

Umbilical cord and maternal blood red cell fatty acids and early childhood wheezing and eczema

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Background: Few studies have explored whether fetal exposure to n-6 and n-3 fatty acids influences the inception of atopic disease.

Objective: To assess prenatal fatty acid exposures as predictors of early childhood wheezing and eczema.

Methods: In the Avon Longitudinal Study of Parents and Children, late pregnancy maternal blood samples and umbilical cord blood samples were assayed for n-6 and n-3 fatty acids (percentage of total red cell phospholipid), and mothers were asked about wheezing and eczema in their children. We measured associations of 11 n-6 and n-3 fatty acid exposures with wheezing at 30 to 42 months, with wheezing patterns defined by presence (+) or absence (−) of wheezing during 2 periods, 0 to 6 months and 30 to 42 months (transient infant, +/−; later-onset, −/+; persistent, +/+; n = 1191 and n = 2764 for cord and maternal analyses, respectively), and with eczema at 18 to 30 months (n = 1238 and n = 2945 for cord and maternal analyses, respectively).

Results: In cord blood red cells, the ratio of arachidonic:eicosapentaenoic acid was positively associated with eczema (adjusted odds ratio [OR] per doubling, 1.14; 95% CI, 1.00-1.31; *P* = .044), the ratio of linoleic acid:α-linolenic acid was positively associated with later-onset wheeze (OR, 1.30; CI, 1.04-1.61; *P* = .019), and the ratio of α-linolenic acid:n-3 products was negatively associated with later-onset wheeze (OR, 0.86; CI, 0.75-0.99; *P* = .040). However, these associations were no longer significant after adjusting for multiple comparisons.

Conclusions: It seems unlikely that fetal exposure to n-6 and n-3 fatty acids is an important determinant of early childhood wheezing and atopic disease. (*J Allergy Clin Immunol* 2004;114:531-7.)

Key words: n-3/n-6 Fatty acids, wheezing, eczema, birth cohort, prenatal

Black and Sharpe¹ proposed that changing patterns of fatty acid intake in the West have contributed to the rise in asthma and atopic disease in recent decades. In particular, they suggested that substitution of margarine and vegetable oils for butter and lard and a falling consumption of oily fish resulted in a higher intake of n-6 fatty acids such as linoleic acid (LA) and a lower intake of n-3 fatty acids such as α-linolenic acid (ALA) and eicosapentaenoic acid (EPA). They postulated that because n-3 fatty acids compete with n-6 fatty acids for the same desaturation enzymes, a consequence of this shift is greater production of the n-6 product arachidonic acid (AA), leading to increases in prostaglandin E₂ (PGE₂), which, in turn, would inhibit T_H1 cytokine responses and increase production of IgE.¹ Such imbalance might also augment asthma inflammation through increased production of 4-series leukotrienes.²

In keeping with this hypothesis, case-control studies of atopic disease, particularly eczema, have consistently shown that cases have higher blood levels of linoleic acid (n-6 precursor), lower levels of EPA and docosahexaenoic acid (DHA; n-3 products), and an increased ratio of AA to EPA. On the other hand, and contrary to the hypothesis, atopic individuals also have lower blood levels of n-6 products, including dihomo-γ-linolenic acid (DGLA) and AA, an increase in the ratio of LA to AA,²⁻⁴ and evidence of reduced ability of monocytes to make PGE₂.⁵ An alternative hypothesis invoked to explain the reduction in n-6 and n-3 products in relation to their precursors is that an inefficiency in the δ-6-desaturase enzyme is responsible,⁶ although a study in children did not support this possibility.⁷ A limitation of these cross-sectional studies, however, is that changes in fatty acid status may be a consequence of established atopic disease.

Few studies have investigated the possible role of n-6 and n-3 fatty acids in the prenatal inception of atopy and atopic disease. We have therefore investigated, in a population-based birth cohort, whether relative concentrations of red cell n-6 and n-3 fatty acids in cord blood, and in maternal blood in late pregnancy, predict risk of eczema and wheezing in early childhood. In particular, we examined whether high n-6 fatty acid exposure and exposure to a high ratio of n-6 to n-3 fatty acids are associated with an increased risk, and whether high exposure to n-3 fatty acids is associated with decreased risk. A subsidiary hypothesis was to explore whether inefficient δ-6-desaturase enzyme activity, as measured by

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Abbreviations used

AA: Arachidonic acid (20:4n-6)
 ALA: α -Linolenic acid (18:3n-3)
 ALSPAC: Avon Longitudinal Study of Parents and Children
 DGLA: Dihomo- γ -linolenic acid (20:3n-6)
 DHA: Docosahexaenoic acid (22:6n-3)
 EPA: Eicosapentaenoic acid (20:5n-3)
 LA: Linoleic acid (18:2n-6)
 PGE₂: Prostaglandin E₂

an increased n-6 and n-3 precursor:product ratio, is associated with increased risk.

METHODS

The Avon Longitudinal Study of Parents and Children (ALSPAC)^{8,9} is a prospective study of 14,541 pregnancies that resulted in 14,062 live births (born to 13,866 mothers), of whom 13,988 survived to 1 year. Women were enrolled as early in pregnancy as possible on the basis of an expected date of delivery between April 1, 1991, and December 31, 1992, and place of residence within the 3 Bristol-based health districts of the former county of Avon, United Kingdom. It was estimated that 85% to 90% of eligible pregnant women were enrolled. ALSPAC is a multipurpose study of child health and development closely monitored by its own Ethics and Law Committee and by 4 local research ethics committees.

Measurement of fatty acids in maternal and umbilical cord blood red cells

At least 1 blood sample was taken for fatty acid analysis from 5144 mothers during pregnancy, of whom 4136 (with 4202 children) had at least 1 sample taken after 20 weeks of pregnancy. Cord blood samples from 1762 children were taken for fatty acid analysis. Blood samples were collected in heparin-containing tubes and centrifuged at 1500g for 15 minutes at 4°C to separate the red cells, which were then stored at -20°C until 1993 and subsequently at -70°C. Frozen red blood cell samples were shipped to the laboratories of Scotia Pharmaceuticals in Canada in 1996 for fatty acid composition analysis. The frozen samples were thawed, and 0.5 mL water was added to 0.5 mL packed red cells. Then, 3 mL of methanol was added, and the mixture was vortexed. Chloroform (6 mL) was added, and the mixture was vortexed again. The mixture was then filtered through Whatman no. 1 filter paper and centrifuged (1500 rpm, 8 minutes). The aqueous phase was removed and discarded, and the organic phase, containing red cell lipids, was evaporated to dryness under nitrogen. Lipids were redissolved in chloroform (100 μ L) and phospholipids isolated by thin-layer chromatography with a mixture of hexane/diethyl ether/acetic acid (80:20:1 vol:vol:vol) as the elution phase. Fatty acid methyl esters were prepared by incubation with 120 g/L boron trifluoride in methanol at 90°C for 30 minutes. Fatty acid methyl esters were isolated by solvent (hexane) extraction, and gas chromatography was used to measure the relative concentrations of 40 fatty acids as a percentage of total red blood cell membrane phospholipid. Fatty acid methyl esters were identified by comparison with standards run previously. Fatty acid concentrations below the limit of detection of the assay (0.01%) were recoded to that value.

We restricted our statistical analyses of maternal red cell fatty acid data to those obtained from the latest blood sample taken after 20 weeks of pregnancy, because we considered fatty acid status in late pregnancy to be of greater biological importance for the developing

immune system of the fetus than status in early pregnancy, and because relevant information on maternal diet was also collected in late pregnancy. We focused attention on 15 fatty acid exposures relevant to our hypotheses, namely 5 n-3 fatty acid percentages (the n-3 precursor, 18:3n-3, ALA; and 4 n-3 products: 18:4n-3; 20:5n-3, EPA; 22:5n-3, docosapentaenoic acid; and 22:6n-3, DHA); 6 n-6 fatty acid percentages (the n-6 precursor, 18:2n-6, LA; and 5 n-6 products: 18:3n-6, γ -linolenic acid; 20:3n-6, DGLA; 20:4n-6, AA; 22:4n-6; and 22:5n-6); 2 n-6:n-3 ratios (LA:ALA and AA:EPA); and 2 precursor:product ratios (LA:[sum of n-6 products], and ALA:[sum of n-3 products]).

Wheezing and eczema

Information on wheezing in the child at 30 to 42 months was obtained by asking the mother at 42 months, "In the last 12 months has he/she had any periods when there was wheezing with whistling on his/her chest when he/she breathed?" A similar question at 6 months asked about wheezing since birth, and we used the information from these 2 periods to identify children with 4 mutually exclusive patterns of wheezing that we have shown to be associated with different risk factors: children who did not wheeze in either period (nonwheezers), children who wheezed before 6 months but not at 30 to 42 months (transient infant wheezers), children who wheezed at 30 to 42 months but not before 6 months (later-onset wheezers), and children who wheezed in both periods (persistent wheezers).¹⁰

Twelve-month prevalence of eczema at 30 months was defined on the basis of a positive response to the question, "Has your child had an itchy dry skin rash in joints and creases of his/her body (eg behind the knees, under the arms) since he/she was 18 months old?"¹¹

Confounders

In multivariate analyses of asthma and wheezing, we controlled for the child's sex, gestational age at birth, and birth weight, and for the mother's age, education level, housing tenure, parity, ethnicity, and smoking in pregnancy (for variable categories see Table EI in the Journal's Online Repository at <http://www.mosby.com/jaci>), as well as maternal atopic disease (asthma, eczema, rhinoconjunctivitis), child's head circumference at birth (<33 cm, 33-34.99 cm, 35-36.99 cm, 37+ cm, unknown), child's crown to heel length at birth (<48 cm, 48-50.99 cm, 51-53.99 cm, 54+ cm, unknown), mother's body mass index (from prepregnancy self-reported weight and height; <18.5 kg/m², 18.5-24.99 kg/m², 25-29.99 kg/m², 30+ kg/m², unknown), breast-feeding in first 6 months (no, yes, unknown), and day care use in first 6 months (yes, no, unknown). We excluded 722 children from the maternal fatty acid analyses and 216 children from the cord fatty acid analyses who were from multiple pregnancies or who were in small missing value categories for various confounders. This left 3480 children and 1546 children with complete data on fatty acid exposures and confounders in the maternal blood and cord blood data sets, respectively. In the maternal fatty acid analyses, we also adjusted for the week of pregnancy (after 20 weeks) in which the blood sample was taken (as a continuous covariate) by including a per week odds ratio in the model.

Statistical methods

We used logistic regression to analyze associations with wheezing at 30 to 42 months and eczema at 18 to 30 months, and multinomial logistic regression (with nonwheezers as the baseline category) to analyze associations with wheezing patterns. Analyses were performed by using the Stata statistical package and Huber variances throughout.¹² The associations between fatty acid exposures and outcomes were estimated in 2 ways: first as odds ratios per doubling of the fatty acid exposure (by including log₂ concentration as a continuous linear predictor in the logistic model), and second as odds

TABLE I. Descriptive statistics of cord blood and maternal blood red cell fatty acid exposures (median values and 80th/20th interpercentile ratios [IPRs])

Fatty acid exposures	Cord blood (n = 1762†)		Maternal blood (n = 4202†)	
	Median	IPR	Median	IPR
n-3 Percentages*				
18:3 n-3	0.02	5.00	0.14	2.22
18:4 n-3	0.04	7.00	0.06	2.50
20:5 n-3	0.11	3.00	0.23	2.64
22:5 n-3	0.22	3.67	0.60	4.11
22:6 n-3	2.00	3.92	2.02	3.05
n-6 Percentages*				
18:2n-6	4.65	1.45	11.46	1.44
18:3n-6	0.04	4.00	0.02	3.00
20:3n-6	2.17	1.92	1.42	1.89
20:4n-6	7.80	2.79	5.88	2.73
22:4n-6	1.28	3.24	0.89	3.50
22:5n-6	0.45	3.46	0.23	2.85
n-6:n-3 Ratios				
LA:ALA	265.25	5.36	79.02	2.01
AA:EPA	78.38	2.30	24.32	2.03
Precursor:product ratios				
LA:sum of n-6 products	0.41	2.19	1.42	2.02
ALA:sum of n-3 products	0.01	7.21	0.05	2.18

*Percentage of total red blood cell membrane phospholipid.

†Number of children.

ratios across quintiles of exposure, with the bottom quintile as the reference category. We used per doubling odds ratios because a doubling of fatty acid exposure was similar in magnitude to the observed ratios between fatty acid concentrations in the top and bottom quintiles (Table I). Because there were 5 outcomes and 15 fatty acid exposures of interest for cord blood and for maternal blood in the main analyses, the number of odds ratios measured was very large (150 adjusted per doubling odds ratios). We therefore used the Simes procedure, controlling the false discovery rate at 0.05,¹³ in an attempt to discover a subset of per doubling odds ratios that could be considered statistically significant, given the multiple statistical tests performed.

Validation of assays as biomarkers of prenatal exposure

Because oily fish is a major source of the n-3 fatty acids, EPA and DHA, we measured trend in relative red cell concentrations of these fatty acids in maternal and cord blood with mother's consumption of oily fish during late pregnancy (as ascertained by food frequency questionnaire at 32 weeks across 4 categories of increasing frequency) by using Somers' D, with fish consumption as the predictor variable.

RESULTS

We examined the distribution of selected maternal and birth characteristics of children with and without cord and maternal blood fatty acid data (see Table EI in the Online Repository). Children with cord blood fatty acid data were born between January 1991 and January 1992, and children without data were born between February 1991 and February 1993. The ranges of birth dates for children with and without maternal blood fatty acid data were more

similar. The main difference between children with and without fatty acid data was that children without data were more likely to have missing information on maternal characteristics.

For each fatty acid exposure, Table I gives the median value and the ratio between the 80th percentile and the 20th percentile as a measurement of variability. These ratios compared the bottom of the top quintile with the top of the bottom quintile and ranged from 1.44 to 4.11 for maternal LA and docosapentaenoic acid, respectively, and from 1.45 to 7.21 for cord LA and the ratio of ALA:n-3 products, respectively. The predominant n-3 fatty acid in cord and maternal blood red cells was DHA, and the predominant n-6 fatty acids were LA and AA.

Maternal oily fish consumption during pregnancy was positively associated with the relative red cell concentrations of EPA and DHA in the blood of 3811 mothers with complete dietary information (Somers' D, 0.09; 95% CI, 0.06-0.12; $P = 1.8 \times 10^{-8}$ for EPA; and Somers' D, 0.09; 95% CI, 0.06-0.12; $P = 2.2 \times 10^{-8}$ for DHA), and negatively associated with the AA:EPA and ALA:n-3 product ratios. No associations were seen between maternal oily fish consumption and EPA or DHA in the cord blood red cells of 1751 children of 1732 mothers (Somers' D, -0.02; 95% CI, -0.07 to 0.02; $P = .32$ for EPA; and Somers' D, -0.04; 95% CI, -0.08 to 0.01; $P = .11$ for DHA).

Of 1546 children with data on the 11 fatty acids of interest in cord blood and information on confounders, the main analyses were restricted to 1238 with information on eczema at 18 to 30 months and 1191 with information on wheezing at 6 months and 30 to 42 months. Of 3480

TABLE II. Prevalence of eczema and wheezing in children with complete data on fatty acid exposures and confounders in cord blood and maternal blood red cell data sets

	Cord blood dataset		Maternal blood dataset	
	n	%	n	%
Children with data on fatty acid exposures, confounders and eczema	1238		2945	
Eczema at 18-30 months				
No	937	75.7	2156	73.2
Yes	301	24.3	789	26.8
Children with data on fatty acid exposures, confounders and wheezing	1191		2764	
Wheezing at 30-42 months				
No	1028	86.3	2404	87.1
Yes	163	13.7	360	13.0
Wheezing patterns				
Nonwheezers	814	68.4	2055	74.4
Transient infant wheezers	214	18.0	349	12.6
Later-onset wheezers	87	7.3	229	8.3
Persistent wheezers	76	6.4	131	4.7

TABLE III. Per doubling odds ratios (95% CI) for eczema at 18 to 30 months with respect to cord blood and maternal blood red cell fatty acid exposures (n = 1238 and n = 2945, respectively) after controlling for potential confounders

Fatty acid exposure	Cord blood OR (95% CI)	Maternal blood OR (95% CI)
18:3n-3	1.00 (0.90-1.11)	1.00 (0.91-1.09)
18:4n-3	1.01 (0.90-1.12)	0.99 (0.92-1.07)
20:5n-3	0.95 (0.86-1.06)	1.03 (0.94-1.12)
22:5n-3	0.97 (0.87-1.07)	1.02 (0.94-1.10)
22:6n-3	1.00 (0.91-1.10)	1.02 (0.94-1.12)
18:2n-6	0.90 (0.65-1.25)	1.10 (0.91-1.32)
18:3n-6	1.01 (0.90-1.13)	0.97 (0.89-1.05)
20:3n-6	1.09 (0.87-1.36)	1.06 (0.92-1.22)
20:4n-6	1.08 (0.93-1.25)	0.98 (0.88-1.10)
22:4n-6	1.01 (0.87-1.17)	1.00 (0.93-1.09)
22:5n-6	1.08 (0.96-1.22)	1.00 (0.93-1.07)
LA:ALA	0.99 (0.89-1.10)	1.03 (0.93-1.14)
AA:EPA	1.14 (1.00-1.31)	0.95 (0.85-1.05)
LA:sum of n-6 products	0.89 (0.74-1.08)	1.04 (0.91-1.18)
ALA:sum of n-3 products	1.00 (0.92-1.09)	0.98 (0.88-1.08)

TABLE IV. Per-doubling odds ratios (95% CI) for wheezing at 30 to 42 months with respect to cord blood and maternal blood red cell fatty acid exposures (n = 1191 and n = 2764, respectively) after controlling for potential confounders

Fatty acid exposure	Cord blood OR (95% CI)	Maternal blood OR (95% CI)
18:3n-3	0.99 (0.85-1.15)	1.03 (0.91-1.16)
18:4n-3	0.98 (0.85-1.14)	0.99 (0.89-1.09)
20:5n-3	1.01 (0.87-1.17)	0.94 (0.84-1.06)
22:5n-3	0.99 (0.87-1.13)	1.03 (0.93-1.14)
22:6n-3	1.05 (0.93-1.19)	0.99 (0.86-1.13)
18:2n-6	1.31 (0.87-1.97)	1.08 (0.82-1.42)
18:3n-6	0.95 (0.82-1.11)	1.03 (0.93-1.15)
20:3n-6	0.97 (0.72-1.31)	1.00 (0.82-1.22)
20:4n-6	1.05 (0.86-1.27)	1.02 (0.86-1.20)
22:4n-6	1.01 (0.84-1.22)	1.03 (0.92-1.15)
22:5n-6	0.99 (0.84-1.16)	1.04 (0.94-1.16)
LA:ALA	1.04 (0.89-1.22)	0.99 (0.86-1.13)
AA:EPA	1.01 (0.85-1.21)	1.11 (0.95-1.30)
LA:sum of n-6 products	1.08 (0.84-1.39)	0.98 (0.82-1.15)
ALA:sum of n-3 products	0.98 (0.87-1.10)	1.03 (0.90-1.19)

children with data on maternal blood fatty acids and confounders, the main analyses were restricted to 2945 with information on eczema and 2764 with information on wheezing. Table II shows the prevalences of eczema and wheezing in children in the cord blood and maternal blood data sets.

Tables III and IV show the odds ratios for eczema at 18 to 30 months and for wheezing at 30 to 42 months per doubling of cord blood and maternal blood fatty acid exposures, after controlling for potential confounders. The ratio of AA:EPA in cord blood red cells was positively associated with eczema ($P = .044$), but there were no

other conventionally significant associations. Table V shows associations with wheezing patterns after controlling for potential confounders. The only associations of note were between cord blood fatty acid exposures and later-onset wheeze. There was weak evidence for a negative association with ALA ($P = .062$), the ratio of LA:ALA was positively associated ($P = .019$), and the ratio of ALA:n-3 products was negatively associated ($P = .040$). No significant associations were seen for maternal blood fatty acid exposures. The unadjusted odds ratios for eczema, wheezing, and wheezing patterns (not shown) were similar to the adjusted results.

TABLE V. Per doubling odds ratios (95% CI) for wheezing patterns with respect to cord blood and maternal blood red cell fatty acid exposures (n = 1191 and n = 2764, respectively) after controlling for potential confounders

Wheezing pattern	Fatty acid exposure	Cord blood OR (95% CI)	Maternal blood OR (95% CI)
Transient infant wheeze	18:3n-3	0.92 (0.81-1.05)	0.92 (0.83-1.03)
	18:4n-3	0.97 (0.85-1.10)	0.94 (0.84-1.05)
	20:5n-3	1.00 (0.88-1.13)	0.99 (0.88-1.12)
	22:5n-3	0.98 (0.87-1.11)	0.94 (0.85-1.05)
	22:6n-3	0.99 (0.87-1.13)	0.98 (0.87-1.10)
	18:2n-6	0.79 (0.53-1.17)	0.87 (0.70-1.10)
	18:3n-6	1.05 (0.91-1.20)	1.03 (0.91-1.16)
	20:3n-6	0.93 (0.72-1.21)	0.91 (0.75-1.10)
	20:4n-6	1.01 (0.84-1.20)	0.97 (0.85-1.12)
	22:4n-6	1.06 (0.90-1.26)	0.97 (0.87-1.09)
	22:5n-6	1.11 (0.97-1.27)	0.99 (0.89-1.09)
	LA:ALA	1.06 (0.93-1.19)	1.08 (0.95-1.24)
	AA:EPA	1.01 (0.86-1.18)	0.99 (0.86-1.14)
	LA:sum of n-6 products	0.89 (0.70-1.12)	0.98 (0.82-1.18)
	ALA:sum of n-3 products	0.96 (0.87-1.06)	0.93 (0.82-1.05)
Later-onset wheeze	18:3n-3	0.82 (0.67-1.01)	0.99 (0.86-1.15)
	18:4n-3	0.92 (0.75-1.13)	0.98 (0.86-1.11)
	20:5n-3	1.03 (0.84-1.26)	0.90 (0.79-1.03)
	22:5n-3	1.04 (0.88-1.22)	1.00 (0.88-1.12)
	22:6n-3	1.08 (0.93-1.26)	0.97 (0.82-1.13)
	18:2n-6	1.43 (0.92-2.22)	1.04 (0.70-1.54)
	18:3n-6	0.93 (0.76-1.15)	1.09 (0.96-1.24)
	20:3n-6	1.08 (0.73-1.59)	0.98 (0.77-1.24)
	20:4n-6	1.10 (0.85-1.42)	0.96 (0.78-1.18)
	22:4n-6	1.05 (0.83-1.32)	0.98 (0.85-1.12)
	22:5n-6	1.08 (0.90-1.31)	1.03 (0.91-1.17)
	LA:ALA	1.30 (1.04-1.61)	1.02 (0.86-1.21)
	AA:EPA	1.02 (0.79-1.31)	1.14 (0.92-1.40)
	LA:sum of n-6 products	1.03 (0.76-1.41)	1.01 (0.81-1.25)
	ALA:sum of n-3 products	0.86 (0.75-0.99)	1.02 (0.87-1.21)
Persistent wheeze	18:3n-3	1.15 (0.93-1.41)	1.05 (0.87-1.26)
	18:4n-3	1.04 (0.84-1.28)	0.97 (0.83-1.13)
	20:5n-3	0.99 (0.82-1.21)	1.02 (0.85-1.23)
	22:5n-3	0.92 (0.75-1.13)	1.07 (0.90-1.28)
	22:6n-3	1.00 (0.84-1.21)	1.03 (0.81-1.32)
	18:2n-6	1.04 (0.51-2.10)	1.08 (0.76-1.53)
	18:3n-6	1.00 (0.81-1.24)	0.95 (0.80-1.14)
	20:3n-6	0.84 (0.54-1.32)	1.01 (0.75-1.36)
	20:4n-6	0.99 (0.75-1.31)	1.13 (0.89-1.42)
	22:4n-6	0.98 (0.72-1.33)	1.13 (0.94-1.36)
	22:5n-6	0.92 (0.70-1.21)	1.07 (0.90-1.26)
	LA:ALA	0.87 (0.69-1.08)	0.97 (0.80-1.17)
	AA:EPA	1.01 (0.80-1.27)	1.08 (0.90-1.29)
	LA:sum of n-6 products	1.07 (0.73-1.57)	0.91 (0.72-1.16)
	ALA:sum of n-3 products	1.10 (0.94-1.30)	1.01 (0.80-1.26)

When we performed the Simes procedure on the 150 adjusted per doubling odds ratios, none of the null hypotheses was rejected. Similarly, none was rejected when we performed the procedure on the 150 adjusted 5th versus 1st quintile odds ratios. Also, when we repeated the main analyses, stratifying by maternal atopic history and several other factors, and applied the Simes procedure, there were no more nominally significant results than would be expected by chance.

DISCUSSION

In this population-based follow-up study, we have not found convincing evidence that higher exposure to n-6 versus n-3 fatty acids *in utero* promotes the development of eczema or wheezing in early childhood, although the cord blood findings for the AA:EPA ratio and eczema and for the LA:ALA ratio and later-onset wheeze were in keeping with this hypothesis.

Previous studies

Few studies have examined the relation between cord or maternal blood n-6 and n-3 fatty acids and atopic outcomes in the offspring. One study of newborns found that levels of n-6 products (DGLA and AA) in cord blood serum were lower, and the LA:AA ratio was higher, in babies who developed atopic disease¹⁴ than in babies who did not, supporting the δ -6-desaturase hypothesis. However, our data do not support the findings of that study, which included only babies at high risk for atopic disease. Our observations are in keeping with a study by Yu and Bjorksten¹⁵ that found no relation between maternal serum phospholipid fatty acids and atopic outcomes in the offspring to 6 years of age. However, in contrast to our study, those authors found no associations between cord blood fatty acids and atopic outcomes.¹⁶ All of these previous studies were very small and therefore lacked statistical power compared with ours, and measured serum (rather than red cell) phospholipid fatty acids, which could account for the conflicting results.

Measurement of fatty acid exposure

Red cell phospholipid concentrations are considered to reflect exposure as long as 120 days before the blood sample being taken (the average half-life of a red cell). Although the preparation of samples and storage conditions may not have been optimal, and this may have led to partial loss through oxidation, we found, as expected, that higher maternal fish consumption was associated with increased levels of EPA and DHA, and reductions in the AA:EPA ratio and the ALA:n-3 product ratio, in maternal blood red cells. This finding suggests that the assays were providing valid estimates of fatty acid exposure. In contrast, cord blood red cell fatty acids were not correlated with maternal fish consumption in pregnancy. However, although a high intake of n-3 fatty acids during late pregnancy (through daily supplementation with fish oil) can lead to a reduction in the AA:EPA ratio in cord blood red cells,¹⁷ n-3 fatty acid intake in ALSPAC mothers is likely to have been substantially lower (only 1% of mothers ate fish more than 3 times a week) and may have been insufficient to influence cord blood red cell fatty acid concentrations. Furthermore, cord blood fatty acid concentrations will depend not only on availability of maternal supply but also on the level of fetal demand, the selectivity and efficiency of placental transfer, and fetal metabolic processes. Hence, we would suggest that fatty acid concentrations in cord blood are likely to be a better biomarker of fetal exposure in late gestation than concentrations in maternal blood, and that the lack of any significant associations with maternal fatty acids, despite the greater statistical power of the maternal blood data set, is perhaps not surprising. Further support for the validity of the fatty acid assays comes from the descriptive data (Table I), which showed that the predominant n-3 and n-6 fatty acids in maternal blood red cells in late pregnancy and in cord blood red cells were DHA, LA, and AA, in keeping with previous studies.¹⁸⁻²⁰

Noncausal interpretations of cord findings

Given the multiple analyses performed and the *P* values it is possible that the few conventionally significant cord fatty acid associations occurred by chance. Also, because cord blood red cell fatty acids were measured in only a small subset of the cohort (12.5%), we cannot rule out the possibility that effect estimates may have been biased in this group, although the selection and analysis of cord blood samples were performed blind to the outcomes of interest. Confounding seems a less likely explanation for the cord fatty acid findings because we controlled for numerous potential confounders in the analyses. However, it is possible that effects of prenatal exposure to n-6 and n-3 fatty acids, particularly on later-onset wheeze, might be confounded in part by postnatal intake of these fatty acids or other nutrients, especially because atopy is thought to result primarily from a failure of maturation of T_H1 responses after birth.²¹ For example, a positive association between the AA:EPA ratio in maternal milk and atopic disease in children at 18 months of age has been reported.²²

Is a causal link plausible?

One interpretation of the, albeit weak, positive associations between the AA:EPA ratio and eczema, and between the LA:ALA ratio and later-onset wheeze, is that a high n-6:n-3 ratio promotes the development of atopy, as proposed by Black and Sharpe.¹ However, if this were so, one might have expected the same fatty acid exposures to have been related to both outcomes. Furthermore, we would challenge the central tenet of the Black and Sharpe¹ hypothesis, that n-6:n-3 imbalance promotes atopic disease through increases in PGE₂ and IgE. Although several studies have shown that PGE₂ may inhibit T_H1 responses and enhance T_H2 responses,²³⁻²⁵ it can also inhibit IgE production by B cells,²⁶ and in animals *in vivo*, the net effect of PGE₂ appears to be one of inhibition of T_H2 responses.²⁷ These contradictory observations, and evidence that PGE₂ may protect against airway inflammation and bronchoconstriction,^{28,29} raise questions about the plausibility of the PGE₂ mechanism suggested by Black and Sharpe¹ in relation to atopy and asthma.

CONCLUSION

It seems unlikely that prenatal n-6:n-3 fatty acid exposure is an important determinant of wheezing and atopic disease in early childhood; however, further exploration of our cord findings in other studies would be of interest. In future analyses, we will examine relations of maternal and cord fatty acid exposures with atopic outcomes at school age and will also explore the role of maternal diet. Furthermore, the possible role of postnatal fatty acid intake in the inception, persistence, and severity of atopic disease deserves consideration.

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