 (6)	47 (9)	
>$150,000	29 (4)	6 (3)	23 (4)		10 (4)	2 (2)	8 (5)		31 (4)	6 (3)	23 (4)	
Refused to say or unknown	176 (24)	53 (26)	123 (23)		59 (24)	24 (29)	35 (22)		196 (24)	57 (26)	123 (23)	
BMI at age 3 y, mean (SD)	16.6 (1.8)	17.0 (2.0)	16.5 (1.6)	**.003**	16.8 (2.0)	17.1 (2.2)	16.6 (1.8)	.06	16.6 (1.8)	17.0 (2.0)	16.5 (1.6)	**.003**

P values are for *t* tests for gestational age, cord blood 25OH and BMI; and for χ^2 tests for all other comparisons. Bold indicates statistical significance ($P < .05$).

Missing data: asthma/wheeze status was missing for 58 subjects. Gestational age was unavailable for 4 subjects. Cord blood 25OH was missing for 190 subjects. BMI was missing for 149 subjects.

25-OH, 25-Hydroxyvitamin D; *BMI*, body mass index; *SD*, standard deviation; *VDAART*, Vitamin D Antenatal Asthma Reduction Trial.

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TABLE II.

Associations of plasma and dietary total PUFA, omega-3 PUFA, and omega-6 PUFA with asthma/recurrent wheeze, total IgE, and allergic sensitization

	Asthma/recurrent wheeze (n = 245)		Allergic sensitization (n = 235)		Total IgE (n = 235)	
	OR (95% CI)	***P* value**	**OR (95% CI)**	***P* value**	**Beta (95% CI)**	***P* value**
Plasma						
Total PUFA	**0.93 (0.88, 0.98)**	**.01**	**0.94 (0.90, 0.99)**	**.03**	−0.04 (−0.07, 0.00)	.06
Omega-3	**0.83 (0.73, 0.95)**	**.01**	**0.87 (0.76, 0.99)**	**.03**	−0.07 (−0.16, 0.03)	.16
Omega-6	**0.87 (0.77, 0.97)**	**.01**	**0.89 (0.79, 0.99)**	**.03**	**−0.09 (−0.17, −0.01)**	**.03**
	Asthma/recurrent wheeze (n = 737)		**Allergic sensitization (n = 541)**		**Total IgE (n = 534)**	
Dietary						
Total PUFA	0.65 (0.38, 1.09)	.10	0.98 (0.59, 1.61)	.92	−-0.11 (−0.50, 0.28)	.58
Omega-3	0.61 (0.36, 1.02)	.06	1.23 (0.75, 2.02)	.40	0.13 (0.25, 0.52)	.50
Omega-6	**0.53 (0.31, 0.90)**	**.02**	1.04 (0.63, 1.72)	.87	−0.15 (−0.55, 0.24)	.44

For plasma PUFA metabolites, relative abundances were analyzed as continuous variables; for dietary PUFA intake, the 4th quartile was compared with the 1st quartile. Logistic, conditional logistic, and linear regression models were adjusted for sex, race/ethnicity, gestational age, study center, maternal education, BMI, and total daily caloric intake.

Bold indicates statistical significance ($P < .05$).

BMI, Body mass index; *CI*, confidence interval; *OR*, odds ratio; *PUFA*, polyunsaturated fatty acid.

TABLE E1.

Top 10 FFQ items by average daily intake in grams of total PUFA, omega-3 PUFA, and omega-6 PUFA

Total PUFA intake		Omega-3 PUFA intake		Omega-6 PUFA intake	
Food	**Mean intake (g)**	**Food**	**Mean intake (g)**	**Food**	**Mean intake (g)**
Chips	0.86	Other fish	0.10	Nuts	0.83
Nuts	0.84	Fried fish	0.03	Chips	0.80
Fried chicken	0.77	Other chicken	0.02	Fried chicken	0.72
Other chicken	0.76	Whole eggs	0.01	Other chicken	0.69
French fries	0.66	Canned tuna	0.01	Peanut butter	0.62

A total of 87 FFQ items were analyzed and 738 subjects contributed to this analysis.

FFQ, Food frequency questionnaire; *PUFA*, polyunsaturated fatty acid.

TABLE E2.

Top 10 food frequency questionnaires with strongest positive correlations with plasma total PUFA, omega-3 PUFA, and omega-6 PUFA metabolites

Total plasma PUFA			**Plasma omega-3**			**Plasma omega-6**		
Food	**Rho**	***P* value**	**Food**	**Rho**	***P* value**	**Food**	**Rho**	***P* value**
Other soup	0.18	**.01**	Cream cheese	0.21	**.001**	Baked beans/chili	0.20	**.002**
Baked beans/chili	0.16	**.01**	Other soup	0.13	**.04**	Other soup	0.19	**.003**
Vegetable soup	0.16	**.01**	Baked beans/chili	0.11	.07	Vegetable soup	0.18	**.005**
Cream cheese	0.15	**.02**	Vegetable soup	0.11	.08	Whole eggs	0.18	**.01**
Whole eggs	0.14	**.02**	Whole eggs	0.11	.09	Cookies or brownies	0.14	**.02**
Cookies or brownies	0.12	.07	Carrots	0.11	.10	Orange or grapefruit	0.13	**.05**
Orange juice	0.12	.07	Other fish	0.10	.13	Orange juice	0.13	**.05**
Other fish	0.11	.08	Canned tuna	0.09	.14	Other fish	0.13	**.05**
Canned tuna	0.11	.09	Orange juice	0.09	.17	Jello	0.12	.05
Cornbread/tortilla	0.10	.11	Cornbread/tortilla	0.08	.19	Canned tuna	0.12	.06

Spearman rho for correlation between estimated number of daily servings and metabolite relative abundances. A total of 87 FFQ items were analyzed and 245 subjects contributed to this analysis.

Bold indicates statistical significance ($P < .05$).

FFQ, Food frequency questionnaire; *PUFA*, polyunsaturated fatty acid.

TABLE E3.

Pearson correlation of dietary and plasma PUFAs

	Variable 1	Variable 2	Rho	*P* value
Plasma-dietary PUFA correlations	Plasma PUFA	Dietary PUFA	0.09	.16
	Plasma PUFA	Dietary omega-3	**0.15**	**.02**
	Plasma PUFA	Dietary omega-6	0.08	.21
	Plasma omega-3	Dietary PUFA	0.05	.48
	Plasma omega-3	Dietary omega-3	**0.14**	**.03**
	Plasma omega-3	Dietary omega-6	0.04	.58
	Plasma omega-6	Dietary PUFA	0.12	.06
	Plasma omega-6	Dietary omega-3	**0.15**	**.02**
	Plasma omega-6	Dietary omega-6	0.11	.08
Dietary-dietary PUFA correlations	Dietary PUFA	Dietary omega-3	**0.47**	**<.001**
	Dietary PUFA	Dietary omega-6	**0.999**	**<.001**
	Dietary omega-3	Dietary omega-6	**0.45**	**<.001**
Plasma-plasma PUFA correlations	Plasma PUFA	Plasma omega-3	**0.95**	**<.001**
	Plasma PUFA	Plasma omega-6	**0.96**	**<.001**
	Plasma omega-3	Plasma omega-6	**0.85**	**<.001**

Dietary PUFA estimates were log-transformed before correlation analysis.

Bold indicates statistical significance ($P < .05$).

PUFA, Polyunsaturated fatty acid.

TABLE E4.

Associations of individual plasma PUFAs with asthma/recurrent wheeze, total IgE (log-transformed), and allergic sensitization

	Asthma/recurrent wheeze		Total IgE		Allergic sensitization	
Metabolite	**OR (95% CI)**	***P* value**	**Beta (95% CI)**	***P* value**	**OR (95% CI)**	***P* value**
Omega-3						
DHA	**0.45 (0.24, 0.84)**	**.01**	−0.39 (−0.80, 0.03)	.07	0.61 (0.34, 1.08)	.09
EPA	**0.50 (0.28, 0.90)**	**.02**	−0.21 (−0.62, 0.19)	.30	**0.48 (0.27, 0.85)**	**.01**
DPA	**0.41 (0.23, 0.74)**	**.003**	−0.28 (−0.67, 0.10)	.15	0.63 (0.37, 1.08)	.10
Docosatrienoic acid	0.91 (0.58, 1.42)	.68	−0.13 (−0.44, 0.19)	.43	0.85 (0.55, 1.30)	.44
Stearidonic acid	**0.48 (0.29, 0.80)**	.005	−0.11 (−0.47, 0.25)	.55	**0.57 (0.35, 0.93)**	**.02**
Omega-6						
Linoleic acid	**0.49 (0.29, 0.86)**	**.01**	**−0.39 (−0.78, −0.01)**	**.04**	0.66 (0.39, 1.12)	.12
Arachidonic acid	**0.50 (0.26, 0.96)**	**.04**	−0.20 (−0.65, 0.25)	.38	**0.48 (0.26, 0.90)**	**.02**
Dihomolinoleic acid	**0.44 (0.24, 0.81)**	**.01**	−0.37 (−0.78, 0.05)	.08	0.72 (0.41, 1.26)	.25
Docosadienoic acid	**0.46 (0.24, 0.90)**	**.02**	**−0.56 (−1.01, −0.11)**	**.02**	0.56 (0.30, 1.04)	.07
Adrenic acid	0.88 (0.57, 1.35)	.57	−0.26 (−0.57, 0.06)	.11	0.66 (0.43, 1.02)	.06
DPA	**0.39 (0.21, 0.75)**	**.005**	**−0.49 (−0.92, −0.06)**	**.03**	**0.50 (0.27, 0.92)**	**.03**
Omega-3 or 6						
Linolenic acid	**0.48 (0.29, 0.79)**	**.004**	**−0.39 (−0.73, −0.05)**	**.03**	0.65 (0.41, 1.03)	.07
Dihomolinolenic acid	**0.33 (0.17, 0.66)**	**.001**	−0.21 (−0.66, 0.23)	.35	0.64 (0.35, 1.18)	.15

Each PUFA was analyzed as a continuous variable. Models were adjusted for gender, race/ethnicity, gestational age, study center, maternal education, BMI, and total daily caloric intake.

Bold indicates statistical significance ($P < .05$).

BMI, Body mass index; *CI*, confidence interval; *DHA*, docosahexaenoic acid; *DPA*, docosapentaenoic acid; *EPA*, eicosapentaenoic acid; *OR*, odds ratio; *PUFA*, polyunsaturated fatty acid.

TABLE E5.

Estimated median (interquartile range) daily dietary intake in grams of total PUFA, omega-3 PUFA, and omega-6 PUFA by quartile

	Total PUFA	Omega-3	Omega-6
1st quartile	11.1 (8.4, 14.4)	0.03 (0.01, 0.04)	9.7 (7.4, 12.8)
2nd quartile	14.2 (10.7, 18.1)	0.09 (0.07, 0.12)	12.6 (9.7, 15.9)
3rd quartile	16.8 (12.7, 21.9)	0.15 (0.12, 0.22)	14.7 (10.7, 19.3)
4th quartile	19.2 (15.6, 26.5)	0.37 (0.21, 0.52)	16.7 (13.7, 22.9)

PUFA, Polyunsaturated fatty acid.

TABLE E6.

Associations of intakes of individual dietary PUFAs with asthma/recurrent wheeze, total IgE (log-transformed), and allergic sensitization

	Asthma/recurrent wheeze		Total IgE		Allergic sensitization	
Metabolite	**OR (95% CI)**	***P* value**	**Beta (95% CI)**	***P* value**	**OR (95% CI)**	***P* value**
Omega-3						
DHA	**0.57 (0.34, 0.96**	**.03**	0.05 (−0.34, 0.44)	.79	1.04 (0.63, 1.70)	.89
EPA	0.69 (0.41, 1.17)	.17	0.19 (−0.20, 0.57)	.35	1.35 (0.82, 2.22)	.23
Omega-6						
Arachidonic acid	0.84 (0.50, 1.42)	.52	0.08 (−0.31, 0.47)	.69	0.94 (0.57, 1.54)	.80
Linoleic acid	**0.57 (0.33, 0.97)**	**.04**	−0.15 (−0.55, 0.24)	.45	1.03 (0.62, 1.70)	.92
Omega-3 or 6						
DPA	0.74 (0.43, 1.24)	.25	0.28 (−0.11, 0.66)	.16	1.35 (0.82, 2.21)	.23

For each PUFA, the 4th quartile was compared with the 1st quartile. Models were adjusted for gender, race/ethnicity, gestational age, study center, maternal education, BMI, and total daily caloric intake.

Bold indicates statistical significance ($P < .05$).

BMI, Body mass index; *CI*, confidence interval; *DHA*, docosahexaenoic acid; *DPA*, docosapentaenoic acid; *EPA*, eicosapentaenoic acid; *OR*, odds ratio; *PUFA*, polyunsaturated fatty acids.

TABLE E7.

Associations between early childhood PUFA exposure/antenatal vitamin D and asthma/recurrent wheeze, allergic sensitization, and total IgE at age 3 y

	Associations with plasma PUFA and vitamin D (n = 241)						Associations with dietary PUFA and vitamin D (n = 569)					
	Asthma/recurrent wheeze		Allergic sensitization		Total IgE		Asthma/recurrent wheeze		Allergic sensitization		Total IgE	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value	Beta (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	Beta (95% CI)	*P* value
Low total PUFA Low vitamin D	1.00 (Reference)	n/a	1.00 (Reference)	n/a	(Reference)	n/a	1.00 (Reference)	n/a	(Reference)	n/a	(Reference)	n/a
High total PUFA Low vitamin D	0.45 (0.20, 1.00)	.05	0.48 (0.21, 1.08)	.07	−0.19 (−0.77, 0.39)	.52	1.28 (0.62, 2.63)	.50	1.36 (0.65, 2.89)	.41	0.13 (−0.45, 0.71)	.67
Low total PUFA High vitamin D	0.57 (0.24, 1.36)	.21	**0.39 (0.17, 0.91)**	**.03**	0.03 (−0.59, 0.65)	.92	0.79 (0.38, 1.61)	.51	0.51 (0.25, 1.03)	.06	−0.41 (−0.96, 0.13)	.14
High total PUFA High vitamin D	**0.37 (0.16, 0.84)**	**.02**	**0.26 (0.11, 0.60)**	**.001**	−0.57 (−1.15, 0.01)	.06	0.50 (0.22, 1.12)	.10	**0.40 (0.19, 0.83)**	**.02**	**−0.68 (−1.24, −0.11)**	**.02**
Low omega-3 Low vitamin D	1.00 (Reference)	n/a	1.00 (Reference)	n/a	(Reference)	n/a	1.00 (Reference)	n/a	(Reference)	n/a	(Reference)	n/a
High omega-3 Low vitamin D	0.45 (0.20, 1.00)	.05	0.58 (0.26, 1.29)	.18	−0.01 (−0.59, 0.58)	.99	0.60 (0.29, 1.20)	.15	0.94 (0.46, 1.93)	.87	−0.02 (−0.58, 0.54)	.94
Low omega-3 High vitamin D	0.51 (0.21, 1.23)	.14	**0.42 (0.18, 0.96)**	**.04**	0.12 (−0.49, 0.73)	.70	0.64 (0.32, 1.24)	.18	**0.31 (0.15, 0.62)**	**.001**	−0.43 (−0.97, 0.10)	.11
High omega-3	**0.39 (0.17, 0.90)**	**.03**	**0.29 (0.13, 0.66)**	**.003**	−0.50 (−1.09, 0.09)	.10	**0.43 (0.19, 0.92)**	**.03**	**0.33 (0.15, 0.69)**	**.004**	−0.33 (−0.91, 0.25)	.26

	Associations with plasma PUFA and vitamin D (n = 241)						Associations with dietary PUFA and vitamin D (n = 569)					
	Asthma/recurrent wheeze		Allergic sensitization		Total IgE		Asthma/recurrent wheeze		Allergic sensitization		Total IgE	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value	Beta (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	Beta (95% CI)	*P* value
High vitamin D												
Low omega-6 Low vitamin D	1.00 (Reference)	n/a	1.00 (Reference)	n/a	(Reference)	n/a	1.00 (Reference)	n/a	(Reference)	n/a	(Reference)	n/a
High omega-6 Low vitamin D	0.48 (0.22, 1.06)	.07	0.56 (0.25, 1.23)	.15	−0.19 (−0.77, 0.38)	.51	1.28 (0.63, 2.61)	.50	1.44 (0.68, 3.03)	.34	0.21 (−0.38, 0.80)	.48
Low omega-6 High vitamin D	0.59 (0.24, 1.43)	.24	0.43 (0.19, 1.02)	.06	0.10 (−0.53, 0.73)	.75	0.88 (0.43, 1.80)	.73	0.57 (0.28, 1.17)	.13	−0.30 (−0.86, 0.26)	.29
High omega-6 High vitamin D	**0.40 (0.18, 0.89)**	**.02**	**0.28 (0.13, 0.64)**	**.002**	**−0.58 (−1.16, −0.01)**	**.04**	0.57 (0.25, 1.24)	.16	**0.41 (0.19, 0.85)**	**.02**	**−0.59 (−1.16, −0.03)**	**.04**

Subjects were cross-classified into 1 of 4 categories based on whether they had high or low antenatal vitamin D exposure (ie, above or below median for umbilical cord blood 25-hydroxyvitamin D) and whether they had high or low PUFA at age 3 y (ie, above or below median for plasma PUFAs or 3rd or 1st tertile for dietary PUFAs). Logistic, conditional logistic, and linear regression models were adjusted for sex, race/ethnicity, gestational age, study center, maternal education, BMI, and total daily caloric intake.

Bold indicates statistical significance ($P < .05$).

BMI, Body mass index; *CI*, confidence interval; *n/a*, not applicable; *OR*, odds ratio; *PUFA*, polyunsaturated fatty acid.

TABLE E8.

Associations between PUFA/vitamin D at age 3 y and asthma/recurrent wheeze, allergic sensitization, and total IgE

	Associations with plasma PUFA and vitamin D (n = 243)						Associations with dietary PUFA and vitamin D (n = 384)					
	Asthma/recurrent wheeze		Allergic sensitization		Total IgE		Asthma/recurrent wheeze		Allergic sensitization		Total IgE	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value	Beta (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	Beta (95% CI)	*P* value
Low total PUFA Low vitamin D	1.00 (Reference)	n/a	1.00 (Reference)	n/a	(Reference)	n/a	1.00 (Reference)	n/a	(Reference)	n/a	(Reference)	n/a
High total PUFA Low vitamin D	0.48 (0.21, 1.09)	.08	**0.36 (0.16, 0.81)**	**.01**	−0.54 (−1.11, 0.03)	.06	1.34 (0.64, 2.84)	.44	1.26 (0.67, 2.39)	.48	0.10 (−0.40, 0.60)	.69
Low total PUFA High vitamin D	1.04 (0.44, 2.46)	.92	0.80 (0.34, 1.86)	.60	−0.18 (−0.79, 0.43)	.56	1.23 (0.60, 2.56)	.57	1.46 (0.79, 2.74)	.23	0.39 (−0.09, 0.88)	.11
High total PUFA High vitamin D	0.53 (0.21, 1.33)	.18	0.73 (0.30, 1.80)	.50	−0.44 (−1.09, 0.21)	.19	0.93 (0.44, 1.98)	.86	1.16 (0.61, 2.20)	.66	0.13 (−0.37, 0.63)	.61
Low omega-3 Low vitamin D	1.00 (Reference)	n/a	1.00 (Reference)	n/a	(Reference)	n/a	1.00 (Reference)	n/a	(Reference)	n/a	(Reference)	n/a
High omega-3 Low vitamin D	**0.39 (0.17, 0.89)**	**.03**	**0.38 (0.26, 1.29)**	**.02**	−0.39 (−0.97, 0.18)	.18	0.91 (0.46, 1.79)	.79	1.01 (0.55, 1.84)	.98	0.09 (−0.39, 0.58)	.71
Low omega-3 High vitamin D	0.78 (0.32, 1.85)	.57	0.75 (0.32, 1.75)	.50	−0.11 (−0.71, 0.50)	.73	1.30 (0.67, 2.56)	.45	1.16 (0.63, 2.14)	.63	−0.16 (−0.64, 0.33)	.53
High omega-3 High	0.61 (0.25, 1.48)	.27	0.84 (0.34, 2.05)	.70	−0.35 (−1.01, 0.31)	.30	0.66 (0.29, 1.46)	.31	1.55 (0.80, 3.03)	.19	−0.12 (−0.65, 0.41)	.66

	Associations with plasma PUFA and vitamin D (n = 243)						Associations with dietary PUFA and vitamin D (n = 384)					
	Asthma/recurrent wheeze		Allergic sensitization		Total IgE		Asthma/recurrent wheeze		Allergic sensitization		Total IgE	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value	Beta (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	Beta (95% CI)	*P* value
vitamin D												
Low omega-6 Low vitamin D	1.00 (Reference)	n/a	1.00 (Reference)	n/a	(Reference)	n/a	1.00 (Reference)	n/a	(Reference)	n/a	(Reference)	n/a
High omega-6 Low vitamin D	0.61 (0.27, 1.35)	.22	0.48 (0.22, 1.06)	.07	−0.46 (−1.02, 0.11)	.12	1.36 (0.66, 2.88)	.41	1.30 (0.68, 2.46)	.43	0.12 (−0.39, 0.63)	.64
Low omega-6 High vitamin D	1.23 (0.52, 2.88)	.64	1.03 (0.45, 2.38)	.94	−0.06 (−0.66, 0.54)	.85	1.27 (0.62, 2.62)	.52	1.46 (0.78, 2.73)	.24	0.38 (−0.11, 0.87)	.13
High omega-6 High vitamin D	0.52 (0.20, 1.35)	.18	0.77 (0.31, 1.88)	.56	−0.50 (−1.16, 0.15)	.13	0.95 (0.45, 2.00)	.90	1.12 (0.59, 2.13)	.72	0.18 (−0.33, 0.68)	.49

Subjects were cross-classified into 1 of 4 categories based on whether they had high or low vitamin D at age 3 y (ie, above or below median 25-hydroxyvitamin D) and whether they had high or low PUFA at age 3 y (ie, above or below median for plasma PUFAs or 3rd or 1st tertile for dietary PUFAs). Logistic, conditional logistic, and linear regression models were adjusted for sex, race/ethnicity, gestational age, study center, maternal education, BMI, and total daily caloric intake. Age 3 y, 25-hydroxyvitamin D concentration was available for 560 subjects.

Bold indicates statistical significance ($P < .05$).

BMI, Body mass index; *CI*, confidence interval; *n/a*, not applicable; *OR*, odds ratio; *PUFA*, polyunsaturated fatty acid.