

Fatty acid composition of serum lipid classes in mice following allergic sensitisation with or without dietary docosahexaenoic acid-enriched fish oil substitution

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Dietary fatty acids have been shown to influence allergic sensitisation. Both *n*-3 and *n*-6 PUFA are involved in targeted mediation of inflammatory responses during allergic sensitisation and manifestation of atopic diseases. In the present experiments we investigated whether supplementation of DHA-enriched fish oil partly substituting dietary sunflower-seed oil, in comparison with sunflower-seed oil, supplemented to mice influences fatty acid composition of serum lipid classes. The effects of the two different diets were also investigated depending on allergic sensitisation. Supplementation of DHA and EPA in doses of 2 and 0.12% (w/w) to non-sensitised and sensitised mice resulted in significantly increased percentile contributions of DHA to all lipid classes. In contrast, serum values of the *n*-6 PUFA arachidonic acid (AA) were significantly lower, both in non-sensitised and sensitised mice fed the DHA-enriched diet. The fatty acid composition of serum lipids also reflected allergic sensitisation: the EPA:AA ratio in TAG, cholesteryl esters and phospholipids in non-supplemented animals fell to 23, 29 and 29% respectively of the original value after allergic sensitisation, whereas it decreased to 70, 80 and 76% respectively only in the animals supplemented with DHA. In summary, allergic sensitisation alone decreased significantly the EPA:AA ratios in serum TAG, while concomitant supplementation of DHA-enriched fish oil ameliorated this decrease. We postulate from the present results that the amelioration of the severity of allergic sensitisation after DHA supplementation may be linked to altered ratios of the eicosanoid precursors EPA and AA as well as DHA needed for further metabolic activation to pro- or anti-inflammatory bioactive lipids.

Docosahexaenoic acid: Arachidonic acid: Eicosapentaenoic acid: Dihomo- γ -linolenic acid: Allergic sensitisation: *n*-3 Polyunsaturated fatty acids

Fatty acids in the mammalian organism are mainly present in esterified form, and in lower concentrations as NEFA. Phospholipid- and cholesteryl esters are the most abundant forms of fatty acid esters in membranes and they occur in addition to TAG also in the serum. PUFA are important nutrients in the human diet^(1–3). Various plant oils, such as sunflower-seed and soyabean oil, as well as meat products, contain mainly *n*-6 PUFA with linoleic acid, γ -linolenic acid and arachidonic acid (AA) as major constituents, whereas fish and linseed oil contain mainly the *n*-3 PUFA: α -linolenic acid in linseed oil and EPA and DHA in marine oils such as fish oil.

Three PUFA (EPA from the *n*-3 series and AA as well as dihomogamma-linolenic acid (DHGLA) from the *n*-6 series) are substrates for various lipo-oxygenases (LOX) and cyclo-oxy-

genases (COX). They can be further metabolised to highly bioactive eicosanoids such as leucotrienes and PG (Miles *et al.*⁽⁴⁾; for a review, see Rustan & Drevon⁽⁵⁾), as well as lipoxins, neuroprotectins and resolvins which mainly originate from DHA and EPA (for reviews, see Serhan⁽⁵⁾ and Serhan & Savill⁽⁶⁾). Although DHA is not directly involved in eicosanoid synthesis, retroconversion of DHA to EPA has been reported⁽⁷⁾.

Increased dietary supplementation with plant oils rich in *n*-6 PUFA has been associated with increased production of pro-inflammatory mediators^(8,9), whereas dietary supplementation with oils rich in the *n*-3 PUFA EPA and DHA induced anti-inflammatory effects in *in vitro* as well as *in vivo* experiments^(2,8–10). Moreover, a higher dietary intake of *n*-3

Abbreviations: AA, arachidonic acid; COX, cyclo-oxygenase; CTRL, control (basal diet); DHGLA, dihomogamma-linolenic acid; LOX, lipo-oxygenase; OVA, ovalbumin.

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PUFA was associated with lower incidences of allergic sensitisation and atopic diseases in several studies^(11–13). Supplementation with various forms of oils rich in DHA or fish oil ameliorated symptoms of allergic sensitisation in atopic children⁽¹⁴⁾, mixed population of atopic children and adults⁽¹⁵⁾, atopic dogs^(16–18) and in mouse allergy models^(19,20).

Allergic sensitisation is reflected by an altered T-cell phenotype and cytokine responses (for reviews, see Worm & Henz⁽²¹⁾ and Bacharier & Geha⁽²²⁾). In particular, PG of the PgE class and leucotrienes of the 4-series were associated with a shift from T-helper cell 1 to T-helper cell 2 response (Th1 → Th2 shift)^(4,23,24). Leucotrienes originating from *n*-3 PUFA are, in general, less potent pro-inflammatory substances than their analogues originating from *n*-6 PUFA⁽²⁵⁾, while PG originating from different fatty acids are almost equipotent⁽⁴⁾.

The aim of the study was to find out how allergic sensitisation, DHA-enriched fish oil supplementation and allergic sensitisation with concomitantly DHA-enriched fish oil supplementation influence the serum percentile distribution of cholesteryl-, TAG- and phospholipid esters of the fatty acids and various ratios of eicosanoid precursor PUFA such as EPA, DHA and AA.

Materials and methods

Animal experiments

The animal experiments were performed in the facilities of the Max Rubner Laboratory of the German Institute of Human Nutrition (Dife) (Potsdam-Rehbrücke, Germany). The respective ethical authorities from the Land Brandenburg approved the experiment. A second set of experiments, for investigation of the influence of Al(OH)₃ on lipid distribution, was performed in the Laboratory Animal Core Facility of the University of Debrecen (Debrecen, Hungary). The experiments were performed according to the ethical guidelines of the Republic of Hungary.

All mice were female Balb/c adult (age 10–12 weeks) mice (*Mus musculus*) and were obtained from Charles River (Sulzfeld, Germany). The animals were kept under controlled conditions at room temperature (21 ± 1°C) and constant relative humidity (55 ± 5%) and a 12 h light–12 h dark cycle with light between 06.00 and 18.00 hours.

Food and water were administered *ad libitum*. The composition of the basal diet was 20% casein, 8% sucrose, 50% wheat starch, 10% sunflower-seed oil, 5% cellulose, 5% mineral mix (Mineral-Spuren-elemente-Vormischung C1000; Altromin, Lage, Germany) and 2% vitamin mix (Vitamin-Vormischung C1000; Altromin)⁽²⁶⁾. In the diet supplemented with DHA-enriched fish oil, 40% of the sunflower-seed oil was substituted by DHA-enriched fish oil (DHA 500TG SR from tuna; Croda GmbH, Nettetal, Germany) yielding a mass content of 2% DHA and 0.12% EPA in the animals' diet. The composition of the two diets is displayed in Table 1. The group fed the basal diet and the group fed the DHA-supplemented diet each contained twelve mice; for the second experiment using aluminium hydroxide (Al(OH)₃) treatment for the group fed the basal diet and the DHA-supplemented group, six mice per group were used.

Before supplementation with the basal and DHA-enriched fish oil diet the mice were fed with a standard mouse diet

Table 1. Percentage fatty acid composition of the administered food*

Fatty acid (%)	Control diet	Diet supplemented with DHA-enriched fish oil
Sum of SFA	12.4	8.3
Sum of MUFA	22.7	17.6
Sum of <i>n</i> -6 PUFA	64.3	42.7
Linoleic acid	64.0	40.9
Arachidonic acid	0.01	0.56
Sum of <i>n</i> -3 PUFA	0.63	31.4
EPA	0.12	3.6
DHA	0.26	23.4

*Data are percentages (w/w) of dry animal food.

from Sniff (Soest, Germany) and for the second set of experiments using Al(OH)₃ treatment the mice were fed with a standard mouse diet from Altromin (Lage, Germany). At 1 week after the start of the supplementation with the basal and the DHA-enriched fish oil diet, six mice per group were sensitised with ovalbumin (OVA) and six mice were treated with PBS. In the second set of experiments just Al(OH)₃ treatment was performed in a comparable manner. Each group was treated either with three intraperitoneal injections of OVA or PBS (or Al(OH)₃ in PBS) after a time interval of 1 week. OVA injections were made according to the protocol previously described⁽²⁷⁾; briefly, 10 µg OVA absorbed to 1.5 mg Al(OH)₃ in 100 µl PBS was given to each animal. The mice were killed by decapitation 4 weeks after the dietary and 3 weeks after the OVA or PBS treatments. At 20–30 min after the collection the blood was centrifuged at 1300g for 3 min and serum was obtained. Serum was stored at –80°C until analysis.

Fatty acid analysis

For the analysis of plasma fatty acid profiles, frozen plasma samples were thawed, and then the three internal standards (dipentadecanoylphosphatidylcholine, cholesteryl pentadecanoate and cholesteryl tripentadecanoate) were added. Lipids were extracted by the addition of 3 ml chloroform and 1 ml methanol according to the method of Folch *et al.*⁽²⁸⁾. The mixture was vortexed at 2000 rpm for 10 min, and then the lower layer was aspirated into vials and evaporated under an N₂ stream. Lipid extracts were reconstituted in 70 µl chloroform and lipid classes were separated by TLC. The solvents for TLC of plasma lipids were as follows: hexane–diethyl ether–chloroform–acetic acid (21:6:3:1, by vol.) followed by chloroform–methanol–water (65:25:4, by vol.). The bands were stained with dichlorofluorescein, removed by scraping and transesterified in 3 M-HCl–methanol solution at 84°C for 45 min.

Fatty acids were analysed by high-resolution capillary GC using a Finnigan 9001 gas chromatograph (Finnigan/Tre-metrics Inc., Austin, TX, USA) with split injection (ratio 1:25), automatic sampler (A200SE; CTC Analytic, Zwingen, Switzerland) and flame ionisation detector with a DB-23 cyanopropyl column of 40 m length (J & W Scientific, Folsom, CA, USA). The temperature program was the following: temperature of injector at 80°C for 0.1 min, temperature increase by 180°C/min up to 280°C, temperature of detector

at 280°C, temperature of column area at 60°C for 0.2 min, temperature increase by 40°C/min up to 180°C, 5 min isothermal period, temperature increase by 1.5°C/min up to 200°C, 8.5 min isothermal period, temperature increase by 40°C/min up to 240°C and 13 min isothermal period. The constant linear velocity was 0.3 m/s (referred to 100°C). Peak identification was verified by comparison with authentic standards. Fatty acid results were expressed as percentages (w/w) of fatty acids detected with a chain length between twelve and twenty-four carbon atoms.

Enzyme-linked immunosorbent assay analysis

Plates were coated overnight with anti-mouse EM95-3 (5 µg/ml; all monoclonal antibodies used were kindly provided by Dr Lamer, MPI, Freiburg, Germany) diluted in 0.1 M-bicarbonate buffer. After blocking with 3% milk powder-PBS, sera (diluted in 1% milk powder-PBS) were incubated overnight and were detected with biotin-conjugated anti-mouse 84 1-C (1 µg/ml). The reaction was developed with streptavidin peroxidase and tetramethylbenzidine (both from Sigma, Dreieich, Germany) and was stopped with 1 M-sulfuric acid. The plates were measured at 450/490 nm and the amount of total IgE was calculated according to the standard curve. The validity was assessed by using a standard with known concentrations for total IgE and by determination of 50% saturation for OVA-specific IgE ELISA. Furthermore, all sera were measured in serial dilutions.

Statistics

Standard deviations were calculated with SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) software for Windows using

a two-factor independent Mann-Whitney test; a value of $P < 0.05$ was used to determine statistical significance.

Results

Here we discuss separately the effects of diet (basal diet (control; CTRL) and PBS injections (CTRL-PBS) v. DHA-enriched fish oil supplementation and PBS injections (DHA-PBS); basal diet and OVA injections (CTRL-OVA) v. DHA-enriched fish oil supplementation and OVA injections (DHA-OVA)) and the effect of allergic sensitisation without dietary supplementation (basal diet with PBS injections (CTRL-PBS) v. basal diet with OVA injections (CTRL-OVA)) and with dietary DHA supplementation (DHA-enriched fish oil supplementation with PBS injections (DHA-PBS) v. DHA-enriched fish oil supplementation with OVA injections (DHA-OVA)). In a second set of experiments we performed Al(OH)₃ treatments to the animals fed the basal diet and the animals supplemented with the DHA-enriched fish oil. Fatty acid composition of plasma cholesteryl esters, phospholipids and TAG are shown in Tables 2, 3, 4 and 6, respectively. Ratios of selected *n*-3 and *n*-6 PUFA such as EPA, DHA and DHGLA TAG are shown in Tables 5 and 6.

Effect of dietary docosahexaenoic acid-enriched fish oil supplementation without allergic sensitisation (control diet and phosphate-buffered saline treatment v. docosahexaenoic acid-enriched diet and phosphate-buffered saline treatment)

The sum of SFA and MUFA were comparable between both groups, except for higher values of MUFA in TAG in the control animals. *n*-3 PUFA levels were strongly increased after DHA-enriched fish oil supplementation in all the three lipid classes. Inconsistent results were seen in the individual

Table 2. Fatty acid composition of plasma cholesteryl ester lipids in mice fed either a diet supplemented with docosahexaenoic acid-enriched fish oil or a control diet (CTRL) and subsequently treated either with ovalbumin (OVA) or phosphate-buffered saline‡ (Mean values and standard deviations)

Treatment...	CTRL-PBS		DHA-PBS		CTRL-OVA		DHA-OVA	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sum of SFA	8.96	0.34	11.47	0.63	6.09†	0.18	12.78*	0.68
Sum of MUFA	12.00	0.51	11.36	0.44	10.97	0.31	13.24	0.27
18:2 <i>n</i> -6 (LA)	31.53	15.44	43.07*	4.10	32.73	9.22	43.02*	2.07
18:3 <i>n</i> -6 (GLA)	0.51	0.32	0.29*	0.06	0.42	0.04	0.55	0.73
20:2 <i>n</i> -6	0.30	0.17	0.16*	0.19	0.13	0.02	0.19	0.17
20:3 <i>n</i> -6 (DHGLA)	0.58	0.17	0.95*	0.10	0.48	0.06	1.13*	0.25
20:4 <i>n</i> -6 (AA)	42.51	12.14	20.16*	5.00	46.61	7.86	18.84*	5.49
22:4 <i>n</i> -6	0.63	0.25	0.15*	0.06	0.54	0.15	0.14*	0.04
22:5 <i>n</i> -6	0.91	0.53	0.18	0.12	0.08	0.03	0.58	0.69
Sum of <i>n</i> -6 PUFA	76.98	0.77	64.96*	1.02	80.99	0.42	64.44*	0.65
18:3 <i>n</i> -3 (ALA)	0.05	0.01	0.24	0.39	0.03	0.03	0.19*	0.27
20:3 <i>n</i> -3	0.29	0.26	1.04*	1.35	0.12†	0.06	0.53*	0.17
20:5 <i>n</i> -3 (EPA)	0.05	0.05	4.21*	1.53	0.02	0.01	3.12*	1.13
22:5 <i>n</i> -3 (DPA)	0.03	0.01	0.15*	0.02	0.03	0.01	0.15	0.08
22:6 <i>n</i> -3 (DHA)	1.64	0.75	6.58*	2.84	1.75	0.34	5.55*	2.61
Sum of <i>n</i> -3 PUFA	2.07	0.15	12.20*	0.63	1.95	0.08	9.54*	0.67

LA, linoleic acid; GLA, γ -linolenic acid; DHGLA, dihomogamma-linolenic acid; AA, arachidonic acid; ALA, α -linolenic acid; DPA, docosapentaenoic acid.

* Mean value is significantly different from that of the CTRL animals ($P < 0.05$).

† Mean value is significantly different from that of the PBS-treated animals ($P < 0.05$).

‡ Data are percentages (w/w) calculated on the basis of six independent samples.

Table 3. Fatty acid composition of plasma phospholipids in mice fed either with a diet supplemented with docosahexaenoic acid-enriched fish oil or a control diet (CTRL) and subsequently treated either with ovalbumin (OVA) or phosphate-buffered saline‡
(Mean values and standard deviations)

Treatment...	CTRL-PBS		DHA-PBS		CTRL-OVA		DHA-OVA	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sum of SFA	49.01	0.72	45.97	0.51	46.06	0.29	52.99*†	0.44
Sum of MUFA	9.13	0.32	10.01	0.33	10.08	0.27	11.70	0.32
18:2 <i>n</i> -6 (LA)	22.75	1.58	24.72	2.85	22.11	1.98	20.82†	1.23
18:3 <i>n</i> -6 (GLA)	0.10	0.04	0.67	0.59	0.14	0.12	0.05*	0.02
20:2 <i>n</i> -6	0.31	0.04	0.27	0.04	0.33	0.04	0.30	0.03
20:3 <i>n</i> -6 (DHGLA)	1.00	0.19	1.63*	0.30	1.03	0.12	1.64*	0.32
20:4 <i>n</i> -6 (AA)	14.01	3.63	6.10*	1.78	15.88	1.98	5.48*	1.46
22:4 <i>n</i> -6	1.50	0.70	0.44*	0.25	1.69	0.56	0.25*	0.07
22:5 <i>n</i> -6	0.03	0.01	0.49*	0.81	0.09	0.10	0.13	0.15
Sum of <i>n</i> -6 PUFA	39.69	0.70	34.32	0.82	41.26	0.51	28.68*†	0.34
18:3 <i>n</i> -3 (ALA)	0.02	0.01	0.24	0.22	0.04	0.03	0.05	0.03
20:3 <i>n</i> -3	0.12	0.08	0.40	0.22	0.13	0.08	0.18*	0.12
20:5 <i>n</i> -3 (EPA)	0.007	0.005	0.94*	0.32	0.003†	0.001	0.64*	0.17
22:5 <i>n</i> -3 (DPA)	0.02	0.01	0.27*	0.08	0.03	0.00	0.23*	0.04
22:6 <i>n</i> -3 (DHA)	2.00	1.02	7.86*	3.78	2.40	0.40	5.54*	2.74
Sum of <i>n</i> -3 PUFA	2.17	0.21	9.70*	0.79	2.60	0.07	6.64*	0.57

LA, linoleic acid; GLA, γ -linolenic acid; DHGLA, dihomogamma-linolenic acid; AA, arachidonic acid; ALA, α -linolenic acid; DPA, docosapentaenoic acid.

* Mean value is significantly different from that of the CTRL animals ($P < 0.05$).

† Mean value is significantly different from that of the PBS-treated animals ($P < 0.05$).

‡ Data are percentages (w/w) calculated on the basis of six independent samples.

n-6 PUFA. Values of linoleic acid increased significantly in cholesteryl esters, but decreased in TAG. Similarly, γ -linolenic acid values were higher in phospholipids, but significantly lower in TAG and cholesteryl esters in the animals treated with *n*-3 PUFA than in the controls. Values of DHGLA were significantly higher in both cholesteryl esters and

phospholipids in animals treated with *n*-3 PUFA than in control mice. In contrast, percentages (w/w) of AA were significantly lower in all three serum lipid classes of animals receiving *n*-3 PUFA supplementation than in the controls. The sum of *n*-6 PUFA was always lower in animals receiving *n*-3 PUFA supplementation.

Table 4. Fatty acid composition of plasma triacylglycerol lipids in mice fed either with a diet supplemented with docosahexaenoic acid-enriched fish oil or a control diet (CTRL) and subsequently treated either with ovalbumin (OVA) or phosphate-buffered saline‡
(Mean values and standard deviations)

Treatment...	CTRL-PBS		DHA-PBS		CTRL-OVA		DHA-OVA	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sum of SFA	25.21	0.30	26.91	0.70	23.18	0.34	34.12*†	0.83
Sum of MUFA	36.24	0.46	30.39*	0.51	40.39	0.52	29.70*	0.56
18:2 <i>n</i> -6 (LA)	33.20	2.58	27.19	4.47	31.51	4.42	22.06*	2.34
18:3 <i>n</i> -6 (GLA)	0.27	0.05	0.16*	0.04	0.27	0.03	0.27	0.20
20:2 <i>n</i> -6	0.53	0.20	0.26*	0.04	0.47	0.12	0.25*	0.03
20:3 <i>n</i> -6 (DHGLA)	0.64	0.29	0.40	0.17	0.49	0.11	0.36	0.09
20:4 <i>n</i> -6 (AA)	2.53	0.91	1.02*	0.26	2.44	0.31	0.99*	0.64
22:4 <i>n</i> -6	0.54	0.21	0.49	0.20	0.47	0.13	0.43	0.12
22:5 <i>n</i> -6	0.41	0.26	0.39	0.30	0.19	0.19	2.19*	1.84
Sum of <i>n</i> -6 PUFA	38.11	0.49	29.91*	1.14	35.84	1.11	26.54*	0.59
18:3 <i>n</i> -3 (ALA)	0.04	0.03	0.19*	0.02	0.11†	0.05	0.24*	0.04
20:3 <i>n</i> -3	0.26	0.19	0.32	0.17	0.26	0.27	0.57	0.34
20:5 <i>n</i> -3 (EPA)	0.02	0.01	1.47*	0.42	0.01†	0.01	1.20*	0.55
22:5 <i>n</i> -3 (DPA)	0.09	0.03	0.87*	0.45	0.03†	0.01	0.72*	0.25
22:6 <i>n</i> -3 (DHA)	0.03	0.01	9.94*	2.64	0.18†	0.02	6.92*	3.64
Sum of <i>n</i> -3 PUFA	0.43	0.05	12.80*	0.62	0.59	0.07	9.64*	0.83

LA, linoleic acid; GLA, γ -linolenic acid; DHGLA, dihomogamma-linolenic acid; AA, arachidonic acid; ALA, α -linolenic acid; DPA, docosapentaenoic acid.

* Mean value is significantly different from that of the CTRL animals ($P < 0.05$).

† Mean value is significantly different from that of the PBS-treated animals ($P < 0.05$).

‡ Data are percentages (w/w) calculated on the basis of six independent samples.

Table 5. Ratios of selected *n*-3 and *n*-6 polyunsaturated fatty acids‡ (Mean values and standard deviations)

	EPA:AA		EPA:DHGLA		EPA:DHA	
	Mean	SD	Mean	SD	Mean	SD
TAG						
CTRL-PBS (%)§	100	41	100	73	100	42
CTRL-OVA (%)	23*	26	20*	19	3*	4
DHA-PBS (%)§	100	26	100	37	100	19
DHA-OVA (%)	70	31	92†	51	119†	11
Cholesteryl esters						
CTRL-PBS (%)§	100	106	100	91	100	105
CTRL-OVA (%)	29	6	42	15	26	6
DHA-PBS (%)§	100	21	100	34	100	16
DHA-OVA (%)	80†	18	66	26	88†	17
Phospholipids						
CTRL-PBS (%)§	100	92	100	79	100	122
CTRL-OVA (%)	29	15	36	22	19	9
DHA-PBS (%)§	100	21	100	23	100	46
DHA-OVA (%)	76†	17	68*†	13	93†	26

AA, arachidonic acid; DHGLA, dihomo- γ -linolenic acid; CTRL, control diet; OVA, treatment with ovalbumin.

* Mean value is significantly different from that of the PBS-treated animals ($P < 0.05$).

† Mean value is significantly different from that of the CTRL animals ($P < 0.05$).

‡ Data are calculated on the basis of six independent samples.

§ Ratios detected in PBS-treated groups were set as 100%.

Effect of dietary docosahexaenoic acid-enriched fish oil supplementation with allergic sensitisation (control diet and ovalbumin treatment v. docosahexaenoic acid-enriched diet and ovalbumin treatment)

Values of the relevant *n*-3 PUFA were all significantly higher in the animals supplemented with DHA and EPA. The sums of SFA were significantly higher in all serum lipid classes in the animals receiving *n*-3 PUFA supplementation. The sums of MUFA were higher in phospholipids and cholesteryl esters, but were significantly lower in TAG in the animals treated with *n*-3 PUFA than in the controls. Values of linoleic acid were significantly higher in cholesteryl esters, but significantly lower in TAG in the animals receiving DHA and EPA supplementation. AA values as well as the sum of *n*-6 PUFA

were significantly lower in all serum lipid classes in the animals treated with *n*-3 PUFA than in the controls. In general, all *n*-3 PUFA levels were higher after DHA supplementation.

Effect of allergic sensitisation without dietary docosahexaenoic acid-enriched fish oil supplementation (control diet and phosphate-buffered saline treatment v. control diet and ovalbumin treatment)

The sums of SFA were comparable in phospholipids and TAG in sensitised and non-sensitised animals, while in cholesteryl esters they were significantly lower in the sensitised animals. The sums of MUFA were similar in phospholipids, TAG and cholesteryl esters. Allergic sensitisation did not result in significant changes in *n*-6 PUFA values. *n*-3 PUFA values exhibited significant changes upon sensitisation mainly in TAG: values of α -linolenic acid and DHA significantly increased, whereas those of EPA and docosapentaenoic acid (22: 5*n*-3) significantly decreased. EPA was significantly lower in TAG and phospholipids and borderline significantly lower in cholesteryl esters.

Effect of allergic sensitisation with dietary docosahexaenoic acid-enriched fish oil supplementation (docosahexaenoic acid-enriched diet and phosphate-buffered saline treatment v. docosahexaenoic acid-enriched diet and ovalbumin treatment)

Sensitisation with OVA with dietary interventions resulted in similar SFA levels in cholesteryl esters but significantly increased levels in TAG and phospholipids. The sums of MUFA were similar in all lipid classes. *n*-6 PUFA values were similar in cholesteryl esters and TAG, while in phospholipids the sum of *n*-6 PUFA as well as linoleic acid levels were significantly lower in the sensitised animals.

Inter-comparison of lipid classes

DHA supplementation affected the fatty acid compositions of all three lipid classes v. control. The increase in the sum of *n*-3 PUFA expressed as DHA-PBS/CTRL-PBS was the most pronounced in serum TAG (thirty times), in comparison with phospholipids (four times) and cholesteryl esters (six times).

Table 6. Fatty acid composition of plasma phospholipids in mice fed either with a diet supplemented with docosahexaenoic acid-enriched fish oil or a control diet (CTRL) and subsequently treated either with ovalbumin (OVA) or phosphate-buffered saline and ratios of selected *n*-3 and *n*-6 polyunsaturated fatty acids‡

(Mean values and standard deviations)

Fatty acid...	AA		DHGLA		DHA		EPA		EPA:AA (%)§		EPA:DHGLA (%)§		EPA:DHA (%)§	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CTRL-PBS	14.0	3.6	1.00	0.19	2.00	1.02	0.007	0.005	100	92	100	79	100	122
CTRL-AI(OH) ₃	11.2	0.8	0.88	0.08	2.08	0.30	0.010	0.006	128	59	155	83	82	44
CTRL-OVA-AI(OH) ₃	15.9	2.0	1.03	0.12	2.40	0.40	0.003	0.001	29	15	36	22	19	9
DHA-PBS	6.10*	1.78	1.63*	0.30	7.86*	3.78	0.94*	0.32	100	21	100	23	100	46
DHA-AI(OH) ₃	4.69*	0.36	1.61*	0.20	7.29*	1.17	0.68*	0.11	94	10	75	9	68	7
DHA-OVA-AI(OH) ₃	5.48*	1.46	1.64*	0.32	5.54*	2.74	0.64*	0.17	76*	17	68*†	13	93*	26

AA, arachidonic acid; DHGLA, dihomo- γ -linolenic acid; OVA, treatment with ovalbumin.

* Mean value is significantly different from that of the PBS-treated animals ($P < 0.05$).

† Mean value is significantly different from that of the CTRL animals ($P < 0.05$).

‡ Data are percentages (w/w) calculated on the basis of six independent samples.

§ Ratios detected in PBS-treated groups were set as 100%.

Eicosapentaenoic acid:arachidonic acid, eicosapentaenoic acid:dihomo- γ -linolenic acid and eicosapentaenoic acid:docosahexaenoic acid ratios

The ratios in the PBS-treated animals were always set as 100 % (Table 5) for better comparison of the OVA sensitisation values after the basal diet or the diet supplemented with DHA-enriched fish oil. The EPA:AA ratio for TAG decreased significantly and just non-significantly in cholesteryl esters and phospholipids in non-supplemented animals to 23 (SD 26), 29 (SD 6) and 29 (SD 15) % after allergic sensitisation, whereas the decrease was only to 70 (SD 31), 80 (SD 18) and 76 (SD 17) % after accompanying DHA-enriched fish oil supplementation.

The EPA:DHGLA ratio also decreased in non-supplemented animals to 20 (SD 18) % for TAG (significantly), 42 (SD 15) % for cholesteryl esters and 36 (SD 22) % for phospholipids after allergic sensitisation, whereas the decrease was much smaller (to 92 (SD 51) % for TAG, 66 (SD 26) % for cholesteryl esters and 68 (SD 13) % for phospholipids) after DHA-enriched fish oil supplementation.

The EPA:DHA ratio decreased after sensitisation without DHA-enriched fish oil supplementation to 3 (SD 4) % significantly for TAG, 26 (SD 6) % for cholesteryl esters and 19 (SD 9) % for phospholipids of the original value, while after DHA-enriched fish oil supplementation it was significantly higher after sensitisation with 119 (SD 11) % for TAG, 88 (SD 17) % for cholesteryl esters and 93 (SD 26) % for phospholipids.

Influence of aluminium hydroxide treatment on the fatty acid composition of plasma phospholipids

Concentrations of AA, DHGLA, DHA and EPA were not significantly altered after Al(OH)₃ or Al(OH)₃-OVA treatments in the animals fed the basal diet as well as in the *n*-3 PUFA-supplemented animals. Concentrations of EPA were always lower (non-significantly) in OVA-Al(OH)₃-treated mice in comparison with PBS-treated mice.

EPA:AA, EPA:DHGLA and EPA:DHA ratios were in the same range after Al(OH)₃ treatment in animals fed the basal diet as for animals with PBS treatment, while the ratios were much, but non-significantly, lower in OVA-Al(OH)₃-treated animals. EPA:AA, EPA:DHGLA and EPA:DHA ratios were just slightly lower after Al(OH)₃ treatment; EPA:AA and EPA:DHA ratios were lower and just EPA:DHGLA ratios were significantly lower in the *n*-3 PUFA diet-supplemented mice.

The *n*-3 PUFA-supplemented diet significantly ameliorated the decrease of the EPA:AA, EPA:DHGLA and EPA:DHA ratios in plasma phospholipids after OVA-Al(OH)₃ treatment but not after Al(OH)₃ treatment alone in comparison with animals fed the basal diet.

Allergic sensitisation

Serum IgE levels increased significantly after OVA sensitisation in the group fed the control diet from 0.4 (SD 0.2) μ g/ml to 3.2 (SD 2.0) μ g/ml and in the DHA-enriched fish oil-supplemented group from 0.7 (SD 0.1) μ g/ml to 2.8 (SD 1.3) μ g/ml. There was no significant difference between the IgE levels of the group fed the control diet and the DHA-enriched fish oil-supplemented group. Additionally the specificity of the sensitisation

was supported by measuring OVA-specific IgE titres in sensitised mice whereas in non-sensitised mice no OVA-specific IgE was detected (data not shown; C Koch, S Dölle, M Metzger, C Rasche, H Jurydas, R Rühl, H Renz and M Worm, unpublished results).

Discussion

In the present study, we demonstrated that both DHA-enriched fish oil supplementation and allergic sensitisation significantly influence the fatty acid composition of different serum lipid classes. Allergic sensitisation after three intraperitoneal injections of OVA associated to Al(OH)₃ has been shown in various publications of our group^(27,29,30) and additionally in the present study we determined an increase of total IgE after OVA sensitisation. The levels of IgE were just slightly lower in the DHA-enriched fish oil-supplemented group in comparison with the control diet-fed group. A following publication will describe more deeply the effects of DHA on immuno-relevant parameters (C Koch, S Dölle, R Rühl and M Worm, unpublished results). In the present paper we first describe the alteration of ratios of individual fatty acids being precursors of pro-inflammatory or anti-inflammatory PUFA metabolites changed after allergic sensitisation in a manner depending on DHA-enriched fish oil supplementation.

Supplementation of DHA has been shown in various studies carried out in various organisms to yield in increased concentrations of DHA as well as EPA in serum lipids^(17,18,31). Our data support this aspect of previous reports in a mouse model investigating the fatty acid composition of serum TAG, phospholipids and cholesteryl esters. The effect of DHA-enriched fish oil supplementation on EPA levels could be mediated *via* three pathways: (1) increased concentrations of EPA in the DHA-enriched fish oil; (2) retroconversion of DHA to EPA⁽⁷⁾; (3) decreased conversion of EPA to DHA⁽⁷⁾. In addition to increased levels of the principal *n*-3 long-chain PUFA DHA and EPA, the levels of the *n*-6 long-chain PUFA AA markedly decreased. This finding is in accordance with previous observations^(17,31), and may reflect the inhibitory effects of abundance of DHA on Δ -6-desaturase, the rate-limiting step also in AA biosynthesis (for a review, see Horrobin⁽³²⁾), based on higher concentrations of AA precursors in sunflower-seed oil in comparison with DHA-enriched fish oil (Table 1).

The exact interrelationship of fatty acids in circulating serum lipids to tissue fatty acid metabolism remains to be clarified^(33,34). However, fatty acid composition of serum lipid classes is still considered an important indicator of fatty acid status⁽³⁵⁾. In the present study, effects of dietary intervention were detectable in all the three serum lipid classes analysed, whereas a marked effect of allergic sensitisation on *n*-3 long-chain PUFA was seen mainly in serum TAG esters.

The major outcome of the present study was the influence of the allergic sensitisation on the fatty acid composition of various serum lipid classes. To the best of our knowledge, no previous study has focused on the effect of allergic sensitisation on serum fatty acid patterns, whereas several studies have compared fatty acid status in human subjects with and without allergic disease^(13,36) (for recent reviews, see Devereux & Seaton⁽¹¹⁾ and Mickleborough & Rundell⁽¹²⁾). Possibly these alterations are partly due to an altered lipoprotein distribution after the acute-phase response (for a review, see Schweigert⁽³⁷⁾).

In the present study, we investigated the effects of allergic sensitisation induced by triple intraperitoneal OVA injections in adult mice, and observed several significant alterations of plasma phospholipids fatty acid levels. Our experiments showed that Al(OH)₃ has no or just marginal effects on lipid composition. This biochemical inactive Al(OH)₃ adjuvant is used to potentiate the immune responses to vaccines by adsorbing the antigen^(38,39). Al(OH)₃ does not alter serum lipid concentrations if given intraperitoneally and absorbed to allergen, while Al or Al ions could be quite active in the alteration of lipid metabolism⁽⁴⁰⁾. No relevant amounts of Al and Al ions will be reached in the blood circulation when biochemically stable Al(OH)₃ is used under physiological pH conditions after intraperitoneal injections⁽⁴¹⁾.

After allergic sensitisation but without accompanying DHA supplementation, the levels of mainly the *n*-3 PUFA, α -linolenic acid, EPA, docosapentaenoic acid and DHA were significantly altered in TAG. EPA and docosapentaenoic acid were significantly down regulated, whereas DHA and α -linolenic acid were significantly up regulated. For better visualisation of the results obtained, the ratios of precursors of pro- and anti-inflammatory bioactive lipids EPA, DHGLA and AA were calculated in Table 5.

EPA:AA and EPA:DHGLA ratios were significantly reduced upon allergic sensitisation and without accompanying DHA-enriched fish oil supplementation. After accompanying DHA-enriched fish oil supplementation, however, the reduction of the ratios was much lower. Consequently, the ratios were significantly lower in the non-supplemented than in supplemented animals.

Metabolism *via* COX and LOX pathways has been shown to be highly dependent on the availability of lipid precursors for further metabolism⁽⁴²⁾. It has been convincingly demonstrated that *n*-3 PUFA were better substrates for the conversion by LOX⁽⁴³⁾, but much weaker for the COX-2-mediated pathways to PgE derivatives⁽⁴⁴⁾. In addition, leucotrienes originating from EPA have been shown to be much less active in comparison with analogues from the AA cascade⁽⁴⁵⁾. EPA and other *n*-3 PUFA were also found to inhibit COX and LOX activity^(46–48).

Our data strongly support the hypothesis that DHA-enriched fish oil supplementation significantly alters the levels and ratios of *n*-3 : *n*-6 precursor fatty acids for further bioactivation to pro-inflammatory PG, which are mainly T-helper cell 2 skewing, and leucotrienes^(4,49). Various immune-competent cells such as lymphocytes, macrophages, dendritic cells, etc could alter lipid metabolism *via* various enzymes such as LOX, COX, cytochromes, etc, as well as various immune reactions could be influenced by dietary lipids and their active metabolites (summarised in various reviews^(3,50)). Further animal studies are in progress as well as the establishment of sensitive MS-based analytical techniques to identify bioactive eicosanoids in *in vitro* as well as *in vivo* studies in the serum and various immunocompetent cells at various stages during allergic sensitisation. In addition, novel studies by Serhan's group support that EPA and DHA *via* COX- and LOX-driven pathways serve as precursors for anti-inflammatory bioactive lipids such as lipoxins, neuroprotectins and resolvins (for reviews, see Serhan⁽⁵⁾ and Serhan & Savill⁽⁶⁾). Higher levels of DHA and EPA may lead to increased production of these novel-described derivatives in the mammalian organism after DHA-enriched fish oil supplementation and

accompanying allergic sensitisation, possibly resulting in reduced severity of the allergic phenotype.

In summary, the data obtained in the present study indicate that fatty acid levels and especially the ratios of fatty acids representing precursors of bioactive lipids after allergic sensitisation highly depend on accompanying DHA-enriched fish oil supplementation. This DHA-enriched fish oil supplementation-mediated alteration of lipid ratios of bioactive-precursor lipids may explain the allergy-ameliorating effects of DHA in particular and/or *n*-3 PUFA in general^(14–20); unfortunately in our experimental set up we could not observe any allergy-ameliorating effect. Further studies of our group will focus on lipid metabolism and molecular mechanisms of possible allergy-preventive effects of DHA in animal studies, human supplementation trials and human cohort studies.

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