Modification of lipid-related atherosclerosis risk factors by ω3 fatty acid ethyl esters in hypertriglyceridemic patients

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The efficacy of ω3 fatty acid ethyl esters was evaluated in 10 mildly hypertriglyceridemic patients in this randomized, placebo-controlled, double-blind, crossover trial. Patients were given capsules (1 per 10 kg body weight) containing 640 mg/g of ω3 fatty acids or an olive oil placebo for two 4-week treatment periods separated by a 1-week washout phase. Plasma lipids, lipoproteins, and apolipoproteins; phospholipid FA composition; the susceptibility to oxidation of the apolipoprotein B-100 containing lipoproteins; and bleeding times were determined at the end of each period. Plasma triglyceride levels were reduced by 37% ($P < 0.001$), whereas low density lipoprotein cholesterol and the cholesterol content of subfraction 2 of high density lipoproteins increased by 23 and 56%, respectively (both $P < 0.02$). Changes in plasma lipid parameters and in phospholipid FA patterns occurred rapidly, usually stabilizing within 1 week, and returned to baseline levels within 10 days after stopping supplementation with ω3 fatty acids. Bleeding times were not changed. However, the susceptibility of lipoproteins to oxidation was increased during the ω3 fatty acid period. We conclude that ω3 fatty acid ethyl esters are effective hypotriglyceridemic agents, and that they impact lipoprotein metabolism very quickly. How they may alter the atherogenic process is not clear from this study because some risk factors worsened and others improved. (J. Nutr. Biochem. 4:706–712, 1993.)

Keywords: fish oil; ω3 fatty acids; hypertriglyceridemia; bleeding time; phospholipid fatty acids; lipoprotein oxidation; eicosapentaenoic acid; docosahexaenoic acid

Introduction

Although fish oils (containing relatively low amounts of ω3 fatty acids (FAs) in the triglyceride form) have been widely used to treat elevated triglyceride levels,[1] the effects on lipid metabolism of products containing high levels of ω3 FA as ethyl esters are not well described. The latter would have obvious advantages over the former as a means of supplying ω3 FAs because lower intakes would theoretically be needed to achieve similar effects.

There were several reasons for conducting this trial. First, we wanted to evaluate a product provided by the Fish Oil Test Material Program of the National Institutes of Health (NIH) and the Department of Commerce (DOC) containing 41% eicosapentaenoate (EPA) and 23% docosahexaenoate (DHA) as ethyl esters. By comparison, one of the most thoroughly tested and widely used fish oil concentrates (MaxEPA, Seven Seas, Hull, UK) contains 18% EPA and 12% DHA. Thus the ethyl ester preparation contained more than twice the ω3 FAs of MaxEPA. Secondly, it was of interest to know how rapidly serum lipoprotein levels and FA compositions in hypertriglyceridemic patients changed after starting ω3 FA supplementation, and how quickly they returned to normal after stopping. Finally, we evaluated how ω3 FAs altered the susceptibility of lipoproteins of copper-induced oxidation.

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Methods and materials

Patients

Ten otherwise healthy, mildly hypertriglyceridemic patients were recruited from among the population of the Lipid and Arteriosclerosis Prevention Clinic at the University of Kansas Medical Center for this study. They were made up of nine men and one (postmenopausal) woman, ranging in age from 34 to 68 years (mean 52 years). Their mean body mass index at baseline was 27.1 ± 3.5. None was taking any medications known to affect lipid metabolism, and all were instructed to avoid aspirin and aspirin-containing medications within 10 days of the end of each treatment phase (see below). Baseline lipids, lipoproteins, and apolipoprotein levels are given in *Table 1*. Each patient gave informed consent before entering the study, which had been approved by the Human Subjects Committee.

Protocol

This was a double-blind, placebo-controlled, randomized, crossover study with two 4-week treatment periods separated by a washout period of about 1 week. The washout phase was intentionally short (averaging 5.6 days, with a range of 2–12 days) because previous work in our laboratory suggested that a rapid return to baseline conditions occurred after discontinuing supplementation with these low levels of ω3 FAs.[2] Subjects were assigned to take one capsule per 10 kg body weight daily of either the ω3 FA concentrate or a placebo (olive oil ethyl esters), both provided by the NIH/DOC. The capsules contained 1 gram of FA ethyl esters, the compositions of which are given in *Table 2*. Blood samples were obtained in the fasting state twice at baseline, and then at 2,4,8, and 28 days of each period. Plasma cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, triglyceride levels, and phospholipid FA composition were determined in each blood sample; all other tests were done at each baseline and at the end of each treatment period. Diet diaries were completed once during each phase to document stability of background diets. The diaries were analyzed by Professional Nutrition Systems (Kansas City, KS USA).

Laboratory methods

Plasma (1 mg/mL EDTA) was analyzed at each visit for lipids and lipoproteins as previously described using enzymatic methods on an ABA 200 Bichromatic Analyzer (Abbott Diagnostics, Irving, TX, USA).[3] Plasma HDL cholesterol levels were measured following precipitation of the apolipoprotein B (apoB)-containing lipoproteins with heparin/manganese chloride.[4] Quality control was maintained, in part, by our participation in the Lipid Standardization Program of the Centers for Disease Control.[5] LDL cholesterol was determined by subtraction of HDL cholesterol from the d>1.006 fraction that was obtained by ultracentrifugation of the plasma for 2 hours at 100,000 rpm in a TLA 100.3 rotor using a TL100 Ultracentrifuge (Beckman Instruments, Palo Alto, CA USA).[6] HDL_2 and HDL_3 cholesterol levels were measured by precipitating the former from HDL with dextran sulfate.[7] ApoB and apoA1 levels were determined in serum (not plasma) by rate immunonephleometry on the Beckman ICS analyzer using the manufacturer's reagents. Plasma lipoprotein(a) [Lp(a)] levels were measured by an enzyme-linked immunoabsorbant assay (ELISA) at Medical Research Laboratories (Cincinnati, OH USA).

Table 1 Effects of ω3 fatty acid ethyl esters on blood lipids and lipoproteins (mM) and apolipoproteins (g/L) in 10 hypertriglyceridemic patients

	Baseline	Placebo	ω3 FAs
Total cholesterol	4.92 ± 0.85	5.00 ± 0.68	5.10 ± 0.43
Total triglycerides	3.05 ± 1.30	3.36 ± 1.08	2.11 ± 0.85[a]
LDL cholesterol	2.55 ± 0.63	2.45 ± 0.53	3.01 ± 0.51[a]
HDL cholesterol	0.81 ± 0.20	0.85 ± 0.20	0.91 ± 0.30
HDL_2 cholesterol	0.18 ± 0.08	0.23 ± 0.10	0.35 ± 0.20[a]
HDL_3 cholesterol	0.66 ± 0.15	0.61 ± 0.10	0.56 ± 0.13
Lipoprotein (a) (g/L)	0.13 ± 0.11	0.12 ± 0.11	0.15 ± 0.12[b]
Apoprotein A-I	1.09 ± 0.26	1.07 ± 0.29	1.09 ± 0.26
Apoprotein B	0.95 ± 0.17	0.97 ± 0.19	1.07 ± 0.14[a]
LDL apoprotein B	0.68 ± 0.16	0.72 ± 0.17	0.88 ± 0.12[a]

[a] $P < 0.02$ versus placebo.
[b] $P < 0.05$ versus placebo.
FA, fatty acid.

Table 2 Fatty acid content of test oils (mg/g)*

	Fatty acid	mg/g
Olive oil placebo		
	16:0	106
	18:0	29
	18:1 ω9	667
	18:1 ω7	18
	18:2 ω6	60
ω3 FA concentrate		
	16:3 ω4	45
	16:4 ω1	44
	18:4 ω3	90
	20:4 ω6	16
	20:5 ω3	411
	21:5 ω3	15
	22:5 ω3	14
	22:6 ω3	229

*All fatty acids making up at least 1% of total fatty acids.
FA, fatty acid.

Lipoprotein oxidation susceptibility

This method[8] utilized a 500 μL plasma sample to which was added 50 μL of a solution containing 0.2 mM dextran sulfate mol. wt. 50,000, Genzyme, Cambridge, MA USA) and 0.5 M $MgCl_2 \cdot 6H_2O$ to precipitate the apo B-containing lipoproteins (LDL and very low density lipoprotein (VLDL)) according to Bachorik and Albers.[9] After centrifugation at 3,000 rpm at 20° C for 10 minutes, the supernatant was removed, and 1 mL of 6% bovine serum albumin (BSA) and another 50 μL of the dextran sulfate-magnesium solution was added. The solution was vortexed and recentrifuged as above to wash away any residual HDL. The supernatant was again removed and the remaining precipitate (containing LDL and VLDL) was dissolved in 2.5 mL of 4% NaCl. A volume of redissolved precipitate containing 100 mg of non-HDL cholesterol was combined with sufficient 4% NaCl to give a total volume of 500 μL. Fifty μL of a 0.5 mM $CuCl_2 \cdot 2H_2O$ solution was added (final copper concentration was 46 μM), and the samples were incubated at 37° C in a shaking water bath for 3 hours. Next, the thiobarbituric acid reactive substances (TBARS) generated were measured according to Buege and Aust[10] by adding two mL of the TBARS reagent (26 mM TBA and 0.92 M trichloroacetic acid in 0.25 N HCl) to each tube. The mixture was placed in a boiling water bath for 15 minutes. After remov-

ing and cooling the tubes, 2.5 mL n-butanol was added, the tubes were vortexed, and centrifuged for 15 minutes at 3,000 rpm at room temperature. The pink upper layer was removed and its optical density determined in a spectrophotometer at 532 nm. A standard curve was constructed with 1,1,3,3-tetraethoxypropane (0.5–16 nmol/mL), and the results were expressed as nmol of malondialdehyde (MDA) produced per mg of non-HDL cholesterol. The coefficient of variation of the method was 4% intraassay and 9% interassay. Recent modification in the method include the use of serum instead of plasma and removing albumin from the precipitate washing solution.

Plasma phospholipid fatty acids

Plasma lipids were extracted from 0.5 mL of plasma by addition of 2.5 mL methanol, 5 mL of methylene chloride, and 7.5 mL of saline.[11] After vortexing, the mixture was centrifuged to separate the layers, and the bottom fraction containing the lipids was recovered. The latter was dried under nitrogen and applied to a silica gel G plate, which was developed in hexane:ethyl ether:formic acid (70:30:1). The origin containing the phospholipids was scraped off and methylated by heating for 10 minutes at 100° C in 1 mL of boron trifluoride/methanol according to Morrison and Smith.[12] The FA methyl esters were extracted by addition of 1 mL of water and 1 mL of hexane, vortexing, and centrifuging to separate the layers. The hexane was removed, evaporated under nitrogen, and the lipids were re-dissolved in a small volume of hexane and injected into a GC9A Gas Chromatograph (Shimadzu Corp., Columbia, MD USA), equipped with a 30-m, 0.32-mm i.d., SP2330 capillary column. Nitrogen was used as a carrier gas with injector and detector temperatures of 225° C, and a temperature program that began after a 2-minute hold at 160° C, ramped up a 3° C/minute to 260° C, and held there for 1 minute.

Bleeding times

These were done with standard transverse cuts using the Simplate II method according to the manufacturer's instructions (Organon Tenika Corp, Durham, NC, USA).

Statistical analysis

When values were available from the beginning and the end of each treatment period, an analysis of variance with repeated measures with post-hoc evaluation by the Newman-Keuls test was done to assess the changes caused by the test oils. Otherwise, a paired t test was utilized, and $P < 0.05$ was required to assign statistical significance to a difference.

Results

Compliance, tolerability, body weight and background diets

All subjects tolerated the supplements well with no reported unpleasant side effects. The average number of capsules assigned to be taken per day was 8.8, compliance (by pill count) in the placebo period was 91%, and during the ω3 FA phase, 100%. These intakes provided about 5.7 g of ω3 FAs per day in the active treatment phase. There was no statistically significant change in body weight observed throughout the study (baseline, 88.1; placebo, 88.3; ω3 FA, 88.5 kg). Background diets remained stable during both periods with no significant change in saturated fat and cholesterol intakes detected (*Table 3*).

Lipids, lipoproteins, apolipoproteins

The placebo produced no statistically significant changes (versus pre-placebo baseline) for any parameter under investigation (*Table 1*). Therefore, values at the end of the placebo phase were compared with those at the end of the ω3 FA phase. The ω3 FA ethyl esters lowered plasma triglyceride levels by 37% ($P < 0.001$) and raised levels of LDL cholesterol by 23%, LDL apo B 22%, whole plasma apo B 10%, and HDL_2 cholesterol by 56% (all at least $P < 0.02$; *Table 1*). Although the change in Lp(a) levels from baseline to the end of the ω3 FA period was not statistically significant, levels did increase slightly but significantly versus the placebo period. There were no statistically significant changes in the other parameters.

Rate of change in lipoprotein and fatty acids

The time course of the change in plasma levels the analytes most affected (triglycerides and LDL cholesterol, and EPA and DHA) are given in *Figures 1–3*. The impact of the ω3 FA supplements was seen quickly, becoming nearly complete for triglycerides (*Figure 1*) and LDL cholesterol (*Figure 2*). The ω3 FAs caused significant increases (versus placebo) in plasma phospholipid EPA (0.6% to 6.3%) and DHA (1.9% to 4.5%) levels ($P < 0.001$ for both)(*Figure 3*). Plasma phospholipid ω3 FAs changed nearly as rapidly as the lipoproteins, with EPA levels rising rapidly from less than 1% to over 4% at day 4. DHA levels stabilized at day 8 at about 4%. Despite supplementation with about 6 g of oleic acid per day during the placebo phase, plasma phospholipid oleic acid levels did not change significantly versus baseline (*Table 4*). There was, however, a significant decrease (versus placebo) in oleic acid levels during the ω3 period (10.4 versus 8.9%, $P = 0.019$). Linoleic acid levels, which were stable during the placebo phase, decreased from 21.5 to 17.6% ($P =$

Table 3 Nutrient composition of background diets

Nutrient	Placebo period	ω3 FA period
Kcalories	2,486 ± 695	2,290 ± 748
Percent of kcalories as:		
Protein	17 ± 3	17 ± 4
Carbohydrate	48 ± 3	48 ± 5
Fat	36 ± 3	36 ± 5
Saturated FA	11 ± 2	12 ± 3
Monounsaturated FA	14 ± 2	13 ± 2
Polyunsaturated FA	8 ± 2	7 ± 2
Cholesterol (mg)	304 ± 120	317 ± 110
RISCC rating*	19 ± 3	21 ± 7

*RISCC, Ratio of Ingested Saturated fat and Cholesterol to Calories. Serves as a summary statistic describing the hyperlipidemic potential of the diet.[37]
FA, fatty acid.

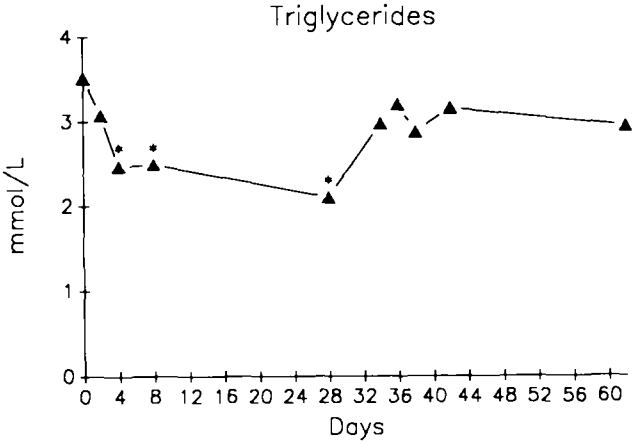

Figure 1 Time course of the change in plasma triglyceride levels in hypertriglyceridemic patients. Although six patients took the ω3 fatty acid (FA) supplement first, and four took it second, the results for all 10 are displayed here by placing all the ω3 FA data first, followed by the placebo data. The ω3 FA period was day 0 through day 28, and the placebo period from day 33 through 62. * $P < 0.02$ compared with day 0 values.

0.008) with ω3 FA supplementation. Arachidonic acid levels were remarkably stable during both phases with no significant changes noted (8.6 versus 7.9%, placebo versus ω3 FA).

Bleeding times

There was no effect on the ω3 FA supplement on Simplate bleeding times. Entering baseline, placebo, and ω3 FA values were 6.4 ± 1.7, 6.3 ± 1.6, and 6.1 ± 0.8 minutes, respectively.

Lipoprotein oxidation susceptibility

The susceptibility of apoB-containing lipoproteins to ex vivo, copper-induced oxidation was increased significant ($P < 0.05$) during the ω3 FA period compared with the placebo period. Entering baseline, placebo, and ω3 FA values for this parameter were 100 ± 11, 93 ± 14, and 157 ± 37 nmol MDA/mg non-HDL cholesterol.

Discussion

Compared with fish oils, ω3 FA ethyl esters are a relatively new and more concentrated form of marine FAs that have been used in only a limited number of studies.[13-16] This study was conducted with an average of less than nine capsules per day and produced similar effects to those seen with 18 capsules of MaxEPA.[13] Because we fed 5.7 g of ω3 FA in this trial and 6 g of EPA + DHA in the previous trial,[13] the finding of similar effects were not unexpected.

In addition to supporting the equivalency of ω3 FA ethyl esters and triglycerides, other principal findings of this trial were (1) ω3 FAs altered serum lipid and lipoprotein levels very rapidly (within 4–8 days) and washout occurred nearly as fast; (2) ω3 FAs increased HDL_2 cholesterol levels; (3) ω3 FAs increased the susceptibility of apoB-100–containing lipoproteins to ex vivo oxidation; and (4) ω3 FAs did not prolong bleeding times. The first point confirms a preliminary observation made in our laboratory[2] and suggests that future studies aimed at determining the mechanism of ω3 FA-induced alteration in lipids could be carried out with relatively short periods of intervention.

The changes seen here (increased LDL and HDL_2, decreased triglycerides) have been reported by others previously,[1,17-19] and the change in triglycerides appears to persist for as long as the patient consumes ω3 FAs.[20,21] The question of how these changes (some adverse, some beneficial) influence the atherosclerotic risk cannot be answered by studies such as this and must await clinical trials evaluating the effects of ω3 FAs on total and cardiac mortality. The recent observation by Burr et al.[22] that an increased intake of oily fish significantly reduced mortality in a group of myocardial infarction survivors by 30% suggests that whether ω3 FAs produce pro- or anti-ath-

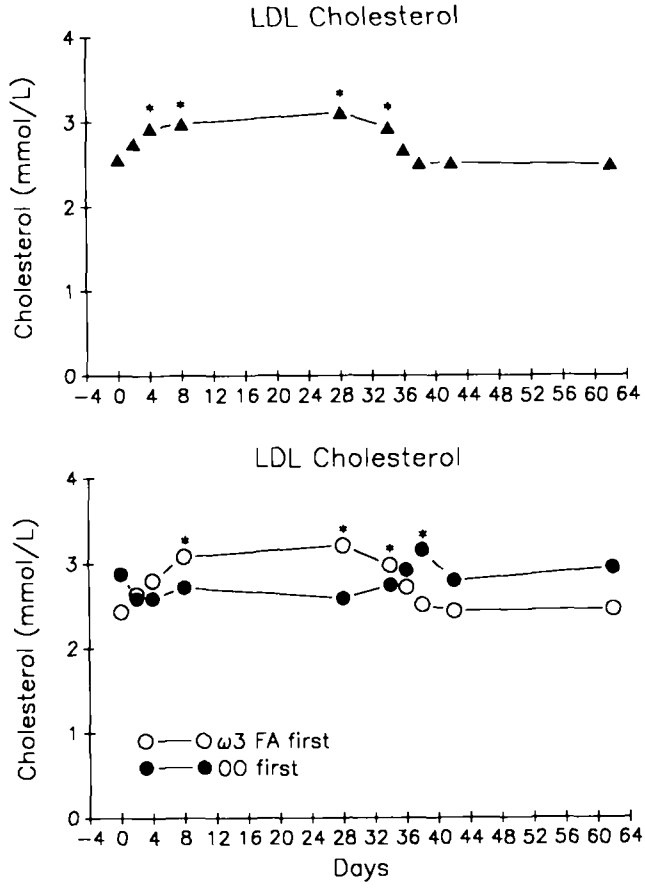

Figure 2 (Top) Time course of the change in plasma LDL cholesterol levels in hypertriglyceridemic patients. Although six patients took the ω3 fatty acid (FA) supplement first, and four took it second, the results for all 10 are displayed here by placing all the ω3 FA data first, followed by the placebo data. The ω3 FA period was from day 0 through day 28, and the placebo period from day 34 through 62. (Bottom) The changes in LDL cholesterol levels displayed in true sequences for the two groups (not combined as in the top graph). Asterisks note the time points at which the FA values were significantly different (at least $P < 0.05$) from their respective baseline values at day 0 or day 28.

erogenic changes in lipid metabolism, their overall effect on the disease process may be palliative. This conclusion may also be drawn from the many studies carried out in pigs and monkeys, which have, almost without exception, shown anti-atherogenic effects.[23]

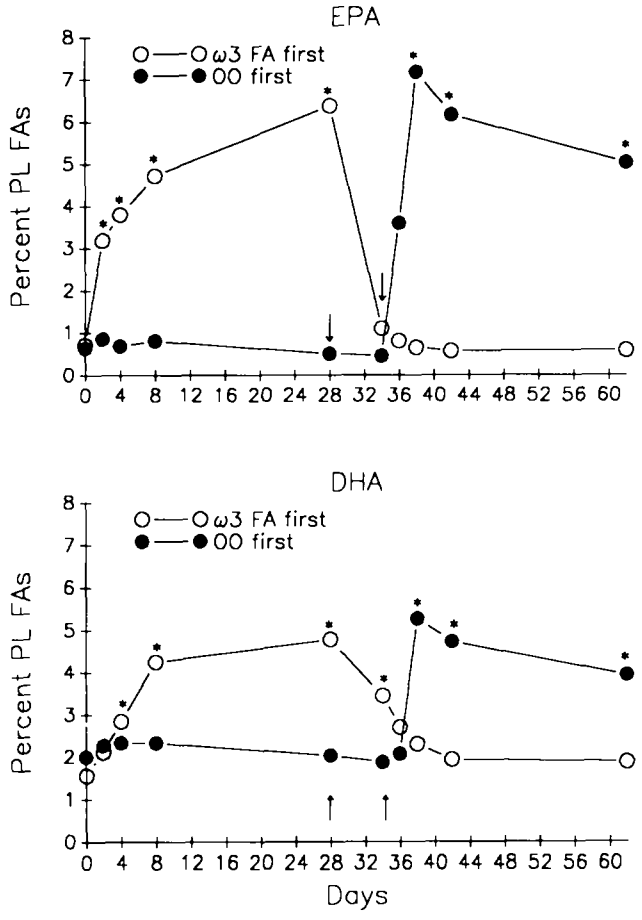

Figure 3 (Top) The changes in EPA levels (as a percentage of total phospholipid fatty acids displayed in true sequences with data from five patients who took ω3 FAs first and three who took the placebo olive oil (OO) first. Asterisks note the time points at which the fatty acid (FA) values were significantly different (at least $P < 0.05$) from their respective baseline values at day 0. The arrows note the end of the first phase (day 28) and the beginning of the next phase (day 34). (Bottom) The same as Top, but for DHA.

The same point must be invoked with respect to the enhanced susceptibility of lipoproteins to oxidation with ω3 FA feeding. This finding is not surprising in view of the highly polyunsaturated nature of ω3 FAs and their marked incorporation in plasma phospholipids. However, our findings here failed to confirm the report of Nenseter et al.,[24] who found that LDL oxidation was not accelerated by ω3 FA supplementation. Certainly the different methods used to assess oxidation susceptibility may have played a role in these discrepant findings, but the most likely explanation is that the control oil for their study was corn oil, a highly polyunsaturated oil. Accordingly, they found that fish oil and corn oil affected oxidation susceptibility equally. Whether these oils altered normal oxidation susceptibility was not evaluated in their study.

We used the TBARS assay to measure lipoprotein lipid peroxidative decomposition because of its ease, its long history of usefulness in this area, and because in 1989 (when this study was carried out), many of the newer methods for assaying lipoprotein oxidation had not yet been published. Although the TBARS method is not as specific or direct as other methods currently available, it has been shown to correlate strongly with macrophage LDL uptake, lipid peroxide generation, and altered LDL electrophoresis on agarose in studies with antioxidants.[25–27] However, in one study in which the FA composition of LDL was modified by feeding mono- or polyunsaturated FAs, the TBARS method gave discordant results to the conjugated diene or macrophage uptake data.[28] Because the TBARS assay as conducted here appears to measure degradation products from polyunsaturated FAs containing three or more double bonds (primarily arachidonic acid),[10] the increases in EPA and DHA in the plasma lipoproteins would be expected to stimulate TBARS generation, as we observed.

Steinberg and colleagues[29] have provided compelling evidence that under normal conditions lipoprotein oxidation can play a role in the initiation and propagation of the atherosclerotic lesion. However, it does not necessarily follow that any agent enhancing ex vivo oxidation will be atherogenic. Again, the observations that in both animals and humans, fish oil has beneficial effects on cardiovascular disease suggests that the overall in vivo impact may be antiatherogenic despite their tendency to increase ex vivo oxidative susceptibility.

Table 4 Plasma phospholipid fatty acid compositions after placebo and ω3 FA supplementation (percent of total FAs)

	Placebo		ω3 FA	
	Pre	Post	Pre	Post
Oleic acid	9.7 ± 1.9	10.4 ± 2.3	11.6 ± 1.5	8.9 ± 2.0*
Linoleic acid	21.6 ± 4.3	21.5 ± 1.7	21.5 ± 2.9	17.6 ± 2.8*
Arachidonic acid	8.4 ± 2.0	8.6 ± 1.5	8.5 ± 2.3	7.9 ± 2.5
Eicosapentaenoic acid	0.9 ± 0.4	0.6 ± 0.2	0.6 ± 0.4	6.3 ± 3.2*
Docosahexaenoic acid	2.9 ± 1.0	1.9 ± 0.5	1.7 ± 0.6	4.5 ± 1.1*

*$P < 0.02$ versus post-placebo value.
FA, fatty acid.

Olive oil ethyl esters were chosen as a placebo for this study primarily because they are in the same chemical state at the ω3 FAs, and because they are rich in oleic acid, a relatively neutral FA easily synthesized in the body. During the placebo period, our subjects took an additional 6 g of oleic acid per day from the capsules. Their diets already contained about 39 grams of oleic acid, making a total intake of 45 g/day. In one study that reported an increased resistance of LDL to oxidation with a high oleic acid diet,[28] intake of oleate on the "high-mono" diet was 93 g/day; on the "low-mono" diet, it was 29 g/day. Linoleate (the more easily oxidized diunsaturated FA) intakes were 15 and 75 g/day. They found about a 40% reduction in oxidation susceptibility with this extremely large difference in FAs. Thus, going from 39 to 45 g of oleate per day would hardly be expected to have any impact on LDL oxidizability. Our data support the appropriateness of olive oil as a placebo because no baseline lipid parameter differed significantly at the end of the placebo period, including lipoprotein oxidation susceptibility, which decreased by only 7% (NS).

In this study, there were six patients assigned to the ω3 FA phase first. Comparison of values from the pre-ω3 (entering) baseline and pre-placebo baseline (the latter being drawn 5–6 days after finishing the ω3 FA phase) revealed the extent of a carry over and how rapidly lipid levels returned to normal after stopping ω3 FAs. Triglyceride levels recovered very rapidly, with mean pre-placebo values being nearly identical to the pre-ω3 FA values (3.33 versus 3.24 mmol/L) (*Figure 1*). LDL cholesterol levels, however, did not return to normal as rapidly and remained significantly elevated at the 6-day-post-ω3 FA point for these six subjects (2.44 versus 2.97 mmol/L, $P = 0.016$). This phenomenon is illustrated in *Figure 2* (top) where the pre-placebo LDL levels (day 34 in *Figure 2*) were higher than at the pre-ω3 FA values (day 8). It is also clear from *Figure 2* that after 5 days of placebo treatment (about 10 days post-ω3 FAs, day 38), LDL levels had returned to normal.

Plasma phospholipid EPA levels behaved like triglycerides in that they returned rapidly to normal after ω3 FA supplementation stopped (*Figure 3*). DHA levels responded more like LDL cholesterol, taking a few days longer to return to baseline (*Figure 3*). We conclude from these observations that it takes about 5 days for EPA and 10 days for DHA in plasma phospholipids to normal after 1 month of 5 g of ω3 FA/day.

We found no effect of the ω3 FA supplement on bleeding times. Although several past studies using larger amounts of fish oil found a statistically significant although minor increase in bleeding times,[30] more recent investigations found using fish oil supplements to have minimal effect on this parameter. For example, in a recent dose-response study with hyperlipidemic patients, neither the 4.5 nor the 7.5 g/day dose of ω3 FAs altered bleeding times.[31] No significant change in bleeding times was observed in 10 hypertriglyceridemic patients given 18 g of MaxEPA/day for 6 weeks,[32] nor were bleeding times changed in 14 hypercholesterolemic patients taking 5–6 of ω3 FA ethyl esters daily for 6 weeks.[33] It should be noted here that the value of the bleeding time test has recently been questioned by Rodgers,[34] who found it to be a very poor predictor of abnormal bleeding tendencies in humans. Indeed, feeding ω3 FA to patients undergoing coronary artery bypass grafting resulted in increased bleeding times but no increase in blood loss at surgery.[35] If ω3 FAs alter hemostatic pathways,[36] their effect appears to be either subtle or difficult to measure. To what extent effects on eicosanoid-mediated processes play a role in the previously reported antiatherosclerotic effects of ω3 FAs is not clear.

In conclusion, ω3 FA ethyl esters (5 g/day) impacted lipid-related risk factors for coronary heart disease in a variety of ways in this group of hypertriglyceridemic patients. Potentially adverse effects were the increases in LDL cholesterol levels and the susceptibility of lipoproteins to ex vivo oxidation. Potentially beneficial effects were the increases in HDL_2 cholesterol and decreases in triglyceride levels. How these balance out is unknown. Because earlier reported increases in bleeding times have often been cited as adverse effects of ω3 FAs, the lack of any change in this parameter here may be considered positive. Finally, care must be taken to not interpret the results of interventional studies only in the light of epidemiological findings. Using an intervention to change an epidemiologically defined risk factor does not necessarily change true risk for the disease in question. Only prospective studies with clinical endpoints can answer the question of how an intervention alters the disease process itself.

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