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Cord blood LC-PUFA composition and allergic diseases during the first 10 yr. Results from the LISAplus study

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Abstract

Background: It has been suggested that n-6 and n-3 long-chain polyunsaturated fatty acids (LC-PUFAs) in blood are associated with risk of allergic diseases, although results are inconclusive. Low levels of n-6 LC-PUFA and high levels of n-3 LC-PUFA are anticipated to have beneficial effects. Pregnancy is considered a critical time period for imprinting the developing immune system. We examined whether n-6 LC-PUFA, n-3 LC-PUFA concentrations or the n-6/n-3 ratio in cord blood (CB) serum are associated with allergic diseases up to the age of 10 yr.

Methods: This analysis included 436 children from the Munich LISAplus birth cohort study. Information on doctor-diagnosed asthma, hay fever/allergic rhinitis, and eczema was collected using questionnaires completed at the ages 6 and 10 yr, and for eczema additionally at 2 yr. Specific immunoglobulin E (IgE) against inhalant allergens was measured at 6 and 10 yr. Fatty acid composition was measured by gas chromatography in serum from CB and from blood collected at 2, 6, and 10 yr. Associations between n-3, n-6 LC-PUFA concentrations, and the n-6/n-3 ratio in CB serum and allergic diseases or atopy were assessed using generalized estimating equations (GEE) considering the longitudinal structure. Models were adjusted for LC-PUFA concentrations at follow-up and potential confounding factors.

Results: There was no significant association between n-3 LC-PUFA, n-6 LC-PUFA, or the n-6/n-3 ratio in CB serum with eczema, asthma, hay fever/allergic rhinitis, or aeroallergen sensitization.

Conclusions: There is no indication of a beneficial effect of increased n-3 LC-PUFA in CB serum on the development of any of the disease entities.

Studies suggest that dietary changes, especially an increased consumption of n-6 polyunsaturated fatty acids (PUFAs) and a decreased intake of n-3 PUFA, may contribute to the increasing prevalence of allergic diseases in children observed in recent decades (1, 2).

Arachidonic acid (20:4n-6, AA), a product of the essential n-6 PUFA linoleic acid (18:2n-6, LA), can act as substrate and be converted to pro-inflammatory eicosanoids (3). Using the same enzymatic pathway, the essential n-3 PUFA α-Linolenic acid (18:3n-3, ALA) is transformed to the long-chain PUFA (LC-PUFA) eicosapentaenoic acid (20:5n-3, EPA), and then docosahexaenoic acid (22:6n-3, DHA) (3). EPA can also be metabolized into eicosanoids; however, this form has less potent inflammatory effects compared to that derived from AA (3). Additionally, E-series resolvins can be generated from EPA. D-series resolvins and protectins can be derived from DHA. Protectins and resolvins have anti-inflammatory and inflammation resolving effects (3).

Pregnancy has been suggested to be a critical period for developmental programming, which may influence the later development of allergic diseases in childhood (4). Maternal diet, especially maternal PUFA intake, during pregnancy may modify neonatal immune response through epigenetic mechanisms and therefore alter disease susceptibility and predisposition (4, 5). It is proposed that a higher intake of n-3 PUFA, for example, by fish oil supplementation, may have protective effects against the development of atopic diseases in the offspring, although not all studies show conclusive results (5–7).

Cord blood (CB) LC-PUFA composition reflects the LC-PUFA supply of the offspring around birth (8).

Therefore, we hypothesize that higher n-3 LC-PUFA, lower n-6 LC-PUFA concentrations, and a lower n-6/n-3 ratio in CB serum are associated with lower prevalence of doctor-diagnosed asthma, eczema, hay fever/allergic rhinitis, as well as aeroallergen sensitization up to 10 yr of age, even after accounting for later LC-PUFA status.

Methods

Study population

The German LISAplus (Life-style Related Factors on the Immune System and the Development of Allergies in Childhood PLUS the influence of traffic emissions and genetics) is a population based birth cohort study in which a total of 3097 neonates were recruited between 1997 and 1999 from the German cities of Munich, Leipzig, Wesel, and Bad Honnef. Details of the study design have been described elsewhere (9). During recruitment in maternity wards, cord blood samples were collected, serum was prepared and deep frozen at −18°C during the first 3 months at recruitment centers and then at −80°C until measurement of fatty acids. Questionnaires were completed by the parents at birth, 6, 12, 18, and 24 months and 4, 5, 6, and 10 yr, and physical examinations took place at 2, 6, and 10 yr. During the physical examinations, blood samples were collected.

This substudy was restricted to children from the Munich study center. The participants flow is illustrated in Fig. S1. Of 1467 successfully recruited children, 814 CB serum samples could be collected. Total immunoglobulin E (IgE) concentrations were measured in these CB serum samples (9), and fatty acids were measured in the 681 samples with sufficient remaining sample volume. Of these 681 children with information on CB serum fatty acid composition, 481 children participated in at least one the fatty acid measurement at 2, 6, or 10 yr of age (2 yr: 351 children; 6 yr: 286 children; 10 yr: 249 children). One child, who participated only in the 2-yr follow-up, was excluded because of insufficient information on eczema. Further, 44 children were excluded due to missing covariables. Therefore, 436 children remained in the analysis. Of these 436 children, 280 children participated in the 2-yr follow-up, 277 children participated in the 6-yr follow-up, and 243 children participated in the 10-yr follow-up.

Approval by the local Ethics Committees (Bavarian Board of Physicians, University of Leipzig, Board of Physicians of North-Rhine-Westphalia) and written consent from participant's families were obtained.

Definition of allergic diseases

Information on having physician-diagnosed asthma, eczema, hay fever, and allergic rhinitis was collected using self-administered questionnaires completed by the parents. Each questionnaire asked for information pertaining to the time-frame since the previous follow-up. The questions on physician-diagnosed hay fever and allergic rhinitis were combined to a single variable.

Based on these questions for each year of age up to 10 yr, combined variables were defined. For eczema, three variables were defined for having physician-diagnosed eczema between birth and 2 yr, 3–6 yr and 7–10 yr of age. For asthma and hay fever/allergic rhinitis, only the last two variables were defined, due to the low prevalence between birth and 2 yr. Controls were defined by a negative answer, or if no answer was given, as long as the questionnaire was returned.

Blood tests

The analysis of fatty acids was performed by transesterification of glycerophospholipid fatty acids into methyl esters and their gas chromatographic separation and quantification (10). This procedure has been previously described in detail for the analysis of serum from CB and from blood samples collected at the ages of 2 and 6 yr (11, 12), and was also applied for blood samples collected at the age of 10 yr. Total n-6 LC-PUFA and n-3 LC-PUFA was calculated by summing up all measured n-6 fatty acids and n-3 fatty acids, respectively, with 20 carbon atoms or more. The n-6/n-3 ratio was calculated by dividing total n-6 LC-PUFA by total n-3 LC-PUFA.

Specific serum IgE concentrations were assayed by the CAP-RAST FEIA system (Pharmacia Diagnostics, Freiburg, Germany) according to the manufacturer's instructions. Screening tests were used to test allergic sensitization against inhalant allergens (sx1: Dermatophagoides pteronyssinus, cat, dog, rye, timothy grass, Cladosporium herbarum, birch, and mugwort). Children were assigned as IgE positive, if their IgE values exceeded 0.35 kU/l for at least one of the measured inhalant allergens, otherwise as controls.

Statistical analysis

N-3, n-6 LC-PUFAs, or n-6/n-3 ratio measured at birth, 2, 6 or 10 yr of age with values exceeding mean ± 4 SD were defined as outliers and treated like missing values in subsequent analysis (Fig. S1). Differences between LC-PUFA levels stratified by case-control status were tested using Student's *t*-test for two independent samples.

Considering the longitudinal data structure, generalized estimating equations (GEE) were applied to model the effect of n-3, n-6 LC-PUFA concentrations, and the n-6/n-3 ratio in CB serum on eczema, asthma, hay fever/allergic rhinitis, as well as aeroallergen sensitization (13). An autoregressive working correlation structure was assumed (AR-1).

The GEE model returns one estimate for the variables, which were measured once (CB serum LC-PUFA composition) and one combined estimate for the variables, which were measured at the various follow-up time points (LC-PUFA composition at follow-up).

The GEE models were additional adjusted for the time-dependent variables time of follow-up (2, 6, and 10 yr for eczema; 6 and 10 yr for asthma, hay fever/allergic rhinitis, and aeroallergen sensitization), age, n-3, n-6 LC-PUFA, and n-6/n-3 ratio, respectively, at follow-up and the total sum of fatty acids as well as parental education, sex, maternal age at birth, and parental atopy. Additionally, the child's BMI, birth

weight, and exclusive breastfeeding for more than 4 months, as well as maternal factors such as smoking during pregnancy, BMI before pregnancy, atopy and parity were tested as potential confounders, but not included in the final models, because there was no significant effect. The GEE model does not require that each subject have the same number of data points. To rule out a potential bias that may be introduced by non-random loss to follow-up, additional sensitivity analyses were conducted. This analysis was restricted to children with complete information on eczema, asthma, hay fever/allergic rhinitis, or aeroallergen sensitization and fatty acid measurements at all time points.

Further, general linear models for each follow-up were conducted, regressing the n-3, n-6 LC-PUFA concentrations, and the n-6/n-3 ratio in CB serum on eczema, asthma, hay fever/allergic rhinitis, or aeroallergen sensitization. The models were adjusted for age, n-3, n-6 LC-PUFA, and n-6/n-3 ratio, respectively, at follow-up and the total sum of fatty acids as well as parental education, sex, maternal age at birth, and parental atopy.

The effect estimates are presented as odds ratio (OR) with corresponding 95% confidence interval (95%-CI). Significance was defined as a two-sided alpha level of 5%.

Statistical analyses were performed using R, version 2.15.2 (www.R-project.org) (14) and the function geeglm() from package 'geepack' to fit GEE models.

Results

Information on CB serum n-3, n-6 LC-PUFAs, or the n-6/n-3 ratio, at least one of the diseases entities and LC-PUFA composition in blood at one or more follow-ups at 2, 6, or 10 yr of age was available for 436 children. The participant flow is illustrated in Fig. S1.

Basic characteristics of the study population are presented in Table 1. Of the 436 children with CB serum fatty acid measurements, 280 children were examined at 2 yr, 277 children at 6 yr, and 243 children at 10 yr. The period prevalence of eczema was constantly high up to age 6 and decreased afterwards, whereas the prevalence of hay fever/allergic rhinitis, asthma, and aeroallergen sensitization increased after age 6 yr. The mean percentage of n-3 as well as n-6 LC-PUFA was higher in CB serum than in blood at later follow-ups, but the n-6/n-3 ratio remained stable.

All investigated variables were tested for differences between children who participated in this analysis with the remaining children from the Munich study center who were not included (Table S1). There was a lower percentage of girls, and a higher parental education level in study participants ($p = 0.0394$ and $p = 0.0229$, respectively).

Table 2 shows mean and SD of n-3 LC-PUFA, n-6 LC-PUFA concentrations, and n-6/n-3 ratio in CB serum in children with (cases) and without (controls) eczema, asthma, hay fever/allergic rhinitis, or aeroallergen sensitization, respectively. It was tested whether the mean of n-3 LC-PUFA, n-6 LC-PUFA concentrations, and n-6/n-3 ratio in CB serum differs significantly between cases and controls. Children with aeroallergen sensitization at 10 yr had lower n-6 LC-PUFA concentrations in CB serum (mean in controls: 25.6 vs. mean in cases: 25.2, $p = 0.0326$).

Table 3 shows mean and SD of n-3 LC-PUFA, n-6 LC-PUFA concentrations and n-6/n-3 ratio cross-sectional at each follow-up in children with (cases) and without (controls) eczema, asthma, hay fever/allergic rhinitis, or aeroallergen sensitization, respectively. Children with eczema at 2 yr have significant lower concentrations of n-3 LC-PUFA (4.6 vs. 4.4, $p = 0.0337$) and a significant higher n-6/n-3 ratio (2.9 vs. 3.1, $p = 0.0074$). Additionally, lower n-3 LC-PUFA concentrations were observed in children with asthma at 6 yr (4.5 vs. 4.1, $p = 0.0032$) and aeroallergen sensitization at age 6 yr (4.5 vs. 4.3, $p = 0.0205$).

The results of GEE on allergic diseases are presented in Table 4. There was no significant association between n-3 LC-PUFA, n-6 LC-PUFA concentrations, or the n-6/n-3 ratio in CB serum with any of the investigated outcomes. Thus, the CB serum LC-PUFA concentrations do not appear to have any effect on allergic disease risk at any time point investigated.

Table 1 Basic characteristics of the study population. Values are presented as mean (SD) or percentage

	Birth	2 yr	6 yr	10 yr
N	436	280	277	243
Eczema, %		15	14	7
Asthma, %			3	5
Hay fever/allergic rhinitis, %			3	13
Aeroallergen sensitization, %			24	43
n-6 LC-PUFA *	25.3 (1.5)	13.3 (1.5)	13.6 (1.6)	15 (1.6)
n-3 LC-PUFA *	8.1 (1.6)	4.5 (0.8)	4.5 (0.9)	4.8 (1.1)
n-6/n-3 ratio	3.3 (0.7)	3.0 (0.5)	3.1 (0.6)	3.2 (0.6)
Total sum of fatty acids (mg/l)	644.7 (103.3)	1146.5 (160.9)	1198.8 (182.4)	1257.4 (202.5)
Parental education (% high)	82			
Sex (% female)	43			
Age (yr)		2.01 (0.03)	6.1 (0.2)	10.2 (0.2)
Maternal age (yr)	32.7 (3.9)			
Parental atopy (% yes)	64			

*n-6 and n-3 LC-PUFAs are presented as percentage of total fatty acids

Table 2 Mean and SD of n-3 LC-PUFA, n-6 LC-PUFA concentrations, and n-6/n-3 ratio in CB serum in children with (cases) and without (controls) eczema, asthma, hay fever/allergic rhinitis or aeroallergen sensitization, respectively. Differences were tested using *t*-test

	n-3 LC-PUFA in CB*			n-6 LC-PUFA in CB*			n-6/n-3 ratio in CB		
	Control	Case	p-value	Control	Case	p-value	Control	Case	p-value
Eczema									
2 yr	8.1 (1.6)	8.0 (1.6)	0.4831	25.2 (1.5)	25.4 (1.4)	0.2721	3.2 (0.7)	3.3 (0.7)	0.2717
6 yr	8.1 (1.6)	8.2 (1.5)	0.5270	25.3 (1.5)	25.2 (1.2)	0.7360	3.2 (0.7)	3.2 (0.6)	0.2808
10 yr	8.1 (1.6)	7.7 (1.3)	0.1316	25.3 (1.4)	25.4 (1.6)	0.7504	3.2 (0.7)	3.4 (0.6)	0.2449
Asthma									
6 yr	8.1 (1.6)	7.5 (1.3)	0.0817	25.3 (1.5)	25.7 (1.2)	0.1175	3.2 (0.7)	3.4 (0.5)	0.1325
10 yr	8.1 (1.6)	8.4 (1.3)	0.4137	25.3 (1.4)	25.5 (1.2)	0.3980	3.2 (0.7)	3.1 (0.6)	0.4917
Hay fever or allergic rhinitis									
6 yr	8.1 (1.6)	8.3 (1.6)	0.4957	25.3 (1.4)	24.9 (1.8)	0.2940	3.2 (0.7)	3.1 (0.6)	0.1888
10 yr	8.1 (1.6)	8.0 (1.4)	0.7138	25.3 (1.5)	25.2 (1.3)	0.5704	3.2 (0.7)	3.2 (0.6)	0.8977
Aeroallergen sensitization									
6 yr	8.1 (1.7)	8.0 (1.5)	0.8713	25.3 (1.4)	25.1 (1.5)	0.3079	3.3 (0.8)	3.2 (0.6)	0.6872
10 yr	8.0 (1.7)	8.1 (1.5)	0.5423	25.6 (1.4)	25.2 (1.5)	0.0326	3.3 (0.8)	3.2 (0.6)	0.1701

*n-6 and n-3 LC-PUFAs are presented as percentage of total fatty acids

Table 3 Mean and SD of n-3 LC-PUFA, n-6 LC-PUFA concentrations and n-6/n-3 ratio at each follow-up in children with (cases) and without (controls) eczema, asthma, hay fever/allergic rhinitis or aeroallergen sensitization, respectively. Differences were tested using *t*-test

	n-3 LC-PUFA at follow-ups*			n-6 LC-PUFA at follow-ups*			n-6/n-3 ratio at follow-ups		
	Control	Case	p-value	Control	Case	p-value	Control	Case	p-value
Eczema									
2 yr	4.6 (0.9)	4.4 (0.8)	0.0337	13.2 (1.5)	13.5 (1.6)	0.0715	2.9 (0.5)	3.1 (0.5)	0.0074
6 yr	4.5 (0.9)	4.4 (0.8)	0.3148	13.6 (1.6)	13.6 (1.4)	0.9646	3.1 (0.5)	3.1 (0.6)	0.6738
10 yr	4.5 (1.0)	4.5 (1.0)	0.5952	14.4 (1.9)	14.2 (1.7)	0.4852	3.3 (0.6)	3.3 (0.6)	0.9266
Asthma									
6 yr	4.5 (0.9)	4.1 (0.5)	0.0032	13.7 (1.6)	13.1 (1.3)	0.1217	3.1 (0.5)	3.1 (0.6)	0.8204
10 yr	4.5 (1.0)	4.4 (1.1)	0.5148	14.4 (1.9)	14.5 (2.2)	0.6645	3.3 (0.6)	3.4 (0.6)	0.1650
Hay fever or allergic rhinitis									
6 yr	4.5 (0.9)	4.4 (0.9)	0.6619	13.6 (1.6)	13.8 (1.6)	0.6287	3.1 (0.5)	3.2 (0.5)	0.3884
10 yr	4.5 (1.0)	4.4 (1.1)	0.3204	14.4 (1.9)	14.3 (1.9)	0.7181	3.3 (0.6)	3.3 (0.7)	0.2460
Aeroallergen sensitization									
6 yr	4.5 (0.9)	4.3 (0.8)	0.0205	13.7 (1.5)	13.6 (1.6)	0.8680	3.1 (0.6)	3.2 (0.5)	0.1074
10 yr	4.5 (1.0)	4.6 (1.1)	0.3149	14.3 (1.9)	14.5 (1.9)	0.1193	3.3 (0.6)	3.3 (0.6)	0.9008

*n-6 and n-3 LC-PUFAs are presented as percentage of total fatty acids

Children with eczema at a given age was significantly associated with lower concentrations of n-3 LC-PUFA at this same age [OR = 0.71, 95%-CI = (0.55–0.92), p = 0.0085].

In an additional sensitivity analysis, the GEE models were restricted to children with complete information on eczema, asthma, hay fever/allergic rhinitis, or aeroallergen sensitization and fatty acid measurements at all time points (Table S2). This analysis showed similar results. Additionally, there was a borderline significant inverse association between n-6 LC-PUFA in CB serum and eczema [0.83 (0.68–1.02), p = 0.0784].

In a further sensitivity analysis, general linear models for each follow-up were conducted (Table S3). The results from the GEE models were confirmed by this analysis. There was also no significant association between CB serum n-3, n-6 LC-PUFA concentrations, or the n-6/n-3 ratio with any of the allergic diseases. Children with eczema at the age of 6 yr had significantly lower concentrations of n-3 LC-PUFA at the same age [0.60 (0.38–0.97), p = 0.0364], but not at 2 yr [0.70 (0.45–1.09), p = 0.1189] or 10 yr [0.90 (0.51–1.59), p = 0.7212]. Aeroallergen sensitization at age 6 yr was inverse associated with n-3 LC-PUFA at the same age [0.68 (0.47–0.98), p = 0.0407], but not at 10 yr [0.90 (0.69–1.18), p = 0.4619].

Discussion

This study investigated the association between n-3 and n-6 LC-PUFA composition as well as the n-6/n-3 ratio in CB serum and the development of eczema, asthma, hay fever/allergic rhinitis and allergic sensitization up to 10 yr of age,

Table 4 Results of a GEE in which n-3, n-6 LC-PUFA concentrations, and n-6/n-3 ratio in CB serum are regressed on allergic diseases. ORs are adjusted for parental education, sex, time of follow-up (2 yr, 6 yr or 10 yr for eczema; 6 yr and 10 yr for asthma, hay fever/allergic rhinitis and aeroallergen sensitization), age, maternal age at birth, parental atopy, total sum of fatty acids, as well as LC-PUFA concentrations at follow-up (FU) were included

	n-3 LC-PUFA*				n-6 LC-PUFA*				n-6/n-3 ratio			
	N	OR	95%-CI	p-value	*N*	OR	95%-CI	p-value	*N*	OR	95%-CI	p-value
Eczema												
CB	431	1.01	(0.87–1.16)	0.9055	432	0.94	(0.80–1.11)	0.4936	434	0.96	(0.70–1.31)	0.7829
FU		0.71	(0.55–0.92)	0.0085		1.00	(0.88–1.15)	0.9580		1.51	(0.98–2.33)	0.0600
Asthma												
CB	335	1.00	(0.75–1.35)	0.9884	334	1.31	(0.93–1.84)	0.1266	337	1.17	(0.53–2.59)	0.6903
FU		1.03	(0.55–1.94)	0.9173		0.77	(0.58–1.01)	0.0579		0.71	(0.35–1.46)	0.3526
Hay fever or allergic rhinitis												
CB	334	0.98	(0.76–1.25)	0.8569	333	0.86	(0.67–1.11)	0.2548	336	0.88	(0.52–1.49)	0.6416
FU		0.82	(0.52–1.29)	0.3915		0.96	(0.77–1.19)	0.7014		1.56	(0.85–2.83)	0.1495
Aeroallergen sensitization												
CB	336	1.01	(0.87–1.18)	0.8494	335	0.87	(0.74–1.01)	0.0742	338	0.88	(0.62–1.23)	0.4407
FU		0.82	(0.67–1.00)	0.0514		1.07	(0.94–1.20)	0.3140		1.34	(0.96–1.86)	0.0849

*n-6 and n-3 LC-PUFAs are presented as percentage of total fatty acids

after accounting for LC-PUFA concentrations in blood using longitudinal data from the Munich LISAplus birth cohort.

We could not find a protective effect of elevated n-3 LC-PUFA concentrations in CB serum on any of the investigated disease entities. When fatty acid concentrations measured at the same time point were entered in the model, children with eczema exhibit lower levels of n-3 LC-PUFA.

To our knowledge, this is the first study in which repeated fatty acids measurements starting from birth to 10 yr of age were included using a longitudinal model.

The ALSPAC study is the only observational study which published data on fatty acid concentrations in CB serum and atopic outcomes, but without adjustment for fatty acids later in life. There was some evidence; however, the results were not significant any more after adjustment for multiple testing, and therefore, Newson et al. concluded that it is unlikely that CB serum fatty acid concentrations are an important risk factor for the later development of allergic diseases (15).

Several observational and interventional studies explored the association between atopic diseases and diet (7, 16–18) as well as maternal n-3 LC-PUFA status during pregnancy (19–24) or lactation (25–27).

In an earlier publication based on data from the LISAplus birth cohort, the association between maternal dietary intake during the last 4 wk of pregnancy and allergic diseases in the offspring up to the age of 2 yr was investigated (16). In this analysis, a higher intake of n-6 PUFA rich foods such as margarine and vegetable oils and a lower intake of n-3 PUFA rich foods such as fish were associated with eczema in the offspring. In our analysis, we used exact measurements of the n-3 and n-6 LC-PUFA concentrations in CB serum instead of food intake as proxy but could not replicate this finding. However, in this study, we focused on LC-PUFAs, whereas margarine is rich in linoleic acid, which may mediate the effect, but was not considered in the current analysis. Additionally, our study population was limited to the Munich study center only and the sample size was reduced due to limited blood sample availability.

In fact, the most consistent evidence was found in studies focusing on dietary intake, especially on dietary fish intake as a source of n-3 PUFA (7, 16–18). Therefore, it has been hypothesized that a sufficient n-3 LC-PUFA supply may prevent from atopic diseases, especially during the pre- and early post-natal period when the immature immune system is developing (5, 28).

Some intervention studies have been conducted to investigate the association between n-3 LC-PUFA status and atopic diseases aiming to increase the n-3 LC-PUFA concentrations during pregnancy (19–24) or lactation (25–27). The studies in which cytokines were measured, reported a more favorable immune response (19–21, 25, 27).

Nevertheless, it is not clear whether these immunological changes can avoid clinical manifestation of allergic diseases. The studies which investigated clinical outcomes, reported less or less severe atopic diseases in the intervention group (20, 22–24, 26), although most of these studies had a short follow-up period of 1 year (20, 23, 24, 26), a small sample size with few cases (20, 23) or the results were not significant anymore after adjustment for multiple comparisons (24, 26). Olsen et al. (22) used registry data to follow-up children at 16 yr of age and found a strong reduction of asthma and especially atopic asthma risk in the intervention group.

The findings from observational studies investigating food intake could not be confirmed by interventional studies. This might be due to the above mentioned limitations or due to residual confounding in observational studies, whereas dietary intake is related to a certain life-style. Donahue et al.(29) investigated the associations between n-3 and n-6 PUFAs in maternal diet, maternal blood, and CB plasma and found the strongest correlation for DHA+EPA. Therefore, the assessment of n-3 LC-PUFA in diet and blood should lead to similar

results, whereas the exact measurement in blood is supposed to be more accurate.

In our study, we could not find a protective effect of n-3 LC-PUFA concentration in CB serum on any of the allergic diseases, as seen for the ALSPAC study (15). CB serum fatty acid composition represents the fatty acid supply of the fetus at birth. The models were adjusted for the fatty acid composition in blood at each follow-up to rule out confounding by lifestyle or dietary factors, which may be reflected in fatty acid concentrations in blood. The longitudinal study design increased this study's statistical power, as the maximal number of observations available was included in the models, and allows modeling the effect of LC-PUFA in CB serum as well at the follow-ups at several time points up to 10 yr of age. Due to the constraints of the longitudinal model, the variables for asthma, eczema, and hay fever/allergic rhinitis which were asked yearly had to be combined into the periods between the blood measurements to obtain the same time points. In a sensitivity analysis, general linear models were conducted for each follow-up. The results were similar for all follow-ups, and we could not identify a time-varying effect of CB serum fatty acids. Another limitation of this study is the potential bias associated with non-random loss to follow-up. A comparison of all investigated variables between participants who were included in this analysis and non-participants showed a significantly higher percentage of parents with high education level in the participants group, but no significant differences in all other investigated variables. However, a sensitivity analysis restricted to children with complete data for all times of follow-up showed similar results, but, only in this analysis, there was a borderline significant inverse association between n-6 LC-PUFA in CB serum and eczema. A further limitation is the relatively low habitual fish and seafood intake in Germany, compared with other European countries (30), leading to a limited range of n-3 LC-PUFA intakes, whereas some of the intervention studies found effects on immune response with high dosages of fish oil supplementation.

As observational and interventional studies on dietary intake of fatty acids and allergic diseases are inconclusive (18), we thought that prospectively collected and longitudinally analysed data on fatty acid composition in CB serum could elucidate the protective association. However, this study with data on repeated measurements of fatty acids during a period of 10 yr failed to show strong and consistent associations.

Clearly, further interventional studies with adequate sample size, long-term follow-up, and repeated measurements are required to confirm the role of n-3 and n-6 PUFAs in the development of atopic diseases.

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Conflict of interest

The authors declare no conflict of interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Participant flow.

Table S1. Comparison of all investigated variables between study participants and non-participants.

Table S2. Results of a GEE in which n-3, n-6 LC-PUFA concentrations, and n-6/n-3 ratio in cord blood (CB) serum are regressed on allergic diseases *restricted to children with complete information on all time points*.

Table S3. Results of general linear models in which n-3, n-6 LC-PUFA concentrations, and n-6/n-3 ratio in cord blood (CB) serum are regressed on allergic diseases stratified for each follow-up.