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Associations of cord blood fatty acids with lymphocyte proliferation, IL-13, and IFN- γ

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Abstract

Background. N-3 and n-6 polyunsaturated fatty acids (PUFAs) have been hypothesized to have opposing influences on neonatal immune responses that might influence the risk of allergy or asthma. However, both n-3 eicosapentaenoic acid (EPA) and n-6 arachidonic acid (AA) are required for normal fetal development.

Objective. We evaluated whether cord blood fatty acid levels were related to neonatal immune responses and whether n-3 and n-6 PUFA responses differed.

Methods. We examined the relation of cord blood plasma n-3 and n-6 PUFAs (n = 192) to antigen- and mitogen-stimulated cord blood lymphocyte proliferation (n = 191) and cytokine (IL-13 and IFN- γ ; n = 167) secretion in a US birth cohort.

Results. Higher levels of n-6 linoleic acid were correlated with higher IL-13 levels in response to Bla g 2 (cockroach, $P = .009$) and Der f 1 (dust mite, $P = .02$). Higher n-3 EPA and n-6 AA levels were each correlated with reduced lymphocyte proliferation and IFN- γ levels in response to Bla g 2 and Der f 1 stimulation. Controlling for potential confounders, EPA and AA had similar independent effects on reduced allergen-stimulated IFN- γ levels. If neonates had either EPA or AA levels in the highest quartile, their Der f 1 IFN- γ levels were 90% lower ($P = .0001$) than those with both EPA and AA levels in the lowest 3 quartiles. Reduced AA/EPA ratio was associated with reduced allergen-stimulated IFN- γ level.

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Conclusion. Increased levels of fetal n-3 EPA and n-6 AA might have similar effects on attenuation of cord blood lymphocyte proliferation and IFN- γ secretion.

Clinical implications. The implications of these findings for

Keywords

Asthma; child; cord blood; cytokine; fatty acids; lymphocyte proliferation

Abbreviations used

AA: Arachidonic acid; BMI: Body mass index; CBMC: Cord blood mononuclear cell; CI: Confidence interval; DHA: Docosahexaenoic acid; EPA: Eicosapentaenoic acid; FA: Fatty acid; LA: Linoleic acid; NICU: Neonatal intensive care unit; OVA: Ovalbumin; PG: Prostaglandin; PUFA: Polyunsaturated fatty acid; SI: Stimulation index

Fetal environmental exposures, including maternal diet during pregnancy and fetal nutrition, have formative influences on fetal and postnatal development.¹ Investigators have proposed that the increase in childhood allergy and asthma rates² might be partially attributable to temporal trends in dietary intake, particularly during pregnancy and early childhood. These trends include decreases in intake of foods containing n-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), found in fish, nuts, or seeds and increases in intake of foods containing n-6 fatty acids (FAs), such as linoleic acid (LA) and arachidonic acid (AA), found in vegetable oils and animal products.³ In an Australian dietary intervention trial, children who received n-3 FA supplementation from 6 months or at onset of bottle feeding had less wheeze in the first 18 months of life than control subjects.⁴ An observational study of 4136 children from the United Kingdom found weaker evidence for protective effects of relatively higher levels of cord blood n-3 FAs on the risk of wheeze in early childhood. They concluded that it was doubtful that FAs had a significant influence on early wheeze or atopic disease.⁵

Early-life exposure to n-3 FAs has been hypothesized to decrease asthma or allergy risk through immune modulation, decreasing T_H2 cytokine (eg, IL-13 and IL-4) secretion, subsequent IgE production, and the risk of chronic allergic inflammation, including airway inflammation.⁶ In a recent randomized, double-blind, placebo-controlled trial of 98 atopic pregnant women, Dunstan et al⁷ found a tendency for all neonatal lymphoproliferative responses and cytokine levels to be reduced in neonates whose mothers had fish oil supplementation. Small numbers limited confidence in the associations, which were significant only for IL-10 in response to cat.

A clinical trial of n-6 FA supplementation in adults suggested that some, but not all, n-6 FAs might have effects similar to those of n-3 FAs on the decrease in lymphocyte-proliferative responses to mitogen-stimulated PBMCs.⁸ In a sample of 192 infants from an ongoing birth cohort study, we aimed to evaluate the influence of cord blood n-3 and n-6 FAs on neonatal immune responses.

METHODS

Subjects

Subjects for this study were a subgroup of participants from Project Viva, a pregnancy and birth cohort study underway in eastern Massachusetts. Enrollment for this study occurred from April 1999 through July 2002. Expectant mothers were approached at their initial prenatal visit

for consent. Participants were interviewed and completed questionnaires in the first and second trimesters of pregnancy, as well as at the time of delivery. Umbilical venous cord blood was obtained at the time of delivery from Viva newborns born not in an emergency state.

This study was approved by the Institutional Review Boards of Brigham and Woman's Hospital and Harvard Pilgrim Health Care. Informed consent was obtained from mothers for their participation, including cord blood collection.

Cord blood samples, cell preparation, and lymphocyte proliferation—Cord blood samples were collected from the umbilical vein after delivery. Samples for immune analysis were placed in heparinized tubes and processed within 24 hours. Cord blood mononuclear cells (CBMCs) were isolated from umbilical cord blood by means of density-gradient centrifugation with Ficoll-Hypaque Plus (Pharmacia, Uppsala, Sweden).

For the lymphocyte proliferation assay, 4 replicates of 0.5×10^6 cells/well CBMCs were cultured for 72 hours. At the start of the culture, cells were stimulated with 30 $\mu\text{g/mL}$ cockroach (Bla g 2), 30 $\mu\text{g/mL}$ house dust mite (Der f 1; Indoor Biotechnologies, Charlottesville, Va), 100 $\mu\text{g/mL}$ ovalbumin (OVA), or 5 $\mu\text{g/mL}$ mitogen PHA (Sigma Aldrich, St Louis, Mo). Lymphocyte proliferation was assayed by means of incorporation of tritiated thymidine. By using a β -counter, the mean counts per minute values of stimulated and unstimulated samples were calculated. By using the average counts per minute of replicate values, the lymphocyte proliferation stimulation index (SI) was calculated as the ratio of the mean counts per minute of stimulated lymphocytes divided by the mean counts per minute of unstimulated lymphocytes. SI or counts per minute measurements suggesting nonviability of cells or with technical tags suggesting significant differences from usual laboratory conditions were excluded from analyses.

Cytokine measurement—Supernatants from media and from Bla g 2-, Der f 1-, and PHA-stimulated cells were collected at 72 hours and analyzed for IL-13 and IFN- γ secretion by means of ELISA (Endogen, Rockford, Ill). Cytokines from OVA-stimulated cells were not measured. Assay sensitivities were less than 7 pg/mL for IL-13 and less than 2 pg/mL for IFN- γ .

FA measurement

We measured FAs in the subjects who had lymphocyte proliferation measurements and complete data available on parental atopy, food frequency questionnaires completed during the first and second trimesters, birth weight, and gestational age at birth. After a single freeze-thaw, cord plasma FAs were quantified by means of gas-liquid chromatography, as described previously.⁹ Peak retention times and area percentages of total FAs were identified by injecting known standards (NuCheck Prep, Elysium, Minn) and analyzed with the Agilent Technologies ChemStation A.08.03 software. FA levels are reported as percentages of total FAs measured.

Definitions of predictor variable and covariates

We focused on EPA and DHA, the predominant n-3 FAs measurable in blood, and LA and AA, the predominant n-6 FAs, as our main predictor variables. FAs were considered as continuous and also as discrete categoric variables (see below).

Factors considered as potential independent predictors or confounders of the associations of FAs with our outcomes included parental factors (family income; maternal education; maternal age; number of prior pregnancies; parental history of asthma, hay fever, or eczema; maternal smoking history during pregnancy; maternal prepregnancy body mass index [BMI]; and maternal weight gain during pregnancy adjusted for prepregnancy BMI), perinatal child factors

(gestational age, birth weight, cesarian section, 5-minute Apgar score, and neonatal intensive care unit [NICU] admission), child sex, and race-ethnicity. A record review was conducted to ascertain NICU admission, to evaluate for diagnosis of neonatal sepsis, and to assess diagnoses associated with hospital stay greater than 7 days. Maternal smoking status was determined during the first-trimester questionnaire, which was completed at an average of 10 weeks of gestation, when the mother was asked if she currently smoked. Self-reported maternal prepregnancy weight and height were obtained at the time of enrollment, and BMI was calculated in standard fashion (in kilograms per meter squared). Neonatal race-ethnicity was determined based on parental report of race, which was collected at the first-trimester interview. If both parents were white, the child was classified as being white. If either parent was black, the child was classified as being black. If no parent was black but at least one parent was Hispanic, the child was classified as being Hispanic. If no parent was black or Hispanic but at least one parent was Asian, the child was classified as being Asian. If no parent was black, Hispanic, or Asian but at least one parent was American Indian, the child was classified as being American Indian.

Definition of outcome variables and data analysis

Because SI was not normally distributed, we used Spearman correlation testing and then $\log_{10}$ transformed the data to normalize it for multivariate linear regression modeling that assessed FA associations with log SI. In logistic regression models we also considered SI as a dichotomous categorical outcome because some investigators consider an SI of 2 or 3 or greater to be a biologically meaningful response to antigens in adults and older children.¹⁰ Proliferative responses after stimulation with the antigens Bla g 2, Der f 1, and OVA were dichotomized at 2, whereas proliferative responses after stimulation with mitogen PHA were dichotomized at the median. Additional covariates were entered into logistic or linear multivariate models if there was association ($P \leq .1$) with any of the related outcomes. Estimates for the FA effects were scaled to be within the range of the distribution of the data (by 0.5 percentage points for EPA and by 1 percentage point for AA). For linear regressions with log SI as a continuous outcome, we used the estimates to calculate the percent change in our outcomes for a 0.5-percentage-point change in EPA or a 1-percentage-point change in AA.

To evaluate associations of FA with the cytokines IFN- γ and IL-13, we also first calculated Spearman correlation coefficients and then $\log_{10}$ transformed the outcome data for multivariate linear regression modeling. Because of the nonlinear relation of FAs to cytokine levels, the FAs EPA and AA were treated as categorical variables, beginning with evaluation of quartile and then expressing FA exposures as a dichotomy (see below). We calculated the percentage of difference in cytokine levels between categories of EPA or AA. All analyses were performed with SAS version 8.2 (SAS Institute, Cary, NC).

RESULTS

In this population the gestational age ranged from 30.9 to 42.1 weeks (Table I). Maternal smoking was rare. Of the mothers, 11% were obese, with a BMI of greater than 30 kg/m². Maternal obesity was associated with lower n-3 EPA levels in cord plasma. The median cord EPA level was 0.86 percentage points for children of mothers with a BMI of greater than 30 kg/m² compared with a median level of 0.97 percentage points for children of mothers with a BMI of 30 kg/m² or less. Only 6 of 192 children had a NICU stay documented; 5 stayed for longer than 7 days. All but 2 children in the NICU were well but premature; the median gestational age of the children in the NICU was 32.8 weeks, whereas the median gestational age for those not in the NICU was 40 weeks. Of the 2 children with illness, one had necrotizing enterocolitis, and the second had hyaline membrane disease.

In Table E1 (in the Online Repository at www.jacionline.org) and Figs 1, 2, and E1 (in the Online Repository at www.jacionline.org), we present the distribution of FA, SI, and cytokine levels. EPA and DHA accounted for an average of 92% of total n-3 FAs measured in each subject, and LA and AA accounted for an average of 85% of total n-6 FAs measured in each subject.

FAs and lymphocyte proliferation

Higher levels of n-3 FA EPA levels were strongly associated with reduced lymphoproliferative responses (ie, SI) after antigen stimulation with Bla g 2, Der f 1, and OVA in univariate analyses by using rank-based Spearman correlation coefficients or in logistic regression analyses estimating the odds of an SI of greater than 2 (Table II). AA had similar inverse associations with antigen-stimulated lymphoproliferative responses (Table II). N-3 EPA and n-6 AA levels were moderately correlated with each other (Spearman correlation: $r = 0.43$, $P < .0001$). Most associations of EPA or AA with reduced SI responses remained significant after adjusting for maternal smoking, neonate race-ethnicity, and child sex (Table III). In multivariate analyses none of the other covariates considered, including NICU stay, were independent predictors of SI. In linear models a 0.5-percentage-point increase in EPA was associated with a 26.8% (95% confidence interval [CI], 10.3% to 40.3%) decrease in Der f 1 SI. A 0.5-percentage-point higher EPA level was associated with 0.34 (95% CI, 0.15 to 0.76) times the odds of having a Der f 1 SI of greater than 2 (vs an SI ≤ 2) in multivariate logistic regression analyses. When both EPA and AA were entered into the same multivariate model, analyses with Der f 1 SI as an outcome suggested an independent effect of EPA, but not of AA, on the lymphoproliferative responses (Table III, model 4).

FAs and cytokine secretion

Higher levels of EPA were also associated with lower levels of Bla g 2- and Der f 1- stimulated IFN- γ and with lower levels of baseline IFN- γ in media (Table IV). AA followed a similar pattern. The negative correlations between FAs and IFN- γ were similar in magnitude and precision in sensitivity analyses removing the subject with the highest levels of Bla g 2 and Der f 1 IFN- γ (Table E1 and Fig E2 in the Online Repository at www.jacionline.org). The association of EPA or AA with cytokine levels was nonlinear, with only the highest quartile of each FA being associated with very low or nondetectable levels of IFN- γ (Table E2 and Fig E2 in the Online Repository at www.jacionline.org). For this reason, we grouped the lowest 3 quartiles of FAs (EPA or AA) and compared them with the upper quartile in evaluating their relationship to IFN- γ . In multivariate analyses adjusting for gestational age and maternal prepregnancy BMI, we found that being in the highest quartile of either EPA or AA predicted reduced levels of IFN- γ (Table V). In these multivariate analyses none of the other covariates considered, including NICU stay, were associated with IFN- γ levels. In multivariate models including both FAs, EPA and AA had independent effects on Der f 1 IFN- γ levels. Compared with having EPA levels in the first through third quartiles, having EPA levels in the fourth quartile was associated with a decrease in Der f 1 IFN- γ levels of 74.2% (95% CI, 3% to 93.3%).

Given these findings, we hypothesized that higher amounts of either AA or EPA would result in lower IFN- γ levels. In Table E2 and in model 6 for Bla g 2 and Der f 1 IFN- γ as outcomes (Table V), being in the highest quartile of either AA or EPA was highly predictive of having low IFN- γ levels compared with having both EPA and AA levels in the lower 3 quartiles. For example, being in the highest quartile of either AA or EPA was associated with an 89.6% (95% CI, 67.6% to 96.7%) lower IFN- γ level.

We also hypothesized that not only the absolute level of EPA but also the relative amount of AA/EPA (the n-6/n-3 ratio of these specific PUFAs) would be predictive of IFN- γ . We evaluated the relation of the lowest quartile of AA/EPA (representing lower amounts of AA

relative to EPA) compared with the upper 3 quartiles grouped together and found that being in the lowest quartile of AA/EPA also predicted reduced IFN- γ .

Higher n-6 LA level was associated with increased IL-13 secretion in response to allergen (ie, Bla g 2 and Der f 1) stimulation (Table IV). In contrast, neither EPA nor AA was correlated with IL-13.

DISCUSSION

In this US birth cohort study, we found that both higher levels of cord blood n-3 EPA and n-6 AA were associated with attenuation of cord blood lymphocyte proliferation and decreased IFN- γ secretion in response to allergen stimulation. The evidence for independent effects of both EPA and AA on attenuated immune responses was stronger for IFN- γ than for SI (lymphoproliferative response). Although n-3 EPA and n-6 AA had similar inverse associations with SI and IFN- γ , the ratio of AA/EPA also influenced IFN- γ production, with lower amounts of AA relative to EPA predicting lower levels of IFN- γ . Within class, individual n-6 FAs differed in their influence on allergen-stimulated secretion of IFN- γ and IL-13. Increased levels of the n-6 LA (but not AA) were associated with increases of allergen-stimulated secretion of the T_H2 cytokine IL-13.

Our findings of reduced cord blood lymphocyte proliferation responses with increased n-3 FA levels are qualitatively similar to the findings in the Australian randomized, double-blind, placebo-controlled trial of 98 atopic pregnancy women by Dunstan et al,⁷ although their associations of lymphocyte proliferation with n-3 FAs were not statistically significant. In contrast to the Dunstan group, we found specific n-3 (EPA) FA-associated suppression of IFN- γ rather than overall suppression of allergen-stimulated cytokine secretion (although as with lymphocyte proliferation, in the Dunstan study associations with cytokines were generally not statistically significant).

Although Dunstan et al⁷ had initially hypothesized an influence of n-3 PUFAs on the balance between T_H1 and T_H2 cytokine expression, they interpreted their findings to suggest a more global influence of n-3 PUFAs on T-cell regulation. A number of investigators have demonstrated that increased intake of EPA $\pm$ DHA can lead to diminished lymphoproliferative responses, IFN- γ responses, or both to mitogens (eg, concanavalin A) and antigens (eg, influenza virus and *Listeria monocytogenes*) in rats, mice, or adult human subjects.^{8,11–13}

Our data and data from other studies suggest that the effects of FAs on suppression of lymphocyte proliferation and some cytokine production might not be isolated-specific to the n-3 class of PUFAs. N-6 FAs, like AA and its prostaglandin (PG) byproducts, might have effects similar to those of EPA. Concanavalin A- or LPS-stimulated lymphocyte proliferation and IFN- γ production were inhibited by PG subtypes metabolized either from n-3 EPA (PGE₃) or n-6 AA (PGE₂) in a study by Dooper et al.¹⁴ The investigators concluded that the immunomodulatory effects of PUFAs might not be caused by a shift in the subtype of PGE and that certain n-3 and n-6 PUFAs might have similar immunomodulatory effects. This conclusion would be consistent with our findings of similar effects for n-3 EPA and n-6 AA. Other investigators have demonstrated, as we have, different immunomodulatory effects for AA versus LA, demonstrating that it is also an over-simplification to generalize about the effects of the overall class of n-6 (or n-3) FAs.¹¹

The implications of our findings for the development of respiratory infections or allergic inflammatory diseases have yet to be defined. Newson et al⁵ have questioned the importance of n-3 FAs in the development of wheeze and have challenged the Black and Sharpe hypothesis³ that n-6/n-3 imbalance promotes atopic disease through increases in PGE₂ and IgE levels. Newson et al⁵ argue that although PGE₂ might inhibit T_H1 responses and enhance

T_H2 responses of some sorts, it can also inhibit IgE production by B cells and protect against airway inflammation and bronchoconstriction.

FAs can influence immune responses and airway inflammation through mechanisms other than PGE₂. Our finding that higher levels of n-6 LA were correlated with increased allergen-stimulated IL-13 levels provides weak evidence for increased T_H2 cytokine production with this specific n-6 FA. However, we do not yet know whether increased neonatal IL-13 levels will be predictive of increased risk for later allergic or asthmatic disease. Some studies suggest that increased T_H2 cytokine levels at birth might in fact reflect a normal pregnancy.¹⁵

Similarly, it is as yet uncertain whether FA-associated suppression of lymphocyte proliferation and IFN- γ in fetal life represents “normal” protective T-cell regulation, as suggested by Dunstan et al.⁷ A number of investigators have found reduced IFN- γ levels in early childhood to be predictive of increased risk of allergic disease or asthma.¹⁶ Although the implication of our findings for the risk of allergy or asthma is uncertain, our study and the Dunstan study⁷ suggest that if fetal n-3 or n-6 FAs influence the risk of allergy or asthma through immune modulation, the immunomodulatory pathways are unlikely to be through simple alteration of the T_H1/T_H2 balance of cytokine secretion. If both n-3 EPA and n-6 AA serve similar immunomodulatory functions, it might be wise to take the advice of Herrerra¹ in his review on FAs, placental metabolism, and fetal and postnatal development. He suggested being cautious before recommending long-chain n-3 FA supplementation in pregnancy. Both EPA and AA are needed for normal development, and fish oil EPA supplementation might reduce levels of AA, which is needed for normal fetal development and antioxidant capacity in the fetus.

Our study has several limitations. The moderate correlation between EPA and AA made it possible to distinguish some, but not all, of the independent effects of EPA from AA on lymphoproliferative responses and cytokine levels. In the case of Bla g 2, low power related to the low level of cytokine production in response to allergen stimulation might have limited our potential to separate EPA from AA effects. Potential contamination with LPS was also considered as a limitation in interpretation of proliferative and cytokine responses. The allergens Der f 1, Bla g 2, and OVA, as well as the mitogen PHA, were tested for endotoxin contents by means of Limulus assay. We found low concentrations of endotoxin (<0.01 EU/mL = 0.002 ng/mL). These concentrations did not significantly change lymphocyte proliferation or cytokine secretion in CBMCs. In separate assays we tested the lymphoproliferative response of CBMCs to endotoxin components (eg, LPS and lipid A, an active component of LPS) in a dose-response analysis (ie, 0.01 ng to 100 ng/mL). Increased lymphocyte proliferation could only be detected with doses of lipid A of greater than 1 ng/mL. In addition, we also assessed cytokine secretion of TNF- α and IL-6, 2 innate cytokines typically increased after stimulation with lipid A. These cytokines were not increased at doses of lipid A as low as 0.002 ng/mL, as detected in our reagents. Thus our data suggest that endotoxin content had minimal influence on the outcomes of lymphoproliferation and cytokine secretion. Even if LPS did not influence our allergen-stimulated responses, it is possible that these responses are not antigen-allergen specific because the presence of allergen-specific T-cell memory has been questioned and challenged. In *in vitro* studies of small samples of newborns, others have found that naive T regulatory cells predominate, with a relative or absolute absence of measurable conventional T memory cells in cryopreserved CBMCs.¹⁷ Nevertheless, even if the lymphoproliferative and cytokine responses to *in vitro* antigen-allergen stimulation do not involve conventional T memory cells, it is still possible that these *in vitro* responses to antigen stimulation reflect the neonate's *in vivo* early immune development. And it is still possible that these responses are influenced by cord blood FA levels. Although our study results could have been influenced by perinatal events, only 2 of the babies in the analyses were not healthy after birth, and sensitivity analyses eliminating these children from analyses did not

significantly alter results. We report results only at one time point and one dose of stimulants, but dose-response and time-kinetic experiments lead to the choice of the time point and dose. Cells other than T cells can also be affected by FAs, and it is possible that some of our responses were, in fact, dependent on interaction between the different types of mononuclear cells. We are limited by lack of knowledge about maternal sensitization to dust mite and cockroach, although we did not find that maternal asthma was a confounder of the relation of FAs with cellular responses to allergen stimulation. Although cord blood FA levels are likely to reflect the *in utero* exposure to FAs, it is possible that unmeasured perinatal events influenced those levels.

In conclusion, our data strongly suggest that increased fetal-neonatal levels of EPA or AA attenuate immunologic responses, specifically allergen-stimulated lymphocyte proliferation and IFN- γ production. Follow-up is needed to evaluate whether FA-associated attenuation of immune responses in the neonate can influence the risk of subsequent allergy and asthma in later childhood.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG 1.

CBMC proliferative responses (SI) after stimulation with Bla g 2 (cockroach), Der f 1 (dust mite), OVA, or PHA. The median is represented by the *black bar*. The *upper* and *lower boundaries* of the *box* represent the 25th to 75th percentiles of the data, respectively. Observations less than 1.5 times the height of the box beyond either quartile are displayed within the *whiskers*.

FIG 2.

IFN- γ and IL-13 cytokine levels (in picograms per milliliter) after stimulation with Bla g 2 (cockroach) and Der f 1 (dust mite). At less than the lower limits of detection, all values were assigned a value of 0.01 for the purpose of ranking.

TABLE I

Population characteristics*

Child's sex, n (%)	
Male	109 (57)
Female	83 (43)
Child's race-ethnicity, n (%)	
White	135 (70)
Black/African American	26 (14)
Hispanic	12 (6)
Asian	12 (6)
American Indian	3 (2)
Other	2 (1)
Gestational age (wk)	
Minimum	30.9
25th percentile	39.0
50th percentile	39.9
75th percentile	40.7
Maximum	42.1
Cesarian section, n (%)	
No	153 (80)
Yes	39 (20)
Maternal smoking, n (%)	
No	170 (89)
Yes	7 (4)
Maternal prepregnancy BMI, n (%)	
<25 kg/m ²	124 (65)
25–30 kg/m ²	45 (23)
>30 kg/m ²	22 (11)

* Numbers might not add up to 192 because of missing values. Percentages might not add up to 100% because of rounding or missing values.

TABLE II
Univariate associations of cord plasma n-3 and n-6 FAs with allergen- and mitogen-stimulated cord blood lymphocyte proliferation

FA	Stimulant for proliferation	Correlation coefficient *	Pvalue	OR (95% CI) for SI >2 (Bla g 2, Der f 1, OVA) or for SI >median (PHA) [†]
n-3 EPA	Bla g 2	-.09	.009	0.31 (0.13 to 0.75) [‡]
	Der f 1	-.23	.001	0.35 (0.16 to 0.77) [‡]
	OVA	-.17	.02	0.27 (0.06 to 1.18)
n-3 DHA	PHA	0.03	.72	0.96 (0.49 to 1.86)
	Bla g 2	-.10	.17	0.66 (0.46 to 0.95) [§]
	Der f 1	-.07	.32	0.84 (0.62 to 1.15)
n-6 LA	OVA	-.03	.69	0.59 (0.32 to 1.09)
	PHA	0.13	.07	1.20 (0.88 to 1.63)
	Bla g 2	-.01	.84	1.08 (0.90 to 1.29)
n-6 AA	Der f 1	0.01	.87	1.12 (0.94 to 1.32)
	OVA	-.13	.07	0.88 (0.65 to 1.18)
	PHA	-.03	.72	0.96 (0.81 to 1.13)
	Bla g 2	-.13	.07	0.82 (0.70 to 0.96) [§]
	Der f 1	-.15	.03	0.87 (0.75 to 1.00)
	OVA	-.10	.18	0.71 (0.55 to 0.92) [§]
	PHA	0.05	.48	1.00 (0.87 to 1.15)

* Rank-based (Spearman) correlation coefficients.

[†] Odds that a 0.5-percentage-point increase in EPA or a 1-percentage-point increase in DHA, LA, or AA will be associated with an SI of greater than 2 for Bla g 2, Der f 1, and OVA, or an SI of greater than median (42.69) for PHA.

[‡] $P < .01$.

[§] $P < .05$.

TABLE III
Associations of cord plasma EPA and AA levels with allergen-stimulated cord blood lymphocyte proliferation (n = 191)

Outcome	Model	Predictor	Linear model		Logistic model	
			Percentage change* (95% CI)	Pvalue	OR (95% CI) for SI >2 [‡]	Pvalue
Bla g 2 SI	1 (univariate)	EPA	-23.7 (-36.5 to -8.4)	.004	0.31 (0.13 to 0.75)	.009
	2 [‡]	EPA	-19.0 (-32.4 to -2.9)	.02	0.32 (0.13 to 0.80)	.02
	3 [‡]	AA	-4.6 (-8.2 to -0.9)	.02	0.82 (0.69 to 0.97)	.02
	4 [‡]	EPA	-13.7 (-28.9 to 4.8)	.14	0.43 (0.16 to 1.15)	.09
Der f 1 SI	1 (univariate)	AA	-3.5 (-7.4 to 0.5)	.08	0.88 (0.73 to 1.06)	.17
		EPA	-32.4 (-45.0 to -17.0)	.0003	0.35 (0.16 to 0.77)	.008
	2 [‡]	EPA	-26.8 (-40.3 to -10.3)	.003	0.34 (0.15 to 0.76)	.009
	3 [‡]	AA	-4.2 (-8.2 to 0.06)	.05	0.88 (0.76 to 1.03)	.11
	4 [‡]	EPA	-24.0 (-39.0 to -5.4)	.02	0.38 (0.16 to 0.90)	.03
		AA	-2.1 (-6.5 to 2.5)	.36	0.95 (0.80 to 1.13)	.57

* Percentage change in lymphocyte proliferation for a 0.5-percentage-point increase in EPA levels or a 1-percentage-point increase in AA levels.

[‡] Models control for child race, maternal smoking in pregnancy, and child sex.

[‡] Odds that a 0.5-percentage-point increase in EPA levels or a 1-percentage-point increase in AA levels will be associated with an SI of greater than 2 for Bla g 2 or Der f 1.

TABLE IV
Associations of cord plasma n-3 and n-6 FAs with IFN- γ and IL-13

FA	Stimulant	IFN- γ		IL-13	
		Correlation coefficient [*]	Pvalue	Correlation coefficient [*]	Pvalue
n-3: EPA	Bla g 2	−0.23	.003	−0.02	.82
	Der f 1	−0.24	.002	−0.06	.47
	Media	−0.20	.006	0.03	.70
	PHA	−0.13	.07	0.11	.15
n-3: DHA	Bla g 2	−0.15	.05	−0.14	.07
	Der f 1	−0.13	.10	−0.08	.32
	Media	−0.17	.02	0.07	.36
	PHA	−0.04	.57	0.08	.29
n-6: AA	Bla g 2	−0.23	.003	0.03	.67
	Der f 1	−0.25	.002	−0.08	.31
	Media	−0.21	.004	0.10	.16
	PHA	−0.19	.01	0.11	.15
n-6: LA	Bla g 2	0.03	.69	0.20	.009
	Der f 1	0.04	.64	0.19	.02
	Media	−0.01	.85	−0.12	.09
	PHA	−0.12	.11	−0.16	.03

* Rank-based (Spearman) correlation coefficients.

TABLE V

Associations of cord plasma EPA and AA levels with allergen-stimulated IFN- γ levels

Outcome	Model	Categoric predictors	Percentage difference in IFN- γ level (95% CI)	Pvalue
Bla g 2 IFN- γ	1	EPA (Q1-Q3) (low)	—	
	(univariate)	EPA (Q4) (high)	−78.9 (−93.4 to −32.2)	.01
	2*	EPA (Q1-Q3) (low)	—	
		EPA (Q4) (high)	−75.2 (−92.5 to −18.2)	.02
	3*	AA (Q1-Q3) (low)	—	
		AA (Q4) (high)	−72.8 (−91.4 to −13.9)	.03
	4*	EPA (Q1-Q3) (low)	—	
		EPA (Q4) (high)	−69.6 (−90.9 to 1.5)	.05
		AA (Q1-Q3) (low)	—	
		AA (Q4) (high)	−66.5 (−89.5 to 6.9)	.07
	5*	AA/EPA (Q1-Q3) (high)	—	
		AA/EPA (Q4) (low)	−61.8 (−88.5 to 26.7)	.12
Der f 1 IFN- γ	6*	EPA and AA (Q1-Q3) (low)	—	
		EPA or AA (Q4) (high)	−81.7 (−93.4 to −48.9)	.001
	1	EPA (Q1-Q3) (low)	—	
	(univariate)	EPA (Q4) (high)	−81.6 (−95.1 to −31.2)	.01
	2*	EPA (Q1-Q3) (low)	—	
		EPA (Q4) (high)	−79.9 (−94.8 to −22.6)	.02
	3*	AA (Q1-Q3) (low)	—	
		AA (Q4) (high)	−81.6 (−95.0 to −33.0)	.01
	4*	EPA (Q1-Q3) (low)	—	
		EPA (Q4) (high)	−74.2 (−93.3 to −0.3)	.05
		AA (Q1-Q3) (low)	—	
		AA (Q4) (high)	−77.3 (−93.8 to −16.5)	.03
	5*	AA/EPA (Q1-Q3) (high)	—	
		AA/EPA (Q4) (low)	−80.5 (−94.8 to −26.2)	.02
	6*	EPA and AA (Q1-Q3) (low)	—	
		EPA or AA (Q4) (high)	−89.6 (−96.7 to −67.6)	.0001

* Models control for gestational age and maternal prepregnancy BMI. In addition, model 4 includes both FAs, EPA and AA. See Table E1 for more details on FA quartiles and IFN- γ levels.