Allergic sensitisation and allergic rhinitis are associated with n-3 polyunsaturated fatty acids in the diet and in red blood cell membranes

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Angaben zur Veröffentlichung / Publication details:

Hoff, S, H Seiler, J Heinrich, I Kompauer, A Nieters, N Becker, G Nagel, et al. 2005. "Allergic sensitisation and allergic rhinitis are associated with n-3 polyunsaturated fatty acids in the diet and in red blood cell membranes." *European Journal of Clinical Nutrition* 59 (9): 1071–80. https://doi.org/10.1038/sj.ejcn.1602213.

Allergic sensitisation and allergic rhinitis are associated with n-3 polyunsaturated fatty acids in the diet and in red blood cell membranes

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Objective: Due to inconsistent results based on dietary intake data, unsaturated fatty acids in red blood cell (RBC) membranes and diet were used to investigate their association with allergic sensitisation and allergic rhinitis.
Design: Cross-sectional, population-based study.
Setting: Bavarian Nutrition Survey II (2002–03), Germany.
Subjects: A total of 568 adult participants, 325 women and 243 men.
Methods: By means of logistic regression models, the relation of fatty acids to (i) allergic sensitisation as defined by means of specific serum immunoglobulin E analysis (CAPSX1 class ≥ 2), and (ii) self-reported allergic rhinitis was examined.
Results: A high cell membrane level of eicosapentaenoic acid (EPA, 20:5 n-3) was inversely associated with allergic sensitisation, the adjusted odds ratio (OR) and 95% confidence interval (95% CI) were 0.52 (0.30–0.90) for the highest (*vs* lowest) quartile. A similar effect was observed for allergic rhinitis with an OR (95% CI) of 0.50 (0.24–1.03; $P = 0.027$ for trend). A higher dietary intake of alpha-linolenic acid (ALA, 18:3 n-3) was associated with a decreased risk of allergic sensitisation and allergic rhinitis with ORs (95% CIs) of 0.51 (0.28–0.93) and 0.43 (0.20–0.93), respectively, in the highest quartiles. No other dietary or cell membrane unsaturated fatty acid was significantly associated with the outcome variables, nor was the n-6/n-3 ratio. The strongest effects were observed among subjects under the age of 40 y.
Conclusions: In this cross-sectional study among adults, a high content of n-3 fatty acids in RBC membranes (EPA) or in the diet (ALA) is associated with a decreased risk of allergic sensitisation and allergic rhinitis.
Sponsorship: The study was supported by funds of the Kurt-Eberhard-Bode-Stiftung and the Bavarian Ministry of Environment, Health and Consumer Protection.

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Contributors: SH—red blood cell analysis, analysis of data, and writing the manuscript. HS—collection and processing of data. JH and IK—significant comments on statistical analysis and interpretation of the results. GN, AN and NB—advice and consultation on data analysis and contribution to the interpretation. KG, GK and GW—fund raising and study design. JL—senior author; responsible for study design, collection and analysis of data, writing the manuscript.

Introduction

Over the last decades, the prevalence of atopic and allergic diseases in Western countries increased (Burr *et al*, 1989; Linneberg *et al*, 2000; Heinrich *et al*, 2002). These diseases are supposed to have a multifactorial background with genetic determinants and environmental factors contributing to their development (von Mutius, 2000; Strachan *et al*, 2001). Since the changes in prevalence rates cannot be explained by genetic factors alone, aetiologic research focuses on the subjects' environment. Attention has been paid to nutritional factors as part of the lifestyle of populations with specific focus on fatty acids and polyunsaturated fatty acids

(PUFA) of the n-6 and n-3 families in particular (Black & Sharpe, 1997).

The dietary intake of fatty acids affects the composition of immune cells and, thus, their capacity to produce eicosanoids (Calder, 2001). Eicosanoids are potent immune mediators which are mainly synthesised from arachidonic acid (AA; C20:4 n-6) and eicosapentaenoic acid (EPA; C20:5 n-3). With respect to the underlying mechanism, that is, a dysregulated T-helper cell 2 (Th2) response in atopic disease, eicosanoids may interfere with immune cells shifting the pattern of the cytokine production of Th1 and Th2. AA and its precursor, linoleic acid (LA, C18:2 n-6), increase the formation of the proinflammatory eicosanoids leukotriene B_4 (LTB_4) and prostaglandin E_2 (PGE_2). The latter is considered to suppress Th1-type cytokine production and to promote Th2 response, and subsequently enhances the synthesis of immunoglobulin E (IgE) by B lymphocytes (Black & Sharpe, 1997). Since n-3 PUFA, in particular EPA, potentially antagonise the effects of AA a high amount of available EPA may be associated with decreasing risk of allergic disease and atopy. In this context, the ratio of n-6 to n-3 PUFA was supposed to be predictive.

However, there are also other mechanisms by which unsaturated fatty acids may affect immune responses. PUFA alter T-lymphocyte signalling by changing the molecular composition of special signalling platforms called lipid rafts (Stulnig, 2003). Additionally, PUFA affect gene expression by acting as ligands for nuclear transcription factors, which regulate a multitude of cell responses. These include the peroxisome proliferator-activated receptor family, liver X receptors , hepatic nuclear factor-4 alpha and sterol regulatory element binding proteins (Jump, 2002). Some or all of these mechanisms may be involved in the modulation of immune function by fatty acids.

Although immune response characteristics are thought to be defined in the early phase of life, there is evidence that in childhood and even in adulthood dietary factors can modulate the clinical manifestation of atopic diseases (von Mutius, 2000; Bolte *et al*, 2001). Of the few epidemiological studies in adults that have investigated the effects of fatty acids—largely based on dietary intake data—and the risk of allergic sensitisation and allergic rhinitis, inconsistent results have been provided (Heinrich *et al*, 2001; Wakai *et al*, 2001; Nagel *et al*, 2003; Trak-Fellermeier *et al*, 2004). Therefore, we used the fatty acid composition of red blood cell (RBC) membranes to investigate its association with allergic sensitisation and allergic rhinitis in an adult population. Our main hypotheses referred to the fatty acids, oleic acid, LA, AA, ALA, and EPA as derived from previously reported findings. Intake data were used for confirmatory analyses.

Materials and methods

Data collection

The Bavarian Nutrition Survey II (BVS II) was designed as a representative study of the Bavarian population to investigate dietary and lifestyle habits. Between September 2002 and June 2003, about 1000 subjects of the German speaking population in Bavaria (Germany) aged 13–80 y were recruited by a three-stage sampling procedure. At baseline, the subjects' lifestyle and socio-economic characteristics as well as their health status were assessed by means of a computer-aided personal interview (CAPI). Within 2 weeks after recruitment, the participants were contacted three times by phone in order to collect dietary information. Computer-assisted telephone interviews (CATI) were conducted by trained interviewers to assess the participants' food intake of the previous 24 h using the software EPIC-SOFT (Slimani *et al*, 1999; Slimani *et al*, 2000). The three recalls per subject were distributed over 2 weekdays and 1 day of the weekend. Nutrient intake was calculated based on the German Nutrient Data Base BLS, version II.3 (BgVV, Berlin, Germany). Intake data were weighted for weekday or weekend day to calculate mean daily fatty acid intake per subject. Within 6 weeks after recruitment, all adult ($\geq$18 y) study subjects were invited to their nearest health office for blood sampling and standardised anthropometric measurements.

The overall participation rate in the study was 71% ($n=1050$). Nonresponder were more likely to be overweight, smokers or exsmokers and were more frequently ranked in the lower categories of socio-economic status than responders. All adults (excluding subjects $<$18 y) who completed at least one 24-h dietary recall ($n=879$) were invited for blood sampling. Of these, 65% ($n=568$) provided blood samples for further analysis of serum IgE and RBC membrane fatty acid composition. Only marginal differences by age, socio-economic status or smoking status were observed between study participants invited for blood sampling and those finally providing blood samples; differences by BMI group disappeared after calibration (linear regression) of self-reported data on weight and height. After excluding seven subjects who completed less than two 24-h diet recalls, a total of 561 subjects were included in the evaluation of dietary fatty acids and atopic outcomes. All participants gave their written informed consent. The study was approved by the local ethics committee.

Case definition

To assess the prevalence of atopic sensitisation by common inhalant allergens an *in vitro* screening test, CAPSX1 (Pharmacia Upjohn, Uppsala, Sweden), was applied to a group of 566 individuals from whom serum samples were available. This ELISA-based test, also known as Phadiatop test, detects the presence of specific serum IgE against common allergens (timothy, rye, birch, mugwort pollen, house dust mite (*Dermatophagoides pteronyssinus*), cat and dog epithelia as well as *Cladiosporum herbarum* mould). All serum samples were analysed in a blinded manner according to the manufacturer's recommendations at the Federal State Health Office Baden-Württemberg. The results are expressed

in seven CAP classes (0: <0.35 kU/l, 1: 0.35–0.70 kU/l), 2: 0.70–3.50 kU/l, 3: 3.5–17.50 kU/l, 4: 17.70–50.00 kU/l, 5: 50.00–100.00 kU/l, and 6: >100.00 kU/l) with class ≥2 considered as evidence of allergic sensitisation.

During the baseline interview, participants were asked about physician-diagnosed diseases ('Have you ever had one of the following diseases diagnosed by a physician?'), including a question about allergic rhinitis. 77 out of 568 subjects (14%) reported suffering from allergic rhinitis.

Blood sampling

Venous blood was drawn into EDTA tubes (1.6 mg/l. blood) or serum tubes, chilled at 4°C, and processed subsequently. Plasma and buffy coat were separated from RBC by centrifugation at $2000 \times g$ for 15 min. Samples were cooled for a maximum of 1 day (transportation, aliquoting) until they were stored at −80°C (serum) or −20°C (RBC). The analysis of RBC membrane fatty acids was conducted no later than 6 months after drawing. Serum samples used for IgE determination were stored for a maximum of 1.25 y.

Fatty acid analysis

An aliquot of 0.5 ml RBC suspension was used for membrane fatty acid analysis. The RBC membranes were isolated by centrifugation at $20\,000 \times g$ for 20 min at 4°C and the pellet was suspended with TRIS buffer (11 mmol/l Tris, 1 mmol/l Na-EDTA, pH 7.4). The washing procedure was repeated twice before adding 800 µl aqua dest. (Golik *et al*, 1996). Fatty acid extraction was performed with chloroform/methanol (2/1, v/v) according to a modification of the method of Folch *et al* (1957). Briefly, lipids were extracted twice with chloroform/methanol mixture, containing the antioxidant BHT (50 mg/l) (Wren & Szczepanowska, 1964). The combined extracts were washed with a $CaCl_2$ solution. The organic phase was collected and evaporated to dryness. The dried extract was suspended with chloroform and fatty acid methyl esters (FAME) were obtained by transesterification with trimethylsulfoniumhydroxide (Butte, 1983).

FAME were separated using a 100 m CP-Sil-88 capillary column (Varian-Chrompack, Darmstadt, Germany), installed in a HP 5890 series II gas chromatograph with a flame-ionisation detector (Hewlett Packard, Munich, Germany). For identification and quantification of the FAME peaks, authentic standards were used (Sigma Aldrich, Steinheim, Germany). In total, the content of 22 types of fatty acids were determined and expressed as a percentage of the total fatty acids identified; for each sample the mean result of two injections is given.

By means of a six-fold analysis of pooled RBC, coefficients of variation (CV) for the most prominent fatty acids (>1% of all FAME) were between 0.4 and 5.1%. CVs of the minor fatty acids (<1% FAME) EPA and alpha-linolenic acid (ALA; 18:3 n-3) were 3.6 and 10.5%, respectively.

Statistical methods

RBC membrane fatty acids (in % FAME) as well as dietary fatty acids (in % or ‰ of total energy intake) were categorised in quartiles according to the distribution in the total study population. To determine an association between fatty acids and allergic sensitisation or allergic rhinitis odd ratios (OR) and corresponding 95% confidence intervals (CI) were calculated by logistic regression models. For adjustment, sex, age group (< 40, 40–59, ≥60 y), socio-economic status (low, middle, high), and smoking status (never, ex, current) were included in the model. Socio-economic status was categorised as low, middle or high based on the subjects' educational level, their social position, and the households' net income (Winkler & Stolzenberg, 1999). Adjustment for body mass index (BMI) did not significantly influence the estimates and, therefore, was not included in the models. Tests for trends were computed using the quartile-based scores as a continuous variable. All statistical analyses were performed using the SPSS 11.0 software package (SPSS Inc., Chicago, USA).

Results

The basic characteristics of the study population are summarised in Table 1. Women were of younger age, had a lower BMI, and smoked less frequently than men. The prevalence of allergic sensitisation was higher in men, whereas symptoms of allergic rhinitis were more often reported by women. Sensitised subjects were younger, had a lower BMI, were of higher socio-economic status, and were more often nonsmokers than the entire study group. Similar differences were evident between subjects suffering from allergic rhinitis and all study participants. In all, 75% of the subjects with symptoms of allergic rhinitis were classified as sensitised according to the CAPSX1 test.

The predominant n-6 PUFAs (% FAME) in RBC membranes were LA and AA with an average membrane content of 11.42 ± 2.38 and 14.48 ± 5.48% FAME in the total study group, respectively (Table 2). EPA and docosahexaenoic acid (DHA; C22:6 n-3) represented the most important n-3 PUFA with proportions of 0.73 ± 0.58 and 2.91 ± 1.58% FAME in the total study population, respectively. Dietary PUFA were mainly comprised of LA (n-6) and ALA (n-3) intake. The content of PUFA in membranes was independent of the intake of the respective fatty acid from diet (in g/day), except for LA ($r = 0.09$, $P = 0.033$). Spearman's correlation coefficients were below 0.03 for oleic acid, AA, and ALA and slightly higher ($r = 0.07$) for EPA and DHA. Similar correlation coefficients were found when dietary fatty acids were expressed as percent of their contribution to the total energy intake.

Table 1 Subject characteristics

	Total study population			*Allergic sensitisation*			*Allergic rhinitis*		
	Total (n = 568)	*Women (n = 325)*	*Men (n = 243)*	*Total (n = 150)*	*Women (n = 79)*	*Men (n = 71)*	*Total (n = 77)*	*Women (n = 57)*	*Men (n = 20)*
Allergic sensitisation (%)[a]	26.5	24.4	29.3				75.3	73.7	80.0
Allergic rhinitis (%)[b]	13.6	17.5	8.2	38.7	53.2	22.5			
Age (%)									
<40 y	33.1	37.2	27.6	46.7	54.4	38.0	42.9	43.9	40.0
40–59 y	37.1	40.0	33.3	32.0	35.4	28.2	36.4	36.8	35.0
≥60 y	29.8	22.8	39.1	21.3	10.1	33.8	20.8	19.3	25.0
Body mass index (%)[c]									
<25 kg/m^2	41.5	47.4	33.7	48.0	53.2	42.3	46.8	52.6	30.0
25 to <30 kg/m^2	38.4	33.2	45.3	38.7	31.6	46.5	37.7	31.6	55.0
≥30 kg/m^2	20.1	19.4	21.0	13.3	15.2	11.3	15.6	15.8	15.0
Socio-economic status (%)[d]									
Low	37.9	38.8	36.6	26.0	21.5	31.0	27.3	26.3	30.0
Middle	31.3	31.7	30.9	30.0	31.6	28.2	35.1	31.6	45.0
High	30.8	29.5	32.5	44.0	46.8	40.8	37.7	42.1	25.0
Smoking status (%)									
Never	51.9	60.5	40.3	59.3	70.9	46.5	63.6	75.4	30.0
Ex	23.5	18.8	29.6	20.7	13.9	26.8	19.5	12.3	40.0
Current	24.7	20.7	30.0	20.0	15.2	26.8	16.9	12.3	30.0

[a]Allergic sensitisation was defined as specific IgE concentration of ≥0.7 kU/l (CAP-class ≥2); total $n = 566$ (women $n = 324$, men $n = 242$).
[b]Defined as self-reported allergic rhinitis diagnosed by a physician.
[c]Body mass index (BMI) was calculated as weight in kg/height in m^2.
[d]Defined as low, middle, and high based on the value of three characteristics on a point scale which include subjects' educational level, social position, and households' net income.

Table 2 Fatty acid composition of red blood cell (RBC) membranes (% FAME[a]) and diet (% of total energy intake) (mean ± s.d.)

RBC membranes (% FAME[a])		*Total (n = 568)*	*Women (n = 325)*	*Men (n = 243)*	*P-value*[b]
MUFA	C 18:1	15.12 ± 2.48	14.84 ± 2.35	15.49 ± 2.61	0.000
n-6 PUFA	C 18:2	11.06 ± 2.25	11.14 ± 2.17	10.94 ± 2.36	0.146
	C 20:4	13.98 ± 5.22	14.38 ± 5.14	13.45 ± 5.30	0.003
n-3 PUFA	C 18:3	0.09 ± 0.05	0.10 ± 0.05	0.09 ± 0.05	0.089
	C 20:5	0.91 ± 0.72	0.89 ± 0.70	0.92 ± 0.75	0.853
	C 22:6	4.66 ± 2.51	4.87 ± 2.46	4.39 ± 2.56	0.014
Dietary intake (% of energy intake)		*Total (n = 561)*	*Women (n = 324)*	*Men (n = 237)*	*P-value*
MUFA	C 18:1	11.39 ± 3.03	11.23 ± 2.92	11.63 ± 3.17	0.075
n-6 PUFA	C 18:2	5.38 ± 2.19	5.45 ± 2.25	5.28 ± 2.12	0.429
	C 20:4	0.09 ± 0.07	0.08 ± 0.06	0.09 ± 0.07	0.024
n-3 PUFA	C 18:3	0.58 ± 0.19	0.59 ± 0.21	0.55 ± 0.18	0.005
	C 20:5	0.04 ± 0.06	0.03 ± 0.06	0.04 ± 0.06	0.005
	C 22:6	0.08 ± 0.12	0.08 ± 0.14	0.08 ± 0.09	0.100

MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
[a]Values for fatty acids are expressed as percentage of total fatty acid methyl esters (FAME) (mean ± s.d.).
[b]Mann–Whitney-*U* test;

Associations between RBC membrane fatty acids and allergic sensitisation

There was a statistically significant inverse association between allergic sensitisation and the membrane content of EPA (Table 3). The ORs for the third and fourth quartiles compared with the lowest were 0.47 (95% CI, 0.28–0.81) and 0.59 (0.35–0.99), respectively. Adjustment for sex, age group, socio-economic status, and smoking status strengthened the association with ORs (95% CI) of 0.44 (0.25–0.78) for the third and 0.52 (0.30–0.90) for the highest quartile, respectively ($P = 0.012$ for trend). Allergic sensitisation was not associated with the n-6/n-3 ratio. However, the risk was significantly increased in the highest quartile of the ratio of AA to EPA, reflecting mainly the EPA effect. No other unsaturated fatty acid significantly affected the risk of allergic sensitisation.

After stratification for age, higher percentages of EPA decreased the risk of allergic sensitisation especially in the youngest group (<40 y), being significantly lower in the highest *vs* lowest quartile ($OR_{adj} = 0.36$, 95% CI = 0.15–0.87;

Table 3 Crude and adjusted[a] odds ratios (OR) and 95% confidence intervals (95% CI) for the association between selected unsaturated fatty acids in RBC membranes and allergic sensitisation[b]

Fatty acid	*First quartile*	*Second quartile*	*Third quartile*	*Fourth quartile*	P *for trend*
C 18:1n-9					
No. of cases	39	39	37	39	
Range (% FAME)	<13.40	13.40–14.33	14.33–16.32	>16.32	
Crude OR (95% CI)	1	1.00 (0.59–1.69)	0.86 (0.50–1.45)	0.92 (0.54–1.56)	0.638
Adjusted OR (95% CI)[a]	1	1.07 (0.62–1.85)	0.92 (0.52–1.62)	1.16 (0.66–2.05)	0.733
C 18:2n-6					
No. of cases	43	37	36	34	
Range (% FAME)	<10.28	10.28–11.45	11.45–12.44	>12.44	
Crude OR (95% CI)	1	0.80 (0.48–1.35)	0.77 (0.46–1.30)	0.72 (0.43–1.23)	0.233
Adjusted OR (95% CI)[a]	1	0.64 (0.37–1.10)	0.65 (0.38–1.14)	0.59 (0.34–1.04)	0.088
C 20:4n-6					
No. of cases	39	34	36	41	
Range (% FAME)	<12.15	12.15–15.97	15.97–17.60	>17.60	
Crude OR (95% CI)	1	0.84 (0.49–1.43)	0.90 (0.53–1.52)	1.08 (0.65–1.82)	0.716
Adjusted OR (95% CI)[a]	1	0.74 (0.42–1.29)	0.74 (0.43–1.30)	0.87 (0.50–1.51)	0.645
C 18:3n-3					
No. of cases	34	41	42	33	
Range (% FAME)	<0.06	0.06–0.09	0.09–0.12	>0.12	
Crude OR (95% CI)	1	1.32 (0.77–2.24)	1.33 (0.79–2.26)	0.96 (0.56–1.66)	0.914
Adjusted OR (95% CI)[a]	1	1.09 (0.62–1.90)	1.03 (0.59–1.79)	0.84 (0.47–1.49)	0.513
C 20:5n-3					
No. of cases	50	37	29	34	
Range (% FAME)	<0.39	0.39–0.77	0.77–1.29	>1.29	
Crude OR (95% CI)	1	0.65 (0.39–1.08)	0.47 (0.28–0.81)	0.59 (0.35–0.99)	0.021
Adjusted OR (95% CI)[a]	1	0.57 (0.33–0.97)	0.44 (0.25–0.78)	0.52 (0.30–0.90)	0.012
n-6/n-3 ratio[c]					
No. of cases	31	40	41	38	
Range	<3.16	3.16–3.81	3.81–5.26	>5.26	
Crude OR (95% CI)	1	1.41 (0.82–2.41)	1.44 (0.84–2.47)	1.30 (0.75–2.24)	0.373
Adjusted OR (95% CI)[a]	1	1.28 (0.72–2.26)	1.40 (0.80–2.47)	1.43 (0.81–2.55)	0.207
AA/EPA ratio[d]					
No. of cases	34	32	34	50	
Range	<11.31	11.31–18.38	18.38–30.42	>30.42	
Crude OR (95% CI)	1	0.91 (0.52–1.57)	0.98 (0.57–1.69)	1.69 (1.01–2.84)	0.040
Adjusted OR (95% CI)[a]	1	0.87 (0.49–1.55)	0.99 (0.56–1.75)	1.76 (1.01–3.06)	0.035

[a]Adjusted for sex, age group (<40, 40–59, ≥60 y), socio-economic status (low, middle, high) and smoking status (never, ex, current).
[b]Allergic sensitisation was defined as specific IgE concentration of ≥0.7 kU/l (CAP-class ≥2); cases $n=150$.
[c]Ratio (C 18:2 n-6, C 20:3 n-6, C 20:4 n-6, C 22:4 n-6) to (C 18:3 n-3, C 20:5 n-3, C 22:5 n-3, C 22:6 n-3).
[d]Ratio (C 20:4 n-6) to (C 20:5 n-3).

$P=0.012$ for trend). This age group also showed the highest prevalence of allergic sensitisation, particularly in females (cf. Table 1). A similar point estimate was observed for subjects aged 40–59 y ($OR_{adj}=0.38$, 95% CI = 0.13–1.09, $P=0.042$ for trend). There was no effect beyond the age of 60 y. We found no evidence for differences between genders (data not shown).

Associations between RBC membrane fatty acids and allergic rhinitis

The risk for allergic rhinitis decreased as the percentage of EPA in RBC membranes increased (Table 4), with the relative risk being lower in the highest EPA quartile relative to the lowest ($OR_{adj}=0.50$, 95% CI = 0.24–1.03; $P=0.027$ for trend). Age was not significantly associated with disease outcome in this model, and stratification by gender was not performed due to the low number of cases. As seen for allergic sensitisation, a higher AA/EPA ratio was positively associated with increasing risk of allergic rhinitis. No associations were found for MUFA, n-6 PUFA or other n-3 PUFA.

Associations between dietary fatty acids and allergic sensitisation

As listed in Table 5, ALA intake was inversely associated with the risk of allergic sensitisation and reached significance in

Table 4 Crude and adjusted[a] odds ratios (OR) and 95% confidence intervals (95% CI) for the association between selected unsaturated fatty in red blood cell membranes and allergic rhinitis[b]

Fatty acid	*First quartile*	*Second quartile*	*Third quartile*	*Fourth quartile*	P *for trend*
C 18:1n-9					
No. of cases	22	18	21	16	
Crude OR (95% CI)	1	0.79 (0.41–1.55)	0.95 (0.50–1.81)	0.69 (0.38–1.38)	0.411
Adjusted OR (95% CI)[a]	1	0.84 (0.42–1.67)	1.29 (0.66–2.54)	0.95 (0.46–1.96)	0.807
C 18:2n-6					
No. of cases	19	20	20	18	
Crude OR (95% CI)	1	1.06 (0.54–2.09)	1.06 (0.54–2.09)	0.94 (0.47–1.88)	0.869
Adjusted OR (95% CI)[a]	1	0.90 (0.45–1.81)	0.85 (0.42–1.71)	0.76 (0.37–1.54)	0.440
C 20:4n-6					
No. of cases	18	21	17	21	
Crude OR (95% CI)	1	1.20 (0.61–2.35)	0.94 (0.46–1.90)	1.20 (0.61–2.35)	0.784
Adjusted OR (95% CI)[a]	1	1.14 (0.57–2.28)	0.79 (0.38–1.63)	0.91 (0.45–1.84)	0.572
C 18:3n-3					
No. of cases	16	22	19	20	
Crude OR (95% CI)	1	1.44 (0.72–2.88)	1.22 (0.60–2.47)	1.29 (0.64–2.60)	0.622
Adjusted OR (95% CI)[a]	1	1.31 (0.64–2.66)	1.01 (0.49–2.11)	1.09 (0.53–2.25)	0.974
C 20:5n-3					
No. of cases	25	22	16	14	
Crude OR (95% CI)	1	0.86 (0.46–1.60)	0.59 (0.30–1.17)	0.51 (0.25–1.03)	0.034
Adjusted OR (95% CI)[a]	1	0.86 (0.45–1.65)	0.55 (0.27–1.11)	0.50 (0.24–1.03)	0.027
n-6/n-3 ratio[c]					
No. of cases	13	23	25	16	
Crude OR (95% CI)	1	1.92 (0.93–3.96)	2.12 (1.04–4.34)	1.26 (0.58–2.73)	0.547
Adjusted OR (95% CI)[a]	1	1.78 (0.85–3.73)	2.19 (1.05–4.57)	1.43 (0.65–3.18)	0.307
AA/EPA ratio[d]					
No. of cases	13	17	19	28	
Crude OR (95% CI)	1	1.35 (0.63–2.89)	1.53 (0.73–3.24)	2.44 (1.21–4.93)	0.011
Adjusted OR (95% CI)[a]	1	1.18 (0.54–2.57)	1.40 (0.65–3.00)	2.33 (1.12–4.85)	0.017

AA, arachidonic acid; EPA, eicosapentaenoic acid.
[a]Adjusted for sex, age group (<40, 40–59, ≥60 y), socio-economic status (low, middle, high) and smoking status (never, ex, current).
[b]Allergic rhinitis was defined as reported allergic rhinitis diagnosed by a physician; cases $n=77$.
[c]Ratio (C 18:2n-6, C 20:3n-6, C 20:4n-6, C 22:4n-6) to (C 18:3n-3, C 20:5n-3, C 22:5n-3, C 22:6n-3).
[d]Ratio (C 20:4n-6) to (C 20:5n-3).

the highest intake quartile with an adjusted OR (95% CI) of 0.51 (0.28–0.93) compared with the lowest quartile ($P=0.036$ for trend). No other dietary fatty acid or the n-6/n-3 ratio was found to be related to disease risk.

After stratification for age groups, only in the youngest group (<40 y) the relative risk significantly decreased with increasing intake of ALA. The adjusted OR in the highest quartile (*vs* lowest) was 0.35 (0.13–0.92) with *P* for trend of 0.020.

Associations between dietary fatty acids and allergic rhinitis

An inverse association could also be detected between ALA intake and the risk of allergic rhinitis (Table 6). The OR for subjects in the highest quartile (*vs* lowest) was 0.48 (95% CI = 0.23–1.00, $P=0.070$ for trend) in the crude model and was slightly strengthened in the adjusted model ($OR_{adj}=0.43$, 95% CI = 0.20–0.93, $P=0.050$ for trend). No other dietary fatty acids were significantly associated with disease outcome.

Discussion

This is the first cross-sectional study in which associations between RBC membrane fatty acid composition and allergic sensitisation and allergic rhinitis have been investigated in an adult population. For both end points, an inverse association with increasing portions of EPA was observed. This effect is supported by the dietary fatty acid intake results, showing a negative association between the intake of ALA, the main dietary n-3 PUFA and precursor of EPA, and disease risk.

Besides the consistent findings for EPA in RBC, positive associations were seen for the AA/EPA ratio; an increased OR in the fourth quartile compared with the first mainly reflects the effect of EPA on the risk of allergic sensitisation and

Table 5 Crude and adjusted[a] odds ratios (OR) and 95% confidence intervals (95% CI) for the association between the dietary intake of selected unsaturated fatty acids and allergic sensitisation[b]

Fatty acid	*First quartile*	*Second quartile*	*Third quartile*	*Fourth quartile*	P *for trend*
C 18:2n-6					
No. of cases	42	36	41	28	
Range (% of energy)	<3.82	3.82–5.05	5.05–6.59	>6.59	
Crude OR (95% CI)	1	0.82 (0.48–1.38)	0.96 (0.57–1.60)	0.59 (0.34–1.02)	0.115
Adjusted OR (95% CI)[a]	1	0.82 (0.47–1.42)	0.98 (0.57–1.68)	0.62 (0.35–1.10)	0.188
C 20:4n-6					
No. of cases	44	31	37	35	
Range (‰ of energy)	<0.46	0.46–0.68	0.68–0.99	>0.99	
Crude OR (95% CI)	1	0.62 (0.36–1.06)	0.78 (0.47–1.32)	0.73 (0.44–1.24)	0.385
Adjusted OR (95% CI)[a]	1	0.65 (0.37–1.14)	0.79 (0.46–1.38)	0.70 (0.40–1.21)	0.308
C 18:3n-3					
No. of cases	44	40	39	24	
Range (‰ of energy)	<4.72	4.72–5.41	5.41–6.32	>6.32	
Crude OR (95% CI)	1	0.89 (0.53–1.49)	0.83 (0.50–1.39)	0.45 (0.26–0.80)	0.008
Adjusted OR (95% CI)[a]	1	0.93 (0.54–1.61)	0.88 (0.51–1.52)	0.51 (0.28–0.93)	0.036
C 20:5n-3					
No. of cases	43	36	31	37	
Range (‰ of energy)	<0.05	0.05–0.11	0.11–0.44	>0.44	
Crude OR (95% CI)	1	0.77 (0.46–1.30)	0.63 (0.37–1.09)	0.80 (0.48–1.35)	0.307
Adjusted OR (95% CI)[a]	1	0.92 (0.53–1.60)	0.74 (0.42–1.31)	0.84 (0.48–1.46)	0.419

[a]Adjusted for sex, age group (<40, 40–59, ≥60 y), socio-economic status (low, middle, high) and smoking status (never, ex, current).
[b]Allergic sensitisation was defined as specific IgE concentration of ≥0.7 kU/l (CAP-class ≥2); cases $n=150$.

Table 6 Crude and adjusted[a] odds ratios (OR) and 95% confidence intervals (95% CI) for the association between the dietary intake of selected unsaturated fatty acids and allergic rhinitis[b]

Fatty acid	*First quartile*	*Second quartile*	*Third quartile*	*Fourth quartile*	P *for trend*
C 18:2n-6					
No. of cases	24	17	23	13	
Crude OR (95% CI)	1	0.67 (0.34–1.31)	0.94 (0.50–1.76)	0.50 (0.24–1.02)	0.137
Adjusted OR (95% CI)[a]	1	0.70 (0.35–1.39)	0.98 (0.052–1.87)	0.50 (0.24-1.05)	0.160
C 20:4n-6					
No. of cases	23	18	14	22	
Crude OR (95% CI)	1	0.75 (0.39–1.46)	0.56 (0.28–1.14)	0.95 (0.50–1.80)	0.695
Adjusted OR (95% CI)[a]	1	0.83 (0.42–1.66)	0.66 (0.32–1.37)	1.05 (0.55–2.02)	0.964
C 18:3n-3					
No. of cases	23	21	21	12	
Crude OR (95% CI)	1	0.90 (0.47–1.71)	0.89 (0.47–1.70)	0.48 (0.23–1.00)	0.070
Adjusted OR (95% CI)[a]	1	0.76 (0.38–1.47)	0.80 (0.41–1.58)	0.43 (0.20–0.93)	0.050
C 20:5n-3					
No. of cases	23	19	15	20	
Crude OR (95% CI)	1	0.80 (0.41–1.54)	0.61 (0.30–1.22)	0.85 (0.44–1.63)	0.471
Adjusted OR (95% CI)[a]	1	1.00 (0.51–1.99)	0.76 (0.37–1.55)	1.01 (0.52–1.97)	0.834

[a]Adjusted for sex, age group (<40, 40–59, ≥60 y), socio-economic status (low, middle, high) and smoking status (never, ex, current).
[b]Allergic rhinitis was defined as reported allergic rhinitis diagnosed by a physician; cases $n=77$.

allergic rhinitis. Owing to the high correlation coefficient between AA and EPA, no interaction terms were investigated. On the other hand, the n-3/n-6 ratio (RBC, diet) did not affect disease risk. Therefore, we cannot confirm the hypothesis of Black & Sharpe (1997) proposing an increased risk of developing allergic diseases as a consequence of an unbalanced dietary n-3/n-6 ratio. This is also supported by other recent work applying serum phospholipid fatty acids as biomarkers (Kompauer *et al*, 2004).

Previous studies on fatty acids and the risk of allergic sensitisation or hay fever mainly relied on dietary intake data (Wakai *et al*, 2001; Nagel *et al*, 2003; Trak-Fellermeier *et al*,

2004). Such data may be subject to (recall) bias (Arab & Akbar, 2002) and cannot consider interindividual differences in fatty acid metabolism. Therefore, we used the fatty acid composition of cell membranes as a measure of the biologically available amount of PUFA in cellular membranes. Owing to the low correlation coefficients for the relationship between dietary fatty acid intake and membrane fatty acid content, the latter can hardly be called a biomarker of dietary intake. However, it has to be reminded that the chosen dietary assessment method do not necessarily reflect an individual's long-term dietary intake. On the other hand, incorporation of n-3 PUFA, in particular EPA, in RBC membranes is well studied and can be influenced in a time- and dose-dependent manner. Intervention studies have shown that changes in EPA occur mainly at the expense of AA and LA (von Schacky *et al*, 1985; Katan *et al*, 1997). Similar observations were made for peripheral blood mononuclear cells (PBMC) (Gibney & Hunter, 1993; Yaqoob *et al*, 2000). Owing to the usually low amount of available immune competent cells in epidemiological studies we chose RBC membranes as a proxy. With respect to the life span of RBCs of about 120 days a long time frame was covered. In contrast, the fatty acid composition of serum phospholipids, which is frequently used as a biomarker of intake is expected to reflect the fatty acid intake within the past days or weeks (Arab, 2003) but does hardly represent the biologically important source of precursors for eicosanoid formation, that is, membrane PUFA.

Concerning the available epidemiologic evidence in adults, a protective role of dietary EPA intake on hay fever risk was reported in a German prospective study (Nagel *et al*, 2003) while evaluations of a Finnish cross-sectional study have shown lower concentrations of EPA in plasma cholesteryl esters in subjects who developed allergic dermatitis but not rhinitis after 6-y follow-up (Dunder *et al*, 2001). Our findings do not support most other results in the literature. In a cross-sectional study no associations were observed between any n-3 and n-6 PUFA in serum phospholipids and allergic sensitisation in young adults as assessed by skin-prick test (Woods *et al*, 2004). The latter and specific IgE measurement may be considered complementary to one another (Droste *et al*, 1996). Using data from the Second National Health and Nutrition Survey (USA), even a positive association between fish intake as a source of n-3 PUFA and wheezing was observed among adults (Schwartz & Weiss, 1990). In a cross-sectional study among Japanese women, (Wakai *et al* (2001) found a positive association between n-6 PUFA intake and symptoms of seasonal allergic rhinoconjunctivitis. Also, a recently published cross-sectional study reported a positive relationship between AA intake and allergic sensitisation among females (Trak-Fellermeier *et al*, 2004). Concerning monounsaturated fatty acids, Heinrich *et al* (2001) saw a positive association between monounsaturated fatty acid intake and the prevalence of allergic sensitisation in an ecological study among adults. Additionally, in a German subsample of the prospective EPIC cohort, a high intake of oleic acid was found to be positively associated with hay fever (Nagel *et al*, 2003). However, in the present study no relationship between atopy or allergic rhinitis and oleic acid by means of the RBC membrane fatty acid data or dietary intake data was detected.

A possible explanation for the discrepancy between our results and other reports from epidemiologic studies among adults may be due to the methodological differences in fatty acid assessment. There is a major difference between dietary intake of n-3 and n-6 PUFA and their proportions in cell membranes, such as RBC membranes. Low quantities of EPA and AA are found in the typical Western diet (eg 0.04 and 0.6%, respectively, in BVS II) but may represent about 1 and 15% or more in the membranes of RBCs, respectively (Sanders, 2000; Linseisen *et al*, 2003). It cannot be excluded that small differences in dietary intake of PUFA, especially EPA and AA, cannot be detected with sufficient accuracy when using protocol methods to explore associations between the intake of fatty acids and allergy and atopy, respectively. The metabolism of absorbed fatty acids contributes substantially to the differences between dietary and membrane fatty acid composition. This also included individual variation, for example, in genes encoding for enzymes involved in fatty acid transport, oxidation, desaturation, and elongation. In the present study, the results drawn from dietary intake data confirm the membrane-based findings: A higher intake of n-3 PUFA is associated with a lower prevalence of allergic sensitisation and rhinitis. Owing to an ongoing low intake of fish in Southern Germany, EPA intake is low and ALA represents the main dietary n-3 PUFA (Linseisen *et al*, 2003). It has been shown that dietary supplementation of ALA leads to an increase in plasma EPA concentrations (Valsta *et al*, 1996), thereby confirming results in fetal/newborn rats on ALA *vs* DHA supplementation and its accretion in cells and tissues (Valenzuela *et al*, 2004). ALA intake decreased over the past decades, which in part may be due to a lower consumption of ALA-providing plant food but also due to a reduced concentration in food for technological reasons in food production and processing (Sanders 2000).

Our findings of an inverse association between long-chain n-3 PUFA and atopic disease are supported by results obtained among young individuals. Higher proportions of long-chain n-3 PUFA were found in umbilical cord serum phospholipids of nonallergic compared with allergic mothers. Also, a higher n-3 PUFA concentration in breast milk was associated with a decreased likelihood of atopy in the infant (Yu *et al*, 1996; Duchen *et al*, 1998). Moreover, proportions of DHA and total n-3 long-chain PUFA in serum phospholipids of school children were higher among nonatopic controls than among children with atopy (Yu & Bjorksten, 1998). It is discussed whether an intervention at an earlier age, before allergic immune responses are established, might provide clearer health benefits (Calder, 2003). With respect to asthma, intervention studies with n-3 PUFA seem to support a protective effect of n-3 PUFA on the onset

of allergic diseases (asthma) in childhood (Nagakura *et al*, 2000; Mihrshahi *et al*, 2003). In a Norwegian study, early introduction of fish in the diet was inversely associated with the risk of allergic rhinitis (Nafstad *et al*, 2003). Also an Australian study that offered fish oil supplementation to pregnant women provided indications for a reduction in subsequent allergy risk among infants (Dunstan *et al*, 2003).

Some methodological issues should be kept in mind when interpreting these results. We cannot rule out the possibility of reverse causation in this cross-sectional study. This may apply to subjects suffering from allergic rhinitis if fatty acid intake is affected by modified food selection patterns (eg fish avoidance). In sensitised subjects without clinical symptoms, it seems unlikely that diet–disease relationships were modified as a result of reverse causation. However, we could not find a distinct difference in fish intake between cases and the population sample. On average, fish intake was even higher in sensitised subjects (37 *vs* 32 g/day) and subjects suffering from allergic rhinitis (36 *vs* 33 g/day) as compared to nonaffected participants.

Self-reports of allergic rhinitis (during the face-to-face interview) may be biased, although this problem should be minimised since we asked for a physician's diagnose of the disease. The observed frequency of sensitisation among subjects suffering from allergic rhinitis is in concordance with the expected number and, therefore, gives further reassurance for the validity of the self-reports. Another aspect is the definition of allergic rhinitis, which can be divided into perennial, occupational, and seasonal rhinitis based on the time of exposure (Bousquet *et al*, 2001). The latter is also known as hay fever. However, perennial allergic rhinitis may not necessarily be excluded by the criteria we used. It would be difficult to completely differentiate seasonal allergic rhinitis from nonseasonal disease due to their frequent coexistence (Sibbald & Rink, 1991).

The prevalence of allergic rhinitis and allergic sensitisation in our study is in good agreement with previously published data with younger subjects being more often affected (Filipiak *et al*, 2001; Nagel *et al*, 2003; Trak-Fellermeier *et al*, 2004). However, the differences between genders are hard to explain. After stratification for age, associations between allergic sensitisation and allergic rhinitis on the one hand and EPA on the other hand were seen for the age groups up to 60 y. Therefore, we included age groups as a confounding factor in the logistic regression model. We also adjusted for smoking and socio-economic status because these characteristics are known as possible confounders for the tested associations (von Mutius, 2000).

Conclusion

The strength of this study was the use of RBC membranes to assess biologically available fatty acids at the cellular level and its association with allergic sensitisation and allergic rhinitis in a cross-sectional population-based study. Increasing proportions of EPA in RBC membranes were associated with a lower risk of allergic sensitisation and allergic rhinitis. In good agreement, a higher dietary ALA intake was associated with a decrease in allergic sensitisation and clinical symptoms of allergic rhinitis. The consistency found for both exposure variables and both health outcomes seems to reflect a valid pattern of association and, therefore, gives support to the hypothesis of a favourable effect of increased n-3 fatty acid consumption even in adulthood. However, with the present study design no causal relationship can be established.

Acknowledgements

We acknowledge the cooperation of the study participants as well as the work of all co-workers involved in the sampling of data and biological specimens. We especially thank the physicians from the health offices in Bavaria for providing study rooms and for drawing the blood samples and Drs Weidner and Gabrio, Landesgesundheitsamt Baden-Württemberg, for IgE measurements.

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