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RESPIRATORY DISEASES

Association of fatty acids in serum phospholipids with lung function and bronchial hyperresponsiveness in adults

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Abstract *Background* The dietary intake of certain fatty acids might have an impact on inflammatory processes in the lung and therefore contribute to the development of lung diseases like asthma or COPD. *Methods* In this study data from a population based cross-sectional study on respirator y health including measurement of fatty acids in serum phospholipids of 593 adults between 20 and 64 years of age were analyzed. *Results* Statistically significant positive associations were found between percentage predicted FEV_1 ($P = 0.0085$) and FVC ($P = 0.0267$) and docosahexaenoic acid concentration in serum phospholipids in men. Dihomo-γ-linolenic acid content in serum phospholipids was significantly negatively associated with percentage predicted FEV_1 ($P = 0.0003$) and FVC ($P = 0.0045$) and transformed dose-response slopes ($P = 0.0488$) in men. Palmitoleic acid was negatively associated with percentage predicted FEV_1 ($P = 0.0037$) and FVC ($P = 0.0029$) in men. Other fatty acids in serum phospholipids did not consistently affect lung function parameters or bronchial hyperreactivity. *Conclusion* A high concentration of docosahexaenoic acid in serum phospholipids may have a protective effect on lung function. Because this long-chain n-3 polyunsaturated fatty acid is almost exclusively derived from marine oils, fish might have a beneficial effect on lung diseases.

Keywords Bronchial hyperresponsiveness · Lung function · Fatty acids · ECRHS

Abbreviations

BHR	Bronchial hyperresponsiveness
COPD	Chronic obstructive pulmonary disease
LTB_4	Leukotriene B_4
PGE_2	Prostaglandin E_2
EPA	Eicosapentaenoic acid

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Iris Kompauer did the data analysis and wrote the paper.
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DHA	Docosahexaenoic acid
PUFA	Polyunsaturated fatty acids
FEV_1	Forced exspiratory volume in one second
FVC	Forced vital capacity
ECRHS	European community respiratory health survey
DRS	Dose-response slope
TDRS	Transformed dose-response slope
OR	Odds ratio
β	Regression coefficient
SE	Standard error
PUFA	Polyunsaturated fatty acids
wt/wt	Weight/weight

Introduction

Over the last two decades associations between impaired lung function, bronchial hyperresponsiveness (BHR), asthma and diet have received increasing attention. In particular, a link between the consumption of long chain polyunsaturated fatty acids and the risk of these disorders was suggested. Linoleic acid, the fatty acid which contributes the highest fraction of polyunsaturated fatty acids in a typical Western diet, is a precursor of arachidonic acid, which can be converted to the proinflammatory eicosanoids leukotriene B_4 (LTB_4) and prostaglandin E_2 (PGE_2). In contrast, eicosanoids derived from the n-3 fatty acid eicosapentaenoic acid (EPA) can down-regulate the production of PGE_2 [1–3]. Therefore, a high ratio of n6/n3 fatty acids in the diet might promote inflammatory diseases like asthma and chronic obstructive pulmonary disease (COPD) [4, 5].

There is an ongoing discussion on the relationship between the composition of fatty acids in the diet, serum and blood cells, respectively, and the occurrence of BHR and asthma. However, most studies are restricted to the n6/n3-ratio and deal with intake of fish and fish oil. We think that other fatty acids may also have an impact on lung diseases. Therefore, our approach is more explorative and relates the contents of fatty acids in serum phospholipids as a marker of intake and subsequent metabolism of fatty acids to the lung function parameters percentage predicted forced expiratory volume in one second (FEV_1 % of predicted), percentage predicted forced vital capacity (FVC % of predicted), the ratio FEV_1/FVC and hyperresponsiveness to methacholine as markers for asthma and BHR.

Methods

Study subjects

The present study is based on a sample from one of the two surveys conducted in Erfurt, East Germany as part of the European Community Respiratory Health Survey (ECRHS). This Erfurt survey was conducted in 1991–92. The age range of 20–44 years of the international project was extended to adults until age of 64 years. Study design and population sampling are described in detail elsewhere [6, 7]. In brief, a total of 1282 participants answered the main questionnaire. Blood samples were drawn from 1258 participants and serum samples were stored at −80°C for later analyses. Lung function measurement by spirometry and a bronchial challenge test to methacholine were carried out on 932 participants. A subset of the participants was invited to participate in a dietary survey with three-day weighted records on their diet [8]. From this subset, unthawed serum samples were still available for 740 participants, 313 women and 427 men. For the present study, these samples were used for fatty acid determination in their serum phospholipids.

The Ethics committee of the Medical School at Erfurt approved the study.

Spirometry and bronchial challenge

Baseline forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) were measured with Masterlab Spirometers (Jaeger, Wuerzburg, Germany) and the best of five satisfactory manoeuvers was used in the analysis. Methacholine was not given to participants whose FEV_1 was less than 70% of predicted values or less than 1.5 l, or participants who reported taking beta blockers, having a heart disease, epilepsy, or were pregnant or breastfeeding. No participant had to be excluded from methacholine challenge because FEV_1 was less than 70% of predicted. Main exclusion criteria were serious heart diseases and missing informed consent.

Bronchial challenge started with the inhalation of saline diluent, and the maximum post diluent FEV_1 recorded was used as the control value. Participants whose post-saline FEV_1 was less than 90% of initial FEV_1 were not challenged further. A Mefar dosimeter (Mefar, Bovezzo, Italy) was used for the administration of methacholine. Challenge started with a dose of 0.0078 mg methacholine; then quadrupling doses were used. FEV_1 was recorded two minutes after each inhalation. After a fall in FEV_1 of 10% from the control value doubling doses were used. Methacholine challenge was stopped if FEV_1 had dropped by 20% or more from the post-saline value, until the maximum cumulative dose of 2 mg methacholine had been reached, a participant was not able to perform two technically satisfactory manoeuvres or the participant did not wish to continue [9–12].

Participants whose FEV_1 fell by 20% or more relative to post-saline values or whose FEV_1 rose more than 11% after

bronchodilator challenge with salbutamol were classified as bronchial hyperreactive.

Analysis of fatty acids

Serum was kept frozen at −80°C until analysis in 2003 and was never thawed before the measurement of fatty acids. Serum lipids were extracted with hexane/ isopropanol (3:2). Phospholipids were isolated by thin layer chromatography and fatty acid methyl esters were obtained by acid catalysed trans-esterification in methanol. Methyl esters were extracted into hexane and frozen until analysis in a gas chromatograph [13, 14] (Hewlett Packard, 5890 Series II). In total, 36 fatty acids were measured and the results were expressed as percentage (wt/wt) of the total fatty acid methyl esters measured in serum phospholipids.

Statistical analysis

Differences in categorical variables between women and men were assessed by χ^2 test, differences in continuous variables by the Wilcoxon test. A two-tailed *P*-value of <0.05 was required for statistical significance.

The dose-response slope (DRS) was calculated by fitting the line fall in

$$FEV_1 = \delta + DRS * dose$$

to all data points using the least-squares method. The fall in FEV_1 was taken as the relative change of FEV_1 with respect to postsaline FEV_1. The DRS was calculated if at least two doses of methacholine had been administered. Because the distribution of DRS was skewed and some negative values were found, it was transformed. We used the reciprocal transformation 1/(DRS + 0.1) to get the transformed dose-response slope (TDRS) for linear regression analysis. This transformation best fulfilled the requirements for statistical analysis, normal distribution and homogeneity of variance. Due to the reciprocal transformation, a lower value of TDRS indicates higher responsiveness to methacholine.

From all analyzed 36 fatty acids we chose 15 to assess the association with percentage predicted FEV_1 and FVC, the dose-response slopes and bronchial hyperresponsiveness. We selected the quantitative major saturated fatty acids palmitic and stearic acid as markers of saturated fatty acids in serum phospholipids. The monounsaturated fatty acids palmitoleic acid, oleic acid and *trans* C18:1 (*t*9, *t*11 combined) were chosen because they are the quantitatively major monounsaturated and trans fatty acids, respectively, and they were associated with asthma, hay fever and allergic sensitisation in other studies [15–17]. Linoleic acid and its desaturation and elongation products were chosen because eicosanoids derived from the n-6 fatty acid arachidonic acid might promote allergic diseases, whereas eicosanoids derived from the n-3 fatty acid eicosapentaenoic acid might have a beneficial effect. Additionally we analyzed the sum of n-6 and n-3 polyunsaturated and the ratio of n6/n3 polyunsaturated fatty acids.

The association between the percentage content of certain fatty acids in serum phospholipids and BHR was assessed by using logistic regression models. Fatty acid values were divided into tertiles. Odds ratios (OR) were adjusted for age, body mass index, education and smoking status. The association between the concentration of certain fatty acids in serum phospholipids and percentage predicted FEV_1 and FVC, the ratio FEV_1/FVC and dose-response slope was assessed by using linear regression models. Associations between the three lung function parameters and fatty acids in serum were only calculated for participants who took part in the methacholine challenge and for whom DRS could be calculated. We conducted a sex stratified analysis, because BHR was significantly more frequent in women in our study, similar to findings of previous studies [18].

We did not analyze asthma as an outcome variable, because only 14 participants in our study population answered "yes" to the question "Have you ever had asthma".

We did not adjust for family history of atopy because this confounder showed no effect on the point estimates and many participants were not aware of the atopic status of their parents, who were born long before 1950.

SAS version 8.2 was used for all calculations (SAS Institute, Cary, NC).

Results

Table 1 shows the basic characteristics of the study population included in this analysis. Baseline FEV_1, baseline FVC and FVC (% predicted) were significantly lower in women than in men ($P < 0.0001$), whereas FEV_1 (% predicted) and the ratio FEV_1/FVC did not differ significantly between sexes. Prevalence of BHR and dose-response slopes were significantly higher in women than in men ($P = 0.0044$ and $P < 0.0001$). Women were significantly younger, had a significantly lower body mass index and a higher proportion of non-smokers than men.

The largest fatty acids fractions in serum phospholipids were saturated (43.9%), followed by n-6 polyunsaturated (34.9%), monounsaturated (14.3%) and n-3 polyunsaturated (6.2%) fatty acids (Table 2). Among single fatty acids, palmitic acid contributed the largest proportion with 26.9%, followed by linoleic acid C18:2n-6 (20.2%), stearic acid C18:0 (13.4%), arachidonic acid C20:4n-6 (9.6%) and oleic acid C18:1n-9 (9.5%). All single fatty acids differed significantly between sexes except for dihomo-γ-linolenic acid (Table 2).

Table 1 Characteristics of the study population

	Total ($n = 593$)	Women ($n = 236$)	Men ($n = 357$)	P-value
BHR (PD_{20})[a]	118 (19.9)	61 (25.9)	57 (16.0)	0.0044[b]
Mild BHR (PD_{10})[a]	245 (41.3)	123 (52.2)	122 (34.2)	<0.0001[b]
Asthma[a]	14 (2.4)	6 (2.5)	8 (2.2)	0.7902[b]
Dose-response slope[e]	0.123 ± 0.360	0.162 ± 0.437	0.097 ± 0.295	<0.0001[c]
Transformed dose-response slope[e]	6.78 ± 2.32	6.14 ± 2.40	7.20 ± 2.17	<0.0001[c]
Baseline FEV_1[e]	3.73 ± 0.87	3.14 ± 0.49	4.12 ± 0.85	<0.0001[c]
Baseline FVC[e]	4.56 ± 1.00	3.81 ± 0.56	5.06 ± 0.90	<0.0001[c]
FEV_1 (% predicted)[e]	107. 0 ± 15.4	107.5 ± 14.5	106.7 ± 16.0	0.6239
FVC (% predicted)[e]	109.8 ± 14.7	112.9 ± 15.9	107.8 ± 13.4	<0.0001[c]
FEV_1/FVC[e]	0.82 ± 0.07	0.82 ± 0.07	0.81 ± 0.08	0.1870
Age (years)[e]	40.4 ± 11.8	37.8 ± 10.9	41.9 ± 12.1	<0.0001[c]
Body mass index[e]	25.2 ± 3.9	24.2 ± 4.1	25.8 ± 3.7	<0.0001[c]
Smoking status[a]				0.0002[b]
Never smokers	227 (38.3)	113 (47.9)	114 (31.9)	
Exsmokers	165 (27.8)	49 (20.8)	116 (32.5)	
Current smokers	201 (33.9)	74 (31.4)	127 (35.6)	
Educational level[d]				0.0732[b]
High	277 (46.9)	110 (46.8)	167 (46.9)	
Medium	205 (34.7)	91 (38.7)	114 (32.0)	
Low	109 (18.4)	34 (14.5)	75 (21.1)	

[a] n (%)
[b] χ^2-test
[c] Wilcoxon
[d] High, >10 classes; medium, =10 classes; low, <10 classes
[e] Mean ± SD

Crude and adjusted regression coefficients (β) and standard errors (SE) for the association between 15 selected fatty acids, the sum of n-6 polyunsaturated, the sum of n-3 polyunsaturated and the ratio n6/n3 fatty acids in serum phospholipids (%) and lung function and DRS in women are given in Table 3. The only fatty acid that was significantly associated with the outcome variables in women was palmitoleic acid, showing a positive association with TDRS.

In men, palmitoleic acid and dihomo-γ-linolenic acid were significantly negatively and DHA and the sum of n-3 polyunsaturated fatty acids were significantly positively associated with FEV_1 (% predicted) and FVC (% predicted) (Table 4). Table 5 shows crude and adjusted odds ratios and corresponding 95% confidence intervals for the association between fatty acids in serum phospholipids and BHR in women. Palmitic acid was negatively associated with BHR, whereas the other fatty acids showed no significant association with BHR. Crude and adjusted odds ratios and corresponding 95% confidence intervals for the association between fatty acids in serum phospholipids and BHR in men are presented in Table 6. Docosatetraenoic acid and γ-linolenic acid were positively associated with BHR.

Discussion

We observed an at least marginal statistically significant ($P < 0.10$) positive association between docosahexaenoic acid (DHA) in serum phospholipids and percentage predicted FEV_1 and FVC both in women and in men, whereas the ratio FEV_1/FVC and DRS were not associated with this fatty acid. Palmitoleic acid was negatively associated with percentage predicted FEV_1 and FVC in men. In women, this fatty acid was positively associated with TDRS, indicating a protective effect on BHR. Dihomo-γ-linolenic acid was negatively associated with lung function parameters and TDRS in men, whereas in women no association was observed.

To our knowledge, our study is the first that investigates the association between fatty acids in serum and lung function parameters. Therefore, only limited comparisons with the results of other published studies are possible. In a group of current or former smokers, Shahar et al. reported stronger associations of DHA in plasma phospholipids with the spirometric indicators $FEV_1 \leq 65\%$ predicted and $FEV_1/FVC \leq 0.65$ respectively than with clinical indicators of smoking-related COPD, which is consistent with our results [19]. In an Australian study, Woods et al. found DHA significantly negatively associated with log-transformed dose-response slope, suggesting a protective effect on BHR [20]. Both studies did not stratify for sex.

These two studies of Shahar et al. and Woods et al. are the only ones that analyzed fatty acid levels in plasma. Other published studies were conducted on the association between dietary intake of fish oil and n-3 polyunsaturated fatty acids (PUFA) respectively and asthma and BHR.

Table 2 Fatty acid composition[a] in serum phospholipids of 593 adults

Fatty acid	Total (n = 593)	Women (n = 236)	Men (n = 357)	P-value[b]
Saturated				
Palmitic acid (C16:0)	26.88 ± 1.76	27.41 ± 1.67	26.53 ± 1.73	<0.0001
Stearic acid (C18:0)	13.38 ± 1.37	12.88 ± 1.43	13.71 ± 1.23	<0.0001
Sum saturated fatty acids[c]	43.85 ± 1.71	43.73 ± 1.03	43.93 ± 2.03	0.4794
Monounsaturated				
Palmitoleic acid (C16:1n-7)	0.52 ± 0.22	0.48 ± 0.17	0.55 ± 0.24	0.0050
Oleic acid (C18:1n-9)	9.50 ± 1.29	9.30 ± 1.03	9.64 ± 1.42	0.0246
Trans-C18:1	0.36 ± 0.15	0.37 ± 0.15	0.35 ± 0.16	0.0472
Sum monounsaturated fatty acids[d]	14.32 ± 1.52	14.01 ± 1.14	14.53 ± 1.69	0.0001
n-6 Series polyunsaturated				
Linoleic acid (C18:2n-6)	20.29 ± 2.68	20.79 ± 2.55	19.96 ± 2.72	0.0004
γ-Linolenic acid (C18:3n-6)	0.10 ± 0.04	0.09 ± 0.03	0.11 ± 0.04	<0.0001
Dihomo-γ-linolenic acid (C20:3n-6)	2.83 ± 0.57	2.86 ± 0.58	2.81 ± 0.56	0.3002
Arachidonic acid (C20:4n-6)	9.63 ± 1.50	9.45 ± 1.40	9.75 ± 1.55	0.0150
Adrenic acid (C22:4n-6)	0.34 ± 0.07	0.32 ± 0.06	0.35 ± 0.07	<0.0001
n6-Docosapentaenoic acid (C22:5n-6)	0.24 ± 0.13	0.26 ± 0.12	0.22 ± 0.13	<0.0001
Sum n-6 fatty acids[e]	34.50 ± 2.38	34.85 ± 1.96	34.26 ± 2.60	0.0137
n-3 Series polyunsaturated				
α-Linolenic acid (C18:3n-3)	0.18 ± 0.06	0.19 ± 0.06	0.18 ± 0.06	0.0106
Eicosapentaenoic acid (C20:5n-3)	1.10 ± 0.61	1.03 ± 0.61	1.14 ± 0.60	<0.0001
n3-Docosapentaenoic acid (C22:5n-3)	0.95 ± 0.22	0.81 ± 0.20	1.05 ± 0.17	<0.0001
Docosahexaenoic acid (C22:6n-3)	3.83 ± 0.95	4.00 ± 0.91	3.72 ± 0.96	<0.0001
Sum n-3 fatty acids[f]	6.20 ± 1.47	6.17 ± 1.44	6.22 ± 1.48	0.5947

[a] % Fatty acid methyl esters (wt/wt), mean ± SD

[b] Wilcoxon

[c] Sum saturated fatty acids = (C14:0 + C16:0 + C18:0 + C20:0 + C22:0 + C24:0)

[d] Sum monounsaturated fatty acids = (C16:1n-7 + C18:1n-7 + C18:1n-9 + C20:1n-9 + C22:1n-9 + C24:1n-9)

[e] Sum n-6 fatty acids = (C18:2 n-6 + C18:3 n-6 + C20:2 n-6 + C20:3 n-6 + C20:4 n-6 + C22:2 n-6 + C22:4 n-6 + C22:5 n-6)/total fatty acids

[f] Sum n-3 fatty acids = (C18:3 n-3 + C18:4 n-3 + C20:3 n-3 + C20:5 n-3 + C22:5 n-3 + C22:6 n-3)/total fatty acids

Studies on dietary intake of n-3 PUFA and fish oil

Two prospective studies observed no effect of dietary intake of EPA and DHA [21] and sum of n-3 PUFA [22] on adult-onset asthma. In contrast, dietary intake of EPA and DHA was even positively associated with asthma in a case-control study, whereas DHA content in erythrocyte membrane showed no effect [23]. In most epidemiological studies on the association between long chain n-3-PUFA and lung function, BHR and asthma, data on fish intake were collected. Frequency of eating fatty fish was not associated with maximum FEV_1 in a British study [24]. Two studies from Norway [25] and Australia [26] showed no association of asthma-like respiratory symptoms [25] and asthma or BHR [26] respectively among young adults. Analyzing data of the First National Health and Nutritional Examination Survey (NHANES I), Schwartz and Weiss reported a positive association between fish consumption in the last three month, assessed by food frequency questionnaire, and FEV_1 in an US population [27]. Results from NHANES II showed a negative association between fish consumption, assessed by a 24-h dietary recall and wheezing and bronchitis [28]. Clinical trials on fish oil supplementation did not show a consistent effect on lung function, BHR or asthma in adults [29–32]. Other fatty acids on the pathway of n-3 PUFA, which we analyzed (Fig. 1), were not consistently associated with lung function values or BHR in our study, indicating that DHA has a special effect on these outcomes. DHA, as well as EPA, is a precursor to the novel local lipid mediators resolvins and docosatrienes, which possess both anti-inflammatory and immunoregulatory properties [33–35]. Resolvins (resolution phase interaction products) are formed within the resolution phase of inflammation and reduce entrance of neutrophils to sites of inflammation and reduce exsudates [36]. Results of unpublished studies from B.D. Levy et al. show that Resolvin D and 10,17-docosatriene,

Table 3 Crude and adjusted[a] regression coefficients (β) and standard errors (SE) for the association between selected fatty acids in serum phospholipids (%), lung function and dose-response slopes in women ($n = 231$)

	FEV_1 (%predicted)		FVC (%predicted)		FEV_1/FVC		Transformed DRS	
Fatty acid	$\beta \pm$ SE (%/%)	*P* value	$\beta \pm$ SE (%/%)	*P* value	$\beta \pm$ SE ((%/100)/%)	*P* value	$\beta \pm$ SE (%/%)	*P* value
Saturated								
Palmitic acid (C16:0)								
Crude	0.010 ± 0.512	0.9850	−0.666 ± 0.597	0.2653	0.005 ± 0.002	0.0445	0.154 ± 0.094	0.1019
Adjusted	0.479 ± 0.515	0.3536	0.143 ± 0.584	0.8065	0.002 ± 0.002	0.4770	0.149 ± 0.096	0.1226
Stearic acid (C18:0)								
Crude	0.448 ± 0.594	0.4519	1.148 ± 0.691	0.0982	−0.006 ± 0.003	0.0310	−0.250 ± 0.109	0.0227
Aadjusted	−0.136 ± 0.614	0.8255	0.095 ± 0.695	0.8909	−0.002 ± 0.003	0.6040	−0.207 ± 0.114	0.0709
Monounsaturated								
Palmitoleic acid (C16:1n-7)								
Crude	−0.646 ± 5.249	0.9021	−6.190 ± 6.123	0.3131	0.038 ± 0.026	0.1449	2.161 ± 0.958	0.0250
Adjusted	0.860 ± 5.213	0.8691	−3.984 ± 5.892	0.4996	0.034 ± 0.025	0.1726	2.399 ± 0.961	0.0133
Oleic acid (C18:1n-9)								
Crude	−0.704 ± 0.830	0.3974	−1.875 ± 0.964	0.0530	0.008 ± 0.004	0.0429	−0.006 ± 0.154	0.9699
Adjusted	−0.004 ± 0.828	0.9960	−1.017 ± 0.934	0.2772	0.006 ± 0.004	0.1085	0.043 ± 0.155	0.7817
trans-C18:1								
Crude	−1.052 ± 5.831	0.8570	1.876 ± 6.817	0.7834	0.013 ± 0.029	0.6434	−0.263 ± 1.079	0.8075
Adjusted	0.972 ± 5.764	0.8663	4.476 ± 6.514	0.4927	0.003 ± 0.027	0.9066	−0.484 ± 1.077	0.6534
n-6 Series polyunsaturated								
Linoleic acid (C18:2n-6)								
Crude	−0.209 ± 0.337	0.5356	−0.285 ± 0.394	0.4708	0.001 ± 0.002	0.4095	−0.018 ± 0.0624	0.7773
Adjusted	0.112 ± 0.349	0.7490	0.207 ± 0.394	0.6007	−0.001 ± 0.002	0.7350	−0.035 ± 0.0652	0.5951
γ-Linolenic acid (C18:3n-6)								
Crude	4.935 ± 25.463	0.8465	11.841 ± 29.762	0.6911	−0.128 ± 0.125	0.3067	−0.128 ± 4.710	0.9784
Adjusted	−32.056 ± 26.593	0.2293	−44.852 ± 30.030	0.1367	0.080 ± 0.126	0.5282	0.168 ± 4.995	0.9732
Dihomo-γ-linolenic acid (C20:3n-6)								
Crude	−1.650 ± 1.475	0.2645	−1.788 ± 1.725	0.3009	0.0002 ± 0.007	0.9752	0.213 ± 0.2724	0.4343
Adjusted	−1.948 ± 1.500	0.1955	−1.383 ± 1.701	0.4169	−0.005 ± 0.007	0.4888	0.062 ± 0.282	0.8250
Arachidonic acid (C20:4n-6)								
Crude	−0.098 ± 0.613	0.8733	−0.834 ± 0.715	0.2441	0.003 ± 0.003	0.2660	0.045 ± 0.114	0.6906
Adjusted	−0.122 ± 0.601	0.8394	−0.720 ± 0.678	0.2897	0.002 ± 0.003	0.4579	−0.003 ± 0.113	0.9813
Docosatetraenoic acid (C22:4n-6)								
Crude	−15.208 ± 14.880	0.3078	−32.765 ± 17.302	0.0595	0.106 ± 0.073	0.1464	−2.671 ± 2.723	0.3278
Adjusted	−5.276 ± 14.659	0.7193	−17.373 ± 16.547	0.2949	0.053 ± 0.069	0.4506	−3.588 ± 2.730	0.1901

Table 3 continued

Fatty acid	FEV_1 (%predicted)		FVC (%predicted)		FEV_1/FVC		Transformed DRS	
	$\beta \pm$ SE (%/%)	*P* value	$\beta \pm$ SE (%/%)	*P* value	$\beta \pm$ SE ((%/100)/%)	*P* value	$\beta \pm$ SE (%/%)	*P* value
n6-Docosapentaenoic acid (C22:5n-6)								
Crude	−4.484 ± 9.062	0.6212	−8.435 ± 10.586	0.4264	0.018 ± 0.045	0.6800	0.716 ± 1.670	0.6686
Adjusted	−4.199 ± 8.814	0.6343	−6.260 ± 9.967	0.5306	0.005 ± 0.042	0.9127	0.143 ± 1.648	0.9310
Sum n-6 polyunsaturated[b]								
Crude	−0.601 ± 0.438	0.1718	−1.097 ± 0.509	0.0324	0.004 ± 0.002	0.0809	0.015 ± 0.082	0.8507
Adjusted	−0.170 ± 0.459	0.7115	−0.303 ± 0.519	0.5607	−0.0002 ± 0.002	0.9134	−0.057 ± 0.086	0.5115
n-3 Series polyunsaturated								
α-Linolenic acid (C18:3n-3)								
Crude	−4.191 ± 14.269	0.7692	−2.794 ± 16.685	0.8672	−0.035 ± 0.070	0.6203	−0.287 ± 2.662	0.9144
Adjusted	−4.488 ± 13.865	0.7465	−4.689 ± 15.684	0.7652	−0.020 ± 0.066	0.7625	0.164 ± 2.609	0.9500
Eicosapentaenoic acid (C20:5n-3)								
Crude	1.700 ± 1.413	0.2301	5.021 ± 1.624	0.0022	−0.021 ± 0.007	0.0020	−0.122 ± 0.259	0.6365
Adjusted	−0.346 ± 1.465	0.8137	2.324 ± 1.650	0.1603	−0.012 ± 0.007	0.0751	−0.033 ± 0.274	0.9041
n3-Docosapentaenoic acid (C22:5n-3)								
Crude	6.435 ± 4.241	0.1306	13.139 ± 4.907	0.0080	−0.052 ± 0.021	0.0129	−1.553 ± 0.784	0.0488
Adjusted	1.168 ± 4.484	0.7947	3.799 ± 5.067	0.4541	−0.007 ± 0.021	0.7272	−1.366 ± 0.834	0.1029
Docosahexaenoic acid (C22:6n-3)								
Crude	2.163 ± 0.929	0.0208	3.057 ± 1.080	0.0051	−0.005 ± 0.005	0.2650	0.063 ± 0.174	0.7165
Adjusted	1.525 ± 0.921	0.0992	1.998 ± 1.040	0.0558	−0.001 ± 0.004	0.8923	0.114 ± 0.173	0.5095
Sum n-3 polyunsaturated[c]								
Crude	1.267 ± 0.586	0.0316	2.323 ± 0.675	0.0007	−0.007 ± 0.003	0.0192	−0.025 ± 0.109	0.8169
Adjusted	0.593 ± 0.600	0.3242	1.283 ± 0.675	0.0587	−0.002 ± 0.003	0.3868	0.019 ± 0.112	0.8629
Ratio n6/n3								
Crude	−1.268 ± 0.566	0.0262	−2.006 ± 0.656	0.0025	0.005 ± 0.003	0.0597	0.014 ± 0.106	0.8924
Adjusted	−0.577 ± 0.589	0.3286	−0.882 ± 0.666	0.1865	0.0003 ± 0.003	0.9088	−0.043 ± 0.110	0.6962

[a] Adjusted for age (continuous), education (low, middle, high), smoking status (never, ex, current), body mass index (continuous)

[b] Sum n-6 fatty acids = (C18:2 n-6 + C18:3 n-6 + C20:2 n-6 + C20:3 n-6 + C20:4 n-6 + C22:2 n-6 + C22:4 n-6 + C22:5 n-6)/total fatty acids

[c] Sum n-3 fatty acids = (C18:3 n-3 + C18:4 n-3 + C20:3 n-3 + C20:5 n-3 + C22:5 n-3 + C22:6 n-3)/total fatty acids

Table 4 Crude and adjusted[a] regression coefficients (β) and standard errors (SE) for the association between selected fatty acids in serum phospholipids (%), lung function and dose-response slopes in men ($n = 346$)

	FEV_1 (%predicted)		FVC (%predicted)		FEV_1/FVC		Transformed DRS	
Fatty acid	$\beta \pm$ SE (%/%)	*P* value	$\beta \pm$ SE (%/%)	*P* value	$\beta \pm$ SE ((%/100)/%)	*P* value	$\beta \pm$ SE (%/%)	*P* value
Saturated								
Palmitic acid (C16:0)								
Crude	−0.372 ± 0.440	0.3992	−0.238 ± 0.395	0.5472	−0.003 ± 0.002	0.2390	−0.053 ± 0.067	0.4297
Adjusted	−0.263 ± 0.450	0.5594	−0.274 ± 0.401	0.4949	−0.0004 ± 0.002	0.8666	0.017 ± 0.067	0.7991
Stearic acid (C18:0)								
Crude	−0.297 ± 0.621	0.6323	−0.695 ± 0.555	0.2114	0.002 ± 0.003	0.4755	−0.094 ± 0.094	0.3209
Adjusted	−0.561 ± 0.656	0.3932	−0.766 ± 0.584	0.1903	0.002 ± 0.003	0.6029	−0.108 ± 0.098	0.2678
Monounsaturated								
Palmitoleic acid (C16:1n-7)								
Crude	−10.425 ± 3.179	0.0011	−8.909 ± 2.854	0.0020	−0.027 ± 0.016	0.0777	−1.286 ± 0.486	0.0085
Adjusted	−9.637 ± 3.294	0.0037	−8.802 ± 2.934	0.0029	−0.012 ± 0.016	0.4559	−0.703 ± 0.496	0.1573
Oleic acid (C18:1n-9)								
Crude	−1.050 ± 0.556	0.0597	−0.935 ± 0.498	0.0614	−0.001 ± 0.003	0.5890	−0.216 ± 0.084	0.0107
Adjusted	−0.787 ± 0.580	0.1752	−0.933 ± 0.515	0.0711	0.0008 ± 0.003	0.7749	−0.147 ± 0.086	0.0890
trans-C18:1								
Crude	1.545 ± 4.861	0.7508	−1.826 ± 4.356	0.6753	0.038 ± 0.023	0.1089	0.584 ± 0.722	0.4190
Adjusted	1.221 ± 4.929	0.8045	−2.052 ± 4.392	0.6407	0.024 ± 0.023	0.3040	−0.0003 ± 0.735	0.9997
n-6 Series polyunsaturated								
Linoleic acid (C18:2n-6)								
Crude	0.197 ± 0.288	0.4950	0.317 ± 0.258	0.2202	0.0008 ± 0.001	0.5470	0.087 ± 0.043	0.0449
Adjusted	0.089 ± 0.303	0.7693	0.246 ± 0.270	0.3638	−0.0004 ± 0.001	0.7708	0.030 ± 0.046	0.5087
γ-Linolenic acid (C18:3n-6)								
Crude	−30.886 ± 17.906	0.0854	−26.426 ± 16.057	0.1007	−0.119 ± 0.086	0.1713	−4.368 ± 2.720	0.1092
Adjusted	−29.964 ± 18.503	0.1063	−23.962 ± 16.504	0.1475	−0.051 ± 0.087	0.5601	−1.912 ± 2.769	0.4905
Dihomo-γ-linolenic acid (C20:3n-6)								
Crude	−4.503 ± 1.334	0.0008	−3.322 ± 1.202	0.0060	−0.012 ± 0.007	0.0629	−0.524 ± 0.204	0.0108
Adjusted	−5.119 ± 1.416	0.0003	−3.632 ± 1.271	0.0045	−0.013 ± 0.007	0.0596	−0.425 ± 0.215	0.0488
Arachidonic acid (C20:4n-6)								
Crude	0.566 ± 0.493	0.2516	0.371 ± 0.442	0.4026	0.002 ± 0.002	0.4312	0.089 ± 0.076	0.2407
Adjusted	0.576 ± 0.501	0.2516	0.532 ± 0.447	0.2348	−0.0001 ± 0.002	0.9620	0.065 ± 0.075	0.3876
Docosatetraenoic acid (C22:4n-6)								
Crude	−20.003 ± 10.690	0.0622	−5.012 ± 9.628	0.6030	−0.083 ± 0.052	0.1101	−3.816 ± 1.622	0.0192
Adjusted	−16.945 ± 10.759	0.1162	−3.503 ± 9.622	0.7161	−0.092 ± 0.050	0.0704	−3.530 ± 1.600	0.0280

Table 4 continued

Fatty acid	FEV_1 (%predicted)		FVC (%predicted)		FEV_1/FVC		Transformed DRS	
	$\beta \pm$ SE (%/%)	*P* value	$\beta \pm$ SE (%/%)	*P* value	$\beta \pm$ SE ((%/100)/%)	*P* value	$\beta \pm$ SE (%/%)	*P* value
n6-Docosapentaenoic acid (C22:5n-6)								
Crude	−4.501 ± 5.848	0.4420	−4.484 ± 5.241	0.3929	−0.005 ± 0.028	0.8583	−0.927 ± 0.889	0.2977
Adjusted	−4.378 ± 5.871	0.4564	−5.382 ± 5.229	0.3041	0.004 ± 0.028	0.8798	−0.516 ± 0.876	0.5564
Sum n-6 polyunsaturated[b]								
Crude	0.192 ± 0.302	0.5251	0.313 ± 0.270	0.2477	0.001 ± 0.001	0.5126	0.002 ± 0.001	0.0292
Adjusted	0.060 ± 0.316	0.8497	0.287 ± 0.281	0.3074	−0.001 ± 0.001	0.4456	0.033 ± 0.047	0.4895
n-3 Series polyunsaturated								
α-Linolenic acid (C18:3n-3)								
Crude	−8.546 ± 13.033	0.5124	−11.985 ± 11.672	0.3052	0.0008 ± 0.063	0.9902	−0.398 ± 2.003	0.8428
Adjusted	−6.406 ± 13.021	0.6231	−11.203 ± 11.593	0.3346	0.017 ± 0.061	0.7770	0.036 ± 1.964	0.9855
Eicosapentaenoic acid (C20:5n-3)								
Crude	0.669 ± 1.261	0.5961	0.913 ± 1.130	0.4196	−0.007 ± 0.006	0.2401	−0.041 ± 0.192	0.8299
Adjusted	1.036 ± 1.275	0.4167	1.389 ± 1.135	0.2216	−0.005 ± 0.006	0.4450	0.095 ± 0.191	0.6198
n3-Docosapentaenoic acid (C22:5n-3)								
Crude	4.747 ± 4.417	0.2833	7.498 ± 3.946	0.0582	−0.026 ± 0.021	0.2222	0.396 ± 0.671	0.5552
Adjusted	4.831 ± 4.447	0.2781	7.854 ± 3.948	0.0474	−0.021 ± 0.021	0.3072	0.355 ± 0.665	0.5944
Docosahexaenoic acid (C22:6n-3)								
Crude	2.226 ± 0.784	0.0048	1.586 ± 0.706	0.0253	0.002 ± 0.004	0.5946	0.153 ± 0.120	0.2038
Adjusted	2.091 ± 0.790	0.0085	1.571 ± 0.706	0.0267	0.003 ± 0.004	0.4383	0.163 ± 0.119	0.1711
Sum n-3 polyunsaturated[c]								
Crude	1.092 ± 0.510	0.0329	0.906 ± 0.458	0.0486	−0.0008 ± 0.002	0.7532	0.061 ± 0.078	0.4351
Adjusted	1.098 ± 0.513	0.0328	0.982 ± 0.457	0.0323	0.0001 ± 0.002	0.9586	0.089 ± 0.077	0.2517
Ratio n6/n3								
Crude	−0.761 ± 0.553	0.1698	−0.434 ± 0.497	0.3834	0.0003 ± 0.003	0.8875	−0.028 ± 0.084	0.7380
Adjusted	−0.824 ± 0.559	0.1417	−0.504 ± 0.499	0.3135	−0.001 ± 0.003	0.6006	−0.093 ± 0.084	0.2711

[a] Adjusted for age (continuous), education (low, middle, high), smoking status (never, ex, current), body mass index (continuous)

[b] Sum n-6 fatty acids = (C18:2 n-6 + C18:3 n-6 + C20:2 n-6 + C20:3 n-6 + C20:4 n-6 + C22:2 n-6 + C22:4 n-6 + C22:5 n-6)/total fatty acids

[c] Sum n-3 fatty acids = (C18:3 n-3 + C18:4 n-3 + C20:3 n-3 + C20:5 n-3 + C22:5 n-3 + C22:6 n-3)/total fatty acids

Table 5 Crude and adjusted[a] Odds Ratios (OR) and corresponding 95% confidence intervals (95% CI) for the association between selected fatty acids in serum phospholipids (%) and BHR[b] in women ($n = 236$)

Fatty acid	1st Tertile	2nd Tertile[c]	3rd Tertile[c]	*P* for trend
Saturated				
Palmitic acid (C16:0) cut off for tertiles	23.06–≤26.65	26.65–≤28.11	28.11–32.61	
Crude OR	1	0.53 (0.26–1.06)	0.42 (0.20–0.87)	0.0167
Adjusted OR	1	0.53 (0.25–1.11)	0.37 (0.17–0.84)	0.0158
Stearic acid (C18:0) cut off for tertiles	9.48–≤11.99	11.99–≤13.68	13.68–16.29	
Crude OR	1	0.73 (0.33–1.59)	1.90 (0.94–3.80)	0.0584
Adjusted OR	1	0.69 (0.30–1.57)	1.83 (0.85–3.92)	0.1062
Monounsaturated				
Palmitoleic acid (C16:1n-7) cut off for tertiles	0.21–≤0.40	0.40–≤0.52	0.52–1.15	
Crude OR	1	0.78 (0.39–1.56)	0.61 (0.30–1.25)	0.1733
Adjusted OR	1	0.74 (0.35–1.58)	0.51 (0.24–1.11)	0.0906
Oleic acid (C18:1n-9) cut off for tertiles	6.41–≤8.84	8.84–≤9.65	9.65–12.44	
Crude OR	1	0.72 (0.34–1.53)	1.30 (0.65–2.60)	0.4416
Adjusted OR	1	0.67 (0.30–1.47)	1.15 (0.55–2.40)	0.6628
Trans-C18:1 cut off for tertiles	0.14–≤0.29	0.29–≤0.40	0.40–0.94	
Crude OR	1	1.41 (0.68–2.92)	1.36 (0.66–2.82)	0.4158
Adjusted OR	1	1.46 (0.68–3.13)	1.46 (0.68–3.12)	0.3370
n-6 Series polyunsaturated				
Linoleic acid (C18:2n-6) cut off for tertiles	14.00–≤19.65	19.65–≤22.20	22.20–27.39	
Crude OR	1	1.39 (0.67–2.87)	1.39 (0.67–2.87)	0.3880
Adjusted OR	1	1.49 (0.69–3.36)	1.52 (0.69–3.36)	0.2980
γ-Linolenic acid (C18:3n-6) cut off for tertiles	0.04–≤0.07	0.07–≤0.10	0.10–0.26	
Crude OR	1	1.04 (0.50–2.17)	1.41 (0.69–2.86)	0.3416
Adjusted OR	1	1.17 (0.52–2.60)	1.65 (0.76–3.62)	0.1980
Dihomo-γ-linolenic acid (C20:3n-6) cut off for tertiles	1.42–≤2.58	2.58–≤3.06	3.06–4.96	
Crude OR	1	0.67 (0.33–1.35)	0.58 (0.28–1.19)	0.1337
Adjusted OR	1	0.80 (0.38–1.68)	0.68 (0.31–1.48)	0.3290
Arachidonic acid (C20:4n-6) cut off for tertiles	6.36–≤8.78	8.78–≤9.91	9.91–12.60	
Crude OR	1	0.57 (0.28–1.15)	0.54 (0.26–1.10)	0.0834
Adjusted OR	1	0.74 (0.35–1.56)	0.59 (0.28–1.25)	0.1665
Docosatetraenoic acid (C22:4n-6) cut off for tertiles	0.16–≤0.29	0.29–≤0.34	0.34–0.55	
Crude OR	1	1.40 (0.67–2.95)	1.69 (0.82–3.51)	0.1596
Adjusted OR	1	1.56 (0.71–3.41)	1.71 (0.79–3.70)	0.1766

Table 5 continued

Fatty acid	1st Tertile	2nd Tertile[c]	3rd Tertile[c]	*P* for trend
n6-Docosapentaenoic acid (C22:5n-6) cut off for tertiles	0.09–≤0.20	0.20–≤0.28	0.28–1.49	
Crude OR	1	1.22 (0.61–2.46)	0.78 (0.37–1.64)	0.5204
Adjusted OR	1	1.24 (0.60–2.58)	0.85 (0.39–1.84)	0.6841
Sum n-6 polyunsaturated cut off for tertiles	28.46–≤34.29	34.29–≤35.71	35.71–39.85	
Crude OR	1	0.70 (0.34–1.45)	0.98 (0.49–1.97)	0.9624
Adjusted OR	1	0.78 (0.36–1.69)	1.29 (0.59–2.82)	0.5440
n-3 Series polyunsaturated				
α-Linolenic acid (C18:3n-3) cut off for tertiles	0.07–≤0.16	0.16–≤0.21	0.21–0.39	
Crude OR	1	1.09 (0.53–2.27)	1.35 (0.66–2.76)	0.4128
Adjusted OR	1	1.05 (0.49–2.26)	1.33 (0.63–2.85)	0.4534
Eicosapentaenoic acid (C20:5n-3) cut off for tertiles	0.13–≤0.72	0.72–≤1.03	1.03–3.57	
Crude OR	1	0.87 (0.43–1.80)	0.97 (0.48–1.96)	0.9252
Adjusted OR	1	0.72 (0.33–1.54)	0.85 (0.38–1.92)	0.6870
n3-Docosapentaenoic acid (C22:5n-3) cut off for tertiles	0.44–≤0.71	0.71–≤0.91	0.91–1.30	
Crude OR	1	1.21 (0.57–2.57)	1.84 (0.89–3.78)	0.0943
Adjusted OR	1	1.07 (0.47–2.41)	1.76 (0.77–4.02)	0.1658
Docosahexaenoic acid (C22:6n-3) cut off for tertiles	2.08–≤3.58	3.58–≤4.22	4.22–6.49	
Crude OR	1	0.80 (0.40–1.60)	0.55 (0.27–1.15)	0.1112
Adjusted OR	1	0.83 (0.40–1.72)	0.49 (0.23–1.08)	0.0788
Sum n-3 polyunsaturated cut off for tertiles	3.70–≤5.35	5.35–≤6.53	6.53–10.29	
Crude OR	1	0.74 (0.36–1.52)	0.89 (0.44–1.80)	0.7462
Adjusted OR	1	0.75 (0.35–1.59)	0.81 (0.37–1.74)	0.5771
Ratio n6/n3 cut off for tertiles	2.85–≤5.28	5.28–≤6.54	6.54–10.55	
Crude OR	1	0.71 (0.34–1.47)	0.97 (0.48–1.94)	0.9252
Adjusted OR	1	0.79 (0.36–1.72)	1.14 (0.53–2.47)	0.7293

[a] Adjusted for age (continuous), education (low, middle, high), smoking status (never, ex, current), body mass index (continuous)

[b] Defined as 20% fall of FEV_1 from baseline after methacholine challenge or a FEV_1 rise of more than 11% after bronchodilator challenge with salbutamol

[c] 1st Quartile was set as the reference category

Table 6 Crude and adjusted[a] Odds Ratios (OR) and corresponding 95% confidence intervals (95% CI) for the association between selected fatty acids in serum phospholipids (%) and BHR[b] in men ($n = 357$)

Fatty acid	1st Tertile	2nd Tertile[c]	3rd Tertile[c]	*P* for trend
Saturated				
Palmitic acid (C16:0) cut off for tertiles	22.89–≤25.80	25.80–≤26.96	26.96–40.64	
Crude OR	1	0.87 (0.41–1.83)	1.60 (0.81–3.14)	0.1585
Adjusted OR	1	0.80 (0.37–1.72)	1.19 (0.58–2.43)	0.5957
Stearic acid (C18:0) cut off for tertiles	9.98–≤13.16	13.16–≤14.05	14.05–19.78	
Crude OR	1	0.47 (0.22–1.00)	0.94 (0.49–1.79)	0.8378
Adjusted OR	1	0.49 (0.22–1.06)	0.95 (0.46–1.95)	0.8721
Monounsaturated				
Palmitoleic acid (C16:1n-7) cut off for tertiles	0.24–≤0.41	0.41–≤0.58	0.58–1.89	
Crude OR	1	1.01 (0.46–2.22)	2.39 (1.19–4.80)	0.0097
Adjusted OR	1	0.76 (0.34–1.73)	1.62 (0.77–3.39)	0.1354
Oleic acid (C18:1n-9) cut off for tertiles	6.94–≤8.92	8.92–≤9.96	9.96–15.63	
Crude OR	1	0.84 (0.38–1.86)	2.19 (1.10–4.34)	0.0167
Adjusted OR	1	0.80 (0.35–1.82)	1.88 (0.91–3.91)	0.0616
Trans-C18:1 cut off for tertiles	0.11–≤0.27	0.27–≤0.38	0.38–1.34	
Crude OR	1	1.12 (0.56–2.25)	1.05 (0.52–2.11)	0.9041
Adjusted OR	1	1.11 (0.54–2.30)	1.35 (0.64–2.82)	0.4309
n-6 Series polyunsaturated				
Linoleic acid (C18:2n-6) cut off for tertiles	10.50–≤18.89	18.89–≤21.08	21.08–28.32	
Crude OR	1	0.82 (0.42–1.61)	0.68 (0.34–1.37)	0.2758
Adjusted OR	1	0.94 (0.47–1.90)	0.96 (0.46–2.03)	0.9145
γ-Linolenic acid (C18:3n-6) cut off for tertiles	0.02–≤0.09	0.09–≤0.12	0.12–0.32	
Crude OR	1	2.93 (1.29–6.63)	3.25 (1.45–7.31)	0.0053
Adjusted OR	1	2.30 (0.98–5.38)	2.45 (1.04–5.76)	0.0518
Dihomo-γ-linolenic acid (C20:3n-6) cut off for tertiles	1.17–≤2.56	2.56–≤2.30	2.30–4.39	
Crude OR	1	1.40 (0.67–2.94)	1.88 (0.92–3.84)	0.0828
Adjusted OR	1	1.29 (0.60–2.78)	1.51 (0.69–3.26)	0.3029
Arachidonic acid (C20:4n-6) cut off for tertiles	4.19–≤8.96	8.96–≤10.47	10.47–14.12	
Crude OR	1	1.61 (0.82–3.18)	0.86 (0.41–1.81)	0.7030
Adjusted OR	1	2.13 (1.03–4.42)	1.06 (0.48–2.37)	0.8616
Docosatetraenoic acid (C22:4n-6) cut off for tertiles	0.13–≤0.32	0.32–≤0.38	0.38–0.63	
Crude OR	1	1.68 (0.78–3.63)	2.44 (1.17–5.11)	0.0167
Adjusted OR	1	1.81 (0.81–4.05)	2.78 (1.26–6.14)	0.0109

Table 6 continued

Fatty acid	1st Tertile	2nd Tertile[c]	3rd Tertile[c]	*P* for trend
n6-Docosapentaenoic acid (C22:5n-6) cut off for tertiles	0.07–≤0.18	0.18–≤0.23	0.23–1.34	
Crude OR	1	1.52 (0.71–3.24)	2.15 (1.04–4.44)	0.0374
Adjusted OR	1	1.55 (0.71–3.40)	1.79 (0.84–3.84)	0.1418
Sum n-6 polyunsaturated cut off for tertiles	22.20–≤33.63	33.63–≤35.42	35.42–39.65	
Crude OR	1	0.66 (0.34–1.29)	0.49 (0.24–1.00)	0.0469
Adjusted OR	1	0.84 (0.41–1.69)	0.73 (0.35–1.55)	0.4065
n-3 Series polyunsaturated				
α-Linolenic acid (C18:3n-3) cut off for tertiles	0.02–≤0.15	0.15–≤0.19	0.19–0.42	
Crude OR	1	0.85 (0.40–1.79)	1.58 (0.80–3.11)	0.1660
Adjusted OR	1	0.88 (0.40–1.93)	1.40 (0.69–2.85)	0.3294
Eicosapentaenoic acid (C20:5n-3) cut off for tertiles	0.18–≤0.86	0.86–≤1.23	1.23–5.90	
Crude OR	1	1.01 (0.50–2.05)	1.19 (0.60–2.37)	0.6161
Adjusted OR	1	0.92 (0.44–1.92)	0.95 (0.46–1.96)	0.8977
n3-Docosapentaenoic acid (C22:5n-3) cut off for tertiles	0.53–≤0.98	0.98–≤1.11	1.11–1.62	
Crude OR	1	1.41 (0.71–2.80)	0.99 (0.48–2.05)	0.9774
Adjusted OR	1	1.58 (0.77–3.25)	1.06 (0.50–2.26)	0.8754
Docosahexaenoic acid (C22:6n-3) cut off for tertiles	1.37–≤3.22	3.22–≤3.98	3.98–7.74	
Crude OR	1	0.78 (0.39–1.56)	0.89 (0.45–1.75)	0.7235
Adjusted OR	1	0.70 (0.33–1.45)	0.81 (0.39–1.65)	0.5460
Sum n-3 polyunsaturated cut off for tertiles	2.90–≤5.45	5.45–≤6.48	6.48–13.94	
Crude OR	1	0.92 (0.46–1.85)	1.05 (0.53–2.09)	0.8815
Adjusted OR	1	0.91 (0.44–1.89)	0.91 (0.44–1.86)	0.7912
Ratio n6/n3 cut off for tertiles	2.02–≤5.22	5.22–≤6.41	6.41–10.18	
Crude OR	1	0.67 (0.33–1.38)	1.00 (0.51–1.95)	1.0000
Adjusted OR	1	0.83 (0.39–1.76)	1.25 (0.62–2.52)	0.5388

[a] Adjusted for age (continuous), education (low, middle, high), smoking status (never, ex, current), body mass index (continuous)

[b] Defined as 20% fall of FEV_1 from baseline after methacholine challenge or a FEV_1 rise of more than 11% after bronchodilator challenge with salbutamol

[c] 1st quartile was set as the reference category

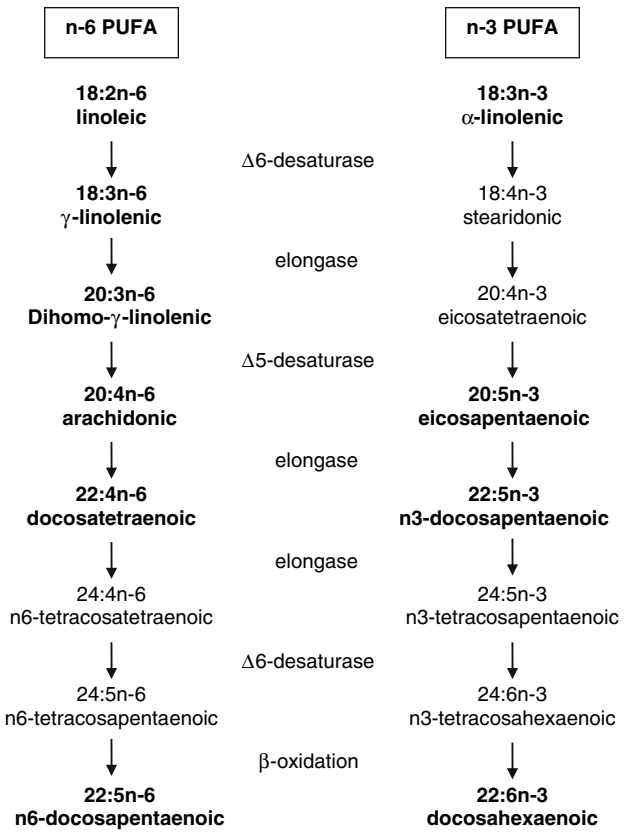

Fig. 1 Biosynthesis of long-chain polyunsaturated fatty acids (PUFA) in mammals. Fatty acids analysed in our study are in bold. Modified from [52]

which are derived from DHA, play a role in the protection of lung-associated tissue damage in murine models of asthma [33].

In our study, dihomo-γ-linolenic acid, the direct precursor of arachidonic acid, showed a consistent negative association with lung function parameters and DRS in men, but not in women. This is consistent with the results of Woods et al. who found a positive association between dihomo-γ-linolenic acid in plasma and log-transformed dose-response slope. These authors also observed a positive association between all analyzed asthma outcomes (current asthma, asthma and doctor diagnosed asthma) and dihomo-γ-linolenic acid [20].

Associations between fatty acids and lung function parameters were slightly different among women and men, respectively. Dihomo-γ-linolenic acid was consistently associated with all lung function parameters and TDRS in men, whereas in women there were no such associations. Palmitoleic acid showed a significant negative association with percentage predicted FEV_1 and FVC in men, whereas it was positively associated with TDRS in women. After adjusting for potential confounders, DHA remained significantly associated with percentage predicted FEV_1 and FVC in men, but in women significance was lost. It seems that there are other determinants for lung function and BHR in women which have stronger effects than fatty acids. Lungs of women are smaller and weigh less than lungs of men [37]. Additionally, lung function and BHR in women seems to be influenced by hormonal determinants [37–40].

A lot of crude effects were significant, but significance was lost after adjustment for confounders. In the linear models for the association between lung function and fatty acids in serum phospholipids, age was by far the greatest confounder, both in women and in men. Another important confounder was BMI in women and smoking in men. The differences between crude and adjusted results in TDRS and BHR were mainly caused by smoking, followed by age in both sexes.

We measured fatty acid contents of serum phospholipids as a biomarker for recent dietary fatty acid intake [41, 42] and metabolic turnover, because we consider fatty acid contents of serum phospholipids to better reflect fatty acid availability in the body than dietary intake estimated with current methodology. In intervention studies that administered n-3 PUFA, n-3 PUFA content in plasma phospholipids increased in a time and dose dependent manner, followed by a somewhat later enrichment in white and red blood cells [43–46]. Similar effects are also observed following high intakes of n-6 polyunsaturated and monounsaturated fatty acids, although somewhat less distinct [47].

Given that the samples analyzed had been stored frozen at −80°C for more than 10 years prior to analysis, it is conceivable that some degree of oxidation of long chain polyunsaturated fatty acids might have occurred, but it is unlikely that n-3 or n-6 polyunsaturated fatty acids would have been preferentially affected. Moreover, Zeleniuch-Jacquotte et al. reported that fatty acids in serum phospholipids remained very well protected during storage of serum samples at −80°C up to 12 years [48].

Some caution appears prudent in interpreting our study results because of the cross-sectional nature of our study design, which does not establish a cause and effect relationship. Lung function tests, methacholine challenge and blood withdrawal took place on the same day, but were measured only once. We conducted an exploratory study with multiple tests. Therefore, some of the statistically significant findings might be attributable to chance. However, relationship between DHA and lung function parameters FEV_1 and FVC (% of predicted) was consistent both in women and in men, although significance was lost in women after adjustment. To confirm our results, further confirmatory studies on the association between lung function and dietary intakes and serum concentrations of DHA are necessary.

In conclusion, DHA is associated with lung function values in our study, indicating that the positive effect of fatty acids derived from marine oils observed in some

studies might be attributable to this fatty acid. One might speculate that the observed sex differences in women and men for the fatty acids palmitoleic acid and dihomo-γ-linolenic are due to stronger determinants of lung function, such as airway calibre or sex hormones.

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