

Polyunsaturated fatty acids in plasma at 8 years and subsequent allergic disease

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Background: Polyunsaturated fatty acids (PUFAs) are hypothesized to modulate the risk of allergic disease. However, evidence from previous studies is inconclusive, and limited longitudinal data exist using circulating biomarkers of PUFA intake and metabolism.

Objective: We aimed to investigate associations between n-3 and n-6 PUFAs at age 8 years and asthma, rhinitis, and aeroallergen sensitization at age 16 years.

Methods: Proportions of n-3 PUFAs (very long-chain n-3 [VLC n-3; sum of eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid] and α -linolenic acid) and n-6 PUFAs (linoleic acid and arachidonic acid [AA]) in blood samples at age 8 years were measured for 940 children from the prospective Swedish birth cohort BAMSE (Children, Allergy, Milieu, Stockholm, Epidemiology). Allergic disease phenotypes were defined by using questionnaires and IgE measures at the ages of 8 and 16 years. Logistic regression was used to examine potential associations.

Results: A higher proportion of total VLC n-3 PUFAs in plasma at age 8 years was associated with a reduced risk of prevalent asthma, rhinitis, and aeroallergen sensitization at age 16 years and with incidence of asthma between 8 and 16 years (adjusted odds ratio, 0.67; 95% CI, 0.47-0.94). AA was associated with a reduced risk of asthma, aeroallergen sensitization, and allergic rhinitis. The findings were most evident for allergic phenotypes of asthma and rhinitis. Additionally, AA was associated with an increased probability of asthma and rhinitis remission between 8 and 16 years of age.

Conclusion: Higher proportions of certain VLC n-3 and very long-chain n-6 PUFAs in plasma phospholipids at age 8 years

were associated with a reduced risk of allergic disease at age 16 years. (*J Allergy Clin Immunol* 2017;■■■■:■■■■-■■■■.)

Key words: Adolescent, allergy, asthma, Children, Allergy, Milieu, Stockholm, Epidemiology study, children, fatty acid, rhinitis, plasma, prospective studies

Allergic disease, including the entities asthma and rhinitis, is one of the most common types of noncommunicable diseases in childhood, and the occurrence is also high in adolescence.^{1,2} Polyunsaturated fatty acids (PUFAs), which are found in, for example, nuts, oily fish, and vegetable oils, are hypothesized to influence the inflammatory response and could thereby modulate the risk of allergic disease.³

When the hypothesis about PUFAs and allergic disease was first launched in the 1990s, 2 main groups of PUFAs (n-3 and n-6) were attributed opposite roles in inflammation: very long-chain n-3 (VLC n-3) to decrease inflammation and n-6 to promote inflammation through the actions of arachidonic acid (AA; 20:4n-6).³ However, the actions of n-3 and n-6 PUFAs on inflammation have been shown to be more complex, with many different mechanisms involved.⁴⁻⁶

Recently, we reported that dietary intake of oily fish and VLC n-3 in childhood was associated with a reduced risk of rhinitis up to adolescence.⁷ For n-6 fatty acids, such as AA, we did not observe an association. Our study was the first prospective study to show that dietary fish at school age might affect the risk of subsequent allergic disease; this finding was in line with most cross-sectional studies on total or oily fish consumption and allergic disease in childhood.⁸⁻¹⁷ The results for n-3 and n-6 consumption have been inconsistent in previous studies, most of which have been cross-sectional in design.¹⁸⁻²²

VLC n-3 fatty acids and AA are present in the diet but can also be synthesized from the essential fatty acids α -linolenic acid (ALA; 18:3n-3, which is found in, for example, canola oil) and linoleic acid (LA; 18:2n-6, which is found in, for example, sunflower and corn oil), respectively. However, the conversion rates of VLC n-3 and AA might be low in human subjects.^{23,24} Among PUFAs measured in plasma lipids or adipose tissue, LA, eicosapentaenoic acid (EPA; 20:5n-3), and docosahexaenoic acid (DHA; 22:6n-3) are generally good biomarkers of dietary intake, whereas AA and, to some extent, ALA show weaker correlations to self-reported dietary intake.²⁵

Blood composition of PUFAs reflects both endogenous synthesis and dietary intake and thereby might provide a more accurate assessment of PUFA levels. Most previous studies on blood proportions of PUFAs and allergic disease have been cross-sectional and have shown conflicting results.^{10,12,19,26-29} However, a recent longitudinal study found an inverse association between blood composition of EPA at preschool age and allergic disease in children followed up to age 5 years.³⁰

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Abbreviations used

AA: Arachidonic acid
 ALA: α -Linolenic acid
 DHA: Docosahexaenoic acid
 DPA: Docosapentaenoic acid
 EPA: Eicosapentaenoic acid
 FFQ: Food frequency questionnaire
 LA: Linoleic acid
 OR: Odds ratio
 PUFA: Polyunsaturated fatty acid
 VLC n-3: Very long-chain n-3

There is a need for longitudinal studies to clarify the role of different PUFAs for the risk of allergic disease after preschool ages. Therefore we aimed to investigate whether n-3 and n-6 PUFAs in plasma phospholipids at age 8 years influenced the risk of asthma, rhinitis, and aeroallergen sensitization up to age 16 years in a prospective population-based cohort.

METHODS**Study design and study population**

This study is based on a Swedish population-based birth cohort, the Children, Allergy, Milieu, Stockholm, Epidemiology (BAMSE) study. Infants were enrolled between 1994 and 1996 ($n = 4089$).³¹ Study participants were followed repeatedly by using questionnaires up to 16 years of age,³² when 76% of the children themselves answered a questionnaire. At ages 4, 8, and 16 years, the participants who answered the respective questionnaires were invited to a clinical examination and provided blood samples that were stored at -80°C . At all 3 examinations, IgE antibodies were analyzed with ImmunoCAP to common aeroallergens by using the Phadiatop mix (cat, dog, and horse dander; timothy, birch, and mugwort pollen; *Cladosporium herbarum*; and *Dermatophagoides pteronyssinus*; Thermo Fisher Scientific, Uppsala, Sweden). A blood sample with positive Phadiatop results was analyzed further for allergen-specific IgE antibodies to the allergens listed above and additionally to *Dermatophagoides farinae* at age 16 years. A technical cutoff at 0.35 kU/L was set, according to the manufacturer's instructions. At the 8-year examination, a food frequency questionnaire (FFQ) was filled out by 2614 children.⁷ The FFQ was nutrient calculated and energy adjusted by using the residual method.³³

In the present study we included children with baseline information on relevant confounders ($n = 3998$), information on fish and nutrient intake from the FFQ at age 8 years ($n = 2540$), information from the questionnaire and clinical examination at age 8 years ($n = 1704$), and data from the questionnaire and clinical examination at age 16 years ($n = 999$, Fig 1). These 999 children were eligible for analyses of fatty acid proportions in plasma phospholipids. Because 59 samples contained too little plasma to be analyzed, the present study consists of 940 children with measured fatty acid composition. The study was approved by the regional Ethics Committee at Karolinska Institutet in Stockholm, Sweden.

Measurement of fatty acids in plasma phospholipids

Blood samples from the 8-year clinical examination were used to quantify fatty acid proportions in plasma phospholipids by using gas chromatography, as previously described.^{34,35} Relative amounts expressed as a percentage of total fatty acids in the sample of 15 different fatty acids were obtained. For the purpose of the current study, we focused on the n-3 fatty acids ALA, EPA, docosapentaenoic acid (DPA; 22:5n-3), and DHA and the major n-6 fatty acids LA and AA. Because of high intercorrelation and shared dietary sources, values for EPA, DPA, and DHA were added together for the total VLC n-3 level.

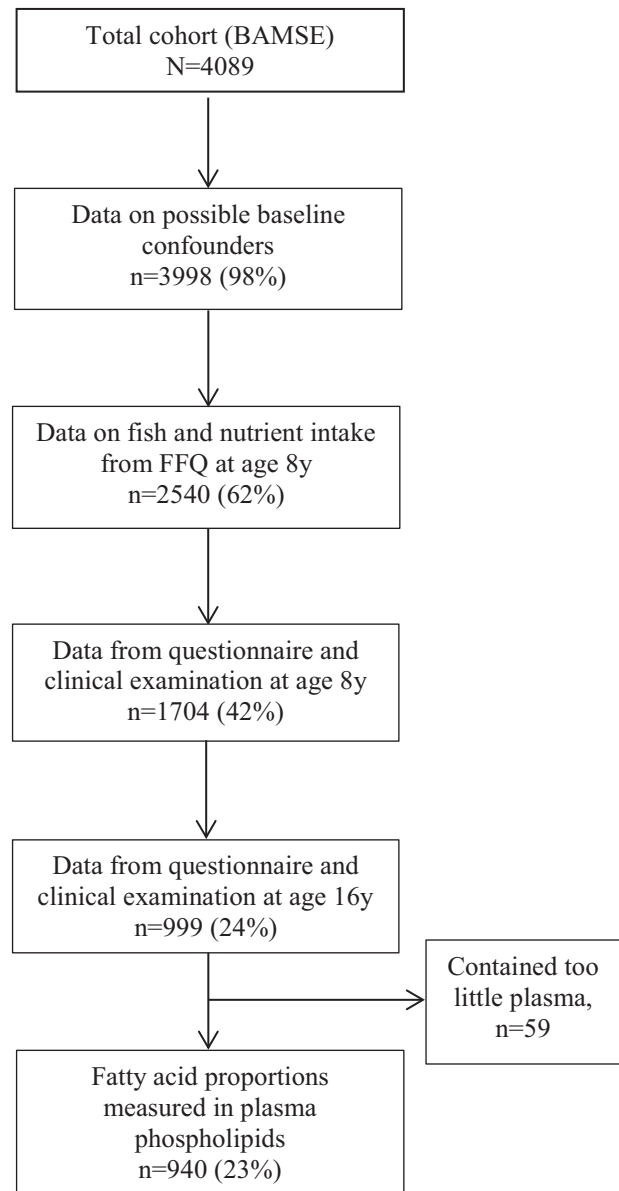


FIG 1. Flow chart of subjects from the birth cohort BAMSE (Children, Allergy, Milieu, Stockholm, Epidemiology) outlining the inclusion for analyses of fatty acid proportions in plasma phospholipids.

Definition of allergic disease outcomes

The definitions of asthma and rhinitis were based on parental questionnaires at ages 4 and 8 years, whereas the children's own reports were used at age 16 years.

Asthma. Asthma was defined as more than 3 episodes of wheeze in the last 12 months or at least 1 episode in combination with prescribed inhaled steroids used occasionally or regularly.³⁶

Rhinitis. Rhinitis was defined as symptoms of hay fever (prolonged sneezing or a runny or blocked nose without common cold in the last 12 months or after exposure to furred pets, pollens, and/or mites).³⁶

Aeroallergen sensitization. Aeroallergen sensitization was defined as a positive Phadiatop result ($\text{IgE} \geq 0.35 \text{ kU/L}$) and at least 1 positive test result to a specific aeroallergen ($\text{IgE} \geq 0.35 \text{ kU/L}$).

Allergic asthma and rhinitis. Allergic asthma and rhinitis were defined as fulfilling the definition of the specific disease and the definition of aeroallergen sensitization.

Nonallergic asthma and rhinitis. Nonallergic asthma and rhinitis were defined as fulfilling the definition of the specific disease but not the definition of aeroallergen sensitization.

Incidence of disease between ages 8 and 16 years.

Incidence of disease between ages 8 and 16 years was defined as fulfilling the definition of the specific disease at age 16 years but not at age 4 years, 8 years, or both.

Remission of disease between ages 8 and 16 years.

Remission of disease between ages 8 and 16 years was defined as fulfilling the definition of the specific disease at age 8 years but not at age 16 years.

Statistical analyses

A 1-sample *t* test was used to compare the distribution of background characteristics between the study population and the whole cohort. Consumption of PUFAs and corresponding food items in the study population was compared with consumption of PUFAs and food items among all children with reported intake to investigate possible selection bias. The plasma proportions of fatty acids are presented as median values with interquartile ranges. Spearman rank correlation with Bonferroni multiple test correction was used to check the correlation between the fatty acids. Log transformation of ALA improved normality, and the transformed variable was used for association analyses. Associations between continuous fatty acids and asthma, rhinitis, and aeroallergen sensitization were analyzed with logistic regression by using children without the specific disease as the reference group. In analyses of remission of asthma and rhinitis, children with persistent specific disease were the reference group. Potential nonlinearity was evaluated by using restricted cubic splines with 4 knots and applying a Wald test. To study associations between fatty acids and allergic and nonallergic phenotypes, multinomial logistic regression was used, and children without the allergic disease and without aeroallergen sensitization comprised the reference group. All results are presented as odds ratios (ORs) with 95% CIs. Potential confounding factors tested are described in the [Methods](#) section in this article's Online Repository at www.jacionline.org. No factor changed the crude OR more than 5%, and therefore we chose to adjust only for sex and allergic heredity. Differences in fatty acid proportions between categories of potential confounding factors and disease status at age 8 years were checked with the Wilcoxon rank-sum test. Sensitivity analyses included control for possible disease-related modification of exposure by adjusting for early symptoms of allergic disease (reported symptoms of wheeze, eczema, or both during the first 2 years of life) and for symptoms of cow's milk allergy at ages 1, 2, 4, and 8 years (reported symptoms to milk, such as vomiting/diarrhea, eczema, swelling of lips/eyes, itchy eyes/stuffy nose, or asthma). Spearman rank correlations between fatty acid proportions in plasma and fatty acid intake levels derived from the FFQ at the same age were calculated. *P* values of less than .05 were considered statistically significant in all analyses. All analyses were executed with Stata software (version 11.2; StataCorp, College Station, Tex).

RESULTS

The study population (*n* = 940) was comparable with the original cohort apart from a somewhat higher prevalence of white collar workers (86.8% vs 81.6%) and allergic heredity (33.7% vs 29.7%) and a lower proportion of boys (45.7% vs 50.5%, see [Table E1](#) in this article's Online Repository at www.jacionline.org). Estimated consumption of the different PUFAs was similar between the included children and all children with information on PUFA intake in the cohort (*n* = 2614), as was reported consumption of the major contributing food items (see [Table E2](#) in this article's Online Repository at www.jacionline.org).

The median proportions of fatty acids in plasma as a percentage of total fat are presented in [Table I](#).³⁷ The proportions of these fatty acids were comparable in relation to background characteristics of the study population, with some exceptions. For example,

children with high oily fish intake had the highest proportion of total VLC n-3 in plasma (median, 3.7% vs 3.3%). In the study population total VLC n-3 and AA proportions were highly correlated (spearman *r* = 0.80, see [Table E3](#) in this article's Online Repository at www.jacionline.org). The correlation between plasma proportions and reported intake of fatty acids varied from 0.02 for LA to 0.29 for DHA (see [Table E4](#) in this article's Online Repository at www.jacionline.org). The correlation for total VLC n-3 proportion was 0.23 (*P* < .001), and the correlation with reported oily fish intake was 0.24 (*P* < .001). Correlations did not differ between children with or without allergic disease or aeroallergen sensitization at age 8 years (see [Table E4](#)).

Asthma, rhinitis, and aeroallergen sensitization were present in 88 (9.4%), 192 (20.5%), and 258 (27.5%) children at age 8 years, respectively. The prevalence of asthma at 16 years of age (*n* = 86 [9.2%]) was similar to the prevalence at age 8 years, whereas both rhinitis and aeroallergen sensitization were more common at age 16 years (*n* = 413 [44.3%] and *n* = 419 [44.6%], respectively). Between ages 8 and 16 years, there were 43, 241, and 158 incident cases of asthma, rhinitis, and aeroallergen sensitization, respectively.

Associations with prevalent allergic disease at age 8 years

The total VLC n-3 proportion in plasma phospholipids at age 8 years was inversely associated with asthma (adjusted OR, 0.64; 95% CI, 0.50-0.82), rhinitis (adjusted OR, 0.73; 95% CI, 0.61-0.87), and aeroallergen sensitization (adjusted OR, 0.77; 95% CI, 0.66-0.90) at the same age, whereas no apparent associations were observed for ALA and LA proportions (see [Table E5](#) in this article's Online Repository at www.jacionline.org). Inverse significant associations were observed between AA proportions and asthma (adjusted OR, 0.80; 95% CI, 0.67-0.96), rhinitis (adjusted OR, 0.78; 95% CI, 0.68-0.88), and aeroallergen sensitization (adjusted OR, 0.76; 95% CI, 0.68-0.86).

Associations with prevalent and incident allergic disease at age 16 years

Associations between proportions of the different PUFAs and prevalent and incident allergic disease at age 16 years are displayed in [Table II](#). The total VLC n-3 proportion at age 8 years was inversely associated with prevalent asthma (adjusted OR, 0.67; 95% CI, 0.52-0.86), rhinitis (adjusted OR, 0.87; 95% CI, 0.77-0.99), and aeroallergen sensitization (adjusted OR, 0.83; 95% CI, 0.73-0.95) at age 16 years. To study incident disease, we excluded children with the respective disease at ages 4 or 8 years (eg, asthma in analyses of asthma) and observed a similar OR for incident asthma (adjusted OR, 0.67; 95% CI, 0.47-0.94). However, associations for rhinitis and aeroallergen sensitization were attenuated and became nonsignificant. For ALA, we observed inverse associations with both prevalent and incident aeroallergen sensitization at age 16 years (adjusted OR, 0.61 [95% CI, 0.38-0.97] and 0.41 [95% CI, 0.22-0.79], respectively) but not with asthma or rhinitis. We did not observe any consistent associations between LA and asthma, rhinitis, or sensitization to aeroallergens. In contrast, AA was inversely associated with prevalent asthma (adjusted OR, 0.74; 95% CI, 0.62-0.88) and

TABLE I. Distribution of PUFAs as a percentage of total fat among the study population (n = 940)

	n-3 FA				n-6 FA			
	ΣVLC n-3*		ALA		LA		AA	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR
Study population	3.3	1.5	0.2	0.09	21.2	2.6	5.6	1.9
Sex								
Female	3.3	1.4	0.2	0.09	21.3	2.5	5.5	1.9
Male	3.3	1.5	0.2	0.1	21.1	2.7	5.8	2.0
Parental origin†								
Scandinavia	3.3	1.4	0.2	0.09	21.2	2.6	5.7	1.9
Outside Scandinavia	3.4	1.6	0.2	0.1	20.9	2.4	5.5	2.3
Socioeconomic status								
Blue collar worker	3.1	1.4	0.2	0.1	21.2	2.8	5.4	1.9
White collar worker	3.3	1.5	0.2	0.09	21.2	2.5	5.7	1.9
Allergic heredity‡								
No	3.3	1.5	0.2	0.09	21.2	2.7	5.7	2.0
Yes	3.2	1.3	0.2	0.08	21.2	2.4	5.5	1.9
Maternal smoking§								
No	3.3	1.4	0.2	0.09	21.2	2.6	5.7	1.9
Yes	3.1	1.4	0.2	0.1	21.3	2.6	5.5	1.8
Maternal age								
>25 y	3.3	1.5	0.2	0.09	21.2	2.53	5.6	1.9
≤25 y	3.2	1.5	0.2	0.1	21.5	2.9	5.5	2.1
Breast-feeding¶								
<4 mo	3.3	1.3	0.2	0.09	21.2	2.4	5.5	1.9
≥4 mo	3.3	1.5	0.2	0.09	21.2	2.7	5.7	1.9
Infant fish intake								
<2 times/mo	3.3	1.4	0.2	0.09	21.2	2.4	5.7	1.8
≥2 times/mo	3.3	1.5	0.2	0.09	21.2	2.7	5.6	2.0
At age 8 y								
Overweight#								
No	3.3	1.4	0.2	0.09	21.3	2.6	5.6	1.9
Yes	3.5	1.3	0.2	0.07	20.9	2.4	5.8	1.9
Oily fish intake								
<1 time/wk	3.3	1.4	0.2	0.09	21.3	2.6	5.6	2.0
≥1 time/wk	3.7	1.7	0.2	0.1	20.8	2.4	5.7	1.7

Boldface text shows *P* values of less than .05 from the Wilcoxon rank sum test.

ALA, α-linolenic acid, 18:3; FA, fatty acid; IQR, interquartile range; LA, linoleic acid, 18:2.

*ΣVLC n-3 is the sum of the very long-chain omega 3 fatty acids EPA (20:5), DPA (22:5), and DHA (22:6).

†Father and/or mother born outside of Scandinavia.

‡Doctor-diagnosed asthma, hay fever, or both in combination with reported allergy to pollen or pets in 1 or both parents.

§Mother smoked at least 1 cigarette per day at any point of time during pregnancy or child's first months.

||Mother's age at the child's birth.

¶Exclusive breast-feeding.

#isoBMI ≥ 25 kg/m² defined based on International Obesity Task Force cut-offs in Cole et al.³⁷

TABLE II. Adjusted ORs of prevalent and incident allergic disease at age 16 years in relation to continuous plasma proportions of PUFAs (percentage of total fatty acids) at age 8 years

	Asthma (n/N)		Rhinitis (n/N)		Aeroallergen sensitization (n/N)	
	Prevalent (86/940)	Incident† (43/819)	Prevalent (413/933)	Incident† (241/692)	Prevalent (419/940)	Incident† (158/665)
	OR‡ (95% CI)	OR‡ (95% CI)	OR‡ (95% CI)	OR‡ (95% CI)	OR‡ (95% CI)	OR‡ (95% CI)
n-3 FA						
ΣVLC n-3*	0.67 (0.52-0.86)	0.67 (0.47-0.94)	0.87 (0.77-0.99)	0.98 (0.84-1.15)	0.83 (0.73-0.95)	0.88 (0.72-1.06)
ALA§	1.22 (0.55-2.69)	1.28 (0.42-3.86)	1.26 (0.80-2.00)	1.28 (0.73-2.23)	0.61 (0.38-0.97)	0.41 (0.22-0.79)
n-6 FA						
LA	0.95 (0.85-1.07)	1.06 (0.90-1.24)	1.01 (0.94-1.08)	1.02 (0.95-1.11)	0.96 (0.90-1.03)	0.98 (0.90-1.08)
AA	0.74 (0.62-0.88)	0.79 (0.62-1.01)	0.93 (0.84-1.02)	1.05 (0.93-1.18)	0.85 (0.76-0.94)	0.92 (0.80-1.06)

Boldface text indicates *P* values of less than .05.

ALA, α-linolenic acid, 18:3; FA, fatty acid; LA, linoleic acid, 18:2.

*ΣVLC n-3 is the sum of the very long-chain omega 3 fatty acids EPA (20:5), DPA (22:5), and DHA (22:6).

†Children with the specific disease at 4 years, 8 years, or both were excluded.

‡Adjusted for allergic heredity and sex.

§One subject was without data for ALA, and ALA values were log-transformed.

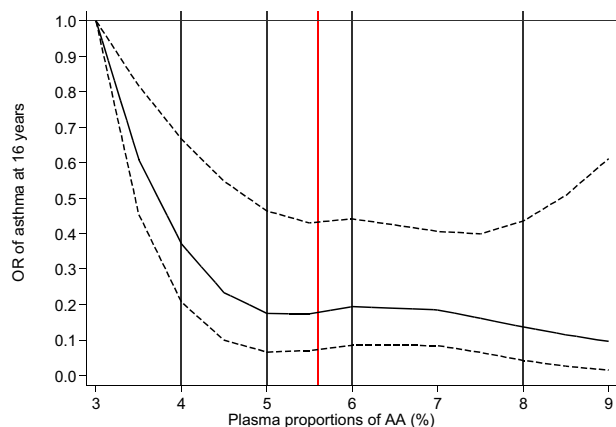


FIG 2. Adjusted ORs for prevalent asthma at age 16 years in relation to continuous proportions of AA in plasma at age 8 years. ORs and 95% CIs were calculated with a restricted cubic spline model with 4 knots (represented by black vertical lines) and adjusted for allergic heredity and sex. The red line represents the median proportion of AA.

aeroallergen sensitization (adjusted OR, 0.85; 95% CI, 0.76-0.94) at age 16 years. A tendency toward inverse associations was observed for prevalent rhinitis at age 16 years (adjusted OR, 0.93; 95% CI, 0.84-1.02) and for incident asthma between ages 8 and 16 years (adjusted OR, 0.79; 95% CI, 0.62-1.01).

We evaluated potential nonlinearity in associations between the studied PUFAs and allergic diseases. Evidence of a nonlinear association was shown between AA proportions and prevalent asthma at age 16 years ($P_{\text{nonlinearity}} = .0495$, Wald test). Fig 2 shows the adjusted association between AA proportions and prevalent asthma at age 16 years obtained by using a restricted cubic spline model. The OR decreased with increasing plasma proportions of AA but seemed to stabilize around the median proportion of AA.

Associations with phenotypes of asthma and rhinitis at age 16 years

Analyses of allergic disease phenotypes were limited to total VLC n-3 and AA proportions. Associations for prevalent phenotypes of asthma and rhinitis are shown in Fig 3. The total VLC n-3 proportion was associated with a reduced risk of allergic asthma (adjusted OR, 0.52; 95% CI, 0.38-0.71) but not with nonallergic asthma (adjusted OR, 1.19; 95% CI, 0.75-1.88; Fig 3, A). The ORs for phenotypes of rhinitis were similar, but only the OR for allergic rhinitis reached statistical significance (adjusted OR, 0.81; 95% CI, 0.69-0.94). Similarly, AA was associated with a reduced risk of prevalent allergic asthma (adjusted OR, 0.61; 95% CI, 0.49-0.76) and allergic rhinitis (adjusted OR, 0.85; 95% CI, 0.75-0.96) but not with nonallergic asthma or rhinitis (Fig 3, B).

Sensitivity analyses

Analyses for prevalent outcomes at age 16 years were adjusted additionally for early symptoms of allergic disease (wheeze, eczema, or both up to 2 years of age) to assess the possible effect of disease-related modification of exposure. The additional adjustment had a marginal effect on the association between total VLC n-3 proportions and prevalent asthma (adjusted OR, 0.71;

95% CI, 0.55-0.91), rhinitis (adjusted OR, 0.88; 95% CI, 0.77-1.01), and aeroallergen sensitization (adjusted OR, 0.84; 95% CI, 0.74-0.97) but produced slightly wider CIs. The same was observed for the associations between AA proportions and asthma (adjusted OR, 0.77; 95% CI, 0.64-0.92) and aeroallergen sensitization (adjusted OR, 0.85; 95% CI, 0.77-0.95).

Cow's milk allergy can affect the child's diet and therefore possibly the proportions of fatty acids in plasma. Additional adjustment for symptoms of cow's milk allergy up to age 8 years did not substantially change the observed ORs between total VLC n-3 proportions and prevalent allergic disease at age 16 years (adjusted OR, 0.68 [95% CI, 0.52-0.90] for asthma, 0.84 [95% CI, 0.73-0.97] for rhinitis, and 0.80 [95% CI, 0.69-0.93] for aeroallergen sensitization). Similarly, associations for AA proportions were also unaffected by additional adjustment for symptoms of cow's milk allergy (adjusted OR, 0.75 [95% CI, 0.62-0.92] for asthma and 0.82 [95% CI, 0.73-0.91] for aeroallergen sensitization).

Remission of asthma and rhinitis between ages 8 and 16 years

Among the 88 children classified as having asthma at age 8 years, 51 did not fulfill the definition at age 16 years. Rhinitis was present among 192 children at age 8 years, and of these children, 40 did not fulfill the definition at age 16 years. The association between increasing proportions of total VLC n-3 and remission of asthma between ages 8 and 16 years was nonsignificant (adjusted OR, 1.18; 95% CI, 0.71-1.97), just as with the association with remission of rhinitis (adjusted OR, 1.35; 95% CI, 0.96-1.91). AA proportions were significantly associated with an increased probability of remission of asthma (adjusted OR, 1.49; 95% CI, 1.05-2.13) and rhinitis (adjusted OR, 1.33; 95% CI, 1.02-1.72).

DISCUSSION

In our longitudinal study of plasma composition of PUFAs in relation to allergic disease, higher proportions of n-3 and n-6 fatty acids in plasma phospholipids at age 8 years were inversely associated with asthma, rhinitis, and aeroallergen sensitization at age 16 years. More specifically, the total VLC n-3 (EPA, DPA, and DHA) proportion was associated with a reduced risk of prevalent asthma, rhinitis, and aeroallergen sensitization, as well as with incident asthma between ages 8 and 16 years. For ALA, we observed a reduced risk of prevalent and incident aeroallergen sensitization. In addition, proportions of the n-6 fatty acid AA were associated with a reduced risk of prevalent asthma and aeroallergen sensitization and with an increased probability of remission of asthma and rhinitis between ages 8 and 16 years. The associations between total VLC n-3 proportions and AA proportions with asthma and rhinitis were most pronounced for the allergic phenotypes.

In line with our findings in this study, higher serum proportions of EPA, DHA, and total VLC n-3 during preschool age were associated with a reduced risk of subsequent asthma up to age 5 years in a recent longitudinal Finish study.³⁰ However, after adjustment for cow's milk allergy, the association for EPA was the only association that was still significant. Symptoms of cow's milk allergy did not affect the results in our study. In addition, our findings were most pronounced for the allergic

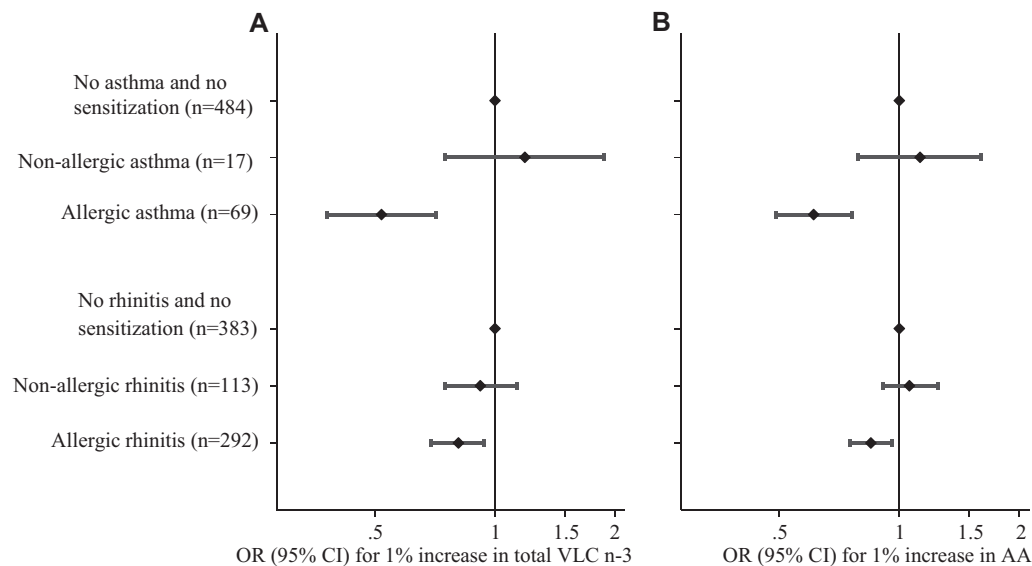


FIG 3. Adjusted ORs for phenotypes of prevalent asthma and rhinitis at age 16 years in relation to continuous proportions of total VLC n-3 (**A**) and AA (**B**) in plasma at age 8 years. ORs and 95% CIs were estimated with multinomial logistic regression models adjusted for allergic heredity and sex.

phenotypes (ie, IgE-associated allergic disease), whereas a significant association between EPA proportions and nonatopic asthma was found in the Finish study. These discrepancies might be explained by the difference in age of the children in the 2 studies as the prevalence of sensitization increases with increasing age.³⁸

The inverse association between plasma proportions of total VLC n-3 and subsequent allergic disease observed in our study is biologically plausible and might be mediated by the anti-inflammatory properties of VLC n-3.^{5,39} These findings are also in line with our previous study showing that children with higher intake of VLC n-3 at age 8 years have reduced risk of rhinitis between ages 8 and 16 years.⁷ In contrast, AA is thought of as a mediator of inflammation through its role as a precursor of proinflammatory eicosanoids, such as prostaglandin D₂, which is involved in determining the immunologic allergic predisposition to asthma. Nevertheless, the actions of AA has been shown to be more complex; for example, AA-derived eicosanoid prostaglandin E₂ might be very important in turning off inflammation.⁴⁰ In the present study we observed an inverse association between AA proportions in plasma phospholipids and allergic disease. In contrast, no association between AA proportions and subsequent rhinitis was observed in our previous study of dietary intake, which might be explained by low consumption of AA.⁷

A major strength of the present study is the longitudinal design. We had the opportunity to study the influence of proportions of different PUFAs in plasma phospholipids in childhood on the risk of allergic disease up to adolescence. Using plasma PUFAs as objective biomarkers, we avoided bias from use of self-reported PUFA intake. We were also able to distinguish between allergic and nonallergic phenotypes. In addition, we adjusted the analyses for a wide range of potential confounders (including diet and overweight), as well as for the influence of disease-related modification of exposure by early symptoms of wheeze or eczema and by cow's milk allergy through childhood.

The study population consisted of 940 children who had data from several different sources, including plasma proportions and dietary intake of PUFAs, repeated IgE measurements, and repeated questionnaires. Although the study population comprises only 23% of the original cohort, selection bias should be a minor problem because there are only small differences in the distribution of characteristics among the study population compared with the total cohort. In addition, the intake of different PUFAs and corresponding food items was similar between the included children and all children with information on food intake in the cohort. We only have PUFA measurements in blood from school age, and therefore we could not study the persistence of PUFA proportions from early life until school age. Definitions of the outcomes were based on parental reporting and self-reporting, which could lead to some misclassification because subjects might perceive and recall symptoms differently. Such a bias is probably nondifferential with regard to the child's proportions of fatty acids in plasma and could lead to a dilution of the observed associations. In analyses of incident asthma, children fulfilling the definition of asthma at 4 or 8 years were excluded. In sensitivity analyses we additionally excluded children with wheeze or eczema up to age 2 years. Despite this, we cannot exclude that the cases of incident asthma might represent resurgence or worsening of preexisting conditions rather than new onset of disease.

Moreover, the blood samples were stored for just over 10 years before the assessment of fatty acid composition in plasma phospholipids. However, the samples were stored at -80°C , and fatty acid composition should be stable.²⁵ Possible degradation should affect all the samples the same and therefore only dilute the associations between fatty acid proportions and allergic disease. Nevertheless, it might explain the moderate correlations we observed between plasma proportions and fatty acid levels obtained from the FFQ.

Plasma proportions of PUFAs reflect both dietary consumption and endogenous metabolism, although the synthetization in

human subjects of the PUFAs included in this study seems to be low.^{23,24} Overall, LA, EPA, and DHA have been shown to be good dietary biomarkers, whereas AA and ALA are weaker biomarkers of dietary intake.²⁵ Our measured correlation for VLC n-3 is lower than what has been presented for adults⁴¹ but in line with other studies on children.⁴² In our study population very few were supplemented with VLC n-3 fatty acids, and therefore our results on total VLC n-3 proportions probably reflect dietary intake to a large extent. Despite this, recommendations of dietary intake cannot be made solely based on studies of plasma proportions of PUFAs.

In conclusion, in our study of 940 children from a population-based cohort, higher proportions of n-3 and n-6 fatty acids in plasma from age 8 years were associated with a reduced risk of asthma, rhinitis, and aeroallergen sensitization at age 16 years. The significant associations were most pronounced for allergic phenotypes of asthma and rhinitis. Our results add to the evidence that PUFAs can influence subsequent allergic disease in childhood.

Clinical implications: Higher plasma proportions of n-3 and n-6 fatty acids at school age were associated with a reduced risk of subsequent allergic disease in adolescence.

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METHODS

Description of the potential confounding factors tested

Allergic heredity: Doctor-diagnosed asthma and/or hay fever in combination with reported allergy to pollen or pets in 1 or both parents (no vs yes).

Maternal smoking: Mother smoked at least 1 cigarette per day at any point of time during pregnancy or the child's first months (no vs yes).

Maternal age: Mother's age at child's birth (>25 vs ≤ 25 years).

Exclusive breast-feeding: Child was exclusively breast-fed (<4 vs ≥ 4 months).

Parental origin: Father and/or mother born outside of Scandinavia (no vs yes).

Socioeconomic status: Socioeconomic status for the household according to dominance order (blue collar worker vs white collar worker).

Fish consumption in infancy: Frequency of fish intake asked for in the 1-year questionnaire (<2 times/month vs ≥ 2 times/month).

Parental smoking: Any parent smoked daily at the time of the 8-year follow-up (no vs yes).

Overweight status: Body mass index adjusted for sex and age (isoBMI < 25 kg/m² vs isoBMI ≥ 25 kg/m²).

Supplement use: Vitamin supplement intake during the last 12 months at the time of the 8-year follow-up (no vs sometimes/regularly).

Total energy intake: Calculated from an FFQ answered at the 8-year follow-up (continuous in kilocalories).

Total fat intake: Calculated from an FFQ answered at the 8-year follow-up and energy-adjusted by using the residual method (continuous).

TABLE E1. Distribution of selected characteristics among children in the cohort (n = 4089) and the study population (n = 940)

	Total cohort*		Study population†		
	No.	Percent	No.	Percent	95% CI‡
Sex: male	2065	50.5	430	45.7	43.0-48.6
Parental origin§: outside of Scandinavia	543	16.0	152	16.2	14.3-18.3
Socioeconomic status: white collar worker	3323	81.6	813	86.8	84.7-88.6
Allergic heredity : yes	1200	29.7	317	33.7	31.1-36.4
Maternal smoking¶: yes	563	13.8	110	11.7	10.0-13.6
Maternal age#: ≤25 y	319	7.8	66	7.0	5.7-8.6
Breast-feeding**: ≥4 mo	3116	79.5	745	80.7	78.4-82.8
Infant fish intake: ≥2 times/mo	3143	80.1	744	80.6	78.3-82.7
Infant eczema: yes	987	25.8	253	27.7	25.2-30.3
Infant wheeze: yes	997	26.1	258	28.3	25.8-30.9

*Original cohort of 4089 children.

†Study population refers to the 940 children who had fatty acid levels measured in plasma.

‡CIs were calculated by applying a finite population correction factor.

§Father, mother, or both born outside of Scandinavia.

||Doctor-diagnosed asthma, hay fever, or both in combination with reported allergy to pollen or pets in 1 or both parents.

¶Mother smoked at least 1 cigarette per day at any time point during pregnancy or the child's first months.

#Mother's age at child's birth.

**Exclusive breast-feeding.

TABLE E2. Comparison of intake of PUFAs and food items reported in the FFQ between all children with data and the study population

Intake (g/d)	All children with FFQ data (n = 2614)		Study population (n = 940)	
	Median	IQR	Median	IQR
VLC n-3*	0.2	0.2	0.2	0.2
ALA	1.1	0.5	1.1	0.5
LA	6.1	2.5	6.1	2.5
AA	0.07	0.04	0.07	0.03
	Percentage		Percentage	
Oily fish (times/wk)				
T1 (0-0.2)	41.1		40.9	
T2 (0.5-0.6)	38.2		38.1	
T3 (0.9-7.5)	20.7		21.0	
Fish finger (times/wk)				
T1 (0-0.5)	61.1		62.9	
T2 (1)	33.1		31.6	
T3 (2-7)	5.8		5.6	
Sausage (times/wk)				
T1 (0-1)	57.2		55.7	
T2 (2)	32.8		33.5	
T3 (3.5-14)	10.0		10.8	
Egg/omelet (times/wk)				
T1 (0)	60.4		61.6	
T2 (0.12)	23.3		22.0	
T3 (0.47-3.5)	16.3		16.4	
Dairy products† (glass/wk)				
T1 (7-21)	53.4		54.1	
T2 (28)	19.5		18.1	
T3 (35-140)	27.1		27.8	
Sandwich with margarine (slices/d)				
T1 (1-2)	52.6		51.0	
T2 (3)	25.3		28.0	
T3 (4-10)	22.1		21.0	

ALA, α -linolenic acid, 18:3; IQR, interquartile range; LA, linoleic acid, 18:2.

* Σ VLC n-3 is the sum of the very long-chain omega 3 fatty acids EPA (20:5), DPA (22:5), and DHA (22:6).

†Dairy products include milk, yogurt, and sour milk.

TABLE E3. Correlations between fatty acids in plasma phospholipids for the study population (n = 940)

	ΣVLC n-3*		ALA		LA	
	<i>r</i> †	<i>P</i> value‡	<i>r</i> †	<i>P</i> value‡	<i>r</i> †	<i>P</i> value‡
ALA	0.17	>.001				
LA	−0.05	1.00	0.37	>.001		
AA	0.80	>.001	0.10	.061	0.02	1.00

ALA, α-linolenic acid, 18:3; LA, linoleic acid, 18:2.

*ΣVLC n-3 is the sum of the very long-chain omega 3 fatty acids EPA (20:5), DPA (22:5), and DHA (22:6).

†Spearman correlation coefficient.

‡Significance of correlation, Bonferroni corrected.

TABLE E4. Spearman rank correlations between plasma proportions of fatty acids (as a percentage of total fatty acids analyzed) and fatty acid intake (as a percentage of total fat intake) obtained from an FFQ stratified by allergic disease status

Fatty acid [‡]	Study population (n = 940)		Allergic disease at age 8 y* (n = 343)		No allergic disease at age 8 y† (n = 596)	
	r§	P value	r§	95% CI	r§	95% CI
ΣVLC n-3	0.23	<.001	0.26	0.16 to 0.36	0.21	0.14 to 0.29
EPA	0.19	<.001	0.23	0.13 to 0.33	0.17	0.09 to 0.24
DPA	0.04	.28	0.06	−0.04 to 0.17	0.01	−0.07 to 0.09
DHA	0.29	<.001	0.32	0.23 to 0.42	0.27	0.20 to 0.34
ALA	0.06	.08	0.06	−0.05 to 0.16	0.06	−0.02 to 0.14
LA	0.02	.46	−0.02	−0.12 to 0.09	0.05	−0.03 to 0.13
AA	0.08	.01	0.10	−0.001 to 0.21	0.08	0.004 to 0.16

ALA, α-linolenic acid, 18:3; LA, linoleic acid, 18:2.

*Asthma, rhinitis, or aeroallergen sensitization.

†No asthma, rhinitis, or aeroallergen sensitization.

‡ΣVLC n-3 is the sum of the very long-chain omega 3 fatty acids EPA (20:5), DPA (22:5), and DHA (22:6).

§Spearman rank correlations.

TABLE E5. Adjusted ORs of prevalent allergic disease at age 8 years in relation to continuous plasma proportions of PUFAs (percentage of total fatty acids) at age 8 years

	Asthma (n = 88/N = 940)	Rhinitis (n = 192/N = 938)	Aeroallergen sensitization (n = 258/N = 940)
	OR† (95% CI)	OR† (95% CI)	OR† (95% CI)
n-3 FA			
ΣVLC n-3*	0.64 (0.50-0.82)	0.73 (0.61-0.87)	0.77 (0.66-0.90)
ALA‡	0.82 (0.38-1.77)	1.02 (0.58-1.80)	0.93 (0.55-1.54)
n-6 FA			
LA	1.00 (0.89-1.12)	1.00 (0.92-1.09)	0.94 (0.87-1.01)
AA	0.80 (0.67-0.96)	0.78 (0.68-0.88)	0.76 (0.68-0.86)

Boldface text indicates *P* values of less than .05.

ALA, α-linolenic acid, 18:3; FA, fatty acid; LA, Linoleic acid, 18:2.

*ΣVLC n-3 is the sum of the very long-chain omega 3 fatty acids EPA (20:5), DPA (22:5), and DHA (22:6).

†Adjusted for allergic heredity and sex.

‡One subject was without data for ALA, and ALA data were log-transformed.