

ORIGINAL PAPER

Fatty acids in serum cholesteryl esters in relation to asthma and lung function in children

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Summary

Background Dietary fatty acid intake has been proposed to contribute to asthma development with n-6 polyunsaturated fatty acids (PUFA) having a detrimental and n-3 PUFA a protective effect.

Objective The aim of our analysis was to explore the relationship between fatty acid composition of serum cholesteryl esters as marker of dietary intake and prevalence of asthma, impaired lung function and bronchial hyper-responsiveness in children.

Methods The study population consisted of 242 girls and 284 boys aged 8–11 years, living in Munich, Germany. Data were collected by parental questionnaire, lung function measurement and skin prick test according to the International Study of Asthma and Allergies in Childhood phase II protocol. Confounder-adjusted odds ratios (OR) with 95% confidence intervals (CI) were calculated for the association between quartiles of fatty acid concentration and health outcomes with the first quartile as reference.

Results n-3 PUFA: levels of eicosapentaenoic acid were not related to asthma and impaired lung function. Linolenic acid levels were positively associated with current asthma (OR for fourth quartile 3.35, 95% CI 1.29–8.66). Forced expiratory volume in 1 s (FEV₁) values decreased with increasing levels of linolenic acid (*p* for trend = 0.057). n-6 PUFA: there was a strong positive association between arachidonic acid levels and current asthma (OR_{4th quartile} 4.54, 1.77–11.62) and a negative association with FEV₁ (*P* = 0.036). In contrast, linoleic acid was negatively related to current asthma (OR_{4th quartile} 0.34, 0.14–0.87) and FEV₁ values increased with increasing levels of linoleic acid (*P* = 0.022). The ratio of measured n-6 to n-3 PUFA as well as levels of palmitic and oleic acid were not consistently related to asthma or lung function.

Conclusion Our data do not support the hypothesis of a protective role of n-3 PUFA. Elevated arachidonic acid levels in children with asthma may be because of a disturbed balance in the metabolism of n-6 PUFA or may be secondary to inflammation in these patients.

Keywords asthma, bronchial hyper-responsiveness, n-3 fatty acids, n-6 fatty acids, wheeze
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Introduction

Changes in diet, especially in its fatty acid composition, have been hypothesized to contribute to the worldwide increase in asthma [1–3]. Typical Western diets are characterized by an imbalance of n-6 to n-3 polyunsaturated fatty acids (PUFA): the ratio has risen considerably from between 1:1 to 2:1 in former times to a ratio of 20:1 to 30:1 recently [4].

n-6 and n-3 PUFA are both precursors of eicosanoids as inflammatory mediators. Whereas linoleic acid (18:2 n-6) can be converted to arachidonic acid (20:4 n-6), a precursor of the highly pro-inflammatory two-series prostaglandins and four-series leukotrienes, α -linolenic acid (18:3 n-3) is metabolized to eicosapentaenoic acid (20:5 n-3), a precursor of the less inflammatory three-series prostaglandins and five-series leukotrienes, and which

competitively inhibits the metabolism of arachidonic acid. Eicosanoids derived from arachidonic acid are important mediators of allergic inflammation. Moreover, prostaglandin E₂ inhibits the production of T-helper type 1 (Th1)-type cytokines without interfering with Th2-type cytokines, and stimulates IgE-production by B lymphocytes [5].

Several studies investigating the impact of fat consumption or fish oil supplementation in children supported the fatty acid hypothesis. A high dietary intake of polyunsaturated fats, mainly n-6 PUFA, has been shown to be associated with an increased risk of wheeze or asthma [6–8]. On the other hand, consumption of fish or fish oil as main source of n-3 PUFA seemed to decrease asthma risk [9, 10].

Fatty acid levels in the blood are biochemical markers of dietary intake and may be used in observational studies to investigate relative patterns of fat intake [11, 12]. The composition of fatty acids in serum cholesteryl esters is insensitive to daily variation in diet and reflects the composition of dietary fat consumed in preceding weeks [13]. Compared with the other lipid fractions of serum, cholesteryl esters have the advantage of a greater stability of its fatty acid composition during storage and are therefore best suited for epidemiological studies [14].

Only a few studies analysed the fatty acid composition of cholesteryl esters in relation to asthma in children and these gave conflicting results. Leichsenring *et al.* [15] compared 17 asthmatic children with 10 healthy controls and observed higher levels of linoleic acid and lower levels of arachidonic acid in plasma cholesteryl esters of the asthmatics. In contrast, Dunder *et al.* [16] found no differences in serum cholesteryl ester fatty acids between 47 asthmatic children and 47 controls.

The objective of our study was to investigate the association between fatty acid composition of serum cholesteryl esters and prevalence of asthma symptoms, physician-diagnosed asthma, impaired lung function as well as bronchial hyper-responsiveness in a population-based sample of children.

Materials and methods

Study subjects

This analysis is based on data of a cross-sectional survey of 8–11 years old children in Munich, Germany, which used phase II modules of the International Study of Asthma and Allergies in Childhood (ISAAC). The methods of the study have been described in detail elsewhere [17]. Based on those children with German nationality, questionnaire data and total serum IgE measurement ($N = 665$), a nested case-control study population was identified for fatty acid measurement and analysis of the association of fatty acid levels with asthma (Fig. 1). All children with

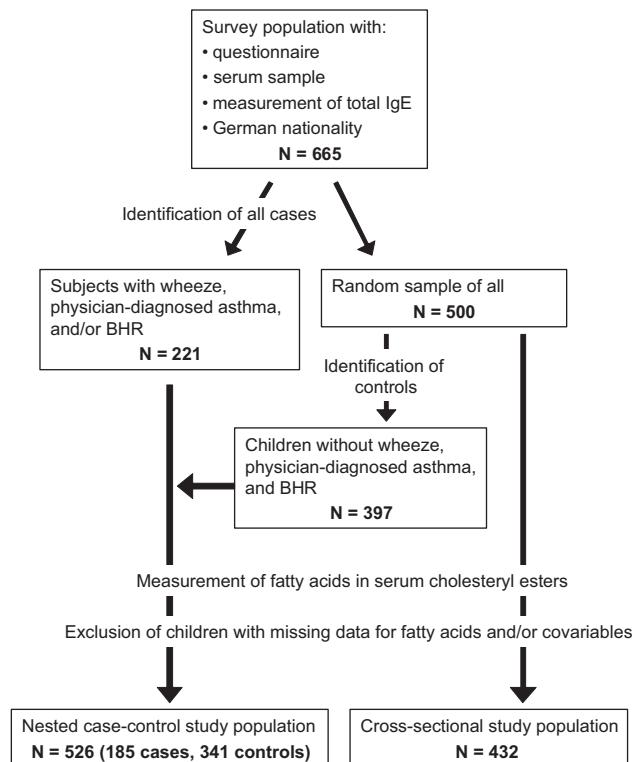


Fig. 1. Flowchart of the definition of the nested case-control and the cross-sectional study population for analyses.

wheeze, physician-diagnosed asthma and/or bronchial hyper-responsiveness were defined as cases ($N = 221$ out of 665). For the identification of controls, a random sample of 500 children out of the survey population of 665 children was drawn first and then within this sample all children without wheeze, physician-diagnosed asthma and bronchial hyper-responsiveness were identified ($N = 397$ out of 500). After exclusion of 92 children with missing data for fatty acids and/or covariables, 526 children (46% girls; 185 cases, 341 controls) remained for analysis. For the analysis of the relationship between fatty acid levels and lung function, all children of the random sample with data for fatty acids and covariables ($N = 432$ out of 500, 47% girls) were eligible (Fig. 1).

Outcome variables

Wheeze was defined as a parent's report of at least one episode of wheezing in the past 12 months. In addition, speech-limiting wheeze and exercise-induced wheeze during the past 12 months were assessed by questionnaire. Current asthma was defined as parent's report of a physician-diagnosis of 'asthma' in the child at least once or 'asthmatic, spastic or obstructive bronchitis' more often than once in combination with wheeze. Children were classified as having frequent asthma attacks if the parents reported at least four attacks during the past 12 months.

Atopic sensitization to six common aero allergens was assessed by skin prick test (SPT). Lung function was measured with a spirometer using the criteria of the American Thoracic Society. Predicted lung function values were standardized for age, height and weight separately for girls and boys. A hypertonic saline challenge for determination of bronchial hyper-responsiveness (BHR) was performed in a random subsample as previously described [17].

Fatty acid composition of cholesteryl esters

Twenty-five microlitres serum to which 100 µL of the internal standard solution (1 mg/mL cholesteryl heptadecanoate/0.001% butylated hydroxyl toluene (BHT) in chloroform) and 500 µL 0.9% NaCl were added, was extracted twice with 500 µL hexane-isopropanol 3:2 (v/v). The combined organic phases were dried under nitrogen (40 °C) and then solubilized with 100 µL of isopropanol/n-heptane/acetonitrile 35:12:52 (v/v). Twenty microlitres of this solution was introduced into the HPLC device (Kontron, Neufahrn, Germany) and cholesteryl esters were analyzed as previously described [18]. Fatty acid concentrations in serum cholesteryl esters were calculated from the measured ester concentrations and expressed as mole percentages of the sum of all analyzed fatty acids.

Statistical analyses

For the comparison of proportions, χ^2 tests were used. To assess differences between continuous variables, *t*-tests were performed. In multivariate analysis, confounder-adjusted effect estimates (odds ratios (OR) or regression coefficients) with 95% confidence intervals (CI) were calculated for the association between quartiles of fatty

acid concentration and outcomes, using the first quartile as reference in multiple logistic and linear regression models. Effect estimates were adjusted for sex, age, parental education and parental asthma. The nested case-control study population was used for separately analysing wheeze, asthma and BHR. The reference group consisted exclusively of children without wheeze, physician-diagnosed asthma and BHR. The random sample was used for analysis of lung function parameters. For analysing trends in multivariate regression models, the median of each quartile of the independent variables was included as an ordinal variable into the models. All analyses were conducted using the SAS software package version 8.2 (SAS Institute Inc., Cary, NC, USA).

Results

Characteristics of the study populations

Demographic characteristics of the nested case-control study population and prevalence of asthma outcomes are given in Table 1. Out of the 526 children of this study population, 185 (35%) were defined as cases having wheeze, physician-diagnosed asthma and/or BHR.

Lung function measurements were analysed in the random sample (*N* = 432). The demographic characteristics did not differ from the case-control study population (data not shown). The prevalence of the main asthma outcomes in that sample was 10% for wheeze, 7% for asthma and 21% for BHR. Data on lung function parameters were available for 221 children. Mean values (standard deviation) were 2.11 L (0.32) [100% (10%) predicted] for the forced expiratory volume in 1 s (FEV₁) and 2.39 L (0.37) [101% (10%) predicted] for the forced vital capacity (FVC).

Table 1. Characteristics of the nested case-control study population (*N* = 526)

	All		Girls		Boys	
	<i>n/N</i>	(%)	<i>n/N</i>	(%)	<i>n/N</i>	(%)
Age (years)						
8–9	291/526	55.3	131/242	54.1	160/284	56.3
10–11	235/526	44.7	111/242	45.9	124/284	43.7
High parental education	293/526	55.7	135/242	55.8	158/284	55.6
Parental asthma	73/526	13.9	34/242	14.1	39/284	13.7
Wheeze during past 12 months	113/526	19.6	38/242	15.7	65/284	22.9
Frequent asthma attacks during past 12 months	32/512	6.3	12/235	5.1	20/277	7.2
Speech-limiting wheeze during past 12 months	35/526	6.7	16/242	6.6	19/284	6.7
Exercise-induced wheeze during past 12 months	65/522	12.5	26/241	10.8	39/281	13.9
Atopic wheeze	49/517	9.5	12/237	5.1	37/280	13.2
Current asthma	66/526	12.6	21/242	8.7	45/284	15.9
Atopic current asthma	35/518	6.8	8/238	3.4	27/280	9.6
Bronchial hyper-responsiveness	50/226	22.1	17/103	16.5	33/123	26.8
Non-atopic wheeze	45/502	9.0	21/231	9.1	24/271	8.9
Non-atopic current asthma	23/502	4.6	9/231	3.9	14/271	5.2

Table 2. Fatty acid composition of serum cholesteryl esters

		All (N = 526)		Girls (N = 242)		Boys (N = 284)		P-value
		Mean	SD	Mean	SD	Mean	SD	
SFA	16:0	10.8	1.3	10.9	1.3	10.8	1.4	0.311
MUFA	18:1	18.7	2.2	18.7	2.3	18.7	2.2	0.759
PUFA	18:2 n-6	51.0	3.6	51.4	3.6	50.6	3.6	0.008
	20:4 n-6	18.2	2.6	17.8	2.5	18.7	2.7	< 0.001
	18:3 n-3	0.5	0.1	0.4	0.1	0.5	0.1	0.335
	20:5 n-3	0.8	0.2	0.8	0.2	0.8	0.2	0.572

Data are presented as mean percentage of all measured fatty acids with standard deviation (SD) for the nested case-control study population. Differences between girls and boys were assessed with the t test.

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

The fatty acid composition of serum cholesteryl esters is given for the nested case-control study population in Table 2. Girls had higher levels of linoleic acid, whereas boys had higher levels of arachidonic acid.

Nested case-control study population: fatty acid levels and asthma

Bivariate analysis. In bivariate analysis, fatty acid serum cholesteryl ester composition varied by health status only in regard to the n-6 PUFA linoleic acid and arachidonic acid. Children with wheeze, physician-diagnosed asthma, and/or BHR (N = 185) had lower linoleic acid values (mean 50.5%, SD 3.1%, vs. 51.2%, SD 3.9%, $P = 0.021$) and higher arachidonic acid values (mean 18.7%, SD 2.4%, vs. 18.0%, SD 2.7%, $P = 0.001$) than children not affected by any of the three outcomes (N = 341).

Multivariate analysis. In multivariate analyses of the several asthma measures, the reference group consisted exclusively of children without wheeze, physician-diagnosed asthma and bronchial hyper-responsiveness (N = 341).

n-6 polyunsaturated fatty acids: linoleic acid. High linoleic acid levels in the fourth quartile were negatively associated with wheeze, speech-limiting wheeze and current asthma (Table 3). By combining the questionnaire data on wheeze and current asthma with the results of the SPT, we further differentiated between atopic and non-atopic outcomes. The negative association with linoleic acid levels was more pronounced for non-atopic wheeze and non-atopic current asthma (data not shown in tables). The OR (95% CI) of the fourth quartile of linoleic acid was 0.77 (0.30–1.96) for atopic wheeze vs. 0.27 (0.07–1.01) for non-atopic wheeze and 0.61 (0.20–1.84) for atopic current asthma vs. 0.20 (0.02–1.77) for non-atopic current asthma.

The results for bronchial hyper-responsiveness are presented in Table 4. Linoleic acid levels were not related to BHR.

n-6 polyunsaturated fatty acids: arachidonic acid. There was a consistent pattern of a positive association of high arachidonic acid levels with wheeze, speech-limiting wheeze, frequent asthma attacks and current asthma (Table 3). Odds ratios were also increasing for exercise-induced wheeze with increasing arachidonic acid levels (OR for the fourth quartile 7.14, 95% CI 2.55–19.99, p for trend < 0.001). In children with physician-diagnosed asthma, but no wheeze during the past 12 months, the association with arachidonic acid levels did not reach statistical significance (OR (95% CI) for the first to the fourth quartile: 1.0 (reference), 1.01 (0.41–2.51), 1.65 (0.71–3.82), 1.98 (0.87–4.52), p for trend = 0.062).

In the separate analysis of atopic and non-atopic outcomes, the positive association between arachidonic acid levels and asthma was more pronounced for non-atopic wheeze and non-atopic current asthma (data not shown in tables). The OR (95% CI) of the fourth quartile of arachidonic acid was 2.38 (0.92–6.15) for atopic wheeze vs. 3.35 (1.12–10.04) for non-atopic wheeze and 3.08 (0.96–9.92) for atopic current asthma vs. 11.36 (1.36–94.51) for non-atopic current asthma.

In contrast to the asthma outcomes defined by questionnaire data, there was no statistically significant association of arachidonic acid with bronchial hyper-responsiveness (Table 4), but the effect estimate increased from the first to the fourth quartile of arachidonic acid levels.

n-3 polyunsaturated fatty acids: linolenic acid. There was the tendency of increased odds for the asthma measures in those children with linolenic acid levels above the first quartile, showing a positive trend in the case of frequent asthma attacks (Table 3). The differentiation between atopic and non-atopic outcomes showed that high levels of linolenic acid were associated with atopic wheeze (OR for the fourth quartile 2.59 (0.96–6.96)) and atopic asthma (5.49 (1.38–21.75)) in contrast to the non-atopic outcomes (non-atopic wheeze: OR 1.43 (0.53–3.86), non-atopic asthma: 1.98 (0.44–8.92)).

Table 3. Adjusted odds ratios with 95% confidence intervals for the association of fatty acids in serum cholesteryl esters with the prevalence of wheeze and asthma during last 12 months

	Quartiles of fatty acid or ratio				
	Q1	Q2	Q3	Q4	p for trend
Wheeze					
MUFA: 18:1	1.00	1.61 (0.82–3.20)	2.23 (1.16–4.31)	1.20 (0.60–2.42)	0.470
PUFA: 18:2 n-6	1.00	1.33 (0.71–2.47)	1.35 (0.73–2.50)	0.41 (0.19–0.87)	0.040
20:4 n-6	1.00	2.56 (1.26–5.23)	2.00 (0.96–4.17)	2.90 (1.41–5.97)	0.014
18:3 n-3	1.00	2.64 (1.33–5.24)	1.78 (0.86–3.66)	1.91 (0.95–3.85)	0.250
20:5 n-3	1.00	1.20 (0.62–2.31)	1.23 (0.64–2.36)	1.14 (0.59–2.20)	0.761
n-6/n-3 PUFA	1.00	1.45 (0.78–2.71)	1.18 (0.62–2.24)	0.79 (0.41–1.55)	0.413
18:2 n-6/18:3 n-3	1.00	1.09 (0.59–2.02)	1.36 (0.75–2.49)	0.38 (0.18–0.81)	0.038
20:4 n-6/18:2 n-6	1.00	3.12 (1.50–6.48)	2.85 (1.37–5.94)	2.53 (1.20–5.35)	0.049
Frequent asthma attacks					
MUFA: 18:1	1.00	1.82 (0.49–6.69)	4.24 (1.30–13.85)	1.22 (0.32–4.69)	0.539
PUFA: 18:2 n-6	1.00	0.96 (0.35–2.57)	0.95 (0.35–2.53)	0.26 (0.07–1.01)	0.070
20:4 n-6	1.00	3.85 (0.99–14.91)	2.16 (0.49–9.48)	5.48 (1.40–21.43)	0.033
18:3 n-3	1.00	3.73 (0.91–15.28)	4.15 (1.02–16.82)	5.00 (1.29–19.36)	0.026
20:5 n-3	1.00	2.49 (0.79–7.81)	0.93 (0.25–3.51)	1.87 (0.59–5.91)	0.559
n-6/n-3 PUFA	1.00	0.81 (0.28–2.31)	1.40 (0.53–3.70)	0.38 (0.11–1.30)	0.243
18:2 n-6/18:3 n-3	1.00	0.95 (0.36–2.48)	1.03 (0.38–2.77)	0.13 (0.03–0.66)	0.018
20:4 n-6/18:2 n-6	1.00	2.66 (0.75–9.46)	2.08 (0.55–7.84)	3.70 (1.07–12.79)	0.060
Speech-limiting wheeze					
MUFA: 18:1	1.00	1.24 (0.45–3.43)	1.60 (0.60–4.27)	0.81 (0.28–2.38)	0.827
PUFA: 18:2 n-6	1.00	1.35 (0.54–3.40)	1.21 (0.48–3.08)	0.17 (0.04–0.81)	0.036
20:4 n-6	1.00	12.59 (1.57–101.3)	13.02 (1.60–106.2)	18.93 (2.36–151.6)	0.002
18:3 n-3	1.00	2.51 (0.80–7.84)	2.34 (0.73–7.49)	2.65 (0.86–8.21)	0.134
20:5 n-3	1.00	4.11 (1.26–13.40)	2.27 (0.64–8.07)	2.35 (0.68–8.15)	0.592
n-6/n-3 PUFA	1.00	1.06 (0.42–2.68)	0.91 (0.34–2.45)	0.50 (0.17–1.45)	0.198
18:2 n-6/18:3 n-3	1.00	0.93 (0.37–2.36)	1.12 (0.45–2.79)	0.20 (0.05–0.76)	0.032
20:4 n-6/18:2 n-6	1.00	8.23 (1.74–38.89)	6.42 (1.31–31.48)	8.30 (1.75–39.40)	0.019
Current asthma					
MUFA: 18:1	1.00	1.13 (0.48–2.69)	2.02 (0.92–4.45)	1.22 (0.53–2.78)	0.440
PUFA: 18:2 n-6	1.00	1.14 (0.55–2.37)	1.03 (0.50–2.15)	0.34 (0.14–0.87)	0.038
20:4 n-6	1.00	3.34 (1.30–8.58)	2.11 (0.79–5.64)	4.54 (1.77–11.62)	0.007
18:3 n-3	1.00	4.50 (1.76–11.50)	2.89 (1.07–7.82)	3.35 (1.29–8.66)	0.082
20:5 n-3	1.00	1.05 (0.47–2.34)	1.08 (0.49–2.38)	1.00 (0.45–2.22)	0.984
n-6/n-3 PUFA	1.00	1.36 (0.64–2.89)	1.21 (0.56–2.65)	0.77 (0.34–1.73)	0.502
18:2 n-6/18:3 n-3	1.00	0.94 (0.45–1.98)	1.34 (0.66–2.71)	0.21 (0.07–0.62)	0.020
20:4 n-6/18:2 n-6	1.00	3.99 (1.56–10.23)	2.51 (0.94–6.67)	3.66 (1.42–9.41)	0.039

Nested case-control study population. Odds ratios were adjusted for sex, age, parental education and parental asthma.

MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Ratio n-6/n-3 PUFA = (18:2 n-6 + 20:4 n-6)/(18:3 n-3 + 20:5 n-3).

Levels of linolenic acid were not associated with BHR (Table 4).

n-3 polyunsaturated fatty acids: eicosapentaenoic acid. Levels of eicosapentaenoic acid were neither related to asthma (Table 3) nor to BHR (Table 4).

Ratios of n-3 and n-6 polyunsaturated fatty acids. In addition to single fatty acids we analysed ratios of n-6/n-3 PUFA and of desaturation and elongation product/essential fatty acid as precursor to disentangle effects of

dietary intake of essential fatty acids and potential imbalances in the metabolism of long-chain fatty acids.

The ratio of arachidonic to linoleic acid (20:4 n-6/18:2 n-6) showed a positive relationship with wheeze and asthma (Table 3), but not with BHR (Table 4) for values above the first quartile.

Children with high ratios of linoleic to linolenic acid (18:2 n-6/18:3 n-3) had decreased odds for wheeze, frequent asthma attacks, speech-limiting wheeze, exercise-induced wheeze and current asthma (Table 3). There was no association with BHR (Table 4).

Table 4. Adjusted odds ratios with 95% confidence intervals for the association of fatty acids in serum cholesteryl esters with the prevalence of bronchial hyper-responsiveness (BHR)

	Quartiles of fatty acid or ratio				p for trend
	Q1	Q2	Q3	Q4	
MUFA: 18:1	1.00	0.81 (0.33–1.96)	0.71 (0.27–1.92)	0.37 (0.13–1.00)	0.051
PUFA: 18:2 n-6	1.00	1.96 (0.74–5.18)	0.89 (0.33–2.46)	1.54 (0.59–4.01)	0.657
20:4 n-6	1.00	0.96 (0.36–2.55)	1.33 (0.50–3.56)	1.87 (0.72–4.84)	0.152
18:3 n-3	1.00	1.58 (0.60–4.18)	1.58 (0.61–4.13)	1.01 (0.38–2.67)	0.934
20:5 n-3	1.00	1.61 (0.63–4.08)	1.14 (0.44–2.96)	0.71 (0.28–1.80)	0.330
n-6/n-3 PUFA	1.00	3.27 (1.16–9.18)	2.28 (0.83–6.26)	2.05 (0.77–5.40)	0.259
18:2 n-6/18:3 n-3	1.00	1.35 (0.50–3.61)	1.08 (0.42–2.81)	1.17 (0.45–3.05)	0.868
20:4 n-6/18:2 n-6	1.00	1.01 (0.38–2.71)	0.96 (0.37–2.51)	1.65 (0.64–4.22)	0.326

Nested case-control study population. Odds ratios were adjusted for sex, age, parental education and parental asthma.

MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Ratio n-6/n-3 PUFA = (18:2 n-6 + 20:4 n-6) / (18:3 n-3 + 20:5 n-3).

Table 5. Adjusted regression coefficients with 95% confidence intervals for the association of fatty acids in serum cholesteryl esters with the lung function parameter FEV₁ (% predicted).

	Quartiles of fatty acid or ratio				p for trend
	Q1	Q2	Q3	Q4	
MUFA: 18:1	0	– 1.40 (– 5.19; 2.38)	– 0.96 (– 5.00; 3.09)	0.17 (– 3.59; 3.93)	0.833
PUFA: 18:2 n-6	0	1.53 (– 2.22; 5.27)	2.77 (– 0.97; 6.50)	4.14 (0.45; 7.83)	0.022
20:4 n-6	0	– 0.28 (– 4.01; 3.45)	– 2.60 (– 6.43; 1.24)	– 3.43 (– 7.07; 0.21)	0.036
18:3 n-3	0	– 0.30 (– 4.12; 3.53)	– 1.54 (– 5.35; 2.27)	– 3.24 (– 6.85; 0.37)	0.057
20:5 n-3	0	– 0.47 (– 4.20; 3.27)	– 1.76 (– 5.53; 2.01)	– 1.05 (– 4.76; 2.66)	0.523
n-6/n-3 PUFA	0	0.71 (– 3.24; 4.65)	– 0.91 (– 4.65; 2.83)	2.55 (– 1.03; 6.14)	0.216
18:2 n-6/18:3 n-3	0	1.16 (– 2.74; 5.06)	2.61 (– 1.09; 6.31)	4.28 (0.69; 7.88)	0.014
20:4 n-6/18:2 n-6	0	– 2.31 (– 6.11; 1.48)	– 2.32 (– 6.01; 1.36)	– 5.38 (– 9.02; – 1.74)	0.005

Cross-sectional study population. Regression coefficients were adjusted for sex, age, parental education and parental asthma. FEV₁, forced expiratory volume in 1 s. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Ratio n-6/n-3 PUFA = (18:2 n-6 + 20:4 n-6) / (18:3 n-3 + 20:5 n-3).

No consistent associations with wheeze and asthma were observed for the ratios of all measured n-6 to n-3 PUFA, of eicosapentaenoic to linolenic acid (20:5 n-3/18:2 n-3), and of arachidonic to eicosapentaenoic acid (20:4 n-6/20:5 n-3).

The ratio of n-6 to n-3 fatty acids above the first quartile was positively associated with BHR, reaching statistical significance for the second quartile (OR 3.27, 95% CI 1.16–9.18). Correspondingly, the OR tended to increase with higher ratios of arachidonic to eicosapentaenoic acid (OR (95% CI) for the first to the fourth quartile: 1.0 (reference), 2.23 (0.78–6.34), 2.46 (0.91–6.65), 2.58 (0.96–6.91), *p* for trend = 0.063).

Monounsaturated fatty acids: oleic acid. Oleic acid levels (18:1) were not related to wheeze or asthma (Table 3). However, children with high levels of oleic acid had decreased odds for BHR (Table 4).

Saturated fatty acids: palmitic acid. Levels of palmitic acid (16:0) were not associated with wheeze or asthma

(data not shown). From the lowest to the highest quartile of palmitic acid levels, the OR (95% CI) of BHR was 1.0 (reference), 0.48 (0.19–1.24), 0.36 (0.14–0.94) and 0.38 (CI 0.15–0.97), respectively (*p* for trend = 0.036).

Random sample: fatty acid levels and lung function parameters

In accordance with the results for wheeze and asthma, linoleic acid and the ratio of linoleic to linolenic acid showed a protective association with FEV₁, while arachidonic acid, linolenic acid and the ratio of arachidonic to linoleic acid showed a detrimental association (Table 5).

The ratio of arachidonic to linoleic acid was also associated with FEV₁/FVC (regression coefficient for the fourth quartile – 2.44 (95% CI – 4.85; – 0.04), *p* for trend = 0.077) and with the maximal mid-expiratory flow (– 10.92 (– 19.72; – 0.86), *p* for trend = 0.042). The relationships between other fatty acid levels or ratios and the lung function parameters investigated (FEV₁/FVC,

peak expiratory flow, MMEF) were weaker or inconsistent (data not shown).

We tested all regression models for effect modification by sex. Further potential confounders such as type of heating and keeping pets were not associated with fatty acid levels and therefore not considered in multivariate analysis. As sensitivity analysis, we also adjusted the effect estimates for environmental tobacco smoke exposure but did not observe any change in the estimates (data not shown).

Discussion

There has been the widespread assumption of a detrimental effect of n-6 PUFA on asthma and of a protective effect of n-3 PUFA [1, 2]. But up to now results of studies on the relationship between fatty acids and asthma are inconsistent. Recent studies on asthma in adults gave no evidence for a protective role of n-3 PUFA and questioned the proposed detrimental effect of n-6 PUFA [19, 20]. We analysed the relationship between the fatty acid composition of serum cholesteryl esters as a crude marker of dietary intake and the prevalence of asthma, impaired lung function and bronchial hyper-responsiveness in a population-based random sample of children living in the south German city of Munich. Our study does not support the hypothesis of a protective role of n-3 PUFA and of a detrimental effect of a high dietary intake of linoleic acid or a high n-6/n-3 PUFA ratio. We observed, however, positive associations of elevated serum levels of arachidonic acid with asthma and reduced lung function.

n-6 polyunsaturated fatty acids

Our results showed on one hand a negative association between asthma prevalence and high levels of linoleic acid and of the ratio linoleic/linolenic acid. Thus our study does not support the hypothesis of a detrimental effect of a high dietary intake of n-6 PUFA. On the other hand, an important finding of our study was the strong association of increased arachidonic acid levels with asthma, which was more pronounced in children with non-atopic asthma, and of high values of the ratio of arachidonic to linoleic acid with decrements in FEV₁.

A previous small study with 47 case-control pairs reported no differences in serum cholesteryl ester fatty acid concentrations between children with and without asthma [16]. Woods et al. [19] analysed fatty acid levels in plasma phospholipids in adults and did not detect an association of asthma with levels of linoleic and arachidonic acid. In contrast to our data, a case-control study of 17 children with asthma and 10 age-matched controls showed higher plasma cholesteryl ester levels of linoleic

acid and lower levels of arachidonic acid in children with asthma [15]. Beside the much lower sample size, this study differed from ours by restriction to children with asthma, multiple atopic sensitization, total IgE levels > 1000 U/mL and no continuous systemic therapy with corticosteroids as cases and by usage of another method for measurement of fatty acids.

Our results for linoleic acid are in accordance with the study of Troisi et al. [21] who found an inverse association between dietary intake of linoleic acid and asthma incidence among participants of the Nurses' Health Study. A recent analysis of erythrocyte membrane fatty acids in adults also demonstrated reduced odds for asthma in relation to high linoleic acid membrane levels [20].

There are several possible explanations for our findings. Whereas linoleic acid is an essential fatty acid which cannot be endogenously synthesized, the level of the long-chain PUFA arachidonic acid reflects not only dietary intake but also depends on metabolic interactions during their formation. Conversion of linoleic acid into arachidonic acid may be enhanced in asthmatics. This may explain the association of high levels of arachidonic acid, but not of linoleic acid, with asthma in our study. In support of this suggestion, it has been proposed that atopy may be related to a disturbed balance in the metabolism of arachidonic acid and eicosapentaenoic acid, leading to decreased synthesis of the biologically less active leukotriene B₅ from eicosapentaenoic acid and to an increased synthesis of prostaglandin E₂ and leukotriene B₄ from arachidonic acid [22]. Our data on the ratio of arachidonic to linoleic acid, however, do not support the earlier hypothesis of an impairment of the $\delta 6$ -desaturase activity in atopic diseases, which would lead to higher plasma levels of linoleic acid and lower levels of arachidonic acid in atopic subjects [22].

The observed associations could be a consequence of disease, if asthma patients adhere to a special diet or if asthma therapy interferes with fatty acid composition of serum cholesteryl esters. Only a small proportion of children in our study were treated with anti-inflammatory drugs [23]. None of the children received leukotriene antagonists as medication. We did not observe differences in fatty acid levels between children with or without treatment with anti-inflammatory drugs (data not shown). Picado et al. [24] also found no differences in the plasma levels of fatty acids between adult asthma patients with and without corticosteroid therapy.

Arachidonic acid levels in serum cholesteryl esters may reflect ongoing airway inflammation rather than differences in dietary intake. Several studies have indicated that plasma levels of arachidonic acid were not correlated with dietary linoleic acid [25, 26]. At the site of chronic airway inflammation, tissue levels of arachidonic acid have been shown to be increased in asthmatic subjects [27].

n-3 polyunsaturated fatty acids

Studies with dietary supplementation of fish oils or n-3 PUFA suggested a beneficial effect in terms of immune parameters and asthma symptoms [28–31]. In line with the recent study by Woods *et al.* [19] our data do not support the hypothesis of a protective effect of n-3 PUFA on asthma in children. We did not observe an association between levels of eicosapentaenoic acid and lung function parameters or asthma manifestations. This may be because of the fact that our study subjects were children who habitually consumed a diet low in fish products, resulting in n-3 long-chain PUFA levels not comparable with those found in intervention studies. The results of observational studies on the effect of fish intake on asthma in children are the subject of controversy at the present time [10, 32–34].

An explanation for the observed association of high levels of linolenic acid with asthma and impaired lung function may be that n-3 PUFA could give rise to eicosanoids with varying biological activities, which tend to augment the actions of arachidonic acid-derived mediators rather than exerting a protective effect [5]. For example, a recent study suggested that high levels of n-3 PUFA in colostrum may be a risk rather than a protective factor for atopy development in infants [35].

Monounsaturated fatty acids

High levels of the monounsaturated fatty acid oleic acid were not related to any asthma measure in our study, but were associated with decreased odds for BHR. Dietary intake as well as endogenous synthesis may contribute to levels of oleic acid in serum cholesteryl esters. Two studies based solely on dietary intake data in children indicated a negative relationship of monounsaturated fatty acids (MUFA) with asthma: A study in Taiwan showed an inverse association of asthma with dietary intake of MUFA fats in adolescents [36]. Significant negative associations of current wheeze in children with per capita MUFA intake from vegetables were observed in an ecological analysis of data from ISAAC phase I [37].

Saturated fatty acids

There are conflicting data on the role of saturated fatty acids. Studies on the intake of butter and margarine have been interpreted as a protective effect on asthma of a high consumption of saturated fatty acids (SFA) in form of butter compared with PUFA in form of margarine [16, 38]. Whereas a study on adults gave no consistent pattern of SFA dietary intake and asthma [19], a study on adolescents showed a positive association with saturated fat intake [36]. We observed no relationship between levels of palmitic acid and any asthma measure in our study, but

a negative association of high palmitic acid levels with BHR.

Limitations and strengths of the study

Our study has some limitations. The cross-sectional design gives no information on the temporal sequence and therefore does not allow a causal interpretation of the observed associations between fatty acid levels and asthma, BHR and lung function in children.

Serum cholesteryl ester fatty acids expressed as proportions of total measured fatty acids may be interpreted as a crude marker of dietary intake during the preceding weeks [11, 13, 39]. The fatty acid composition of cholesteryl esters in our study was within the range described for children in other studies [13–16]. However, the relative proportion of arachidonic acid was considerably higher than in other studies. This may reflect our analytical method, which takes special precautions to prevent degradation of sensitive analytes such as addition of antioxidant or rapid and careful sample preparation [18]. Our observations of significantly higher values of linoleic acid in cholesteryl ester from girls than boys and higher values of arachidonic acid in boys compared with girls are in accordance with data obtained previously in adults [40].

Fatty acid composition of serum cholesteryl ester is not only a surrogate marker of recent dietary intake but reflects also fatty acid metabolism [11]. We could not disentangle the contribution of dietary intake and metabolic changes to the measured fatty acid levels. In addition, we had no further data to be able to control for total dietary energy intake.

We used quartiles of fatty acid levels as mathematical categorization method because no information on biologically meaningful category boundaries was available. The power of our study was dependent on the fatty acid and the outcome to be analysed. In the case of wheeze for example, when comparing only the children in the highest quartile of eicosapentaenoic acid levels with those in the first quartile as reference our study had a power of 80% to detect an OR of 2.53 with $\alpha = 0.05$. For the analysis of a potential dose–response relationship across the quartiles, we used category medians as less distorting method [41].

The strengths of our study are that we had comprehensive and valid data on asthma symptoms and diagnosis, measurements of BHR and lung function as well as data on atopic sensitization. We were therefore able to compare several measures of asthma including severity and atopic as well as non-atopic outcomes. The data were collected by validated procedures in the framework of the ISAAC study. There was no indication of selection bias since participation in lung function measurement and blood sampling was not related to disease status [17]. We used a highly sensitive method for quantification of fatty acid composition of serum cholesteryl esters [18]. To our

knowledge this is the first study on the relationship between serum fatty acids and asthma, BHR and lung function in children.

In conclusion, our study adds to the body of evidence suggesting that PUFA are related to asthma and impaired lung function. However, our data do not support the hypothesis of a protective role of n-3 PUFA. Whether the observed differences in fatty acid composition of serum cholesteryl ester are a reflection of etiologic factors in terms of a disturbed balance in the metabolism or a consequence of the disease remains to be elucidated.

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