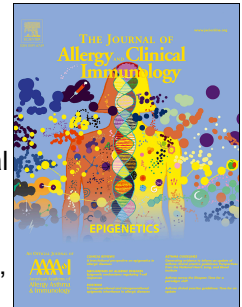


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Prenatal polyunsaturated fatty acids and child asthma: effect modification by maternal asthma and child sex

Maria José Rosa, DrPH, Terryl J. Hartman, PhD, MPH, RD, Margaret Adgent, MSPH, PhD, Kourtney Gardner, MD, Tebeb Gebretsadik, MPH, Paul E. Moore, MD, Robert L. Davis, MD, MPH, Kaja Z. LeWinn, ScD, Nicole R. Bush, PhD, Frances Tylavsky, DrPH, Rosalind J. Wright, MD, MPH, Kecia N. Carroll, MD, MPH



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Prenatal polyunsaturated fatty acids (PUFAs) and child asthma: effect modification by maternal asthma and child sex



the CANDLE study

n=1019



n-6 PUFAs
Interquartile range
increase



Maternal Asthma



RR:1.70
95%CI (1.17, 2.48)



Child Asthma
at 4-6 years



Prenatal polyunsaturated fatty acids and child asthma: effect modification by maternal asthma and child sex

Maria José Rosa, DrPH¹, Terryl J. Hartman, PhD, MPH, RD², Margaret Adgent, MSPH, PhD³, Kourtney Gardner, MD⁴, Tebeb Gebretsadik, MPH⁵, Paul E. Moore, MD⁶, Robert L. Davis, MD, MPH⁷, Kaja Z. LeWinn, ScD⁸, Nicole R. Bush, PhD^{8,9}, Frances Tylavsky, DrPH¹⁰, Rosalind J. Wright, MD, MPH^{1,11,12}, Kecia N. Carroll, MD, MPH³

¹Department of Environmental Medicine and Public Health, Icahn School of Medicine at Mount Sinai, New York, NY

²Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA,

³Division of General Pediatrics, Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN

⁴Department of Medicine, Vanderbilt University Medical Center, Nashville, TN

⁵Department of Biostatistics, Vanderbilt University Medical Center, Nashville, TN

⁶Division of Allergy, Immunology and Pulmonary Medicine, Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN

⁷Center for Biomedical Informatics, University of Tennessee Health Sciences Center, Memphis, TN

⁸Department of Psychiatry, Weill Institute for the Neurosciences, University of California San Francisco, San Francisco, CA

⁹Department of Pediatrics, University of California San Francisco, San Francisco, CA

¹⁰Department of Preventive Medicine, University of Tennessee Health Science Center in Memphis, TN

¹¹Institute for Exposomic Research, Icahn School of Medicine at Mount Sinai, New York, NY,

¹²Kravis Children's Hospital, Department of Pediatrics, Icahn School of Medicine at Mount Sinai, New York, NY

Corresponding Author: Maria José Rosa, One Gustave L. Levy Place, Box 1057 New York, NY 10029, USA. Telephone: +1 (212) 824-7027; Fax: +1 (212) 212-996-0407; E-mail: maria.rosa@mssm.edu

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Abstract

Background: Findings on prenatal polyunsaturated fatty acid (PUFA) intake and child wheeze and asthma have been inconsistent.

Objective: We examined associations between prenatal PUFA status and child wheeze/asthma and modifying effects of maternal asthma/atopy, child sex and maternal race.

Methods: Analyses included 1019 mother–child dyads with n-3 and n-6 PUFAs measured in 2nd trimester plasma; n-6/n-3 ratios were calculated. Child wheeze/asthma outcomes ascertained at age 4–6 years included: ever physician-diagnosed asthma, current wheeze (symptoms past 12 months), current asthma (diagnosis and medication and/or symptoms past 12 months) and current diagnosed asthma. Each PUFA indicator and outcome was analyzed in separate models using modified Poisson regression with interaction terms.

Results: In quartile (Q) analyses, higher n-6 PUFAs were associated with increased risk of ever (relative risk high v. low [RR Q4 v.Q1]: 1.70; 95% CI: 1.07, 2.71) and current (RR Q4 v. Q1: 1.70; 95% CI: 1.07, 2.71) diagnosed asthma while n-3 PUFAs were associated with lower risk (RR Q4 v. Q1 0.59; 95% CI: 0.33, 1.03) of current diagnosed asthma ($p_{\text{trend}} < 0.05$ for all). Higher n-6 PUFAs were associated with higher risk of all respiratory outcomes among children born to women with asthma ($p_{\text{interaction}} < 0.05$ for all outcomes). A significant three-way interaction between child sex, maternal asthma and n-6/n-3 PUFA –indicated that male children born to women with asthma and a higher ratio had the highest risk across wheeze/asthma outcomes ($p_{\text{interaction}} < 0.05$).

Conclusion: Associations between prenatal PUFA status and childhood wheeze/asthma were modified by maternal history of asthma and child sex.

Key messages: Maternal polyunsaturated fatty acids in pregnancy are predictors of asthma risk in childhood and these associations are potentially modified by maternal asthma and child sex.

Capsule summary: Children born to women with a history of asthma and higher prenatal n-6 PUFAs were at increased risk of wheeze/asthma, particularly boys.

Key words: polyunsaturated fatty acid; childhood asthma; sex-specific effects; prenatal

Abbreviations:

AA: arachidonic acid

BMI: body mass index

CANDLE: Conditions Affecting Neurocognitive Development and Learning in Early Childhood

FFQ: food frequency questionnaire

IQR: interquartile range

ISAAC: International Study of Asthma and Allergies in Children

PR: Prevalence ratio

PUFA: polyunsaturated fatty acid

RR: risk ratio

Introduction

Wheezing in early life is associated with significant morbidity and healthcare costs¹ and subsequent asthma development.² Increasing prevalence of asthma and allergic disease has been linked to Western lifestyle changes, including a shift in dietary intake.³ The decrease in consumption of n-3 (omega-3) long-chain polyunsaturated fatty acids (PUFAs), abundant in oily fish, and the concomitant increase in consumption of mostly plant based n-6 (omega-6) PUFAs, has been a particular research focus. Arachidonic acid (AA), one of the n-6 derived PUFAs, is a precursor to important pro-inflammatory markers including leukotrienes, eicosanoids and prostaglandins that can promote Th2 differentiation and IgE production, immune features correlated with particular asthma phenotypes.⁴ Conversely, n-3 PUFAs may have anti-inflammatory properties that counteract the effects of n-6 PUFAs by competing for incorporation into cells and inhibiting the synthesis of pro-inflammatory AA.^{5, 6}

Research has linked maternal intakes of PUFAs during pregnancy to the development of allergic and/or respiratory disorders. Most birth cohort studies examining the association between n-3 PUFA intake and offspring wheeze have relied on food frequency questionnaires to assess usual PUFA consumption, either by estimating intakes for specific PUFAs or relying on self-reported consumption of specific foods, like lean or fatty fish. These studies have reported protective⁷⁻¹⁰, null¹¹⁻¹³ and even detrimental associations between n-3 PUFA intake and wheeze in childhood.¹⁴ Similarly, prospective studies that have measured PUFA biomarkers in cord blood and maternal blood collected in pregnancy have yielded mixed results.¹⁵⁻²¹ In addition to these observational studies, randomized controlled trials in which women received n-3 PUFAs supplementation during pregnancy have also produced mixed results.²²⁻²⁷ A recent systematic review and meta-analysis of observational and trial data concluded that while findings have been inconsistent, they are suggestive that increased maternal consumption of n-3 PUFAs in pregnancy plays a beneficial role in prevention of childhood allergic disease.²⁸ Some

prospective birth cohorts have also shown associations between higher n-6 PUFA intake in pregnancy and higher risk of eczema^{11, 18} and allergic rhinitis in childhood¹², while others did not find an increased risk of allergic disease in relationship to higher n-6 PUFA intakes.^{9, 16} The ratio of n-6 relative to n-3 PUFA has also been associated with increased risk of allergic rhinitis¹² but not asthma or wheeze¹⁶ in 2 different prospective studies.

Few investigations have assessed how factors such as a familial predisposition to asthma, race, or child sex may influence the association between prenatal PUFA status and child wheeze/asthma. Only one cross-sectional investigation of the relationship between n-3 and n-6 PUFA status and wheeze/asthma has been published within a racially-diverse population in the United States (U.S.).²¹ Sex differences in childhood asthma development are well documented²⁹ and previous work also demonstrates sexual dimorphism in fatty acid metabolism.³⁰ The need to consider maternal history of atopic disease as an important effect modifier between the association of prenatal diet and child atopic disease has also been underscored.³¹

We assessed the relationships between 2nd trimester n-3 and n-6 PUFAs and the n-6/n-3 ratio and wheeze/asthma outcomes at age 4-6 years in mother-child dyads enrolled in a primarily African-American, prenatal cohort- the Conditions Affecting Neurocognitive Development and Learning in Early Childhood (CANDLE) study. We hypothesized that higher prenatal n-3 PUFAs would be associated with reduced risk of early childhood wheeze/asthma, while higher prenatal n-6 PUFAs and n-6/n-3 ratios would be associated with increased likelihood of wheeze and asthma. We also examined effect modification by sex, maternal race and maternal asthma.

Methods

Study Population

Pregnant women (n=1503) were recruited during the 2nd trimester of pregnancy from community obstetric practices and an obstetric clinic in Shelby County (Memphis), Tennessee from December 2006 to June 2011.³² The demographic characteristics of the CANDLE Study participants reflect the demographics of Shelby County, which is predominantly low-income and African American. Inclusion criteria included: pregnant women aged between 16 and 40 years old, residing in Shelby County, ability to speak and understand English, and 16–27 weeks of gestation with a singleton and low medical risk pregnancy. Low medical risk pregnancy was defined as participants who did not have the following: chronic hypertension requiring therapy or vascular disease requiring therapy; maternal red-cell alloimmunization except Rhesus (Rh) factor; hemoglobinopathy; insulin-independent diabetes; appreciable renal or cardiopulmonary disease; prolapsed or ruptured membranes; oligohydramnios; complete placenta previa; endocrine disease; collagen disease (e.g., lupus erythematosus or scleroderma); active or chronic hepatitis; renal disease; pulmonary or heart disease requiring therapeutic medication or limitation of physical activity; major fetal anomaly (e.g., aneuploidy, major organ-system defect); and human immunodeficiency virus.³³ The parent study procedures were approved by the Institutional Review Board (IRB) of the University of Tennessee Health Science Center (UTHSC) and this study was approved by the IRBs of UTHSC and Vanderbilt University (VU). Women aged 18 and older provided written informed consent and assent was given by those aged 16–17.9 years with consent provided by their legally authorized representative.

Prenatal PUFA Indicators

The primary exposures were n-3 and n-6 PUFAs measured in maternal plasma (n=1159) obtained in the 2nd trimester and the calculated n-6/n-3 ratios. Whole blood, collected from

women with unknown fasting status, was centrifuged at 300pm for 10 minutes to separate plasma which was then stored in the UTHSC Department of Pathology until being sent in bulk to the VUMC Lipid Services Core where phospholipid PUFA levels were determined by standard techniques.³⁴⁻³⁶ In brief, lipids were extracted using the method of Folch-Lees.³⁵ Extracts were filtered and lipids recovered in the chloroform phase. Individual lipid classes were separated by thin layer chromatography using Silica Gel 60 A plates developed in petroleum ether, ethyl ether, acetic acid (80:20:1) and visualized by rhodamine 6G. Phospholipids, diglycerides, triglycerides and cholesteryl esters were scraped from the plates and methylated using BF₃/methanol as previously described.³⁴ Methylated fatty acids were extracted and analyzed by gas chromatography using an Agilent 7890A gas chromatograph equipped with flame ionization detectors, a capillary column (SP2380, 0.25 mm x 30 m, 0.25 µm film, Supelco, Bellefonte, PA). Fatty acid methyl esters are identified by comparing the retention times to those of known standards. Inclusion of lipid standards with odd chain fatty acids permitted quantitation of the amount of lipid in the sample. Dipentadecanoyl phosphatidylcholine (C15:0), diheptadecanoin (C17:0), trieicosenoin (C20:1), and cholesteryl eicosenoate (C20:1) were used as standards. Measurements for 10 PUFAs were available including 4 n-3 PUFAs and 6 n-6 PUFAs. All assayed n-3 and n-6 PUFAs were expressed as the percentage of total fatty acids and these percentages (%) were summed to calculate the respective total n-3 and n-6 PUFA %s and used to compute the n-6/n-3 ratio. In quantifying lipid classes, the inter-assay coefficient of variation was less than 1.5%. The fatty acid percentage variation was less than 1%. The advantages of using the plasma phospholipid specimen pool include the stability of the sampling method and the minimal variability in relation to postprandial triacylglycerol levels.³⁷

Child Respiratory Outcomes

Women were asked about their child's respiratory health at the 4-6 year postnatal visit. Outcomes were assessed using standardized questionnaires based on the International Study

of Asthma and Allergies in Children (ISAAC)³⁸ and parent-report of provider diagnoses and child asthma-specific medication use.³⁹ A hierarchy of four outcomes were considered in these analyses. Ever asthma was defined as an affirmative response to “Has a physician or other health care provider ever told your family that your child had asthma or reactive airway disease?” Current wheeze was defined as an affirmative response to “Has your child ever had wheezing or whistling in the chest in the last 12 months?” Report of current asthma was defined as an affirmative response to 2 out of 3 of the following questions: 1) “Has a physician or other health care provider ever told your family that your child had asthma or reactive airway disease?” 2) “Has your child ever had wheezing or whistling in the chest in the last 12 months?” and 3) “In the past 12 months, has your child used any type of medicines, liquids, puffers or other medication for wheezing or asthma?” Healthcare provider diagnosed current asthma was defined as an affirmative response to “Has a physician or other health care provider ever told your family that your child had asthma or reactive airway disease?” AND either report of wheezing OR asthma medication use in the past 12 months.

Covariates

Information on maternal age (continuous), race (Black or African American, White and Other), education (<high school, high school or GED and >high school), self-report of woman's history of asthma, parity (primiparous or multiparous) and pre-pregnancy body mass index (BMI, continuous), calculated by dividing weight by height squared (kg/m^2), were self-reported at enrollment. Child sex was obtained from a neonatal summary form at birth. Child's BMI was measured at the 4-6 year visit. Data on women's atopic disease status was also collected on the 4-6 year visit questionnaire and was defined as self-report of the woman ever having any one of the following: asthma, hay fever/allergic rhinitis or eczema/atopic dermatitis.

Statistical Analysis

Dyads with PUFAs, estimated gestational age ≥ 32 weeks, and complete outcome and covariate data from the 4–6 year visit (N=1019) were included in analyses. Modified Poisson regression⁴⁰ was used in order to obtain risk ratios to assess the relationships between prenatal PUFA indicators and wheeze/asthma outcomes. In separate multivariable Poisson regression models, we investigated the association of total n-3 and n-6 PUFAs (continuous and categorical [quartiles]) predictors plus their ratio with wheeze/asthma outcomes. Tests of linear trend were conducted in final multivariable models by assigning the median value from each quartile of fatty acids and modeling this as a continuous variable. Models were adjusted for maternal age at enrollment, race, education, asthma history, parity, pre-pregnancy BMI and child sex and BMI at the 4-6 year visit. We then examined effect modification by maternal asthma at enrollment (yes/no), maternal atopic disease assessed at age 4-6 visit (yes/no), child sex (male/female), and maternal race (African American/white, excluding Other category) with PUFA status (as continuous variable) for child wheeze/asthma outcomes by including the relevant interaction terms. Finally, we considered 3-way interactions between these variables and the 3 PUFA predictor variables (continuous variable) (race x sex x PUFA; race x maternal asthma x PUFA; sex x asthma x PUFA). Analyses were performed in R Version 3.5.1 (Vienna, Austria) and SPSS version 24 (Chicago, IL) with statistical significance set at 0.05.

Results

Maternal and children's characteristics are reported in Table 1 as number and percentage (n [%]) or mean and standard deviation (median [IQR]), as appropriate. The majority of women were African American (67.6%), approximately 60% of women had a high school education or less and approximately 12% reported a history of asthma at enrollment. By age 4-6 years, 14.4% of children were reported to have had healthcare provider-diagnosed asthma.

Table 2 summarizes the plasma PUFAs measured in mid-pregnancy as median (IQR), including the total n-3% and total n-6%, the specific PUFAs that contributed to the summed indicators, as well as the n-6/n-3 ratio. For n-3, docosahexanoic acid (DHA) was the predominant contributor to the sum and for the sum of n-6 PUFA, linoleic acid (LA) and AA were the predominant fatty acids. This table also summarizes the medians across quartiles for the total n-3 and total n-6 summed indicators and the n-6/n-3 ratio.

Main effects

Table 3 shows the linear associations between PUFAs (continuous) and each of the four respiratory outcomes in children with adjustment for covariates. Higher summed n-6 PUFAs were associated with higher risk of caregiver-report of ever asthma diagnosis at age 4-6 years (RR: 1.21, 95%CI: [1.01, 1.46] per an interquartile [IQR] increase). No other statistically significant associations were seen in analyses considering PUFAs as continuous indicators. We see similar results when adjusting for maternal atopic disease instead of maternal asthma (supplemental table E1).

Next, we conducted analyses with the exposures characterized as quartiles. When compared to the first (lowest) quartile of total n6 PUFAs, the third (RR Q3 v. Q1: 1.83; 95% CI: 1.16, 2.91) and fourth quartiles (RR Q4 v. Q1: 1.70; 95% CI: 1.07, 2.71) were associated with higher risk of ever asthma in children at age 4-6 years (Figure 1A). When compared to the first quartile of exposure, the third quartile of total n-6 PUFAs was also associated with higher risk of diagnosed and current asthma; however, the confidence limits for the comparison of the fourth and first quartiles crossed 1.0 (Figure 1B, C)). We observed linear trends for increasing total n-6 PUFAs and higher risk of ever asthma and diagnosed current asthma ($p < 0.05$ for both). When considering total prenatal n-3 PUFAs, increasing quartiles of exposure were associated with

decreased risk of respiratory outcomes with a significant trend for diagnosed current asthma (RR Q4 v. Q1 0.59; 95% CI: 0.33, 1.03, p-trend=0.04).

Interaction models

In two-way interaction models, we did not find evidence for effect modification by race (all interaction p-values>0.25) or sex (all interaction p-values>0.15). We did find evidence for effect modification by maternal asthma on associations between prenatal total n-6 PUFAs and all four child respiratory outcomes (Table 4). Increasing n-6 PUFAs were associated with higher risk of all respiratory outcomes only among children born to women who reported a history of asthma whereas in those born to women without a reported history of asthma, we did not observe statistically significant association between n-6 PUFAs and any of the respiratory outcomes. We observed similar but attenuated patterns when testing for effect modification and stratifying by maternal atopic disease (supplemental table E2). We did not find evidence that maternal asthma modified the association between n-3 PUFAs and n-6/n-3 PUFA ratio and child outcomes.

Exploratory 3-way interactions

We also found evidence of a three-way interaction between child sex, maternal asthma and some prenatal PUFA indicators. Figures depicting all significant 3-way interactions after stratifying by the relevant covariates are shown in the Supplement (Figures E1 and E2). Male children born to women reporting a history of asthma with a higher n-6/n-3 ratio had the highest risk of ever, current and current diagnosed asthma. There were similar patterns for the association between prenatal total n-6 PUFAs and ever asthma in children, in which male children born to asthmatic women with higher n-6 PUFAs have the greatest risk. In contrast, increasing total n-3 PUFAs in the prenatal period were associated with higher risk of diagnosed

asthma in girls born to women with a history of asthma, while the effect was in the opposite direction for all other groups.

Discussion

These analyses leveraged data from a large, diverse US pregnancy cohort to examine the associations between measured prenatal PUFAs and respiratory outcomes in childhood. We found that higher maternal plasma n-6 PUFAs in pregnancy were associated with higher risk of ever being diagnosed with asthma and active asthma in their children at age 4-6 years. While the associations were in the expected directions, we did not find statistically significant associations between n-6/n-3 PUFA ratios and any respiratory outcomes in our main effect models. The effects of n-6 PUFAs were modified by maternal asthma, with their effects being most pronounced in children born to women with asthma compared to those born to women without asthma. In exploratory analyses, a significant three-way interaction between child sex, maternal asthma and n-6/n-3 PUFA indicated that male children born to women with asthma and a higher n-6/n-3 ratio had the highest risk across all outcomes.

Most research on dietary fatty acids and asthma has focused on the potential protective effects of n-3 PUFA intake in pregnancy against asthma development in children, with conflicting results. Prospective birth cohorts examining n-3, n-6 and n-6/n-3 ratio PUFA measures in pregnancy in relation to allergy and asthma development in childhood have similarly produced mixed results. In an analysis of data from the Osaka Maternal and Child Health Study, higher maternal n-6 PUFA intake assessed via a semi-quantitative diet history questionnaire was associated with increased risk of eczema but not wheeze at age 2 years.¹¹ In a similar analysis of dietary data in another Japanese cohort, the authors reported borderline protective associations between n-3 PUFA intakes and lower odds of wheeze at 23-29 months and no associations between total or individual n-6 PUFAs and wheeze.⁹ A Finnish cohort reported associations between higher n-6/n-3 PUFA ratios, estimated via FFQs assessing

intakes during the last month before delivery, and increased risk of allergic rhinitis by age 5 but did not find statistically significant associations with wheeze at that same age.¹² In analyses from a Dutch cohort, authors reported that increasing maternal plasma n-6/n-3 PUFA ratios in maternal plasma collected in pregnancy was associated with decreased risk of eczema in the first 7 months of life but found no associations with asthma or wheeze by age 7 years.¹⁶ The differences in these findings could be due to the different instruments used to assess PUFA intakes, the timing of outcome assessment and underlying differences in demographic and lifestyle characteristics or overall dietary patterns in the samples.

Black and Sharpe first proposed that changes in consumption of n-3 and n-6 PUFAs may be related to the development of asthma and other atopic diseases.⁴¹ In the United States, dietary changes during the 20th century, particularly the increase in availability of dietary fat from soy bean derived oils, have been associated with increased linoleic acid consumption, an n-6 PUFA precursor.⁴² Linoleic acid is a precursor of arachidonic acid, and the principal substrate for the synthesis of prostaglandins, thromboxanes and leukotrienes which are well-characterized mediators of allergic inflammation.⁴³ Prostaglandin E affects the Th1/Th2 balance by promoting the differentiation of naive T cells into Th2 cells, thereby increasing the production of Th2 cytokines, including interleukin (IL)-4, IL-5, and IL-13, and enhancing the production of IgE.^{4, 44-46}

A recent review noted that maternal diet in pregnancy, including PUFA intake, may interact with the genetic predisposition to allergic disease by influencing the developing immune system.⁴⁷ Most prospective studies have not examined maternal asthma/atopic disease as a potential modifier of the relationship between PUFA intakes and asthma in their children. In the Dutch Maastricht Essential Fatty Acid Birth (MEFAB) and the Greek Rhea Mother-Child Study cohorts, the authors reported no evidence of effect modification by parental atopy of the association between n-3/n-6 ratios and current asthma, rhinitis or eczema at age 6-7 years.²⁰

PUFA levels in both cohorts were assessed in umbilical cord blood samples, rather than maternal prenatal plasma, and as expected given the study locations and differences in PUFA consumption, levels of n-3s were lower and n-6s and n-6/n-3 ratios were higher in our cohort when compared to these cohorts. Furthermore, while PUFA measures in maternal plasma and cord blood are correlated, there are differences in the distribution of specific PUFA levels.⁴⁸ In maternal samples, the dominant PUFA is LA, whereas AA is the most abundant in cord blood samples.^{20, 48} While we do not have data on active asthma during pregnancy in the women in the study, higher n-6 intake may also affect their own asthma severity and control in pregnancy. For example, in a study of asthmatics, a higher ratio of n-6/n-3 PUFA intake was associated with greater levels of airway inflammation assessed via exhaled nitric oxide and greater likelihood of uncontrolled asthma.⁴⁹ In overlapping research, active maternal asthma in pregnancy has been associated with higher risk of offspring asthma, with risk being enhanced when asthma is not well controlled. For example, it was recently shown that higher prevalence of early-onset persistent asthma was observed among children born to women with mild uncontrolled (prevalence ratio (PR):1.2, 95% CI 1.1, 1.4), moderate-to-severe controlled (PR:1.3, 95% CI 1.1, 1.6), and moderate-to-severe uncontrolled asthma (PR:1.4, 95% CI 1.2, 1.6) compared to those born to women with mild controlled asthma.⁵⁰ Future studies examining associations between PUFA intakes in pregnancy among women with active asthma in relation to their own asthma outcomes as well as risk in their children would further inform this area of research.

Sex-differences in both fatty acid metabolism and asthma development in childhood asthma have been previously reported.^{29, 30} While we did not find sex differences in the association between PUFA main effects and asthma outcomes, in exploratory analyses we did find evidence of effect modification by both child sex and maternal history of asthma, particularly for the association between n-6/n-3 ratio and asthma outcomes. In examined three-way

interactions, the association between n-6/n-3 ratio and n-6 PUFAs and respiratory outcomes was strongest for male children born to women with asthma. Differential maturation in lung development of males relative to females may predispose male infants to childhood respiratory diseases including asthma⁵¹⁻⁵³ and they may be more susceptible to the pro-inflammatory effects of prenatal n-6 PUFAs. Conversely, we found that there was an inverse association between n-6/n-3 ratio and asthma outcomes in girls born to asthmatic women, with higher n-6/n-3 PUFA ratio being associated with lower risk of asthma in these girls. Lee Sarwar and colleagues also reported inverse associations between concurrent n-6 PUFAs and asthma outcomes in children born to parents with history of atopy.²¹ While the underlying mechanism of these observed effects are unclear, there are data demonstrating that males and females differ in their ability to convert ALA to other longer chain n-3 PUFAs, which may play a role.⁵⁴

Strengths of our study include the prospective design, the reasonably large ethnically and demographically diverse sample facilitating our ability to examine effect modification, the objective assessment of total PUFA status in pregnancy, and our ability to adjust for important confounders. Potential limitations should also be considered. While we only measured plasma PUFAs at a single time point, it has been shown that dietary patterns do not vary much across pregnancy.^{55, 56} Our quartile analysis of n-6 PUFA did not reflect a linear dose response but we detected a statistically significant linear trend. Simple linear dose response relationships for dietary factors, may not be evident, in part because of limits in absorption, transport, metabolism, and storage.³⁷ While plasma PUFAs provide an objective measure, other food components may alter these associations which were not in the realms of this investigation. In addition, we chose to use the plasma phospholipid specimen pool as opposed to alternatives such as total plasma because of the stability of the sampling method and their minimal variability in relation to postprandial levels, which is primarily seen with the triglycerides or free fatty acid fractions. Our cohort is primarily African American and demographically diverse which

fills a research gap in this field, nonetheless our results may not be generalizable to other populations with differing demographics. Potential misclassification of covariates measured at baseline, such as maternal asthma, is likely non-differential relative to the measured PUFA levels and child outcomes and thus likely to move results towards the null. Child outcomes were based on questionnaire data which can lead to misclassification. However, questions were adapted from the ISAAC study and have been widely validated⁵⁷ and we used a hierarchy of wheeze/asthma definitions with consistent findings. While we found significant interactions between maternal asthma, child sex and PUFAs, our sample size within some stratum was limited which may lead to overfitting. Thus these findings warrant replication and should be examined in other populations with larger sample sizes to further explore these complex relationships. There is the possibility of unmeasured confounding or effect modifying variables that may have influenced our results.

This study adds to a growing literature underscoring the need to consider prenatal diet as an important programming factor in the development of asthma in childhood. These results demonstrate that prenatal status of long-chain PUFAs can enhance or reduce risk of children's respiratory outcomes, depending on PUFA category. Moreover, in order to more fully understand the influence of PUFA exposures *in utero* on childhood asthma risk, modifying effects of maternal asthma and child sex need to be taken into account. Indeed, prior conflicting results in studies examining associations between prenatal intakes of PUFAs and child asthma and allergy risk may in part be because potential modifying factors were not considered. Understanding mechanisms underlying modifying effects of maternal asthma as well as child sex on the link between prenatal PUFAs and child respiratory outcomes will further elucidate how respiratory disease is programmed starting prenatally. Moreover, enhanced knowledge of those who may most likely benefit from prevention and intervention strategies such as n-6

consumption reduction strategies in combination with n-3 supplementation, can lead to more efficacious protocols.

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Figure Legends

Figure 1 legend. Association of mid-pregnancy maternal plasma polyunsaturated fatty acids in quartiles with respiratory outcomes at age 4-6 years. Ever asthma defined as physician/medical provider report of asthma ever; current wheeze defined as wheeze in the past 12 months; current asthma defined as 2 out of 3 ever asthma, wheeze and/or medication use in past 12 months; diagnosed current asthma defined as ever asthma + wheeze and/or medication use in past 12 months. *p for linear trend <0.05

607 **Table 1.** Characteristics of mother–child dyads in the CANDLE cohort

	n or median	% or interquartile range
<i>Maternal characteristics</i>		
Race (n,%)		
White	315	30.9
Black	689	67.6
Other	15	1.5
Education (n,%)		
<High School	121	11.9
High School or GED*	484	47.5
>High School	414	40.6
Smoking in pregnancy (n,%)		
Yes	92	9.0
Parity (n,%)		
Primiparous	448	39.6
Multiparous	682	60.4
Ever asthma at enrollment (n,%)		
Yes	118	11.6
Atopic disease [†] (n,%)		
Yes	364	36
Missing	2	
Age at enrollment (years)		
Median, 25 th -75 th percentile	26.0	22.0-31.0
Pre-pregnancy BMI (kg/m ²)		
Median, 25 th -75 th percentile	25.8	22.1-32.1
<i>Child characteristics</i>		
Male sex (n,%)	508	49.9
Birth weight (g)		
Median, 25 th -75 th percentile	3262	2970-3565
Missing	1	
Age at follow-up (years)		
Median, 25 th -75 th percentile	4.17	4.05-4.51
BMI at follow-up (kg/m ²)		
Median, 25 th -75 th percentile	16.00	15.0-17.0
Outcomes (n,%)		
Ever diagnosis of asthma [‡] , yes	147	14.4
Current wheeze [§] , yes	200	19.6
Current asthma , yes	165	16.2

Diagnosed current asthma [#] , yes	121	11.9
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*GED=General Educational Development test

[†] Defined as report of the mother having any one of the following conditions: asthma, hay fever/allergic rhinitis or eczema/atopic dermatitis

[‡] Report of physician/medical provider diagnosis of asthma ever

[§] Caretaker report of wheeze or whistling of the chest in the past 12 months

^{||} At least 2 out of 3 of following: ever diagnosis of asthma, current wheeze and wheeze/asthma medication use in past 12 months

[#] Report of ever diagnosed asthma AND wheeze and/or asthma/wheeze medication use in past 12 months

637 **Table 2.** Maternal plasma polyunsaturated fatty acids in mid-pregnancy (n=1019)

	Weight %, Median	IQR
Total n-3 PUFAs	5.43	1.35
n-3 PUFA Q1	4.36	
n-3 PUFA Q2	5.03	
n-3 PUFA Q3	5.63	
n-3 PUFA Q4	6.72	
α -Linoleic acid (ALA, C18:3n-3)	0.40	0.30
Eicosapentaenoic acid (EPA, C20:5n-3)	0.28	0.16
Clupanodonic/Docosapentaenoic acid (DPA, C22:5n-3)	0.63	0.20
Docosahexaenoic acid (DHA, C22:6n-3)	3.96	1.22
Total n-6 PUFAs	41.1	2.28
n-6 PUFA Q1	39.0	
n-6 PUFA Q2	40.6	
n-6 PUFA Q3	41.6	
n-6 PUFA Q4	43.1	
Linoleic acid (LA, C18:2n-6)	23.4	3.27
γ -Linolenic acid (GLA, C18:3n-6)	0.00	0.00
Dihomo- γ -linolenic acid (DGLA, C20:3n-6)	3.34	0.96
Arachidonic acid (AA, C20:4n-6)	12.5	2.57
Adrenic acid (C22:4n-6)	0.57	0.18
Osbond acid (C22:5n-6)	1.05	0.51
Total n-6/n-3 ratio	7.74	2.02

n-6/n-3 ratio Q1	5.99
n-6/n-3 ratio Q2	7.26
n-6/n-3 ratio Q3	8.20
n-6/n-3 ratio Q4	9.53

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Table 3. Association of mid-pregnancy maternal plasma PUFAs (per IQR increase) with respiratory outcomes at age 4-6 years: Main Effect Models

PUFA measure (IQR difference)	Outcome			
	RR (95%CI)			
	Ever asthma*	Current wheeze [†]	Current asthma [‡]	Diagnosed current asthma [§]
n-3 PUFAs	0.88	0.96	0.91	0.83
(1.35)	(0.72, 1.08)	(0.83, 1.12)	(0.76, 1.09)	(0.65, 1.05)
n-6 PUFAs	1.21	0.95	1.00	1.12
(2.28)	(1.01, 1.46)	(0.82, 1.11)	(0.84, 1.18)	(0.91, 1.37)
n-6/n-3 PUFA ratio	1.14	0.97	1.05	1.17
(2.02)	(0.94, 1.37)	(0.83, 1.13)	(0.88, 1.24)	(0.95, 1.43)

Modified Poisson models adjusted for child sex, mother's educational level at enrollment, mother's asthma ever, parity, mother's age at enrollment, child's BMI at age 4-6 year visit, mother's pre-pregnancy BMI and mother's race. Effect sizes shown per an interquartile range (IQR) increase in PUFA measure.

*Report of physician/medical provider diagnosis of asthma ever

[†]Caretaker report of wheeze or whistling of the chest in the past 12 months

[‡]At least 2 out of 3 of following: ever diagnosis of asthma, current wheeze and wheeze/asthma medication use in past 12 months

[§]Report of ever diagnosed asthma AND wheeze and/or asthma/wheeze medication use in past 12 months

Table 4. Association of mid-pregnancy maternal n-6 PUFAs (continuous) and respiratory outcomes stratified by maternal history of asthma

Child Outcome	Maternal Ever Asthma Yes N=118	Maternal Ever Asthma No N=901	
	RR (95% CI)	RR (95% CI)	P-value for interaction
Ever asthma*	1.65 (1.17, 2.30)	1.10 (0.89, 1.38)	0.050
Current wheeze [†]	1.39 (0.97, 1.98)	0.87 (0.74, 1.03)	0.017
Current asthma [‡]	1.53 (1.07, 2.19)	0.90 (0.74, 1.09)	0.011
Diagnosed current asthma [§]	1.70 (1.17, 2.48)	0.96 (0.77, 1.22)	0.005

Modified Poisson models adjusted for adjusted for child sex, mother's educational level at enrollment, parity, mother's age at enrollment, child's BMI at age 4-6 visit, mother's pre-pregnancy BMI and mother's race. Effect sizes shown per an IQR (2.28) increase in n-6 PUFA measure.

*Report of physician/medical provider diagnosis of asthma ever

[†]Caretaker report of wheeze or whistling of the chest in the past 12 months

[‡]At least 2 out of 3 of following: ever diagnosis of asthma, current wheeze and wheeze/asthma medication use in past 12 months

[§]Report of ever diagnosed asthma AND wheeze and/or asthma/wheeze medication use in past 12 months

