A Long-Term Seal- and Cod-Liver-Oil Supplementation in Hypercholesterolemic Subjects

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ABSTRACT: In this long-term study, we wanted to explore the effect of dietary supplementation of seal oil (SO) as compared cod-liver oil (CLO) on subjects with moderate hypercholesterolemia. The test parameters included fatty acid composition in serum, blood lipids, platelet aggregation, and the activity of blood monocytes. After a run-in period of 6 mon, 120 clinically healthy hypercholesterolemic (7.0–9.5 mmol/L; 270–366 mg/dL) subjects were randomly selected to consume either 15 mL of SO or CLO daily for 14 mon followed by a 4-mon wash-out period. A third group was not given any dietary supplement (control). Consumption of marine oils (SO and CLO) changed the fatty acid composition of serum significantly. Maximal levels were achieved after 10 mon. No further changes were seen after 14 mon. A wash-out period of 4 mon hardly altered the level of n-3 fatty acids in serum. Addition of SO gave 30% higher level of eicosapentaenoic acid, as compared to CLO. Subjects taking SO or CLO had lower whole-blood platelet aggregation than the control group. Neither SO nor CLO had any effects on the levels of serum total cholesterol, high-density lipoprotein cholesterol, postprandial triacylglycerol, apolipoproteins A1 and B100, lipoprotein (a), monocyte function expressed as monocyte-derived tissue factor expression, and tumor necrosis factor.

Paper no. L8549 in *Lipids 36*, 7–13 (January 2001).

During the last two decades it has been established that Greenland Eskimos living on their traditional diet have a lower incidence of coronary heart disease than Danes living on a Western diet (1,2).

The Eskimos have significantly prolonged primary bleeding time, a mild clinical bleeding tendency, increased level of n-3 fatty acids in blood plasma and cell membranes, lower serum total cholesterol, high density lipoprotein (HDL)-cholesterol, lower apolipoprotein (Apo) B100 and higher Apo A1 levels as compared to those regarding the Danes (3–6). These findings have been attributed to their diet, particularly the marine fatty acids.

Dietary intake of fish, fish oil, and n-3 fatty acid-containing capsules does not give similar findings of blood n-3/n-6 ratio, total cholesterol, HDL-cholesterol, Apo A1, and Apo B100 as in Greenland Eskimos.

The Eskimo diet consists mainly of meat and blubber of seal and whale, containing high amounts of monounsaturated acids (MUFA) and n-3 polyunsaturated fatty acids (PUFA) and relatively small amounts of saturated fatty acids (SFA) and fish (7,8).

Eskimos consume the bulk of their food raw or dried, seldom boiled or exposed to excessive heat. The fatty acid composition in seal and whale blubber also deviates from fish oil in the positioning of the n-3 fatty acids in the glycerol (9–11). It has also been shown that PUFA in marine mammals are quite well-protected from oxidation (12).

Seal oil (SO) contains slightly more MUFA as compared to cod-liver oil (CLO); the eicosapentaenoic acid (EPA; 20:5n-3) molecule is mainly in the 1,3-position of the glycerol, and SO also contains a strong natural antioxidant, not yet fully identified. If the positioning of the fatty acids in the glycerol molecule or the antioxidative abilities of seal fat are significant in the prevention of arteriosclerosis, it might be possible to detect effects on parameters related to the development of this condition, by ingesting SO or blubber.

The oils served in the present study were subjected to a traditional CLO refining process. The main objectives of such processes are to remove pesticides and to make an edible and stable product. Removal of molecules that cause off-flavors or taste by "steam stripping" (exposure to excessive heat) to improve sensory attributes may destroy potent antioxidants. Other steps in the process designed to remove impurities (protein, water, and polar lipids) may as well remove components with potential beneficial effects (13,14).

Reduced tendency of developing arteriosclerosis has been related to the lower reactivity of platelets and less production of proinflammatory products, e.g., cytokines, prostaglandins,

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Abbreviations: ALAT, alanine amino transferase; ALP, alkaline phosphatase; Apo, apolipoprotein; AST, aspartate aminotransferase; CLO, cod-liver oil; CRP, C reactive protein; CV, coefficient of variation; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL, high density lipoprotein; Lp(a), lipoprotein (a); MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; SO, seal oil; TF, tissue factor; TNF, tumor necrosis factor.

and leukotrienes (15,16), and the maintenance of prostacyclin production in the endothelial cells (17). Various studies have verified that dietary intake of n-3 fatty acids (fish, fish oil, or fish oil capsules) increases the level of these fatty acids in blood plasma and cellular compartments and prolongs the bleeding time.

The objective of the present work is, by mimicking an aspect of the traditional Eskimo diet, to explore therapeutic and prophylactic possibilities of SO on parameters related to development of cardiovascular disease [n-3 fatty acids in serum, blood cholesterol, platelet activity, blood monocyte activity (tissue factor: TF), inflammatory activity (tumor necrosis factor: TNF)], as compared to CLO.

MATERIALS AND METHODS

Subjects. The study was carried out at Hammerfest General Hospital (situated at 70° N latitude) in northern Norway. It included 120 clinically healthy volunteers from 43 to 66 yr of age (median age 55), 60 males (median age 54) and 60 females (median age 56); 102 subjects finished the study. The inclusion criteria were serum cholesterol 7.0–9.5 mmol/L (270–366 mg/dL), clinically healthy, and no lipid-lowering drugs. They were given general dietary advice (consume more vegetables, fruit and fish, and less saturated fat). All participants were asked to continue their daily habits. The study was approved by the Regional Board of Research Ethics.

Study design. The subjects were randomly divided into three groups. One group received 15 mL/d SO (about 1.1 g EPA, 20:5n-3 and 1.5 g DHA, 22:6n-3); another group received 15 mL CLO (about 1.5 g EPA and 1.8 g DHA); and a third group received no oil (control). The fatty acid composition of the oils is given in Table 1. The study was double-blinded for the groups given the dietary oils. No placebo was given in the control group, and the study of this group could thus not be blinded to test subjects or investigators. The groups were in parallel for a period of 2 yr.

Before the intake of the oils, there was a run-in period of 6 mon where the serum cholesterol level was monitored. The period of oil supplementation lasted 14 mon, followed by a 4 mon wash-out period.

Oil supplements. The oils were processed by a standard procedure. This included heating to minimum 150°C, removal of pesticides and dioxins, vitamins A and D, molecules that cause off-flavor or taste, and addition of vitamin E (tocopherol, 1 mg/mL). The oils were kept frozen until used by the participants. Each test subject was delivered one 250-mL bottle of oil at regular intervals from the start of the study.

Both SO and CLO are relatively rich in n-3 fatty acids (Table 1). SO contains higher amounts of MUFA (16:1, 18:1, 20:1, 22:1). The fatty acid content of both oils, kept in control bottles in a refrigerator at the test laboratory, were controlled several times during the study and did not change (Table 1).

Diet. The test subjects consumed an ordinary Norwegian diet throughout the study (in general about 40% fat with P/S ratio of 0.5, 15% proteins, and 45% carbohydrates). The dietary content was evaluated by a clinical dietician by means of a questionnaire at the entry of the study and repeated twice during the study to discover possible dietary changes. The test subjects were asked to report their weekly physical activity in the same questionnaire.

TABLE 1
The Fatty Acid Composition (weight percent) of Cod-Liver Oil and Seal Oil

	Cod-liver oil[a]		Seal oil[a]	
	Entry	14 mon	Entry	14 mon
Fatty acid				
14:0	3.8	3.6	5	5
16:0	9.4	9.2	7.6	7.6
18:0	2.1	1.9	0.5	1.1
Sum	15.3	14.7	13.1	13.7
16:1n-7	7.9	7.2	12.1	11.6
18:1n-9	17.1	17.2	19.6	19.6
18.1n-7	4.2	3.6	3.2	2.9
20:1n-9	10.9	11.5	9.6	10.2
22:1n-11	5.3	5.9	3.5	3.9
22:1n-9	0.5	0.5	0.4	0.4
Sum	45.9	45.9	48.4	48.6
18:2n-6	1.8	1.7	1.9	1.9
18:3n-3	1	0.9	1	0.9
18:4n-3	3	2.7	3.5	3.2
20:5n-3	10.1	9.7	7.4	7.2
22:5n-3	1.2	1.2	3.9	3.9
22:6n-3	11.9	12.1	10.5	10.5
Sum	27.2	26.6	26.3	25.7
n-3/n-6	15.1	15.6	13.8	13.5
Rest (unidentified)	9.8	11.1	10.3	10.1

[a]The oils were kept at 4°C.

Body weight. Each of the participants was asked to report their body weight three times during the study (6 wk before oil intake, and after 2 and 6 mon of dietary oil supplementation).

Blood sampling. Blood samples were collected at 6 and 3 mon before the start of the oil supplementation (run-in period), at the start after 4, 10, and 14 mon of supplementation, and after the wash-out period (4 mon). The test subjects were informed not to drink alcohol or perform any strenuous exercise for 48 h prior to each blood sampling, and the food intake prior to the sampling (breakfast) in general contained coffee and bread. Blood samples were collected between 9:00 and 11:00 A.M. from nonfasted subjects. This implies that the variation/imprecision in measured triacylglycerol is considerable, and results are difficult to interpret.

Lipids in serum. Serum was prepared by whole blood clotted in a glass tube for 2 h at room temperature, followed by centrifugation (2,500 × *g* for 15 min). Serum was tested for total cholesterol (enzymatic colorimetric test, cholesterol esterase and cholesterol oxidase), HDL-cholesterol [after precipitating chylomicron, very low density lipoprotein (VLDL), low density lipoprotein (LDL) with phosphotungstic acid, and Mg^{2+}], triacylglycerol (enzymatic colorimetric test, lipoprotein lipase/oxidation/-peroxidase). The analyses were done in Axon autoanalyzer, Technicon/Bayer. Apo A1 and Apo B100

were determined in a Beckman Nephelometer Immunonephelometric method, with reagents from Beckman. Lipoprotein (a) [Lp(a)] was measured by enzyme-linked immunosorbent assay-technique, TintElize (Biopool, Ventura, CA).

Fatty acids. Total fatty acids in serum were determined after extraction by modified Folch method (chloroform/methanol/KCl aq., 8:4:3) and methylation (BF_3 in methanol) as described earlier (18,19). The fatty acids were analyzed by capillary gas–liquid chromatography (Hewlett-Packard 5880, SP 2380 capillary column; Palo Alto, CA), at an injection temperature of 250°C, column temperature of 180–225°C, and hydrogen flame-ionization detector temperature of 270°C. The signal was analyzed by a disc integrator and compared with those of assays on mixtures of fatty acids supplied by Nu-Chek-Prep (Elysian, MN). The fatty acid compositions of CLO and SO were expressed as relative percentage (area), and the individual fatty acids in serum were calculated by use of 17:0 as internal standard. The identification was based on retention times. Results were expressed as mmol/L.

Standard clinical chemistry analysis. Creatinine (modified Jaffe method), bilirubin (total), total protein, aspartate aminotransferase (AST) and alanine aminotransferase (ALAT), measured as photometrically determined NADH decrease (international standardized procedure), alkaline phosphatase (ALP; colorimetric assay), and C reactive protein (CRP) in serum were analyzed by Axon Autoanalyser, Technicon, with reagents from Technicon/Bayer. The CRP method did not measure "ultrasensitive" CRP.

Standard hematological parameters. Hemoglobin (modified cyanmethemoglobin-method), white cells, red cells, platelets, and hematocrit were determined by use of Cobas Argos, Roche Diagnostics Systems (Nutley, NJ).

TF and TNF were analyzed as earlier described (13).

Platelet aggregation. Fifteen subjects from each group (control, SO, CLO) were randomly selected, and after a period of 14 mon of oil supplementation, the subjects were tested for platelet aggregation using collagen as platelet agonist. Two concentrations of collagen (Collagen-reagent Horm) were used: 1.0 μg/mL and 10 μg/mL. Platelet aggregation was measured in citrated whole blood using a dual channel Crono-Log aggregometer model 540, Coulter Electronic Ltd., which operates by impedance method (20).

Statistical analyses. To compare the various fatty acids and platelet aggregation in the three groups, paired and two-sample *t*-tests were used. Handling of data was done using Microsoft Excel (Redmond, WA) and SAS statistical system (Cary, NC).

RESULTS

The participants maintained their diet throughout the study, which on average contained 1–2 fish meals per week. The subjects tolerated oil supplementation well, and no adverse effects were reported. There were no effects on liver and kidney function or hematological parameters. The level of CRP remained unchanged. The body weight did not change significantly in any of the groups during the study (Table 2).

TABLE 2
Body Weight (kg) at 6 wk Before and 2 and 6 mon After Dietary Addition of 15 mL Cod-Liver Oil (CLO) and Seal Oil (S)[a]

	Before	2 mon	6 mon	*n*
CLO	70.8 (13.6)	71.3 (13.8)	71.3 (14.0)	38
SO	72.6 (13.8)	72.6 (13.2)	72.4 (13.1)	37
Control	69.8 (10.2)	69.6 (9.9)	69.0 (8.5)	37

[a]Mean and SD.

Lipids in serum. The levels of serum total-cholesterol were unaffected both by CLO [mean (SD); 8.3 (0.8) mmol/L (320 mg/dL) before and 7.8 (0.9) mmol/L (301 mg/dL) after 14 mon of oil supplementation], and by SO [8.2 (0.9) mmol/L (316 mg/dL) before and 8.0 (0.7) mmol/L (309 mg/dL) after] as were HDL-cholesterol [CLO; 1.3 (0.4) mmol/L (50 mg/dL) before and 1.3 (0.4) mmol/L (50 mg/dL) after, SO; 1.3 (0.3) mmol/L (50 mg/dL) before and 1.4 (0.3) mmol/l (54 mg/dL) after], A1 [CLO; 1.6 (0.2) g/L before and 1.7 (0.3) g/L after; SO, 1.6 (0.3) g/L before and 1.8 (0.4) g/L after], B100 [CLO; 2.0 (0.3) g/L before and 2.0 (0.3) g/L after, SO; 2.0 (0.3) before and 2.0 (0.3) g/L after] and Lp(a) [CLO; 185 (181) mg/L before and 185 (194) mg/L after 14 mon of oil supplementation, SO; 163 (170) before and 183 (168) mg/L after]. The great SD of Lp(a) measurements is due to considerable interindividual differences in the Lp(a) level (50–800 mg/L). The intraindividual variation was relatively small (<20%), and the coefficient of variation of the assay (CV) was 4%.

The triacylglycerol level (nonfasting) was analyzed during and after the period of oil supplementation (Table 3). There were no significant differences between the controls and the subjects given the oil supplementation, and the wash-out period had no effect. The intraindividual variation was relatively great (10–40%), and the CV of the assay was 5%.

Fatty acids. The three groups (CLO, SO, control) did not differ in the level of any fatty acid, at the entry of the study (Table 4). No significant changes in the serum fatty acid pattern in the control group during the study were observed. Both the SO and the CLO had a significant effect on the composition of fatty acids in serum. This was more pronounced during the course of the study. The maximum effect was achieved after 10 mon. Analysis after 14 mon of oil supplementation showed no further changes.

In both in the CLO and SO groups, the level of several fatty acids was raised significantly including MUFA and the PUFA (Table 4). There was no significant fall in the absolute levels

TABLE 3
Nonfasting Triacylglycerol Levels (mg/dL) in Hypercholesterolemic Subjects During Dietary Addition of 15 mL of CLO and SO and After a 4-mon Wash-out Period[a]

	Oil supplementation period			Wash-out,	
	4 mon	10 mon	14 mon	4 mon	*n*
CLO	177 (79)	203 (115)	195 (124)	195 (97)	38
SO	186 (97)	195 (88)	168 (79)	212 (159)	38
Control	186 (79)	195 (88)	195 (79)	204 (115)	37

[a]Mean and SD. For abbreviations see Table 2.

TABLE 4
The Fatty Acid Composition (mmol/L) of Serum

	Before, during, and after daily addition of 15 mL cod-liver oil							
	Entry		14 mon with oil			Wash-out, 4 mon		
Fatty acid	Mean (n = 40)	SD	Mean (n = 36)	SD	P^a	Mean (n = 36)	SD	P^a
14:0	0.36	0.16	0.42	0.21		0.36	0.13	
16:0	3.61	1.13	5.14	1.51	0.0001	4.19	0.93	0.0003
18:0	1.14	0.3	1.53	0.37	0.0001	1.55	0.34	0.0001
20.0	0.05	0.05	0.04	0.05		0.15	0.07	0.0001
22:0	0.1	0.03	0.15	0.03	0.0001	0.15	0.03	0.0001
24.0	0.02	0.03	0.06	0.05	0.0001	0.07	0.03	0.0001
Sum	5.28		7.34			6.47		
16:1n-7	0.37	0.23	0.52	0.31	0.0001	0.42		
18:1n-9	2.83	0.9	4.06	1.39	0.0001	3.77		
20:1n-9	0.03	0.11	0.17	0.13	0.0001	0.13		
Sum	3.23		4.75			4.32		
18:2n-6	4.66	0.84	6.45	1.51	0.0001	6.04	1.03	0.0001
20:2n-6	0.06	0.06	0.08	0.08		0.09	0.08	
20:3n-6	0.16	0.07	0.21	0.09	0.0001	0.21	0.07	0.0001
20:4n-6	0.63	0.15	0.84	0.21	0.0001	0.91	0.22	0.0001
22:4n-6	0.07	0.07	0.25	0.08		0.48	0.11	0.0001
Sum	5.58		7.83			7.73		
18:3n-3	0.19	0.12	0.19	0.09		0.18	0.07	
20:5n-3	0.31	0.2	0.73	0.32	0.0001	0.61	0.3	0.0001
22:5n-3	0.07	0.04	0.13	0.05	0.0001	0.11	0.04	0.0001
22:6n-3	0.47	0.17	1.01	0.29	0.0001	1.03	0.26	0.0001
Sum	1.04		2.05			1.93		
n-3/n-6	0.19		0.26			0.27		

	Before, during, and after daily addition of 15 mL seal oil							
	Entry		14 mon with oil			Wash-out, 4 mon		
Fatty acid	Mean (n = 40)	SD	Mean (n = 36)	SD	P^a	Mean (n = 36)	SD	P^a
14:0	0.35	0.14	0.41	0.14		0.39	0.27	
16:0	3.8	1.06	5.15	1.32	0.0001	4.2	1.76	
18:0	1.19	0.31	1.60	0.33	0.0001	1.5	0.48	0.0045
20.0	0.02	0.04	0.04	0.05		0.17	0.1	0.0001
22:0	0.09	0.03	0.15	0.03	0.0001	0.14	0.03	0.0001
24.0	0.03	0.04	0.05	0.04		0.06	0.05	0.0054
Sum	5.48		7.4			6.46		
16:1n-7	0.37	0.18	0.47	0.16	0.0011	0.45	0.21	0.0367
18:1n-9	3.01	0.96	3.83	1.09	0.005	3.76	1.83	
20:1n-9	0.03	0.09	0.14	0.13	0.0003	0.16	0.16	0.0003
Sum	3.41		4.44			4.37		

(*continued*)

of any fatty acid. Although SO is almost 30% (relative) lower in EPA as compared to CLO, the serum level of EPA (20:5n-3), after 14 mon of oil supplementation, is about 30% higher in the SO group (0.96 vs. 0.73 mmol/L).

After the wash-out period of 4 mon, the fatty acid changes, compared to the entry values, of both the SO and CLO groups were not so prominent, but they were still significantly different from the start of the study. For example the level of DHA (22:6n-3) did not fall during the wash-out period.

Platelet aggregation. Collagen-induced platelet aggregation was compared between 15 subjects of each group after ingestion of oils for 14 mon. The CLO group showed the lowest platelet aggregation, statistically significant lower than the control group (P = 0.03) (Fig. 1, collagen 1 μg/mL). The aggregation in the SO

Before, during, and after daily addition of 15 mL seal oil (*cont.*)

	Entry		14 mon with oil			Wash-out, 4 mon		
Fatty acid	Mean (n = 40)	SD	Mean (n = 36)	SD	P^a	Mean (n = 36)	SD	P^a
18:2n-6	4.9	0.98	6.53	1.49	0.0001	5.59	1.13	0.0257
20:2n-6	0.05	0.05	0.08	0.08		0.12	0.12	0.0114
20:3n-6	0.16	0.07	0.19	0.09		0.19	0.08	
20:4n-6	0.6	0.19	0.88	0.25	0.0001	0.83	0.24	0.0001
22:4n-6	0.07	0.04	0.26	0.08	0.0001	0.45	0.13	0.0001
Sum	5.78		7.94			7.18		
18:3n-3	0.19	0.11	0.19	0.07		0.19	0.08	
20:5n-3	0.27	0.18	0.96	0.44	0.0001	0.74	0.29	0.0001
22:5n-3	0.07	0.04	0.15	0.44	0.0001	0.15	0.07	0.0001
22:6n-3	0.48	0.19	1.08	0.31	0.0001	1.09	0.32	0.0001
Sum	1.01		2.38			2.17		
n-3/n-6	0.17		0.3			0.3		

Control group (no dietary addition)

	Entry		14 mon		
Fatty acid	Mean (n = 36)	SD	Mean (n = 36)	SD	P^a
14:0	0.34	0.13	0.31	0.12	
16:0	3.78	1.18	3.76	0.79	
18:0	1.19	0.31	1.28	0.25	
20.0	0.03	0.04	0.07	0.07	0.0022
22:0	0.09	0.04	0.1	0.05	
24.0	0.02	0.03	0.05	0.07	0.0075
Sum	5.45		5.57		
16:1n-7	0.42	0.24	0.43	0.21	
18:1n-9	3.1	1.27	3.02	0.78	
20:1n-9	0.05	0.12	0.13	0.14	
Sum	3.99		3.58		
18:2n-6	4.71	1.03	5	0.83	
20:2n-6	0.07	0.05	0.08	0.08	
20:3n-6	0.16	0.06	0.2	0.1	
20:4n-6	0.62	0.15	0.67	0.14	
22:4n-6	0.11	0.1	0.1	0.05	
Sum	5.67		6.05		
18:3n-3	0.2	0.12	0.17	0.06	
20:5n-3	0.3	0.18	0.31	0.22	
22:5n-3	0.07	0.05	0.07	0.05	
22:6n-3	0.53	0.2	0.67	0.31	
Sum	1.1		1.22		
n-3/n-6	0.19		0.2		

[a]Versus entry values.

group was also reduced as compared with the control group, but the change was not statistically significant (P = 0.07).

No effect of SO or CLO was seen on either TF or TNF (Table 5) after 10 mon of oil supplementation, when compared to the control group. The wash-out period of 4 mon had no effect in any of the groups (control, CLO,SO).

DISCUSSION

The present study focused on subjects with hypercholesterolemia and included a run-in period of 6 mon, and an oil supplementation period of 14 mon, which should be sufficient to observe effect on the parameters studied, and a 4-mon wash-out period.

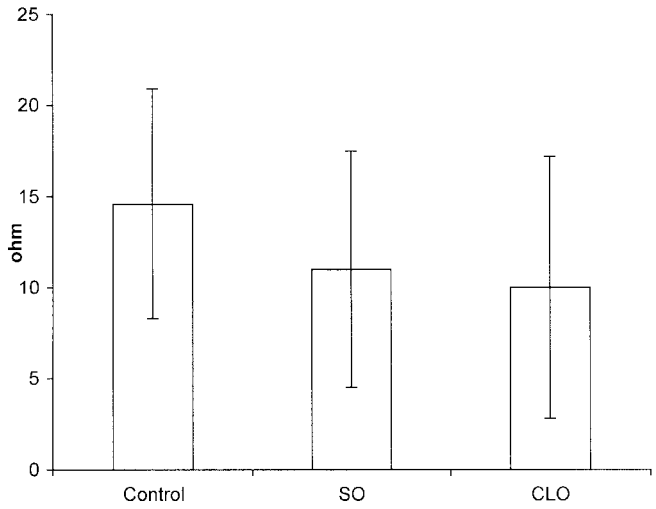

FIG. 1. Collagen (1 μg/mL)-induced platelet aggregation (ohm) in whole blood in subjects taking daily supplementation of 15 mL seal oil (SO; $P = 0.07$) and cod-liver oil (CLO; $P = 0.03$) for 14 mon, vs. control (no dietary addition) (mean and SD).

The n-3 fatty acid content of both CLO and SO represented a considerable daily addition, although well below the intake through original Eskimo diet. The serum level of EPA after intake of SO was about 30% higher as compared to CLO (Table 4), in spite of the fact that the EPA content of SO is 30% lower than in CLO (Table 1). It is an open question whether this implies that SO may be more efficient than CLO in preventing cardiovascular events. The mechanism behind the present observation is not clear, but may be related to the fact that EPA is located in position 1,3 in glycerol in SO, and in position 2 in CLO.

The n-3/n-6 ratio was moderately increased after the intake of both oils (Table 4). Still, this is far from the ratio in plasma of Greenland Inuit ($R = 0.7–1.2$) (4) and implies a different biological setting. During the study, the level of n-3 fatty acids in serum increased with *ca.* 100% in the CLO group and with about 140% in the SO group (Table 4). Also the level of n-6 fatty acids was elevated, although not to a similar degree. Intake of n-3 fatty acids may increase the enteral absorption of n-6 fatty acids (21).

The wash-out period of 4 mon hardly affected the serum levels of n-3 fatty acids. The level of DHA was quite similar at the end of the oil supplementation period and after 4 mon washout. Similar findings were reported in normal individuals (22). This probably reflects that the fatty acids were incorporated into the fat tissues (depot fat) and only slowly released.

One implication of this observation is that all crossover studies on PUFA should be interpreted carefully. This would also have significance for studies in subjects with traditionally high and low fish intake, and the finding also indicates that once the body depot fat is saturated with fatty acids, the need of supplementation is reduced, probably to one or two times a week.

Daily dietary addition of 15 mL of oil (about 180 kcal) might be expected to give a slight increase in the body weight. This did not occur (Table 2). The test subjects did not report increased physical activity, nor any significant change in their dietary habits. The n-3 fatty acids may increase β-oxidation of fatty acids and thereby counteract weight increase (23).

Neither SO nor CLO affected the levels of serum total cholesterol, HDL-cholesterol, and apo [Apo A1, Apo B100, Lp(a)]. This is in accordance with findings in normocholesterolemic subjects (13,24,25). By dietary addition of CLO and SO, SFA as well as PUFA are ingested. The level of SFA in serum is also raised after 14 mon of oil supplementation (Table 4). It might be that the intake of SFA is masking a possible lipid-lowering effect of PUFA. The more beneficial levels of total cholesterol in the Eskimos may be due to lower intake of SFA and possibly the fact that they consume a larger amount of unprocessed foods (higher amounts of biologically active compound, i.e., antioxidants) in their diet compared to a Western diet.

Intake of n-3 fatty acids is known to reduce the level of triacylglycerols in the blood (6). This was not confirmed in the present study, in which nonfasting serum was analyzed. This implies that triaclylglycerol in chylomicrons is included, which results in great intra- and interindividual variation in the level of serum triacylglycerol.

CLO supplementation is known to reduce platelet aggregation (18,26,27). In the present study aggregation was done in whole blood, rather than in platelet-rich plasma, which is a more common method. We found that platelet aggregation was statistically significantly reduced in the CLO group, as compared to the controls. Aggregation in the SO group was also lower than in the controls, although this was not statistically significant. The difference between the oil groups was small. Intra- and interindividual variation is substantial with respect to platelet aggregation, which influences the statistical calculations.

The mechanism of reduction in platelet aggregation is probably the same in both of the oil groups. EPA (20:5n-3) substitutes arachidonic acid (20:4n-6) in the platelet membrane phospholipids, and less potent prostanoids (tromboxane) are synthesized. We did not observe any effect of the oil supplementation on monocyte TF activity or TNF in blood. The level of both variables decreased during the study, in all groups. This may be related to biological and seasonal variations.

TABLE 5
The Monocyte-derived Tissue Factor (TF) Activity in Whole Blood and Tumor Necrosis Factor (TNF, ng/mL) Before, During and After Dietary Addition of 15 mL of CLO and SO[a]

		6 wk before	10 mon of oil supplementation	Wash out, 4 mon	*n*
TF	CLO	16.8 (12.3)	12.2 (7.8)	9.9 (9.4)	38
	SO	16.5 (8.9)	16.2 (11.9)	9.1 (5.8)	38
	Control	18.9 (14.6)	13.3 (10.9)	9.7 (7.8)	37
TNF	CLO	4.4 (1.9)	1.0 (0.7)	1.0 (0.6)	38
	SO	4.5 (1.3)	1.3 (1.1)	1.5 (1.0)	38
	Control	4.9 (1.8)	1.2 (0.9)	1.5 (1.2)	37

[a]The TF activity is expressed as mU/10^6 cells of a standard. Values given as mean and SD. For other abbreviations see Table 2.

The present study mainly focused on certain parameters relevant to the development of cardiovascular disease (n-3 fatty acids in serum, other blood lipids, apo, blood platelets, monocyte activity, inflammation), leaving out aspects of oxidation, although the intake of vitamine E was increased. The oils utilized had been heated to 150°C, which may destroy natural antioxidants. It may well be that the positive influence of the Eskimo diet, and possibly also SO, is acting through such biological systems. This aspect of the Eskimo diet remains to be further explored, as unprocessed food items contain biological active components (antioxidants other than tocopherol), and the relationship between the natural fat and natural antioxidants of Eskimo diet is probably important.

In conclusion we found that dietary addition of SO increased the EPA level considerably more than CLO; after 10 mon of oil supplementation there was no further rise in serum fatty acid levels, and a wash-out period of 4 mon had only a modest effect on these levels.

ACKNOWLEDGMENTS

This study was supported by the program Medical Research in Finnmark County, University of Tromsø. The skillful technical assistance of Jan Ole Olsen, Oddvar Gamst, Roger Johansen, and the staff in Laboratory of Clinical Chemistry of Hammerfest Hospital is gratefully acknowledged.

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[Received June 5, 2000, and in final revised form and accepted November 27, 2000]