Incorporation of n-3 Fatty Acids into Plasma Lipid Fractions, and Erythrocyte Membranes and Platelets During Dietary Supplementation with Fish, Fish Oil, and Docosahexaenoic Acid-Rich Oil Among Healthy Young Men

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ABSTRACT: The effects of n-3 fatty acid supplementation in the form of fresh fish, fish oil, and docosahexaenoic acid (DHA) oil on the fatty acid composition of plasma lipid fractions, and platelets and erythrocyte membranes of young healthy male students were examined. Altogether 59 subjects (aged 19–32 yr, body mass index 16.8–31.3 kg/m^2) were randomized into the following diet groups: (i) control group; (ii) fish diet group eating fish meals five times per week [0.38 ± 0.04 g eicosapentaenoic acid (EPA) and 0.67 ± 0.09 g DHA per day]; (iii) DHA oil group taking algae-derived DHA oil capsules (1.68 g/d DHA in triglyceride form); and (iv) fish oil group (1.33 g EPA and 0.95 g DHA/d as free fatty acids) for 14 wk. The fatty acid composition of plasma lipids, platelets, and erythrocyte membranes was analyzed by gas chromatography. The subjects kept 4-d food records four times during the study to estimate the intake of nutrients. In the fish diet, in DHA oil, and in fish oil groups, the amounts of n-3 fatty acids increased and those of n-6 fatty acids decreased significantly in plasma lipid fractions and in platelets and erythrocyte membranes. A positive relationship was shown between the total n-3 polyunsaturated fatty acids (PUFA) and EPA and DHA intake and the increase in total n-3 PUFA and EPA and DHA in all lipid fractions analyzed. DHA was preferentially incorporated into phospholipid (PL) and triglyceride (TG) and there was very little uptake in cholesterol ester (CE), while EPA was preferentially incorporated into PL and CE. The proportion of EPA in plasma lipids and platelets and erythrocyte membranes increased also by DHA supplementation, and the proportion of linoleic acid increased in platelets and erythrocyte membranes in the DHA oil group as well. These results suggest retroconversion of DHA to EPA and that DHA also interferes with linoleic acid metabolism.

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Recent studies have demonstrated that increased intake of polyunsaturated fatty acids (PUFA) of the n-3 series, in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), may have a favorable effect on serum lipids (1–3), platelet aggregability (4–6), and bleeding time (4) and thus could lead to a reduced risk of atherosclerotic vascular disease and thrombotic complications. Long-term consumption of fish leads to the increased incorporation of n-3 fatty acids into plasma lipids, erythrocytes, and platelets (7,8). After fish oil supplementation, increased content of EPA and DHA has been measured in plasma lipids (6,8–10) and platelet (11,12) and erythrocyte membranes (13,14) with the simultaneous decrease of arachidonic acid and docosatetraenoic acid content, but less is known about the effect of DHA supplementation alone. DHA is a major n-3 fatty acid of most fish, yet most commercial fish oils contain more EPA than DHA (15). The aim of the present study was to examine the long-term effects of the supplementation of three different sources of EPA and DHA on the incorporation of fatty acids in plasma lipids, erythrocyte membranes, and platelets in healthy young men. In particular, interest was focused on the effects of DHA-rich oil (without EPA), which has been shown to have a comparable hypotriglyceridemic effect with fish diet and fish oil supplementation (16).

SUBJECTS AND METHODS

Subjects. Altogether 59 healthy male students aged 19–32 yr participated in this study. Body mass index (BMI) ranged from 16.8 to 31.3 kg/m^2. One subject in the control and one in the fish diet group were excluded because of high plasma triglyceride (TG) levels (>2.5 mmol/L). One subject did not follow the fish diet and was excluded. One subject in the fish oil group was excluded because of low platelet counts in the beginning of the study ($35 \cdot 10^9$/L). Three subjects (one in the control, one in the fish diet, and one in the DHA oil group) missed the analysis of fatty acid composition. Thus, the final analysis included 52 subjects. In addition, erythrocyte sam-

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Abbreviations: BMI, body mass index; CE, cholesterol ester; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MUFA, monounsaturated fatty acids; PL, phospholipid; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TG, triglyceride.

ples from 13 subjects—7 in the control, 1 in the fish diet, 3 in the DHA oil, and 2 in the fish oil groups—and a platelet sample from 1 subject in the control group were discarded because of contamination during sample preparation.

Study design. The present study was part of a study to investigate the long-term effects of moderate amounts of n-3 fatty acids in different forms on plasma lipids and was carried out at the Department of Physiology, University of Kuopio (Kuopio, Finland). Detailed description of the study has been described elsewhere (16). The protocol was approved by the Ethics Committee of the University of Kuopio. The subjects were randomly allocated into one of the four diet groups: (i) control group; (ii) fish diet group; (iii) DHA oil group, and (iv) fish oil group. The subjects started the study stepwise with 2-wk intervals in three groups. The study period was 14 wk. The subjects visited the research unit four times: at baseline and after 4 wk, 9 wk, and 14 wk. Height was measured at baseline only and weight at every visit. Blood samples for laboratory measurements were taken at every visit after 12 h fasting.

Experimental diets and oil supplements. Subjects in the control group were asked to maintain their normal dietary habits and physical activities constant throughout the study. Subjects in the fish diet group consumed their fish meal at the University Restaurant every working day five times per week. The number of fish meals actually eaten was 4.3 ± 0.5 times per week which produced 0.38 g EPA, 0.08 g docosapentaenoic acid, and 0.67 g DHA per day. Fish used for meals were rainbow trout *(Oncorhynchus mykis),* Balting herring *(Clupea harengus),* and vendace *(Coregonus albula)*. Samples of cooked fish meals were taken every day. Meals containing same-fish species were pooled, and the fatty acid compositions of fish meals were analyzed. In the fish diet group, the intake of fish, except at the University Restaurant, was on an average 14 g/d, supplying 0.05 g EPA and 0.15 g DHA per day according to food records. The subjects in the DHA oil group took eight algae-derived DHA oil capsules/d (Martek Biosciences Corporation, Columbia, MD) supplying 210 mg DHA per capsule (1.68 g/d) in TG form. DHA oil capsules did not contain EPA or docosapentaenoic acid. Fish oil supplement (Bio-Marin, Pharma Nord ApS, Vojens, Denmark) provided 166 mg EPA, 19 mg docosapentaenoic acid, and 119 mg DHA per capsule (1.33, 0.15, and 0.95 g/d, respectively) as free fatty acids. Compliance was assessed by asking for the return of the unused capsules. Except for the oil supplement, the subjects in the DHA oil and fish oil groups were asked to maintain their normal food habits. Dietary fish intake in the DHA oil and fish oil groups during the study was 32 g and 26 g, respectively. At baseline, there were no significant differences in the dietary intake of arachidonic acid among the study groups (ranged from 0.14 to 0.22 g/d). Subjects in the fish oil group received 59 mg/d arachidonic acid from fish oil supplement.

Dietary records. Each participant kept a 4-d food record, using household measures, over three weekdays and one weekend day in the beginning of the study, and three times during the study. Food records were checked by a nutritionist. The intake of nutrients was calculated using the Micro-Nutrica dietary analysis program, based on the database of the Finnish Social Insurance Institute (17). In the results on the nutrient intake, data for each group are given at baseline (4 d) and for the whole study period based on the mean of 12-d food records.

Analysis of fatty acid composition of plasma lipids, platelets, and erythrocyte membranes. Blood samples for laboratory analyses were taken into EDTA-containing vacuum tubes after 12 h fasting. Plasma was separated by centrifugation and stored at −70°C until analyzed. In the analysis of the fatty acid composition of plasma lipids, samples were extracted with chloroform/methanol (2:1, vol/vol) (18), and the lipid fractions were separated by solid-phase extraction with an aminopropyl column (19). For fatty acid analysis of platelets and erythrocyte membranes, the blood samples were rotated at low speed (120 × *g* for 10 min at 4°C) to separate platelet-rich plasma. The platelets were separated by centrifugation at 2,000 × *g* for 15 min at 4°C and then washed with Tris-HCl buffer (pH 7.6, 172 mmol/L). Erythrocytes were washed with the same buffer and hemolyzed in the same diluted buffer (11 mmol/L). Erythrocyte membranes were prepared by centrifugation at 20,000 × *g* for 20 min at 4°C. Both platelet and erythrocyte membrane sediments were suspended in the Tris-HCl buffer (172 mmol/L) and stored at −80°C until analyzed. Lipids were extracted with chloroform/methanol and transmethylated with 14% boron trifluoride in methanol at +100°C for 1 h. Fatty acid methyl esters were analyzed with gas chromatography (HP 5890 Series II; Hewlett-Packard Company, Waldbronn, Germany) equipped with an HP-FFAP capillary column. The molar percentage proportions of fatty acids were calculated.

Statistical methods. Statistical analyses were carried out with the use of SPSS-PC (SPSS, Chicago, IL). The Kruskall-Wallis test was used to compare the between-group differences in the fatty acid composition values at baseline, and within group the changes in the fatty acid composition values from baseline to 14 wk. When the analysis indicated significant change ($P < 0.05$), the Mann-Whitney U-test was used to compare the values of the study groups to those of the control group.

RESULTS

The baseline characteristics of the subjects and the nutrient composition of the diets at baseline are presented in Table 1. Age, height, weight, and BMI were comparable among the study groups. There were no significant differences among the diet groups in the nutrient intake values based on the 4-d food record at baseline compared with those based on the 12-d food record during the study (three 4-d food records combined), except the intakes of total fat and monounsaturated fatty acids (MUFA) in the fish oil group which increased from 30.9 ± 5.7 to 33.7 ± 4.9% and from 11.0 ± 2.3 to 12.5 ± 2.5% of total energy, respectively, $P < 0.01$ in both. In addition, the intake of vitamin D decreased from 5.5 ± 2.6 to 3.9 ± 1.0 μg/d in the control group and increased from 5.1 ± 3.2

TABLE 1
Characteristics of the Study Subjects and the Nutrient Intake at Baseline[a]

	Control $n = 13$	Fish diet $n = 12$	DHA oil $n = 13$	Fish oil $n = 14$
Characteristics				
Age (yr)	23 ± 2	23 ± 2	24 ± 4	23 ± 2
Weight (kg)	75 ± 11	75 ± 12	71 ± 12	78 ± 11
Height (cm)	179 ± 8	182 ± 7	181 ± 6	181 ± 4
BMI (kg/m^2)	23.4 ± 2.1	22.8 ± 3.5	21.5 ± 2.5	23.7 ± 3.3
Nutrient intake				
Energy (MJ/d)	9.9 ± 1.6	10.6 ± 1.7	10.5 ± 2.7	11.3 ± 3.4
Fat (% of energy)	33.0 ± 7.3	36.0 ± 5.4	35.3 ± 7.2	30.9 ± 5.7
SFA (% of energy)	12.9 ± 2.5	14.9 ± 2.4	14.0 ± 3.2	11.8 ± 2.8
MUFA (% of energy)	12.3 ± 3.5	12.6 ± 2.9	12.3 ± 3.1	11.0 ± 2.3
PUFA (% of energy)	5.3 ± 1.8	5.9 ± 1.6	6.4 ± 2.2	5.8 ± 1.9
Protein (% of energy)	15.5 ± 3.1	16.1 ± 1.7	16.0 ± 3.5	16.4 ± 3.7
Carbohydrates (% of energy)	48.0 ± 7.0	43.5 ± 4.6	45.3 ± 7.4	48.0 ± 7.9
Cholesterol (mg/d)	295 ± 134	320 ± 75	312 ± 122	290 ± 87
n-3 Fatty acids from fish diet and supplements				
EPA (g/d)		0.38 ± 0.04	—	1.33
DHA (g/d)		0.67 ± 0.09	1.68	0.95

[a]Values are means ± SD. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; BMI, body mass index.

to 9.2 ± 1.8 μg/d in the fish diet group ($P < 0.05$ and $P < 0.01$, respectively).

Fatty acid composition. The fatty acid composition of plasma TG, cholesterol esters (CE) and phospholipids (PL), and platelets and erythrocyte membranes at baseline are shown in Tables 2–6, as well as the changes in the fatty acid values during 14 wk. The baseline levels of fatty acids in the plasma lipid fractions, erythrocyte membranes, and platelets did not differ among the diet groups.

No significant changes were observed in the fatty acid composition of the control group in either plasma lipids, platelets, or erythrocyte membranes during the study period.

Fatty acid composition of plasma lipids. In the fish diet, in DHA oil and fish oil groups, the amount of n-3 fatty acids increased significantly in TG, CE, and PL, and the amount of n-6 fatty acids decreased significantly in PL when compared with those of the control group. In the fish diet group, the proportions of dihomo-γ-linolenic acid in CE and PL and that of

TABLE 2
Fatty Acid Composition (mol% of total) of Plasma Triglycerides[a]

	Control ($n = 13$)		Fish diet ($n = 12$)		DHA oil ($n = 13$)		Fish oil ($n = 14$)	
Fatty acid	Baseline	Change	Baseline	Change	Baseline	Change	Baseline	Change
SFA	33.4 ± 3.5	0.6 ± 5.2	34.4 ± 4.0	1.9 ± 5.1	35.7 ± 4.3	–0.7 ± 5.3	33.4 ± 4.3	–1.0 ± 6.5
MUFA	45.1 ± 3.0	0.0 ± 6.2	44.6 ± 2.1	–2.8 ± 4.0	44.1 ± 3.6	–3.2 ± 3.3	44.8 ± 3.6	–4.7 ± 6.2
18:1n-9	38.4 ± 3.1	0.2 ± 5.3	38.0 ± 2.3	–2.3 ± 3.9	37.6 ± 2.9	–2.5 ± 2.8	38.3 ± 3.1	–3.8 ± 5.0
PUFA	21.5 ± 4.1	–0.6 ± 6.6	21.0 ± 3.5	0.9 ± 6.1	20.9 ± 4.6	4.0 ± 5.6	21.8 ± 5.9	5.7 ± 6.8
18:2n-6	16.9 ± 3.8	–0.5 ± 6.1	15.6 ± 3.1	–0.9 ± 4.7	15.7 ± 3.7	–0.0 ± 2.7	16.8 ± 4.8	1.3 ± 5.4
18:3n-6	0.4 ± 0.2	–0.1 ± 0.2	0.4 ± 0.2	–0.1 ± 0.2	0.4 ± 0.2	–0.1 ± 0.2	0.4 ± 0.2	–0.1 ± 0.2
18:3n-3	1.6 ± 0.6	0.2 ± 0.5	1.7 ± 0.4	0.0 ± 0.7	1.4 ± 0.3	0.7 ± 0.5	2.0 ± 0.8	0.1 ± 0.9
20:3n-6	0.2 ± 0.1	0.0 ± 0.1	0.2 ± 0.1	–0.0 ± 0.1	0.2 ± 0.1	–0.0 ± 0.1	0.2 ± 0.1	–0.1 ± 0.1
20:4n-6	0.8 ± 0.2	–0.1 ± 0.3	0.9 ± 0.1	–0.0 ± 0.2	0.8 ± 0.3	0.0 ± 0.2	0.8 ± 0.2	0.2 ± 0.2
20:5n-3	0.3 ± 0.2	–0.0 ± 0.2	0.4 ± 0.2	0.6 ± 0.9[b]	0.3 ± 0.2	0.2 ± 0.4[a]	0.4 ± 0.2	1.7 ± 0.6[c]
22:5n-3	0.3 ± 0.1	–0.0 ± 0.1	0.4 ± 0.1	0.1 ± 0.2	0.3 ± 0.1	–0.0 ± 0.2	0.3 ± 0.1	0.5 ± 0.2[c]
22:6n-3	0.9 ± 0.6	–0.1 ± 0.7	1.3 ± 0.6	1.3 ± 1.8[a]	1.0 ± 1.0	3.2 ± 2.7[c]	0.9 ± 0.6	2.0 ± 1.0[c]
∑n-6	18.4 ± 3.8	–0.7 ± 6.2	17.2 ± 3.2	–1.2 ± 4.9	17.2 ± 3.9	–0.1 ± 2.9	18.2 ± 4.9	1.4 ± 5.6
∑n-3	3.1 ± 0.8	0.1 ± 0.9	3.8 ± 0.9	2.1 ± 2.9[a]	3.0 ± 1.3	4.1 ± 3.5[c]	3.5 ± 1.3	4.3 ± 2.0[c]
Quantitative amounts (μg/mL)								
	Baseline	After 14 wk	Baseline	After 14 wk	Baseline	After 14 wk	Baseline	After 14 wk
20:5n-3	2.5 ± 1.2	2.9 ± 2.0	3.3 ± 1.5	7.0 ± 4.0	2.5 ± 1.6	3.5 ± 2.5	3.3 ± 2.9	12.9 ± 5.7[c]
22:6n-3	7.0 ± 3.6	8.4 ± 6.6	11.2 ± 6.9	21.2 ± 10.5	7.8 ± 6.6	28.0 ± 18.7[b]	9.1 ± 7.5	19.8 ± 10.8[b]

[a]At baseline and the changes after 14 wk, and quantitative amounts of eicosapentaenoic and docosahexaenoic acids (μg/mL) at baseline and after 14 wk among the study groups. Values are means ± SD. Superscript letters a, b, c indicate a significant difference from control group, $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively. See Table 1 for abbreviations.

TABLE 3
Fatty Acid Composition (mol% of total) of Plasma Cholesterol Esters[a]

	Control (n = 13)		Fish diet (n = 12)		DHA oil (n = 13)		Fish oil (n = 14)	
Fatty acid	Baseline	Change	Baseline	Change	Baseline	Change	Baseline	Change
SFA	14.9 ± 0.8	1.2 ± 2.3	15.3 ± 1.3	0.7 ± 1.6	15.3 ± 1.8	–0.2 ± 1.3	15.1 ± 1.1	1.8 ± 1.7
MUFA	24.9 ± 3.8	0.6 ± 2.6	23.9 ± 3.1	0.5 ± 4.1	24.4 ± 2.8	–2.0 ± 2.3	23.7 ± 2.8	–1.6 ± 2.6
18:1n-9	20.5 ± 2.4	0.6 ± 2.3	19.7 ± 2.5	0.3 ± 3.0	20.0 ± 2.1	–1.5 ± 1.6	19.3 ± 1.8	–1.2 ± 1.6
PUFA	60.2 ± 4.0	–1.8 ± 4.5	60.8 ± 3.8	–1.2 ± 4.5	60.4 ± 3.9	2.2 ± 3.1	61.3 ± 3.2	–0.2 ± 3.2
18:2n-6	51.4 ± 4.0	–1.0 ± 4.6	52.1 ± 4.0	–2.2 ± 5.1	52.6 ± 3.9	1.9 ± 3.8	53.2 ± 4.1	–4.4 ± 4.3
18:3n-6	0.8 ± 0.3	–0.1 ± 0.2	0.7 ± 0.2	–0.2 ± 0.3	0.7 ± 0.2	–0.3 ± 0.2[a]	0.7 ± 0.4	–0.4 ± 0.2[b]
18:3n-3	0.8 ± 0.3	0.0 ± 0.2	0.8 ± 0.2	0.0 ± 0.2	0.8 ± 0.2	0.1 ± 0.2	0.8 ± 0.2	–0.1 ± 0.2
20:3n-6	0.5 ± 0.1	–0.0 ± 0.1	0.5 ± 0.1	–0.1 ± 0.1[a]	0.4 ± 0.1	–0.1 ± 0.1	0.5 ± 0.2	–0.1 ± 0.1[b]
20:4n-6	4.9 ± 1.3	–0.5 ± 0.6	4.8 ± 1.1	–0.5 ± 0.8	4.3 ± 0.9	–0.5 ± 0.7	4.6 ± 1.2	0.2 ± 0.7[a]
20:5n-3	1.2 ± 0.4	–0.2 ± 0.4	1.3 ± 0.5	1.5 ± 1.0[c]	1.0 ± 0.2	0.5 ± 0.5[c]	1.0 ± 0.3	4.0 ± 1.2[c]
22:6n-3	0.6 ± 0.2	0.0 ± 0.2	0.6 ± 0.2	0.3 ± 0.3[a]	0.5 ± 0.2	0.5 ± 0.3[c]	0.6 ± 0.1	0.5 ± 0.2[c]
∑n-6	57.6 ± 4.1	–1.6 ± 4.6	58.1 ± 3.8	–3.0 ± 5.0	58.0 ± 3.8	1.0 ± 3.4	58.9 ± 3.5	–4.6 ± 4.0
∑n-3	2.6 ± 0.6	–0.2 ± 0.6	2.7 ± 0.6	1.8 ± 1.2[c]	2.3 ± 0.4	1.2 ± 0.9[c]	2.4 ± 0.4	4.4 ± 1.4[c]
Quantitative amounts (μg/mL)								
	Baseline	After 14 wk	Baseline	After 14 wk	Baseline	After 14 wk	Baseline	After 14 wk
20:5n-3	13.0 ± 6.3	9.9 ± 3.6	12.0 ± 5.2	27.1 ± 10.5	9.7 ± 2.8	14.2 ± 6.2	10.5 ± 4.5	45.5 ± 16.1[c]
22:6n-3	6.4 ± 3.2	6.0 ± 2.7	6.0 ± 2.2	9.1 ± 2.8	5.7 ± 2.4	11.2 ± 4.7	6.1 ± 1.8	10.5 ± 3.8[b]

[a]At baseline and the changes after 14 wk, and quantitative amounts of eicosapentaenoic and docosahexaenoic acids (μg/mL) at baseline and after 14 wk among the study groups. Values are means ± SD. Superscript letters a, b, c are significantly different from control group, $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively. See Table 1 for abbreviations.

arachidonic acid in PL decreased significantly. The DHA oil-supplemented subjects had significantly decreased values of γ-linolenic acid in CE and lower values of dihomo-γ-linolenic, arachidonic and docosapentaenoic acids in PL. Compared with the control group, the fish oil-supplemented subjects had significant increases in the proportions of docosapentaenoic acid in TG and PL and arachidonic acid in CE, and decreases in the proportions of γ-linolenic and dihomo-γ-linolenic acids in CE and oleic, linoleic, dihomo-γ-linolenic, and docosatetraenoic acids in PL (Tables 2–4, Fig. 1).

Quantitative amounts of EPA and DHA in plasma lipids. Quantitative amounts of EPA and DHA in plasma lipid fractions are presented in Tables 2–4. The 14-wk fish diet elevated most of the quantitative amounts of EPA in PL and CE and the amount of DHA in PL and TG. After DHA oil supplementation, the quantitative amount of DHA in plasma

TABLE 4
Fatty Acid Composition (mol% of total) of Plasma Phospholipids[a]

	Control (n = 13)		Fish diet (n = 12)		DHA oil (n = 13)		Fish oil (n = 14)	
Fatty acid	Baseline	Change	Baseline	Change	Baseline	Change	Baseline	Change
SFA	48.0 ± 1.2	0.1 ± 1.1	48.6 ± 1.4	–0.4 ± 1.4	47.8 ± 0.9	–0.2 ± 1.5	47.6 ± 1.1	0.7 ± 0.9
MUFA	14.6 ± 1.7	0.0 ± 1.5	14.3 ± 1.7	0.1 ± 1.9	14.5 ± 1.3	–0.8 ± 0.9	14.8 ± 1.4	–1.4 ± 1.7
18:1n-9	10.1 ± 1.5	0.2 ± 1.5	10.1 ± 1.7	0.1 ± 1.9	10.3 ± 1.1	–0.8 ± 0.7	10.4 ± 1.2	–1.4 ± 1.3[b]
PUFA	37.9 ± 1.9	–0.1 ± 2.0	37.7 ± 2.4	0.1 ± 2.5	38.3 ± 1.5	0.8 ± 1.5	38.2 ± 1.7	0.4 ± 1.8
18:2n-6	22.4 ± 2.4	0.2 ± 3.0	21.8 ± 2.4	–0.7 ± 3.1	23.6 ± 2.1	–0.3 ± 3.1	23.2 ± 2.6	–3.9 ± 2.5[b]
18:3n-6	0.1 ± 0.1	–0.0 ± 0.1	0.1 ± 0.0	–0.0 ± 0.0	0.1 ± 0.1	–0.1 ± 0.1	0.1 ± 0.1	–0.0 ± 0.1
18:3n-3	0.3 ± 0.1	0.0 ± 0.1	0.4 ± 0.1	0.0 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.4 ± 0.1	0.1 ± 0.1
20:3n-6	2.7 ± 0.5	0.1 ± 0.6	2.8 ± 0.9	–0.9 ± 0.5[c]	2.5 ± 0.5	–0.9 ± 0.5[c]	2.6 ± 0.7	–1.2 ± 0.6[c]
20:4n-6	6.6 ± 1.2	–0.2 ± 1.0	6.7 ± 1.1	–1.2 ± 0.5[b]	6.4 ± 1.0	–1.1 ± 0.8[a]	6.6 ± 1.3	–0.6 ± 0.8
20:5n-3	1.2 ± 0.4	–0.1 ± 0.4	1.4 ± 0.5	1.5 ± 1.4[b]	1.1 ± 0.3	0.4 ± 0.6[a]	1.1 ± 0.4	3.9 ± 1.1[c]
22:4n-6	0.2 ± 0.1	–0.0 ± 0.1	0.1 ± 0.1	–0.1 ± 0.0	0.1 ± 0.1	–0.1 ± 0.8	0.2 ± 0.1	–0.1 ± 0.1[a]
22:5n-3	0.6 ± 0.1	–0.0 ± 0.1	0.6 ± 0.1	0.1 ± 0.2	0.6 ± 0.1	–0.3 ± 0.1[c]	0.6 ± 0.1	0.3 ± 0.2[c]
22:6n-3	3.7 ± 0.5	–0.0 ± 0.7	3.8 ± 0.8	1.4 ± 1.2[b]	3.5 ± 0.9	3.0 ± 1.3[c]	3.4 ± 0.6	2.1 ± 0.8[c]
∑n-6	32.1 ± 2.1	–0.1 ± 2.6	31.5 ± 2.6	–2.8 ± 3.4[a]	32.7 ± 1.6	–2.3 ± 2.6[a]	32.6 ± 1.8	–5.7 ± 2.7[c]
∑n-3	5.9 ± 0.8	–0.1 ± 1.0	6.2 ± 1.3	3.0 ± 2.6[b]	5.6 ± 1.0	3.2 ± 1.8[c]	5.6 ± 0.8	6.2 ± 1.8[c]
Quantitative amounts (μg/mL)								
	Baseline	After 14 wk	Baseline	After 14 wk	Baseline	After 14 wk	Baseline	After 14 wk
20:5n-3	15.5 ± 6.0	13.2 ± 5.1	18.0 ± 7.2	34.4 ± 15.5	13.7 ± 3.0	19.1 ± 8.3[b]	16.5 ± 10.4	54.6 ± 18.8[c]
22:6n-3	51.2 ± 12.6	48.2 ± 11.4	50.0 ± 12.6	67.0 ± 8.4[b]	47.0 ± 12.6	85.1 ± 25.7[c]	49.5 ± 14.9	65.4 ± 20.2[b]

[a]At baseline and the changes after 14 wk, quantitative amounts of eicosapentaenoic and docosahexaenoic acids (μg/mL) at baseline and after 14 wk among the study groups. Values are means ± SD. Superscript letters a, b, c are significantly different from control group, $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively. See Table 1 for abbreviations.

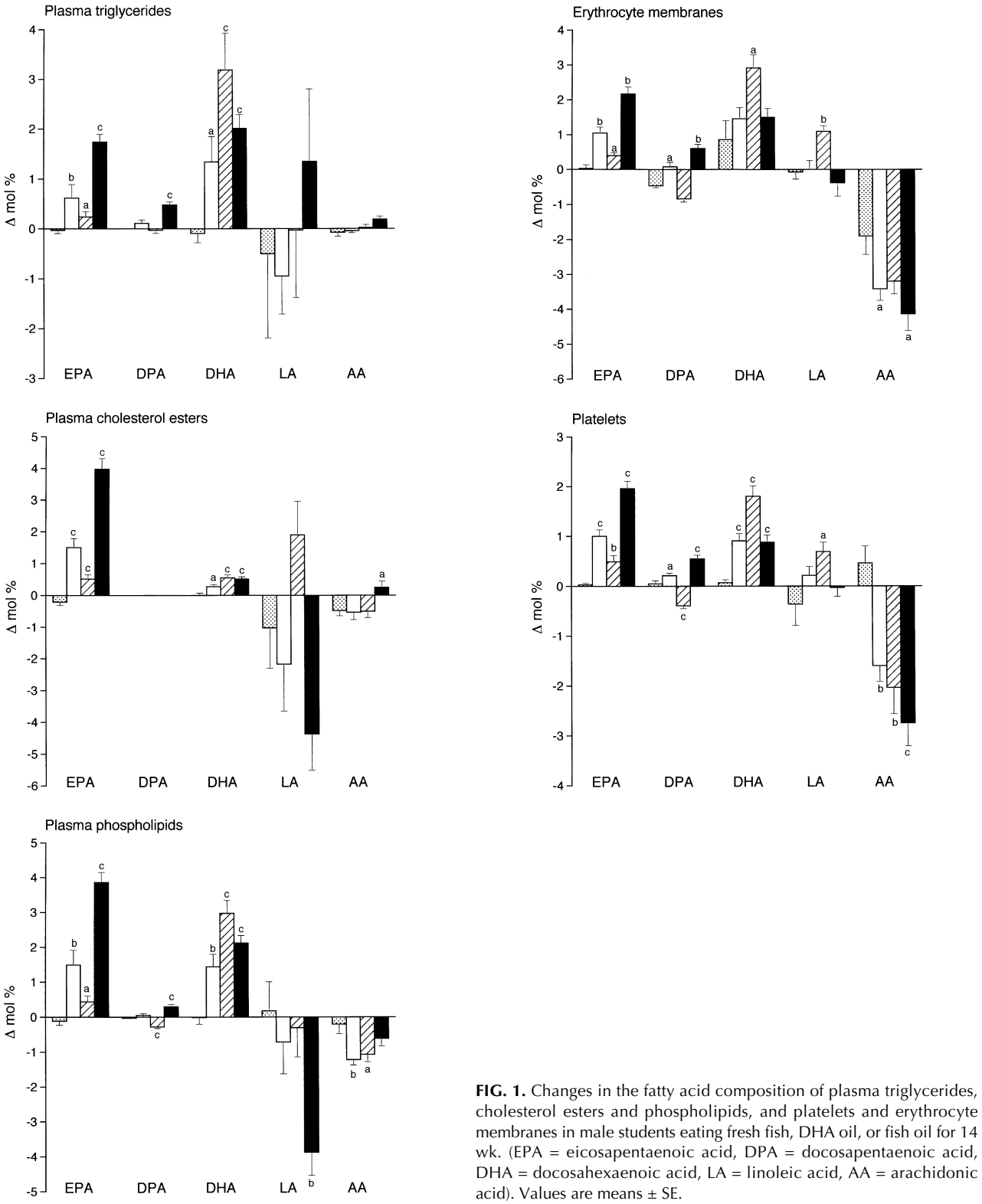

FIG. 1. Changes in the fatty acid composition of plasma triglycerides, cholesterol esters and phospholipids, and platelets and erythrocyte membranes in male students eating fresh fish, DHA oil, or fish oil for 14 wk. (EPA = eicosapentaenoic acid, DPA = docosapentaenoic acid, DHA = docosahexaenoic acid, LA = linoleic acid, AA = arachidonic acid). Values are means ± SE.

lipids increased more than that of EPA, and this increase was greatest in PL and TG. Fish oil supplementation induced the most marked increase of EPA in PL and CE, whereas the increase of DHA was most evident in PL and TG. Figure 2 indicates the relationship between the intake of EPA and DHA and their levels in plasma PL. The same relationship applies to plasma TG, and platelets and erythrocytes membranes, as well (data not shown). Also the dose-response curves calculated on the basis of the results are shown in Figure 2. It should be noted that there are small amounts of EPA and

TABLE 5
Fatty Acid Composition (%mmol) of Platelets

	Control (n = 12)		Fish diet (n = 12)		DHA oil (n = 13)		Fish oil (n = 14)	
Fatty acid	Baseline	Change	Baseline	Change	Baseline	Change	Baseline	Change
SFA	44.1 ± 1.0	–0.2 ± 0.9	44.9 ± 1.7	–1.1 ± 1.2	44.0 ± 1.2	–0.8 ± 1.3	44.1 ± 1.2	–1.2 ± 1.1
MUFA	21.0 ± 0.9	–0.0 ± 0.8	20.6 ± 1.0	0.8 ± 0.8[a]	21.2 ± 1.5	0.5 ± 1.4	21.3 ± 0.8	1.2 ± 1.0[b]
18:1n-9	16.5 ± 0.8	0.0 ± 0.9	16.2 ± 0.8	0.5 ± 0.7	16.7 ± 1.2	0.5 ± 1.1	16.6 ± 0.6	1.1 ± 0.8[b]
PUFA	34.8 ± 0.7	0.3 ± 1.3	34.5 ± 1.9	0.2 ± 1.4	34.8 ± 1.8	0.3 ± 2.2	34.6 ± 1.0	–0.0 ± 1.0
18:2n-6	6.3 ± 1.4	–0.4 ± 1.5	5.9 ± 0.7	0.2 ± 0.6	6.3 ± 0.7	0.7 ± 0.6[a]	6.1 ± 0.6	–0.0 ± 0.7
20:3n-6	1.3 ± 0.2	0.1 ± 0.1	1.2 ± 0.3	–0.0 ± 0.2	1.3 ± 0.3	0.0 ± 0.2	1.3 ± 0.2	–0.0 ± 0.7[b]
20:4n-6	21.7 ± 1.3	0.5 ± 1.2	21.6 ± 1.5	–1.6 ± 1.1[b]	22.0 ± 1.4	–2.0 ± 1.9[b]	21.8 ± 0.9	–2.8 ± 1.7[c]
20:5n-3	0.7 ± 0.1	0.0 ± 0.1	0.7 ± 0.2	1.0 ± 0.5[c]	0.7 ± 0.2	0.5 ± 0.4[b]	0.7 ± 0.2	2.0 ± 0.6[c]
22:4n-6	1.1 ± 0.1	–0.0 ± 0.1	1.3 ± 0.2	–0.4 ± 0.1[c]	1.1 ± 0.2	–0.4 ± 0.2[c]	1.3 ± 0.3	–0.6 ± 0.3[c]
22:5n-3	1.1 ± 0.2	0.1 ± 0.2	1.2 ± 0.2	0.2 ± 0.2[a]	1.1 ± 0.2	–0.4 ± 0.2[c]	1.2 ± 0.2	0.6 ± 0.3[c]
22:6n-3	2.2 ± 0.3	0.1 ± 0.2	2.1 ± 0.5	0.9 ± 0.5[c]	1.9 ± 0.4	1.8 ± 0.8[c]	2.0 ± 0.2	0.9 ± 0.5[c]
∑n-6	30.4 ± 0.9	0.2 ± 1.2	30.0 ± 1.7	–1.8 ± 1.6[b]	30.6 ± 1.7	–1.6 ± 2.5[b]	30.4 ± 1.0	–3.4 ± 1.5[c]
∑n-3	4.0 ± 0.4	0.2 ± 0.4	4.0 ± 0.6	2.1 ± 1.0[c]	3.7 ± 0.5	1.9 ± 1.0[c]	3.8 ± 0.5	3.4 ± 0.9[c]

[a]At baseline and the changes after 14 wk among the study groups. Values are means ± SD. Superscript letters a, b, c are significantly different from control group, $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively. See Table 1 for abbreviations.

DHA derived from α-linolenic acid. Therefore, concentrations of EPA and DHA are clearly measurable even with zero dietary intake of these fatty acids. It seems that the response curves of EPA and DHA are steepest with low intakes of EPA and DHA, and the response curves follow logarithmic equations: the higher the intake the lower the proportional increase in concentration (Fig. 2).

Fatty acid composition of platelets. In platelets, the proportions of EPA and DHA were substantially increased mainly at the expense of arachidonic acid and docosatetraenoic acid in the fish diet, DHA oil, and fish oil groups (Table 5, Fig. 1). Also the proportion of docosapentaenoic acid increased in the fish diet and fish oil groups but was decreased by DHA oil supplementation. Both in the fish diet and fish oil groups the total proportion of MUFA and in the fish oil group the proportion of oleic acid increased significantly. In contrast to other groups, the proportion of linoleic acid increased in the DHA oil group.

Fatty acid composition of erythrocyte membranes. The proportions of EPA in erythrocyte membranes increased in the fish diet, DHA oil, and fish oil groups. The increase of DHA was statistically significant only in the DHA oil group (Table 6, Fig. 1). In the fish diet group, the proportions of arachidonic and docosatetraenoic acids were significantly decreased compared with those of the control group. The DHA oil group had a significantly increased proportion of linoleic acid and decreased proportion of docosatetraenoic acid. In the fish oil group, the proportion of docosapentaenoic acid increased and the proportions of dihomo-γ-linolenic, arachidonic, and docosatetraenoic acids decreased when compared with the control group.

The proportions of EPA and DHA in plasma PL correlated with those in platelets and erythrocyte membranes both at baseline (r = 0.50–0.72, $P < 0.001$ for EPA; and 0.52–0.65, $P = 0.001$ or less for DHA) and after 14 wk (r = 0.83–0.89; $P < 0.001$ and r = 0.40–0.78; $P = 0.015$ or less, respectively)

TABLE 6
Fatty Acid Composition (mol% of total) of Erythrocyte Membranes[a]

	Control (n = 6)		Fish diet (n = 11)		DHA oil (n = 10)		Fish oil (n = 12)	
Fatty acid	Baseline	Change	Baseline	Change	Baseline	Change	Baseline	Change
SFA	43.4 ± 0.5	0.7 ± 1.3	43.4 ± 0.9	1.2 ± 1.8	43.0 ± 0.8	0.3 ± 1.6	43.1 ± 0.7	1.1 ± 1.6
MUFA	22.5 ± 0.9	0.7 ± 0.5	23.1 ± 0.8	0.5 ± 2.0	22.9 ± 1.3	0.0 ± 1.3	23.0 ± 1.4	0.3 ± 2.7
18:1n-9	16.8 ± 1.1	–0.1 ± 1.0	17.5 ± 0.7	–0.2 ± 2.0	17.1 ± 1.2	–0.2 ± 1.2	17.1 ± 1.2	–0.4 ± 2.0
PUFA	34.0 ± 1.0	–1.4 ± 1.3	33.5 ± 0.8	–1.6 ± 1.5	34.1 ± 0.7	–0.3 ± 1.7	33.8 ± 1.1	–1.4 ± 1.7
18:2n-6	8.4 ± 0.7	–0.1 ± 0.5	8.6 ± 0.9	0.0 ± 0.8	9.0 ± 1.0	1.1 ± 0.5[b]	9.3 ± 1.0	–0.4 ± 1.3
20:3n-6	1.5 ± 0.2	0.1 ± 0.2	1.5 ± 0.3	–0.2 ± 0.2	1.5 ± 0.2	–0.2 ± 0.2	1.5 ± 0.3	–0.4 ± 0.2[b]
20:4n-6	16.5 ± 1.0	–1.9 ± 1.3	15.9 ± 1.2	–3.4 ± 1.1[a]	16.1 ± 1.4	–3.2 ± 1.2	15.2 ± 1.6	–4.1 ± 1.7[a]
20:5n-3	0.8 ± 0.2	0.0 ± 0.3	0.8 ± 0.3	1.0 ± 0.6[b]	0.7 ± 0.1	0.4 ± 0.3[a]	0.7 ± 0.2	2.2 ± 0.7[b]
22:4n-6	1.3 ± 0.2	–0.3 ± 0.2	1.5 ± 0.3	–0.6 ± 0.2[a]	1.4 ± 0.2	–0.6 ± 0.3[a]	1.6 ± 0.4	–0.8 ± 0.4[b]
22:5n-3	1.7 ± 0.2	–0.5 ± 0.2	1.7 ± 0.3	0.1 ± 0.4[a]	1.7 ± 0.3	–0.8 ± 0.3	1.8 ± 0.2	0.6 ± 0.4[b]
22:6n-3	3.3 ± 0.6	0.9 ± 1.3	3.0 ± 0.5	1.5 ± 1.0	3.2 ± 0.9	2.9 ± 1.2[a]	3.2 ± 0.6	1.5 ± 0.8
∑n-6	19.3 ± 1.0	2.1 ± 1.4	18.9 ± 1.4	–4.2 ± 1.1[a]	19.0 ± 1.6	–4.0 ± 1.2[a]	18.3 ± 1.8	–5.4 ± 2.0[b]
∑n-3	5.8 ± 0.7	0.4 ± 1.3	5.5 ± 0.9	2.6 ± 1.7[a]	5.6 ± 1.1	2.5 ± 1.4[a]	5.7 ± 0.9	4.3 ± 1.7[b]

[a]At baseline and the changes after 14 wk among the study groups. Values are means ± SD. Superscript letters a, b are significantly different from control group, $P < 0.05$, $P < 0.01$, respectively. See Table 1 for abbreviations.

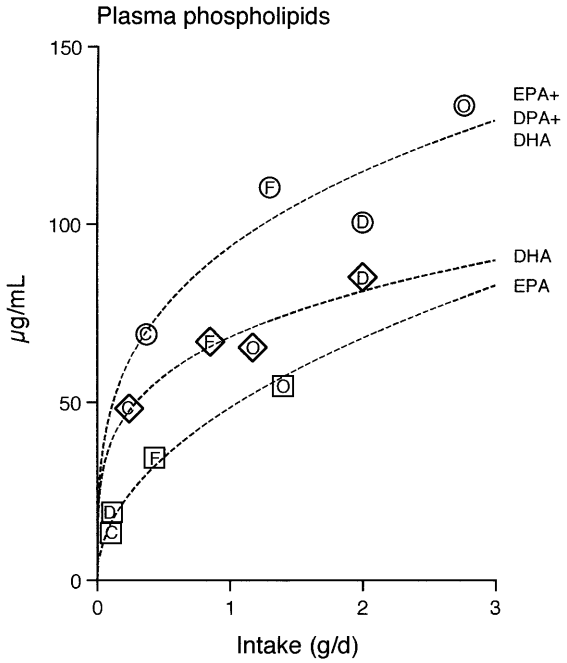

FIG. 2. Relationship between the total intakes (g/d) of EPA, DPA, and DHA (supplementation + dietary intake) and the concentrations in plasma phospholipids (μg/mL). C = control group, F = fish diet group, D = DHA oil group, O = fish oil group. See Figure 1 for other abbreviations.

among the study groups (study subjects pooled together). Correlations between the changes in the levels of EPA in plasma PL with those of EPA in erythrocyte membranes and platelets were $r = 0.91$, $P < 0.001$ and $r = 0.86$, $P < 0.001$, and the corresponding correlations between the levels of DHA were $r = 0.70$, $P < 0.001$ and $r = 0.77$, $P < 0.001$.

DISCUSSION

Fatty acid composition of the diet has a major impact on the fatty acid compositions in plasma lipids, platelets and erythrocyte membranes, but these fractions differ in their response to increased EPA and DHA intakes, as also shown in the present study when examining the effects of n-3 fatty acid supplementation, in the form of fresh fish, fish oil, and DHA oil, on the fatty acid composition of plasma lipid fractions, and platelets and erythrocyte membranes of young healthy male students. In the fish diet and fish oil groups, EPA was preferentially incorporated in plasma CE and PL, as shown in earlier studies, too (13,20,21). In contrast, the increase of DHA was greatest in plasma PL and TG in these groups, whereas only a small increase of DHA was seen in CE. This may be caused by the greater specificity of lecithin-cholesterol acyltransferase (LCAT) to EPA compared with DHA (21). Correlations between the fish intake of Finns (31 g/d in women and 37 g/d in men, respectively) and EPA content of serum CE ($r = 0.60$) and PL ($r = 0.52$) as well as in TG ($r = 0.36$), as reported by Nikkari and coworkers (22), support our results.

EPA and DHA levels in plasma PL have been used as an indicator of fish intake (23,24). Our study subjects were male students habitually consuming diets relatively low in fish products (26–35 g/d). The variability of EPA in plasma PL has been explained by habitual fish consumption, but fish consumption explains a lower proportion of variability in DHA in plasma lipids (25,26). Svensson and coworkers (27) found fourfold higher EPA and twofold higher DHA content of serum phosphatidylcholine in men who consumed fatty fish at an average of 1046 g/wk compared with men who did not consume fish at all. The proportions of both EPA and DHA correlated significantly with fish intake. In the present study, the increase of EPA in plasma PL was about 1.7 times greater compared with that of DHA in the fish diet and fish oil groups. The reason for this could be a more efficient liberation of DHA from chylomicrons (28), leaving more EPA in chylomicron remnants and thereby making more EPA available for PL synthesis in liver.

In addition, the data reported here suggest that the proportions of EPA in platelets reflect well the habitual fish intake over a long term. EPA in platelets (wt%), but not DHA, has been reported to correlate with habitual fish consumption (26). However, in our study the effect of increased DHA intake was seen in platelets, and the change correlated strongly with that of plasma PL. Thus, it is possible that DHA also reflects fish intake, but its less steep dose-response curve compared to EPA makes it more difficult to find strong correlations especially when intakes are estimated from questionnaires. In the fish oil group, the changes in the proportions of EPA and DHA in platelets and erythrocytes were similar as observed after 6-wk cod liver oil supplementation (29), but the change in the proportion of DHA in erythrocytes was greater in the present study. This stresses the importance of the duration of supplementation when erythrocytes are examined.

Our results indicate that the response curves are steepest with low intakes of EPA and DHA (Fig. 2). This view is supported by the results of Bønaa and coworkers (25) comparing plasma PL fatty acids and habitual fish intake, and also by the dose-response study with four different fish intake levels (30). In a dose-response study, the increase of EPA + DHA intake from 3 to 6 g/d caused a very modest increase in their amount in plasma PL compared with the effect of increasing the intake from 1.5 to 3 g/d (31), showing only a weak effect of very high doses. Thus, it seems that the dose-response curves for both EPA and DHA follow logarithmic equations. DHA intake alone does not increase the total amount of n-3 fatty acids as much as the combination of EPA, docosapentaenoic acid, and DHA. In addition, the results of the fish oil group suggest that the high amount of EPA in this product (EPA to DHA ratio 1.4) decreases the effect of DHA when compared with the fish diet group (EPA to DHA ratio 0.6). These results indicate that the relationship between the intake of EPA and DHA and their amounts in plasma and membranes is dependent on the ratio of these fatty acids in the diet, and a reliable equation cannot be calculated on the basis of these results. To apply plasma or membrane EPA and DHA levels for the estimation of fish intake, dose-responses should be determined

using different fish intake levels, and dose-responses for other n-3 fatty acid products should be determined separately.

Interestingly, DHA oil supplementation caused an increase in the proportion of EPA both in plasma lipid fractions and in platelets and erythrocyte membranes, although DHA oil did not contain EPA at all, and the intake of EPA from habitual diet was on average only 0.09 ± 0.04 g/d during the study. This gives evidence of retroconversion of dietary DHA to EPA. Retroconversion of DHA to EPA takes place in peroxisomes by Δ4 enoyl reductase and Δ3, Δ2 enoyl CoA isomerase enzymes (32,33). Retroconversion of DHA to EPA has also been documented in some human (13) and animal studies (34–37), and also *in vitro* (33,37,38). In these studies the increase of docosapentaenoic acid has also been noticed. Docosapentaenoic acid could be a result of a saturation of the DHA or a chain elongation of EPA or both (32,33). However, in the present study the amount of docosapentaenoic acid was unchanged in plasma TG; it decreased in plasma PL, and in platelets and erythrocyte membranes it decreased as much as EPA increased. The decrease of docosapentaenoic acid has also been reported in a short-term study by von Schacky *et al.* (8) in plasma and platelet PL in humans after high DHA ingestion. The decrease of docosapentaenoic acid in the DHA oil group may indicate that the increase of EPA after DHA supplementation is not caused solely by retroconversion but could be a consequence of decreased elongation of EPA as well.

In our fish diet group, a reduced level of arachidonic acid in plasma PL, platelets, and erythrocyte membranes was noticed compared with the control group. Earlier studies have demonstrated that fish consumption decreases the concentration of n-6 PUFA in plasma (26,39), and a negative correlation between fish intake and the proportion of arachidonic acid in serum lipids has been observed (40,41). Eskimos who have a diet high in n-3 fatty acids have low levels of arachidonic acid in serum lipids (7,42). Siess *et al.* (5) reported a considerably reduced arachidonic acid proportion in platelet PL after consumption of a mackerel diet with high doses of EPA (7–11 g/day for 8 d), but also an unchanged proportion of arachidonic acid in plasma (10,32) and platelet PL (10) has been reported. It has been speculated that an unaltered arachidonic acid proportion could be a consequence of transforming linoleic acid to arachidonic acid (40), and the reduced level of arachidonic acid in platelets, but not in serum PL or total serum lipids, could be due to a direct competition between arachidonic acid and n-3 fatty acids for acylation into PL (33).

An interesting finding in our study after DHA supplementation was that the proportion of linoleic acid increased in platelets and erythrocyte membranes and remained unchanged in plasma fractions. DHA has been noticed to decrease Δ6 desaturase activity in rats (43) and to inhibit Δ6 desaturase *in vitro* (44). Our results support these findings and suggest that the transformation of linoleic acid to its metabolites is decreased by DHA. In addition to this step, Δ6 desaturation is also involved in the pathway of DHA from docosapentaenoic acid *via* 24:5n-3 and 24:6n-3. Thus it is possible that the inhibition of this desaturase contributes to the observed increase of the proportion of EPA and linoleic acid and decrease of that of docosapentaenoic acid after DHA supplementation.

In conclusion, fish diet and fish oil supplementation increased the proportions of n-3 fatty acids in plasma lipids, platelets, and erythrocyte membranes. The most striking differences in the distribution of EPA and DHA were seen in plasma CE with preferential incorporation of EPA and in plasma TG with preferential incorporation of DHA. DHA supplementation increased also the proportion of EPA, but in contrast to fish diet and fish oil supplementation, the proportion of docosapentaenoic acid remained unchanged or even decreased in fractions analyzed. Another special aspect of DHA supplementation was the increased proportion of linoleic acid in platelets and erythrocytes. These results indicate that there are marked differences in the effects of EPA and DHA on the composition of plasma and membrane lipids.

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