Effects of Dietary Marine Oils and Olive Oil on Fatty Acid Composition, Platelet Membrane Fluidity, Platelet Responses, and Serum Lipids in Healthy Humans

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ABSTRACT: The influence of various dietary marine oils and olive oil on fatty acid composition of serum and platelets and effects on platelets and serum lipids were investigated as part of an extensive study of the effects of these oils on parameters associated with cardiovascular/thrombotic diseases. Healthy volunteers (266) consumed 15 mL/d of cod liver oil (CLO); whale blubber oil (refined or unrefined); mixtures of seal blubber oil and CLO; or olive oil/CLO for 12 wk. In the CLO, seal oil/CLO, and whale oil groups, serum levels of eicosapentaenoic acid (EPA) were increased. In platelets, EPA was increased in the CLO, seal/CLO, and olive oil/CLO groups. The localization of n-3 polyunsaturated fatty acids in the triacylglycerols did not seem to influence their absorption. Intake of oleic acid is poorly reflected in serum and platelets. No significant differences in triacylglycerols (TG), total cholesterol, or high density lipoprotein cholesterol were observed, even though TG were reduced in the CLO, CLO/seal oil, and whale oil groups. Mean platelet volume increased significantly in both whale oil groups and the CLO/olive oil group. Platelet count was significantly reduced in the refined whale oil group only. Lipopolysaccharide-stimulated blood tended to generate less thromboxane B_2 in CLO, CLO/seal, and CLO/olive groups. The whale oils tended to reduce *in vivo* release of β-thromboglobulin. In conclusion, intake of various marine oils causes changes in platelet membranes that are favorably antithrombotic. The combination of CLO and olive oil may produce better effects than these oils given separately. The changes in platelet function are directly associated with alterations of fatty acid composition in platelet membranes.

Lipids 33, 427–436 (1998).

In the 1970s, Bang, Dyerberg, and coworkers (1–3) suggested that the low incidence of death attributable to coronary heart disease among Greenland Eskimos was due to their high intake of n-3 polyunsaturated fatty acids (PUFA) derived from fish and other seafoods. These studies created the basis for the great increase in the interest in cardiovascular benefits of fish oils, and the effects of the consumption of fish and fish oils have been examined in various important biological systems.

Dietary supplementation with fish and fish oils is reported to modify platelet and leukocyte function in several ways. Suggested modes of action are through their modulation of eicosanoid synthesis (4–6) and reduction in plasma triacylglycerol (TG) concentration (for review see Ref. 7). n-3 Fatty acids as a dietary supplement decrease platelet aggregation and release thromboxane A_2 (TxA_2). Another mechanism for the effect of n-3 PUFA has been suggested to be associated with the change in fluidity by incorporation of PUFA in the cell membranes and an influence on the activities of membrane-associated enzymes or receptors (8). Because of variable results, additional human clinical studies are still needed.

Attention has been directed on n-3 PUFA, although it was evident from Bang and Dyerberg's studies that the traditional Eskimo diet consists of substantial quantities of meat and blubber from seals and whales (1). The fat composition of seal, and of whale in particular, differs significantly from that of fish (9). In marine mammals eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are found mainly in the *sn*-1 and *sn*-3 positions of the TG, whereas in fish these fatty acids are positioned in *sn*-2 (10,11). However, the diet of Greenland Eskimos also differs from the Western diet in other respects, especially in the intake of saturated fatty acids (2). The favorable disease pattern in Eskimos may be caused by the combination of a diet low in saturated fat together with high n-3 PUFA intake, but the beneficial effects of this diet also may be due to components other than the fatty acids. A previous study in our laboratory indicated that oils from the blubber of seal and whale have beneficial effects on some selected parameters thought to play a role in cardiovascular disease, and it was hypothesized that the effect of whale oil is not mediated by n-3 fatty acids alone (12).

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Abbreviations: AA, arachidonic acid; CLO, cod liver oil; CV, coefficient of variation; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FAME, fatty acid methyl ester; HDL, high density lipoprotein; LPS, lipopolysaccharide; MPV, mean platelet volume; PGA_1, $-A_2$, $-D_2$, $-E_2$, $-F_{1\alpha}$, $-F_{2\alpha}$, prostaglandin A_1, $-A_2$, $-D_2$, $-E_2$, $-F_{1\alpha}$, $-F_{2\alpha}$; PUFA, polyunsaturated fatty acid; r_s, steady-state fluorescence anisotropy; TG, triacylglycerol; β-TG, β-thromboglobulin; TMA-DPH, 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene, *p*-toluenesulfonate; TxA_2, $-B_2$, thromboxane A_2, B_2.

The so-called Mediterranean diet is characterized, among other things, by a moderately low intake of PUFA and by a high intake of monounsaturated fatty acids from olive oil. Since Mediterranean populations have shown a low mortality from cardiovascular diseases, which is negatively correlated to the percentage of dietary energy supplied by monounsaturated fatty acids (13,14) in addition to findings of favorable effects of n-3 fatty acid supplementation to a diet rich in olive oil (15), it was of major interest to include olive oil in this dietary study.

With the traditional diets of Mediterranean populations and Greenland Eskimos in mind, we have focused on the effects of marine oils and olive oil on important parameters concerning the physical and functional properties of blood platelets. Platelets play an essential role in arterial thrombosis and atherosclerosis, but the mechanisms for the effects of dietary oils on platelet function are not well understood. In this controlled study we investigated whether any of the dietary oils contributed to changes in structural order (membrane fluidity) of the platelets and related membrane fluidity to platelet function by measuring eicosanoid production thromboxane [(TXB_2 and prostaglandin E_2) (PGE_2)] and release of the α-granule protein β-thromboglobulin (β-TG) from platelets. In addition, we have related these results to plasma lipids and to the corresponding fatty acid composition in serum and platelets and compared the changes in fatty acid patterns of serum and platelet membranes to the composition of the dietary oils. The results are also discussed in relation to the intramolecular TG structures of the oils.

MATERIALS AND METHODS

Subjects and dietary protocol. Healthy volunteers (266) living in Tromsø, Norway (124 women and 149 men), aged 16–69 yr (median 36), were randomly selected to consume 15 mL/d of cod (*Gadus morhua*) liver oil (CLO), olive (*Olea europea*) oil (extra virgin), refined (steam refined by heating to 170°C with N_2) and unrefined oil from the blubber of minke whale (*Balaenoptera acutorostrata*), mixtures of CLO and harp seal (*Phoca groenlandica*) blubber oil (1:1, vol/vol) and CLO and olive oil (1:1, vol/vol), and no oil (control group). Table 1 shows the number of subjects in each group and some characteristics of the groups regarding distribution of gender, age, and smoking habits. The oils were a supplement to their normal dietary habits, and the subjects were counseled to continue eating their regular diet. The study was double-blinded for the groups given various dietary oils. No placebo oil was given in the control group, and the study of this group could thus not be blinded to volunteers or investigators. The seven groups were run in parallel for a period of 12 wk. The procedures followed were in accordance with the Helsinki declaration of 1964 as revised in 1989.

The fatty acid composition of the dietary oils, determined by gas–liquid chromatography as described shortly, is given in Table 2. There were no changes detected during storage over the period of 12 wk. The vitamin E concentrations of the oils were analyzed according to Lie *et al.* (16) and were as follows: CLO: 1.52 mg/g; olive oil: 0.23 mg/g; refined whale oil: 0.17 mg/g; whale oil: 0.25 mg/g; seal oil/CLO: 1.38 mg/g; and olive oil/CLO: 0.85 mg/g.

Blood sampling. Blood samples were taken before the start of the study, after 8 wk, and after 12 wk of supplementation. The data after 12 wk of supplementation are presented in this work. The participants were informed not to drink alcohol or perform any strenuous exercise for 48 h prior to each blood sampling. They fasted overnight, and all the blood samples were taken between 8:00 and 10.00 A.M. Venous blood (25 mL) was drawn into a plastic syringe with a 19-gauge needle and immediately distributed into serum tubes or tubes containing anticoagulants as specified for each procedure described.

Mean platelet volume (MPV) and platelet counts. MPV and platelet counts were measured in whole blood anticoagulated with Na_2-EDTA (final concentration 0.2%) with a Coulter Counter (STRK, Luton, England).

Platelet preparation. Platelet-rich plasma was obtained by centrifugation of citrated blood (5 mL, final concentration of Na_3-citrate: 0.38%) at $180 \times g$ for 15 min at room temperature with the brake off. For fatty acid analysis, the platelets were washed once in 0.15 mol/L NaCl, centrifuged at $1500 \times g$ for 10 min and resuspended in 0.15 mol/L NaCl. The platelet preparations were kept at −70°C until extraction of the fatty acids was performed for the gas chromatographic analysis. For membrane fluidity studies, the platelet pellets were washed twice with 0.15 mol/L NaCl, centrifuged at $1500 \times g$ for 10 min and resuspended in potassium phosphate buffer (0.01 mol/L, pH 7.4). The platelet suspensions were

TABLE 1
Characteristics of the Test Groups Regarding Smoking Habits, Age, and Sex

Oil consumed	Number of females/males	Age (yr)[a]	Smokers (%)
Control (n = 38)	20/18	29.0 (21–57)	18
CLO[b] (n = 37)	14/23	33.0 (20–59)	22
Olive oil (n = 34)	18/16	38.0 (16–66)	26
Whale oil, refined (n = 39)	17/22	36.0 (20–69)	21
Whale oil (n = 40)	17/23	36.5 (22–59)	40
Seal oil/CLO (n = 40)	21/19	35.0 (21–64)	28
Olive oil/CLO (n = 38)	17/21	34.5 (22–59)	39

[a]Age data are median and total range.
[b]CLO = cod liver oil.

TABLE 2
Fatty Acid Profile of the Dietary Oils (wt/wt% of total fatty acids)[a]

Fatty acid	CLO	Olive oil	Whale (refined)	Whale oil (crude)	Seal/CLO	Olive/CLO
14:0	3.9	0.0	5.9	6.2	4.9	2.4
15:0	0.3	0.0	0.4	0.4	0.6	0.2
16:0	9.9	11.1	9.6	9.8	9.4	10.5
18:0	1.9	2.0	1.8	1.9	1.4	2.0
20:0	0.2	0.3	0.0	0.2	0.1	0.1
22:0	0.1	0.1	0.0	0.0	0.0	0.1
Saturated	16.1	13.6	17.6	18.5	16.4	15.3
14:1	0.3	0.0	0.0	0.3	0.0	0.2
16:1n-7	8.1	0.6	9.6	9.6	10.6	5.4
18:1n-9	21.3	77.4	21.9	23.0	20.7	43.3
20:1n-9	11.7	0.6	17.0	16.9	10.7	7.2
22:1n-11	6.5	0.0	10.7	10.6	5.0	3.9
24:1	0.5	0.0	0.3	0.3	0.2	0.3
MUFA	48.6	78.6	59.5	60.7	47.2	60.3
16:2	0.4	0.0	0 4	0.3	0.5	0.3
18:2n-6	1.8	6.0	1.4	1.8	1.7	3.4
20:2n-6	0.3	0.1	0.4	0.4	0.4	0.2
18:3n-6	0.1	0.0	0.3	0.3	0.1	0.1
18:3n-3	1.3	0.7	1.2	1 2	1.3	1.1
16:4	0.1	0.0	0.0	0.1	0.2	0.1
18:4n-3	2.7	0.0	1.7	1 7	3.0	1.7
20:4n-6	0.4	0.0	0.3	0.2	0.3	0.2
20:4n-3	0.7	0.0	0.9	0.9	0.6	0.4
20:5n-3	9.5	0.0	3.9	3.3	7.7	5.8
21:5n-3	0.4	0 0	0 2	0.2	0.4	0.3
22:5n-3	1.0	0.0	1.7	1.7	2.2	0.6
22:6n-3	13.5	0.0	5.9	4.2	11.3	7.8
PUFA	32.2	6.8	18.3	16.3	29.7	22.0
n-3 PUFA	29.1	0.7	15.5	13.2	26.5	17.7
Total	96.8	98.7	95.9	95.7	97.6	97.4

[a]MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids. For other abbreviation, see Table 1.

frozen at −70°C until anisotropy measurements were performed (17). Before labeling with the fluorescent probe, the suspensions were sonicated in an MSE sonicator (Sussex, United Kingdom) (high power, setting 4, 10 s × 2 with 10-s interval, on ice). The platelet preparations were adjusted to a final optical density of 0.03 at 600 nm to minimize the effect of light scattering on the anisotropy measurements (18). Platelet counts were performed both in whole blood and in platelet-rich plasma.

Fatty acid analysis. The fatty acids were extracted from serum and platelet membrane preparations using chloroform/methanol (1:1 vol/vol; Merck, Darmstadt, Germany) by a modified method of Folch and coworkers (19), esterified with 2 mL boron trifluoride in methanol (Supelco, Bellefonte, PA) at 100°C for 90 min (20). Fatty acid methyl esters (FAME) were analyzed by capillary gas–liquid chromatography using a Fisons Carlo Erba 8340 gas chromatograph (Carlo Erba, Rodano, Italy). FAME were separated on a 50-m × 0.25-mm CP-Sil 88 (FAME) capillary column with 0.2 μm film thickness (Chrompack, Middelburg, Holland) after splitless injection of a 1-μL sample at a column temperature of 80°C. The operating conditions were as follows: The initial temperature was maintained for 1 min and then raised by 20°C/min to 160°C, followed by a gradient of 2°C/min to 177°C and 20°C/min to 230°C, and it was kept there for 15 min. The injector and the flame-ionization detector temperatures were 250 and 270°C, respectively. The carrier gas was hydrogen, with an inlet pressure of 110 kPa. The fatty acid composition is expressed as percentage FAME with respect to the total peak surface (area/area).

Analysis of the stereochemistry of TG in the marine oils. The positional distribution of EPA (20:5n-3) and DHA (22:6 n-3) in CLO, seal blubber, and whale blubber oils was analyzed by high-resolution ^{13}C nuclear magnetic resonance spectroscopy as previously described (21).

Measurements of platelet membrane fluidity. The membrane fluidity was assessed by measurements of steady-state fluorescence anisotropy (r_S) of 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene, *p*-toluenesulfonate (TMA-DPH; Molecular Probes. Inc., Eugene, OR) (22–24). A stock solution of TMA-DPH in *N*,*N*-dimethylformamide (Uvasol®; spectroscopy grade, Merck) was prepared at a con-

centration of 10 mmol/L, stored at –20°C and diluted before use. The diluted platelet preparation (2 mL) was incubated for 10 min at 37°C in the dark with TMA-DPH at a final concentration of 1.0 μmol/L. The r_s measurements were carried out with a Perkin-Elmer LS 50 Luminescence Spectrometer (Buckinghamshire, United Kingdom) equipped with polarizers. The measurements were made at excitation and emission wavelengths of 360 and 430 nm, respectively, with slit widths of 5 nm in both excitation and emission. The r_S-value for each sample is an average of five readings (integration time, 10 s), measured at a constant temperature of 37.0 ± 0.1°C, controlled with a digital thermometer. The r_S is automatically calculated from the following equation:

$$r_S = \frac{IV_V - GIv_H}{IV_V + 2GIV_H} \text{ with a correcting factor } G = \frac{IH_V}{IH_H} \quad [1]$$

where r_S is the corrected anisotropy, and IV_V and IH_V are the emission intensities of vertically polarized light parallel and perpendicular to the plane of excitation, respectively. Likewise, IV_H and IH_H are the emission intensities of horizontally polarized light perpendicular and parallel to the plane of excitation. Fluorescence anisotropy values are inversely related to the rotational rate of the probe in the membrane, thus low anisotropy values indicate high fluidity of the membrane.

Lipids in serum. Serum samples were analyzed for TG, cholesterol, and high density lipoprotein (HDL) cholesterol using commercially available enzyme kits from Boehringer (Mannheim, Germany) in combination with an RA-1000 automatic analyzer (Technion RA-100; Bayer Norway, Hagan, Norway).

Quantitation of β-TG. β-TG was measured in heparinized plasma with a β-TG radioimmunoassay kit according to the manufacturer's instructions (Kodak Clinical Diagnostics Ltd., Amersham, United Kingdom). At 45 ng/mL the within-assay coefficient of variation (CV) was 7.5%, and the between-assay CV was 9.9–15.3% in the range 19.6–108 ng/mL.

Stimulation of whole blood in vitro. Lipopolysaccharide (LPS; *Escherichia coli* 026:B6; Difco, Detroit, MI) was added to heparinized blood (1.0 mL) to a final concentration of 5 ng/mL, followed by incubation at 37°C in an incubator shaker (180 rpm) for 2 h. The stimulation was stopped by adding 0.1 mL 2% Na_2-EDTA/mL blood, and the blood was immediately centrifuged at 1400 × *g* for 10 min. The plasma was pipetted off and frozen at −70°C until quantitation of TxB_2 and PGE_2 was performed.

Quantitation of TxB_2. TxB_2, the stable metabolite of TxA_2, was measured in plasma of LPS-stimulated blood with a TxB_2 enzyme immunoassay system (Biotrak, Amersham, United Kingdom), as described by the manufacturer. The cross reactivities to related compounds were: 2,3-dinor-TxB_2: 60.50%; 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$: <0.40%; 6,15-diketo-13,14-dihydro-$PGF_{1\alpha}$; <0.01%; 11-dehydro-TxB_2, 0.10%; 6-keto-PGE_2, <0.01%; PGD_2, 0.18%; PGE_2, <0.01%; $PGF_{1\alpha}$, 1.6%; $PGF_{2\alpha}$, 0.06%; and arachidonic acid (AA), <0.01%. The sensitivity of this assay was 3.6 pg/mL, and the within and between assay CV were 2.5–9.2% (range 21.4–254 pg/mL) and 9.9–13.9% (range 46.2–222 pg/mL), respectively.

Quantitation of PGE_2. PGE_2 was measured in plasma of LPS-stimulated blood in a PGE_2 [^{125}I] RIA (radioimmunoassay) Kit (Du Pont, NEN Research Products, Boston, MA). The following compounds have been tested for cross reactivity: PGE_1, 30%; 13,14-dihydro-15-keto-PGE_2, 0.02%; PGA_2, 0.8%; $PGF_{1\alpha}$, 0.7%; TxB_2, 0.01%; $PGF_{2\alpha}$, 0.9%; AA, 0.01%; 13,14-dihydro-15-keto-$PG_{2\alpha}$, 0.005%; PGA_1; 0.08%; PGB_2, 0.07%; 6-keto-$PGF_{1\alpha}$, 1%; linoleic acid, 0.002%; and PGD_2, 0.3%. The sensitivity of the system was 4.4 pg/mL.

Measurements of antioxidant capacity. Antioxidant capacity was measured in citrated plasma by enhanced chemiluminescent assay as described elsewhere (25). The enhanced chemiluminescent immunoassay signal reagent and horseradish peroxidase conjugate were obtained from Amersham International (Amersham, United Kingdom). The signal reagent consisted of assay buffer and tablets A and B containing luminol, enhancer, and oxidant. The tocopherol analog standard, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), was obtained from Aldrich (Gillingham, United Kingdom). Chemiluminescence was measured in a Biocounter (Schaesberg, The Netherlands) (Lumac/3M, M2010, Switzerland).

Statistical analysis. The data are presented as mean ± standard error of mean (SEM). Differences between gender groups in baseline values of various parameters were determined by Student's unpaired *t*- or Mann-Whitney *U*-test. For each parameter tested, the differences between the values before and after intake of dietary oils were calculated within the groups. One-way analysis of variance followed by Dunnett's *t*-test was used for simultaneous, multiple comparisons of the test groups with the control group. When data are presented as percentages, statistical analysis was performed on absolute values, unless otherwise stated. Correlations were calculated by means of the Spearman's rank correlation coefficient (*r*). *P*-values <0.05 were considered as statistically significant. All analyses were performed on an Apple Macintosh using StatView™ SE+ Graphics version 1.04 statistical software (Abacus Concepts Inc., Berkeley, CA).

RESULTS

Baseline characteristics. Statistical analysis (one-way analysis of variance and Dunnett's *t*-test) of baseline characteristics in the different groups of oils showed that TG in the whale oil group were significantly higher than the values observed in the control group ($P < 0.05$). For the other clinical and biochemical variables, there were no significant differences between the groups of oils.

Comparisons of differences in clinical and biochemical characteristics between the gender groups showed that men had higher mean values for age (37.9 and 34.3 yr for men and women, respectively), TG (1.5 ± 0.1 and 1.1 ± 0.1 mmol/L), and cholesterol (6.3 ± 0.1 and 5.7 ± 0.1 mmol/L). Women had

significantly higher HDL cholesterol (1.6 ± 0.03 and 1.2 ± 0.03 mmol/L) and TxB_2 values (12.5 ± 0.6 and 10.5 ± 0.5 ng/mL) than men.

Fatty acid composition in serum. The composition of total fatty acids in serum before and after 12 wk of supplementation with different dietary oils is given in Table 3. After 12 wk of daily intake, CLO and the mixture of CLO and seal oil both produced highly significant rises ($P < 0.01$) in serum EPA (20:5n-3), by 188 and 115%, respectively, and DHA (22:6n-3), by 56 and 50%. The same groups experienced a significant reduction (50–60%) of dihomo-γ-linolenic acid (20:3n-6) compared with the control group. The changes in EPA (110% increase, $P < 0.01$) and dihomo-γ-linolenic acid (43% decrease, $P < 0.05$) levels were also seen in the whale oil group. In addition, we observed an increase in the serum concentration of oleic acid (18:1n-9) in the group given olive oil (from 18.8 to 20.8%), but this change was not statistically significant.

There was a dose–response relationship between the relative contents of n-3 fatty acids in the various dietary oils and the change in total n-3 fatty acids in serum.

Fatty acid composition in platelets. When comparing the baseline fatty acid composition of serum (Table 3) and platelets (Table 4), we found that the relative composition of fatty acids did not differ much for all except three fatty acids: There were striking differences between stearic acid (18:0), linoleic acid (18:2n-6), and AA (20:4n-6) in serum and platelets. The relative contents of these fatty acids were as follows: 7.5 and 20.4% (18:0), 31.6 and 6.1% (18:2n-6), and 4.9 and 18.1% (20:4n-6) for serum and platelets, respectively. The strongest positive correlation between fatty acids in serum and platelets was found for 20:5n-3 ($r = 0.84$, $P < 0.0001$).

Table 4 shows that in all three groups given CLO (CLO alone or in mixtures with seal oil or olive oil) the concentration of EPA in platelet membrane preparations was significantly raised (by 118, 92, and 64%, respectively). In addition, in the CLO group, there were increases in 22:5n-3 and 22:6n-3 and a reduction in the relative content of AA (20:4n-6) by 21%.

Intramolecular fatty acid distributions in the marine oils. Distribution of the main n-3 PUFA in the TG molecule in the oils of marine origin was dependent on their source. Nuclear magnetic resonance data showed that 81% of EPA and 93.7% of DHA were localized in *sn*-1/3 positions of the TG in minke whale oil. Corresponding values for harp seal oil were 97.7 and 98.1%, and for CLO 58.2 and 22.3% for EPA and DHA, respectively.

Serum lipids. Neither the TG, total cholesterol, nor the HDL-cholesterol concentrations in any of the groups were significantly altered after 12 wk of supplementation with dietary oils, even though the TG were reduced by 10–12% in the CLO, CLO/seal, and whale oil groups (data not shown). Although men and women differed in baseline values of the serum lipids, no difference was observed between the two groups concerning effects of the oils when tested separately. Still, there was a strong trend to reduction of TG in the CLO and seal/CLO groups in women.

Platelet membrane fluidity. The r_S of the platelet membrane preparations labeled with TMA-DPH was unchanged after intake of marine oils and olive oil (data not shown).

MPV and platelet counts. MPV increased in both whale oil groups as well as in the CLO/olive oil group after 12 wk of dietary supplementation (Fig. 1), with the most pronounced effect in the refined whale oil group with a rise from 8.7 to 9.4 μm^3 (8%, $P < 0.01$). Baseline values of MPV correlated negatively with platelet counts ($r = -0.41$, $P < 0.0001$). The platelet count was significantly reduced only in the refined whale oil group (7%, $P < 0.01$).

Platelet products. TxA_2 is generated mainly from platelets in LPS-stimulated blood, and the measurement of its stable metabolite TxB_2 showed no significant changes after intake of dietary oils, both when the results were analyzed for the two genders together and separately. However, there was a tendency to reduction in all groups given CLO: TxB_2 was reduced by 39, 34, and 28% in the CLO, seal/CLO, and olive/CLO groups, respectively.

There were significant changes in the concentration of PGE_2 ($P < 0.01$) in all the groups given CLO [CLO only (28%) or CLO in mixture with seal oil (22%) or olive oil (32%)] when compared with the control group. However, the rise in PGE_2 was most pronounced in the control group (81%), so that the significant changes in PGE_2 in those three groups mean less rise in the concentrations of PGE_2 when compared with the control group.

The release of β-TG from platelets was not affected by any of the dietary oils (data not shown), even though the whale oils tended to reduce the concentrations of β-TG from 7.59 ± 0.41 to 6.43 ± 0.43 ng/mL (15%) in the unrefined whale oil group and from 7.63 ± 0.49 to 5.84 ± 0.34 ng/mL (23%) in the group receiving refined whale oil.

Antioxidant capacity. Analyses of antioxidant capacity in citrated plasma were preliminary studies and only performed on a limited number of samples in some of the test groups. There were no significant changes in the antioxidant capacity following supplementation with CLO, whale oil, or olive oil. In the control group ($n = 15$), the antioxidant capacities were 564 ± 34 (before) and 550 ± 35 μmol/L (after 12 wk). The baseline values were significantly ($P < 0.0001$) higher in men (628 ± 24 μmol/L, $n = 30$) than in women (464 ± l5 μmol/L, $n = 30$), as revealed by Student's unpaired *t*-test.

DISCUSSION

The main interest in the present work was to investigate the effects on physical and functional properties of platelets and possibly find any associations between the parameters measured and the fatty acid composition in serum and platelets. Changes in the distribution of total serum fatty acids partly reflected the fatty acid composition of the ingested dietary oils as seen in Tables 3 and 4. Serum ordinarily contains low levels of EPA (20:5n-3) and DHA (22:6n-3), but after admin-

TABLE 3
Fatty Acid Composition in Serum Before and After 12 wk of Supplementation with Dietary Oils (wt/wt% of total fatty acids)[a]

	Control ($n = 35$)		Cod liver oil ($n = 25$)		Olive oil ($n = 29$)		Whale oil, refined ($n = 38$)		Whale oil ($n = 31$)		Seal oil/CLO ($n = 36$)		Olive oil/CLO ($n = 33$)	
Fatty acid	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
14:0	1.2 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.2 ± 0.1
16:0	23.8 ± 0.3	24.1 ± 0.3	23.4 ± 0.3	23.2 ± 0.3	24.3 ± 0.4	23.4 ± 0.3	23.7 ± 0.4	23.0 ± 0.3	24.0 ± 0.2	24.2 ± 0.3	23.4 ± 0.2	23.3 ± 0.3	23.7 ± 0.3	24.0 ± 0.3
18:0	7.5 ± 0.1	7.4 ± 0.1	7.5 ± 0.2	7.8 ± 0.2	7.8 ± 0.1	7.3 ± 0.1	7.2 ± 0.1	7.7 ± 0.1	7.6 ± 0.1	7.3 ± 0.1	7.5 ± 0.1	7.6 ± 0.1	7.5 ± 0.1	7.6 ± 0.1
16:1n-7	2.1 ± 0.1	1.8 ± 0.2	2.0 ± 0.1	1.6 ± 0.1	2.1 ± 0.1	2.0 ± 0.2	1.9 ± 0.1	1.9 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	1.7 ± 0.1
18:1n-9	17.9 ± 0.5	18.2 ± 0.4	18.2 ± 0.3	16.4 ± 0.5	18.8 ± 0.8	20.8 ± 0.5	17.8 ± 0.6	17.8 ± 0.4	19.6 ± 0.4	18.1 ± 0.4	18.4 ± 0.4	16.7 ± 0.3	17.3 ± 0.6	18.6 ± 0.4
18:1n-7	2.2 ± 0.2	1.9 ± 0.1	2.1 ± 0.2	2.2 ± 0.2	1.7 ± 0.1	2.1 ± 0.2	1.9 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	2.3 ± 0.2	1.8 ± 0.1	2.0 ± 0.1	2.2 ± 0.1	2.0 ± 0.2
20:1n-9	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:1n-7	0.1 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	0.3 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	0.0 ± 0.0	0.4 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	0.1 ± 0.0	0.2 ± 0.0
18:2n-6	31.5 ± 0.7	32.3 ± 0.7	31.9 ± 0.6	31.0 ± 0.7	31.6 ± 0.7	31.5 ± 0.8	31.9 ± 0.6	31.5 ± 0.6	31.2 ± 0.6	30.8 ± 0.6	31.6 ± 0.7	30.6 ± 0.7	31.7 ± 0.8	31.2 ± 0.7
18:3n-6	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.1	0.3 ± 0.0
18:3n-3	0.6 ± 0.0	0.5 ± 0.1	0.6 ± 0.0	0.5 ± 0.1	0.8 ± 0.0	0.5 ± 0.1	0.7 ± 0.0	0.6 ± 0.1	0.7 ± 0.0	0.4 ± 0.1	0.7 ± 0.0	0.4 ± 0.1	0.7 ± 0.1	0.5 ± 0.1
20:3n-6	1.3 ± 0.1	1.0 ± 0.1	1.4 ± 0.1	0.6 ± 0.1[c]	1.3 ± 0.0	0.9 ± 0.1	1.3 ± 0.1	0.8 ± 0.1	1.4 ± 0.0	0.8 ± 0.1[b]	1.3 ± 0.1	0.6 ± 0.1[c]	1.3 ± 0.1	0.8 ± 0.1
18:4	0.2 ± 0.0	0.6 ± 0.5	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.4 ± 0.2	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
20:4n-6	5.1 ± 0.1	4.7 ± 0.2	5.1 ± 0.2	4.4 ± 0.2	4.7 ± 0.1	4.4 ± 0.1	4.6 ± 0.1	4.4 ± 0.1	4.7 ± 0.2	4.5 ± 0.2	5.0 ± 0.2	4.6 ± 0.2	5.0 ± 0.1	4.1 ± 0.1
20:4n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:5n-3	1.9 ± 0.3	1.7 ± 0.3	1.6 ± 0.2	4.6 ± 0.3[c]	1.3 ± 0.1	1.3 ± 0.2	2.1 ± 0.3	2.9 ± 0.2	1.4 ± 0.1	2.5 ± 0.2[c]	2.0 ± 0.3	4.3 ± 0.3[c]	2.1 ± 0.3	2.8 ± 0.2
22:5n-3	0.5 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	0.8 ± 0.2	0.4 ± 0.0	0.5 ± 0.1	0.5 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
22:6n-3	2.9 ± 0.2	2.7 ± 0.2	2.7 ± 0.1	4.2 ± 0.2[c]	2.4 ± 0.2	2.4 ± 0.1	2.7 ± 0.2	3.3 ± 0.1	2.5 ± 0.1	3.1 ± 0.1	2.8 ± 0.2	4.2 ± 0.1[c]	2.9 ± 0.2	3.4 ± 0.1

[a]Mean values ± SEM. The differences between values before and after dietary supplementation were tested by one-factor analysis of variance and Dunnett's *t*-test for multiple comparisons of the test groups with the control group. [b]$P < 0.05$ [c]$P < 0.01$.

TABLE 4
Fatty Acid Composition in Platelets Before and After 12 wk of Supplementation with Dietary Oils (wt/wt% of total fatty acids)[a]

	Control ($n = 36$)		Cod liver oil ($n = 34$)		Olive oil ($n = 29$)		Whale oil, refined ($n = 31$)		Whale oil ($n = 36$)		Seal oil/CLO ($n = 35$)		Olive oil/CLO ($n = 35$)	
Fatty acid	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
14:0	2.0 ± 0.1	2.4 ± 0.2	2.0 ± 0.2	2.4 ± 0.2	2.1 ± 0.3	2.1 ± 0.2	2.1 ± 0.1	2.5 ± 0.2	2.0 ± 0.2	2.4 ± 0.2	2.0 ± 0.1	2.2 ± 0.2	2.1 ± 0.1	2.2 ± 0.2
16:0	23.7 ± 0.3	23.6 ± 0.3	23.6 ± 0.4	23.4 ± 0.4	23.5 ± 0.5	23.0 ± 0.3	22.9 ± 0.3	23.5 ± 0.3	23.5 ± 0.4	23.9 ± 0.2	23.2 ± 0.3	23.5 ± 0.3	24.0 ± 0.6	23.1 ± 0.3
18:0	21.4 ± 0.3	21.6 ± 0.3	21.1 ± 0.2	20.4 ± 0.4	21.3 ± 0.3	21.5 ± 0.3	21.2 ± 0.2	21.2 ± 0.4	21.2 ± 0.3	21.4 ± 0.4	21.5 ± 0.2	20.7 ± 0.3	21.2 ± 0.3	21.2 ± 0.3
18:1n-9	13.7 ± 0.2	13.8 ± 0.3	13.7 ± 0.2	14.1 ± 0.3	13.0 ± 0.6	14.7 ± 0.4	13.9 ± 0.2	13.9 ± 0.5	13.9 ± 0.2	13.8 ± 0.4	13.7 ± 0.2	14.6 ± 0.3	13.6 ± 0.2	14.5 ± 0.3
18:2n-6	6.3 ± 0.2	6.2 ± 0.2	6.3 ± 0.2	5.9 ± 0.1	6.4 ± 0.3	6.3 ± 0.1	6.4 ± 0.2	6.3 ± 0.1	6.5 ± 0.1	6.0 ± 0.2	6.4 ± 0.1	5.8 ± 0.2	6.5 ± 0.2	6.0 ± 0.1
18:3n-6	0.3 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.1
20:1	0.7 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.7 ± 0.1	0.6 ± 0.0	0.6 ± 0.1	0.7 ± 0.0	0.8 ± 0.1	0.7 ± 0.0	0.7 ± 0.1	0.7 ± 0.0	0.7 ± 0.1	0.6 ± 0.0	0.6 ± 0.1
20:2	0.6 ± 0.1	0.4 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.8 ± 0.2	0.5 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.6 ± 0.1	0.5 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.4 ± 0.0
22:0	3.3 ± 0.1	3.2 ± 0.1	3.0 ± 0.1	3.3 ± 0.1	4.4 ± 1.3	3.2 ± 0.2	3.4 ± 0.2	3.0 ± 0.1	2.9 ± 0.1	3.5 ± 0.2	3.2 ± 0.1	3.5 ± 0.2	3.1 ± 0.2	3.2 ± 0.1
20:3n-6	1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.6 ± 0.1	1.4 ± 0.2	1.5 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.4 ± 0.2	1.4 ± 0.1	1.4 ± 0.1
20:4n-6	18.9 ± 0.5	17.1 ± 0.4	19.4 ± 0.3	15.4 ± 0.5[c]	18.6 ± 0.9	17.6 ± 0.5	19.0 ± 0.3	15.8 ± 0.4	18.9 ± 0.4	16.21 ± 0.4	18.8 ± 0.4	15.5 ± 0.4	18.3 ± 0.4	16.2 ± 0.4
20:4n-3	0.1 ± 0.0	1.1 ± 0.2	0.1 ± 0.0	1.3 ± 0.5	0.1 ± 0.1	0.9 ± 0.2	0.0 ± 0.0	0.9 ± 0.2	0.1 ± 0.0	0.8 ± 0.1	0.1 ± 0.0	1.0 ± 0.2	0.1 ± .0.0	1.0 ± 0.2
24:0	0.8 ± 0.1	1.8 ± 0.2	1.0 ± 0.3	1.7 ± 0.1	0.9 ± 0.1	1.6 ± 0.1	0.9 ± 0.1	1.7 ± 0.1	0.7 ± 0.1	1.6 ± 0.1	1.0 ± 0.1	1.4 ± 0.1	0.8 ± 0.1	1.8 ± 0.1
20:5n-3	1.1 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	2.4 ± 0.1[c]	0.9 ± 0.1	1.2 ± 0.2	1.2 ± 0.1	1.8 ± 0.1	1.1 ± 0.1	1.6 ± 0.1	1.3 ± 0.1	2.5 ± 0.1[c]	1.1 ± 0.1	1.8 ± 0.1[b]
24:1	1.0 ± 0.0	1.0 ± 0.2	0.9 ± 0.0	1.4 ± 0.4	0.7 ± 0.1	0.9 ± 0.0	0.9 ± 0.0	1.3 ± 0.2	0.8 ± 0.1	1.3 ± 0.2	0.9 ± 0.1	1.1 ± 0.0	0.9 ± 0.0	1.3 ± 0.2
22:5n-3	1.2 ± 0.0	1.0 ± 0.1	1.2 ± 0.0	1.4 ± 0.1[b]	1.2 ± 0.1	1.0 ± 0.1	1.2 ± 0.0	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.3 ± 0.0[b]	1.1 ± 0.0	1.2 ± 0.1
22:6n-3	2.3 ± 0.1	2.2 ± 0.1	2.4 ± 0.1	2.9 ± 0.1[c]	2.4 ± 0.1	2.3 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.9 ± 0.1	2.5 ± 0.1	2.8 ± 0.2

[a]Mean values ± SEM. The differences between values before and after dietary supplementation were tested by one-factor analysis of variance and Dunnett's *t*-test for multiple comparisons of the test groups with the control group. [b]$P < 0.05$ [c]$P < 0.01$.

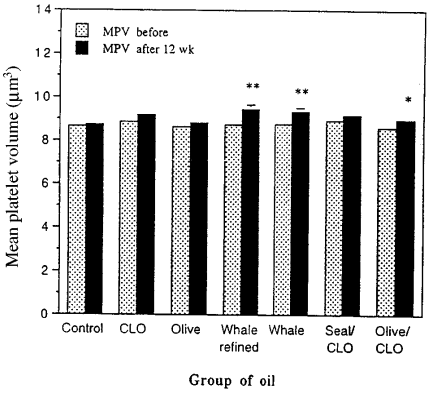

FIG. 1. Mean platelet volume (MPV) before and after 12 wk of supplementation with dietary oils. Values are means ± SEM. MPV was measured with a Coulter Counter in whole blood anticoagulated with Na_2-EDTA. MPV was significantly raised in both whale oil groups (**$P < 0.01$) and the group given a mixture of olive oil and cod liver oil (CLO) (*$P < 0.05$), tested by one-way analysis of variance and Dunnett's *t*-test for comparisons of the test groups with the control group.

istration of oils rich in these fatty acids, the levels were increased by more than 100%. Previous results showed an even stronger effect of similar oils on the levels of EPA and DHA, and in addition, whale oil induced a significant increase in these fatty acids (12). Compared to the high concentrations of n-3 PUFA in CLO and seal oil, the high content of monounsaturated fatty acids in the whale oils did not seem to influence the distribution of fatty acids in serum to the same extent. This may be due to the relatively high concentration of oleic acid (18:1n-9) already present in our regular diet.

It has been suggested that the stereochemistry of TG molecules may influence the digestion and absorption of fatty acids (26). The n-3 PUFA are located mainly in the outer positions *sn*-1 and *sn*-3 of the TG in seal and whale oil, whereas in TG from CLO the *sn*-2 position is enriched in n-3 PUFA. The present long-term study does not indicate differences in absorption of n-3 PUFA from oil of marine mammals or fish oil. Christensen *et al.* (27) demonstrated that the digestibility and lymphatic absorption in rats of n-3 PUFA from native marine oils were not significantly influenced by the localization of the n-3 PUFA within the TG. These findings, however, do not exclude possible differences in digestibility and absorption rates of n-3 PUFA from different sources. In a study in humans it was suggested that finwhale oil (with EPA mainly in positions *sn*-1/3) may provide a moderately more available source of dietary EPA than herring oil (EPA mainly in *sn*-2), as there was observed a higher average accumulation of EPA in platelet phospholipids after whale oil consumption (28). However, retroconversion from DHA (higher level in the whale oil) to EPA may have contributed to these effects (28). In addition, the short duration (10 d) of the study and the low number of subjects ($n = 3$) must be taken into account. The changes in DHA levels in serum and platelets following intake of DHA-containing oils were considerably lower than corresponding values for EPA, supporting differences in digestion and incorporation of these fatty acids, as shown by others (29).

The highly significant, positive correlation between EPA in serum and platelets indicates that the relative content of EPA in platelets is strongly dependent on the availability and the fact that EPA appears to be easily incorporated into phospholipids. Even though the serum concentration of AA (20:4n-6) was not altered significantly after intake of CLO, the increase in n-3 PUFA in platelets seemed to occur at the expense of AA. However, the fatty acid composition of phospholipids in platelets reflects the fatty acid composition of total fatty acids in serum only to some extent.

One objective of this work was to study whether the various dietary oils have any effect on platelet membrane fluidity. The oils had no observed influence on the physical properties of the platelet membrane, and thus membrane fluidity was not associated with alterations in platelet membrane fatty acid composition. This is supported by other studies on human platelets (30, 31), whereas rat platelets have shown increased membrane fluidity under the influence of a marine oil diet (32). Possibly, the modifications of the fatty acid pattern in our study were not extensive enough to alter the membrane fluidity, but this could also be due to mechanisms which are able to buffer changes in fatty acid composition and maintain the physical properties in the hydrocarbon region measured by the fluorescent probe. These findings may also be a consequence of the low sensitivity of the TMA-DPH fluorescence anisotropy technique.

In human intervention studies, the most consistent effect of n-3 PUFA on the plasma lipid profile is that they lower the level of TG in both normolipidemic and hyperlipidemic subjects (7), apparently by reducing hepatic TG synthesis and secretion of very low density lipoprotein TG (33–35). Even though our results did not show significant reductions in any of the groups, the oils with the highest relative contents of n-3 PUFA had the most pronounced reducing effects on the TG concentrations.

The overall results from several fish oil studies (7) showed that normolipidemic subjects responded to n-3 fatty acids with essentially no change in total or low density lipoprotein cholesterol and a slight rise in HDL cholesterol, which is consistent with the findings of our study. Women have a more favorable lipid pattern than men, including higher HDL cholesterol levels (36), but despite gender differences in baseline values in the present study there were no sex-related differences in serum lipids in response to the dietary oils. Lipid data from numerous fish oil studies reviewed by Harris (7) show a mean value of total cholesterol in control groups at about 4.8 mmol/L (range of means 3.4–6.2 mmol/L), and one might ask if the high level of total cholesterol (6 mmol/L) found in our

study reflects high cardiovascular risk in the population of northern Norway (37). More likely, the high cholesterol concentrations reveal the tendency of people with known moderately high cholesterol levels and a familial history of coronary heart disease to volunteer in such studies.

TxB_2 is the main oxygenated end product of the cyclooxygenase pathway in platelets and can be regarded as a parameter of platelet functionality. A nonlinear relationship has been demonstrated between the reduction of Tx production and reduction of platelet aggregation (38). It has been shown that more than 90% inhibition of Tx is necessary to obtain some inhibition of platelet aggregation (38). For practical reasons no aggregation measurements were done in the present study, but the reduced (not significantly) TxB_2 production (30–40%) observed in the CLO groups may indicate no severe impairment of the platelet aggregation caused by TxB_2. Previous studies have shown 30–65% reductions (17,39) in TxB_2 production in response to dietary marine oils, but in the study of Østerud *et al.* (17) only whale oil reduced the TxB_2 significantly. In several other *in vivo* and *in vitro* assays, dietary n-3 PUFA reduced eicosanoid synthesis significantly (40). We have used weak LPS stimulation of whole blood as a model for the reactivity of blood platelets, and we believe that this *ex vivo* system may reflect the weak stimuli for aggregation found in most *in vivo* conditions more than does measurement of maximally stimulated TxB_2 formed in serum. Thus the Tx production probably reflects the platelets' ability to be activated in this model system.

PGE_2, the other cyclooxygenase product measured, was clearly increased in all groups, including the control group during the period of 12 wk. Plasma was analyzed in bulk after each blood sampling, within 1 wk after the start of the dietary supplementation and within 3 wk after the end of the study. This difference in storage time may be the cause of the change observed. Nevertheless, when the results are adjusted for changes in the control group, the pattern is similar to the changes seen in TxB_2, with significant reductions in all CLO groups. As for TxB_2, although not significant, this may be connected to the observed reduction of 20:4n-6 in platelets in the CLO group.

For both TxB_2 and PGE_2, the measured values of the 2-series of these eicosanids may be overestimated owing to cross reactivity in the assay with the corresponding 3-series compounds. This could also possibly explain the failure to see any differences in TxB_2 and PGE_2 after intake of dietary oils.

Platelet factor 4 and β-TG are both released from the α-granules of platelets upon activation and correlate closely with the platelet aggregation score (41). In our study the β-TG concentration in plasma from LPS-stimulated whole blood was reduced in both whale oil groups (15–23%), but the changes were not statistically significant.

A reduction in platelet number associated with increased platelet volume has been observed in subjects given about 10 g of n-3 fatty acids as salmon oil per day (42). In our study the whale oils had the most pronounced effect on these platelet parameters. Large platelets are reported to be more reactive than small platelets (43) and are believed to be an independent risk factor for myocardial infarction (44). However, the reduced platelet number and slightly increased platelet size induced by fish oil supplements have been associated with reduced responsiveness to some aggregating agonists (45). It has repeatedly been observed in one individual that intake of marine oils over several months is associated with an increase in MPV and a decrease in platelet count. This is followed by a transient increase in platelet count and decrease in MPV after end of marine oil intake (Østerud, B., unpublished data); and interestingly, 4–6 wk after end of marine oil intake, there is a rise in MPV and a decrease in platelet count which resembles the platelets before intake of marine oils. Since the platelets from this person in their normal condition are large (MPV 11.0–11.5) and highly reactive, we believe that it is unlikely that the rise in MPV induced by marine oils is related to higher reactivity of platelets but may rather reflect alteration of the platelet membrane by unknown mechanisms.

Preliminary measurements do not indicate any differences between the oil groups regarding effect on the antioxidant capacity in plasma measured by enhanced chemiluminescent assay. Thus, the low levels of antioxidants present in the various oils did not seem to influence the antioxidant capacity. The supplement of PUFA may lead to enhanced oxidation of the fatty acids in plasma and possibly reduce the antioxidants buffering these reactions. However, this needs to be further investigated before any conclusions can be drawn.

In summary, EPA and DHA, both from marine fish and mammals, induced increases in the serum concentrations of the corresponding fatty acids as well as their relative contents in platelets. The absorption and incorporation of EPA were dependent on its availability. The present study indicates that none of the dietary oils induced changes in the plasma lipids, although oils containing CLO tended to induce reductions in the TG concentration. CLO, seal/CLO and olive/CLO also produced significant reductions in PGE_2 and a strong tendency toward reduction of TxB_2 generation in plasma of LPS-stimulated whole blood. β-TG, however, was not affected by any of the oils. In spite of alterations in fatty acid composition in the platelets in some of the groups, we observed no changes in platelet membrane fluidity. MPV was significantly increased and platelet counts reduced by dietary supplementation with refined and unrefined whale oils. Olive oil alone as a dietary supplement did not have significant effects on any of the parameters measured in this study. The doses of the dietary oils in this study (corresponding to 1.5 g EPA and 2.1 g DHA per day in the CLO group) were rather low compared both to other studies and to the complete diet of Greenland Inuits and Mediterranean populations. Based on the *in vivo* parameters measured (β-thromboglobulin, MPV, and platelet count), the whale oils and to some degree the combination of olive oil and CLO may have the best anticoagulant effect. These oils are also found to have antiinflammatory effects (Østerud, B., unpublished data).

The intake of fatty acids through the ordinary diet is not

adjusted for in this study. Possible relationships between the complete diet of the subjects and the parameters measured in the present study may give further explanation to some of the observations, and investigations are in progress in our group.

ACKNOWLEDGMENTS

This study was supported by the Norwegian Research Council, the Norwegian Council on Cardiovascular Diseases, the Nansen Foundation, and the Regional Authority of Northern Norway. The skillful technical assistance of Tone Jacobsen, Karin Lia, and Jan Ole Olsen is gratefully acknowledged.

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[Received September 9, 1996, and in final revised form May 21, 1997; revision accepted December 17, 1997]