Effects of fish-oil ingestion on cardiovascular risk factors in hyperlipidemic subjects in Israel: a randomized, double-blind crossover study[1-3]

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ABSTRACT Effects of a daily fish-oil supplement on serum lipids, apolipoproteins, and some platelet functions and hemorheologic variables were examined in 27 hyperlipidemic subjects in a randomized, controlled, double-blind, crossover fashion with an identically encapsulated vegetable oil serving as the control treatment. Despite the habitual high linoleic acid intake of the study population, significant incorporation of n−3 (ω-3) fatty acids into the serum, platelet, and erythrocyte lipids was observed after the fish-oil supplement. Ingestion of fish oil resulted in a 40% decrease in the triglyceride concentration, a 12% increase in HDL cholesterol, and a significant decrease in plasma viscosity, whereas the vegetable-oil placebo had no significant effect. We conclude that a moderate intake of fish oil (15 g/d) is a feasible treatment for hypertriglyceridemia even in patients with a background of high linoleic acid intake and that it may have a beneficial effect on several cardiovascular risk factors. *Am J Clin Nutr* 1990;52:1118-24.

KEY WORDS Fish oil, n−3 PUFAs, serum lipids, apolipoproteins, hyperlipidemia, platelet functions, plasma viscosity, erythrocyte deformability

Introduction

Fish-oil ingestion as a means of preventing cardiovascular disease has recently attracted much attention (1). Actually, fish oils provide the most effective means of dietary intervention for lowering the serum triglyceride (TG) concentration in hypertriglyceridemic individuals (2-4). The effect is brought about by the n−3 (ω-3) fatty acids characteristically present in these oils. The other polyunsaturated fatty acid (PUFA) family, which comprises linoleic acid and derived n−6 fatty acids, fails to cause such an effect. A comprehensive and critical review of the effects of fish oil on lipoprotein metabolism was recently published (5).

In view of the metabolic interactions taking place between the two fatty acid families (6), the question arises whether the TG-reducing effect of fish oils will also be observed in a population with a background of high linoleic acid intake.

Israel has a considerably higher linoleic acid consumption than do other industrialized countries, as evidenced by the elevated linoleate content of subcutaneous fat, which, according to three reports published since 1976 (7-9), averages between 22% and 27% of total fatty acids. National food balance sheets (10) and family surveys (11) point to linoleic acid–rich vegetable oils as the major separated fat consumed in Israel.

The aims of our study were to examine whether a moderate intake of fish oil under conditions of a high dietary linoleate intake will be as effective as it is in other countries with much lower linoleate consumptions and to test the effects of this intake on several cardiovascular risk factors (including serum lipids, apolipoproteins, platelet functions in vivo as estimated by measurement of circulating aggregated platelets, and hemorheologic variables) in a randomized, controlled, double-blind, crossover fashion.

Subjects and methods

Subjects

We recruited 27 patients at the Lipid Clinic of the Beilinson Medical Center. The following criteria were met by the participants: serum triglyceride concentration > 2.8 mmol/L while on a standard lipid-lowering diet and without any hypolipidemic drug for ≥ 2 mo before the trial and no serious illness or operation during the 3 mo preceding the trial. All participants were required to sign an informed-consent form approved by the hospital Helsinki Committee, which also approved the study procedures. The World Health Organization phenotypes of the patients, their clinical characteristics, and baseline serum lipids are summarized in **Table 1.** A weight-maintenance diet was followed by the patients throughout the study.

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[2] Parts of this work were presented at the NATO Advanced Research Workshop on Dietary ω-3 and ω-6 Fatty Acids: Biological Effects of Nutritional Essentiality, Belgirate, Italy, 1988.

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TABLE 1
Clinical characteristics of patients

	Type IIB (n = 15)	Type IV (n = 12)
Diabetes mellitus (n)	3	6
Hypertension (n)	5	5
Smokers (n)	10	3
Ischemic heart disease (n)	6	5
Peripheral vascular disease (n)	2	2
Serum cholesterol [mmol/L]	8.15 ± 1.40*	6.45 ± 0.55
Serum triglycerides [mmol/L]	6.72 ± 4.14	6.24 ± 2.61
HDL cholesterol [mmol/L]	0.85 ± 0.27	0.81 ± 0.21

* $\bar{x}$ ± SD.

Study design

The patients were randomly divided into two groups. The treatment group received 15 g fish oil/d in an encapsulated form (EPAGIS, Agis Ltd, Tel Aviv, Israel), whereas the control group received a similarly encapsulated mixture of equal amounts of corn and olive oils. The fatty acid composition of both preparations is shown in **Table 2.** The fish oil provided 5.2 g n−3 PUFAs/d, whereas the vegetable-oil mixture supplied a daily dose of 3.7 g linoleic acid 18:2n−6 and 0.15 g α-linolenic acid 18:3n−3. The cholesterol content of the fish oil was 4.2 mg/g, so the total daily cholesterol intake from the fish-oil preparation was 63 mg. After 8 wk of either treatment there was a 4-wk washout period after which each group received the alternative treatment for another 8 wk.

Lipid determinations were made after a 12-h overnight fast at baseline and during the treatments at 2-wk intervals. Blood was drawn by venipuncture of an antecubital vein after brief tourniquet application for vein localization. All of the other determinations described below were performed at the beginning and at the end of each treatment period.

Fatty acid analysis

The fatty acid composition of the total lipids in serum, washed platelets, and erythrocytes was determined by gas-liquid chromatography after preparation and methylation of the fatty acids, essentially as described by Budowski et al (12).

Serum lipid analysis

Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and TGs were measured with commercial reagent kits (Boehringer Mannheim GmbH, Mannheim, FRG). The cholesterol assay was performed with the Boehringer cholesterol-CHOD-PAP kit. It was standardized with Sigma reference serum with a cholesterol value of 1.96 g/L (Sigma, St Louis). The between-days CV was 1.7%. The HDL cholesterol was estimated after MgCl-phosphotungstic acid precipitation of the apolipoprotein B–containing lipoproteins.

The TG assay was performed with the Boehringer Triglyceride-Peridochrom kit. It was standardized with Boehringer Glycerol-Precimat standard corresponding to 2.00 g TGs/L. The between-days coefficient of variation for the method was 2.2%. Low-density-lipoprotein (LDL) cholesterol was estimated by the Friedwald equation (13).

Apolipoprotein determinations

Apolipoproteins A-I and B were determined by an immunoturbidimetric method (IDL, Jerusalem) (14). The assay was standardized by serial dilutions of a Centers for Disease Control (CDC) plasma pool (CDC-IUIS #1883; 1.20 g/L). Within-assay precision was between 1.2% and 4.8%, whereas interassay precision was between 3.1% and 5.2%.

Platelet measurements

Platelet counts were made on a standard Coulter counter (Technicon Instruments Corp, Tarrytown, NY). Circulating aggregated platelets and mean platelet-aggregate size were determined by methods previously described (15, 16). In short, 1 mL of venous blood was equally separated into two test tubes. One contained 2 mL EDTA and formalin (solution F). Assuming that the formalin fixes all platelet aggregates, this solution will contain reversibly plus irreversibly aggregated platelets. The second test tube contained EDTA alone (solution E), in which reversibly aggregated platelets disaggregate; therefore, this solution contains only irreversibly aggregated platelets. Slides were prepared from both solutions and stained with Giemsa. The number of platelets forming aggregates in 1000 counted platelets was determined by direct microscopic readings and expressed as a percentage. Reversibly aggregated platelets were estimated by subtracting the percentage of aggregated platelets in solution E from that in solution F. An index for mean platelet aggregate size was calculated by dividing the number of aggregated platelets in solution F by the number of aggregates/1000 counted platelets. The percentage of big platelets (a big platelet is defined as one with a diameter of > 50% of an adjacent red blood cell) was determined microscopically on slides prepared from solution E by counting 1000 platelets in each determination. All platelet measurements were made in triplicate, with a coefficient of variation not > 2%.

Hemorheologic measurements

Plasma viscosity was determined at 37 °C by the capillary method and a Harkness Coulter viscometer (Coulter Electronics,

TABLE 2
Fatty acid composition of the experimental oils

Fatty acid	Treatment (fish oil)	Control (corn:olive oil, 1:1)
	% of total fatty acids	
14:0	9.2	—
16:0	15.8	11.3
16:1	11.5	0.7
18:0	1.7	2.4
18:1	14.3	59.2
18:2n−6	—	24.8
18:3n−3 and 20:1	3.5	1.0
18:4n−3	3.0	—
20:2	3.5	—
20:5n−3	18.1	—
22:1	2.6	—
22:5n−3	2.8	—
22:6n−3	10.7	—
24:1	0.7	—
Others	2.6	0.6

Ltd, Luton, England). Deformability of red blood cells was determined by a filtration method that uses polycarbonate membranes (Nucleopore Corp, Pleasanton, CA) from a single batch with a pore diameter of 5 μm and negative pressure of 1.96 MPa (17). Routine hematologic and chemistry profiles were performed at the beginning and end of each treatment period and included a complete blood count and analysis by sequential multichannel analyzer (SMA; Technicon Instruments Corp).

Statistical analysis

The data were stored and analyzed by the Statistical Analysis System (18). Continuous variables compared between two classes were tested for statistical significance by use of a paired, two-tailed t test. Because multiple comparisons were performed, the differences were considered significant if $P < 0.005$. For the variables that were measured several times during the study period (ie, cholesterol, TG, and HDL concentrations), an analysis of variance (ANOVA) for repeated measures (19) to test for the overall effect of the fish-oil supplement was used.

Results

The changes in the fatty acid content of the serum lipids, platelets, and erythrocytes after the fish-oil supplementation are shown in **Figure 1.** There was a significant increase in the concentration of 20:5n−3 in all three blood fractions and a significant decrease in n−6 fatty acids in the erythrocyte lipids only. Other n−3 fatty acids underwent similar changes (not shown). The placebo treatment did not affect the fatty acid composition of the platelets, increased the n−6 fatty acids in the erythrocyte lipids, and decreased the serum n−3 fatty acids (not shown; complete data available from the authors). The higher concentrations of the n−3 fatty acids after the fish-oil ingestion indicate good compliance with the trial regimen.

The changes in serum total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and apolipoproteins A-I and B are shown in **Figure 2.** There was a significant decrease of ~40% in the TG level during the fish-oil supplement ($P < 0.005$ by ANOVA). Most of the decrease was achieved as early as 2 wk after starting the supplement. A significant rise in the HDL-cholesterol concentration of ~12% was observed also ($P < 0.001$ on analysis of variance). This rise is largely accounted for by a significant elevation in HDL cholesterol in the IIB patients (**Table 3**). The total- and LDL-cholesterol concentrations as well as the apolipoprotein concentrations did not change significantly with either supplement (Fig 2). However, when the serum lipid changes were analyzed according to phenotype (Table 3), a slight decrease in the total cholesterol concentration was observed in the IIB patients after the fish-oil ingestion. No changes were observed after the ingestion of the vegetable-oil capsules and no significant changes were observed in LDL cholesterol after either supplementation (Fig 2, Table 3).

To examine a possible carry-over effect after crossover from the fish-oil to the placebo treatment, the PUFA values of the erythrocytes at the beginning of the placebo period were grouped according to the supplement that the patient received first (**Table 4**).

A clear carry-over effect was observed; the n−3 fatty acid content of the erythrocyte fatty acids at the beginning of the placebo period was significantly higher in the group that started with fish oil than in the group that started with the placebo.

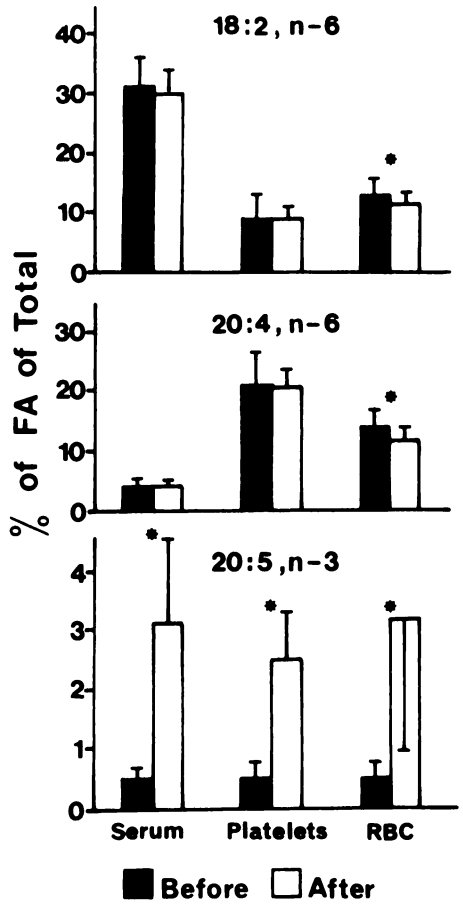

FIG 1. Changes in some fatty acids in the serum, platelet, and erythrocyte (RBC) lipids after fish-oil ingestion. $\bar{x} \pm$ SEM. *Before and after values are significantly different, $P < 0.0005$.

The results of the platelet-function studies are summarized in **Table 5,** which also includes the normal values found in our laboratory. No significant changes were observed after the ingestion of either oil, although the percentage of big platelets was much higher in both groups than in the control ("normal") population, as was the percentage of aggregated platelets.

Hemorheologic changes are shown in **Table 6.** A significant decrease in plasma viscosity was observed after the fish-oil ingestion, but no changes were seen in the filtrability of the erythrocytes. No changes were observed in the routine hematologic and blood chemistry analyses for either treatment (results not shown).

Discussion

The pronounced TG-lowering effect of fish oil (20), which was confirmed in the present study, should be viewed against the background of the very high consumption of linoleic acid in Israel, where linoleic acid–rich vegetable oils and margarines constitute by far the major source of separated fatty acids for culinary use and the food industry (10). This high linoleic acid consumption is reflected in the elevated linoleic acid content of subcutaneous fat in Israelis; a mean value of 24% was reported in 1976 (7) and more recently a value of 27% was found (9).

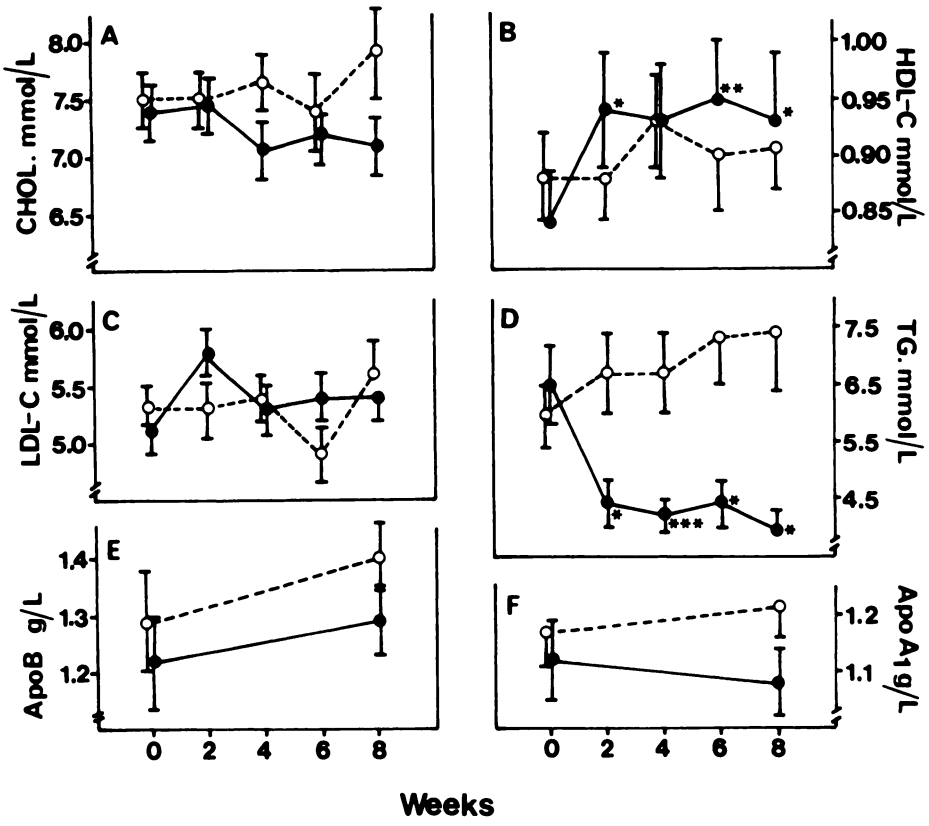

FIG 2. Serum cholesterol (A), HDL cholesterol (B), LDL cholesterol (C), triglycerides (D), apolipoprotein B (E), and apolipoprotein A-I (F) changes during treatment with fish oil (● —— ●) and vegetable oil (○ - - - ○) ($\bar{x} \pm$ SEM). Asterisks denote significant changes compared with baseline values: *P = 0.001, **P = 0.003, ***P = 0.0001.

Figures for the United States have been rising and have lately reached 18% (21). Europeans have less linoleic acid in their body fat, eg, ~12% in Heidelberg, FRG, (22) and 7–9% in Finland and Scotland (23). Our results show that large body reserves of linoleic acid do not interfere with the TG-lowering action of fish oil.

No significant changes were observed in the total cholesterol concentrations after fish-oil ingestion, similar to the observations made in several other investigations (3, 24, 25). The slight non-significant decrease in the total cholesterol concentration found in our IIB patients was also observed in another study in which similar amounts of fish oil were administered to hyperlipidemic

TABLE 3
Serum total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides before and after the supplementation periods according to phenotype*

	Type IIB			Type IV		
	Before	After	P	Before	After	P
	mmol/L			*mmol/L*		
Fish oil						
Total cholesterol	8.16 ± 1.40	7.47 ± 1.23	0.05	6.45 ± 0.55	6.53 ± 1.17	NS
LDL cholesterol	5.62 ± 1.32	5.76 ± 1.06	NS	4.38 ± 0.58	4.86 ± 1.13	NS
HDL cholesterol	0.85 ± 0.27	0.98 ± 0.35	0.002	0.81 ± 0.21	0.86 ± 0.23	NS
Triglycerides	6.72 ± 4.14	3.78 ± 1.92	0.02	6.24 ± 2.61	4.02 ± 1.77	0.01
Vegetable oil						
Total cholesterol	8.08 ± 0.90	8.82 ± 2.37	NS	6.78 ± 2.51	6.73 ± 1.08	NS
LDL cholesterol	5.97 ± 0.86	6.35 ± 1.62	NS	4.54 ± 0.60	4.74 ± 1.10	NS
HDL cholesterol	0.94 ± 0.25	0.92 ± 0.26	NS	0.80 ± 0.14	0.89 ± 0.11	NS
Triglycerides	6.23 ± 3.30	8.54 ± 6.30	NS	5.69 ± 2.44	6.00 ± 2.11	NS

* $\bar{x} \pm$ SD. P values denote significance of difference between before and after oil supplementation.

TABLE 4
The carry-over effect: fatty acid concentrations of erythrocytes at the beginning of the placebo period in patients who started with fish oil and patients who received the placebo first*

Fatty acid	Fish oil first	Vegetable oil first	P
	% of total fatty acids		
16:0	26.00 ± 2.41	26.40 ± 2.47	NS
18:0	17.88 ± 2.08	18.40 ± 2.59	NS
18:1n−9	16.31 ± 2.42	15.56 ± 1.22	NS
18:2n−6	12.71 ± 2.10	12.53 ± 2.10	NS
18:3n−3	0.38 ± 0.17	0.30 ± 0.09	NS
20:3n−6	1.77 ± 0.45	1.87 ± 0.48	NS
20:4n−6	11.31 ± 2.42	12.44 ± 1.68	NS
20:5n−3	1.44 ± 0.53	0.32 ± 0.17	0.0001
22:5n−3	2.09 ± 0.50	1.13 ± 0.30	0.0001
22:6n−3	4.71 ± 1.12	3.30 ± 0.86	0.002

* $\bar{x}$ ± SD.

subjects (26), but, to achieve a more pronounced and significant decrease in the cholesterol concentration, much higher doses of fish oil had to be given (4). HDL-cholesterol values were significantly higher after the fish-oil treatment, a finding that was reported by some (25) but not by others (24). Variable contents of cholesterol and saturated fatty acids in different fish oils as well as in the basic diets might account in part for the discrepancies (5).

The vegetable-oil placebo had no effect on cholesterol concentration, possibly because of the dosage and limited duration of the treatment, but also because of the high background consumption of linoleic acid already mentioned.

No changes were observed in the apolipoprotein concentrations of our subjects after ingestion of either fish oil (providing 5.2 g n−3 PUFAs/d) or vegetable oil. Similar results were reported by investigators who gave fish oils providing 3 and 7.5 g n−3 PUFAs/d to hypertriglyceridemic subjects (25, 27). In some clinical trials in which hypertriglyceridemic subjects were given fish oil providing 4.6 (28) or 6–7 g n−3 PUFAs/d (29), significant increases in apolipoprotein B concentrations were observed, but decreases were noted after ingestion of large amounts of fish oil providing 20–30 g n−3 PUFAs/d (4). However, the time factor and variability in fish-oil composition constitute confounding factors.

The percentage of circulating aggregated platelets was found to be elevated in our study population; most platelets were irreversibly aggregated. Neither ingestion of fish oil nor of vegetable oil had any effect on this variable. A similar percentage and distribution of reversibly and irreversibly aggregated platelets was found in patients with stable angina (15) and this may represent a chronic state of platelet activation. The same observations were made in another group of hyperlipidemic subjects with hypercholesterolemia in our Lipid Clinic (Y Beigel, personal communication, 1989). Our speculation is that the hyperlipidemic state itself, possibly hypercholesterolemia, may be associated with increased platelet aggregability. This possibility remains to be explored.

Another platelet-function variable investigated in this study was the percentage of big platelets. Big platelets have been associated with ischemic heart disease (30) and were shown to produce more thromboxane A_2 (31), therefore being considered more active than the average platelet population. The origin of these platelets is not established entirely and some authors suggest that they may originate from pulmonary megakaryocytes (32). Although big platelets were described only in clinically active ischemic heart disease (30), our study demonstrated an increase in their number in hyperlipidemic patients even without evidence for clinically active ischemic heart disease. It remains to be determined whether these platelets contribute to the increased risk of the development of ischemic heart disease.

Although n−3 PUFAs were incorporated into erythrocytes, no change in their deformability was observed after the ingestion of fish oil. This finding is in contrast to that of Tamura et al (33), who found increased erythrocyte deformability in patients with a variety of thrombotic diseases and hyperlipidemia 16 wk after ingestion of 1.8–2.7 g eicosapentaenoic acid (EPA) ethyl ester/d. Their patient population was similar to ours (34) and red-cell deformability was determined by the same method that we used (34), but the supplement was given for twice as long as in the present study before the change became apparent. The EPA supplement in the study of Tamura et al was given as highly concentrated ethyl ester of EPA, whereas in the present study the supplement was fish oil, which contains other fatty acids and is in the TG form. However, no significant difference was observed in the absorption of these two forms of EPA (35), and

TABLE 5
Platelet functions studied during the treatments*

	Treatment (fish oil)		Placebo (vegetable oil)		
	Before	After	Before	After	Normal values
Platelet count (×10^9/L)	226.8 ± 65.9	217.0 ± 80.6	240.1 ± 69.7	242.0 ± 60.5	350 ± 150
Percent of big platelets (%)	23.3 ± 12.0	25.7 ± 9.4	21.9 ± 9.0	22.4 ± 9.8	6.8 ± 3.5
Percent of aggregated platelets (%)	16.5 ± 4.7	17.4 ± 5.0	18.8 ± 3.7	17.7 ± 4.8	6.0 ± 2.0
Percent of irreversibly aggregated platelets (%)	9.5 ± 4.0	9.4 ± 3.6	10.6 ± 3.1	10.3 ± 4.0	4.0 ± 2.0
Percent of reversibly aggregated platelets (%)	6.8 ± 5.1	8.0 ± 5.8	7.7 ± 3.1	7.0 ± 4.1	2.0 ± 1.0
Mean platelet-aggregate size (*n*)	2.2 ± 0.2	2.2 ± 0.1	3.3 ± 3.9	2.8 ± 3.3	2.2 ± 0.4

* $\bar{x}$ ± SD.

TABLE 6
Hemorheologic changes during treatment*

	Treatment (fish oil)		Placebo (vegetable oil)	
	Before	After	Before	After
Plasma viscosity (mPa·s)	1.4000 ± 0.08552	1.3495 ± 0.088647†	1.3969 ± 0.07707	1.3757 ± 0.07933
Filtrability of RBC (s/mL)	33.6 ± 5.4	34.0 ± 8.3	35.6 ± 6.3	34.0 ± 8.1

* $\bar{x}$ ± SD.
† Significantly different from before-treatment value, $P = 0.0001$.

the incorporation of EPA into the erythrocyte lipids of our patients was of a magnitude similar to that reported by Tamura et al (34) for the major erythrocyte phospholipids.

We observed a significant decrease in the plasma viscosity of the patients' blood after the fish-oil feeding but not after supplementation with vegetable oil. The mechanism of this decrease is not clear, since no changes in the plasma proteins were observed and there was no correlation between the decrease in viscosity and the changes in lipid concentrations. The decrease in plasma viscosity might have been mediated by a decrease in plasma fibrinogen concentration brought about by dietary fish oil (36), although this supposed mechanism is controversial (37).

Our study was designed to be randomized, double-blind, and controlled. However, a significant carry-over effect was demonstrated (Table 4), suggesting that a washout period of 4 wk was not sufficient to achieve a true baseline state. This carry-over could only have caused an underestimation of the effect of the fish oil in this study, but it should be taken into account when variables other than erythrocyte fatty acids are measured. An alternative design of this kind of feeding trial may be a comparison between two matched groups, one serving as the treatment group and the other as the placebo group.

Most patients in whom a significant TG-lowering effect was achieved with ingestion of fish-oil capsules stated that they preferred this form of treatment over their previous treatment with drugs, mostly fibric acid derivatives. Those who can afford to purchase the capsules are continuing this treatment, 10–15 g EPAGIS capsules/d, up to 12 mo after completion of the clinical trial, with good results. The main impediments to implementing this treatment in more hypertriglyceridemic patients remain the high cost of the fish-oil preparation and the need to ingest many capsules per day.

The results of this study suggest that fish-oil ingestion in a moderate dose is a feasible treatment for hypertriglyceridemia even in a population with a high intake of linoleic acid. Furthermore, the improvement in the lipid profile observed in our patients together with the decrease in the plasma viscosity point toward a potential beneficial effect of fish-oil ingestion on the atherosclerotic process.

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