Short-term effects of fish oil on human plasma lipid levels

Mario I. Sztern and William S. Harris

The Lipid and Arteriosclerosis Prevention Clinic, Division of Clinical Pharmacology, Department of Medicine, The University of Kansas Medical Center, Kansas City, Kansas, USA

Fish oils rich in eicosopentaenoic acid (EPA) are potent hypotriglyceridemic agents in both normal subjects and in hypertriglyceridemic patients. However, the rapidity with which fish oil exerts this effect has not been studied systematically, nor has the temporal relationship between decreasing triglyceride levels and increasing EPA and low density lipoprotein cholesterol (LDL-C) levels been defined. To examine these interactions, we recruited 9 normal subjects who were given a single dose of fish oil (0.25 g/kg) and 5 other subjects who were given a single dose of a safflower oil placebo. Fasting plasma triglyceride levels were measured at baseline and at 24, 48, and 72 hr post-dosing. In a second study, a group of 5 hypertriglyceridemic patients were given 12 capsules of fish oil/day for 14 days. Plasma triglyceride and LDL-C levels were measured at baseline and every 2-3 days thereafter, including a 10-day washout period. In the single dose study, triglyceride levels decreased by 15% at 24 hr and 16% at 48hr ($P < 0.001$ both), and began to normalize at 72 hr. Triglyceride levels in the placebo group did not change significantly. Plasma EPA levels increased 4-fold at 24 hr, and then began to return towards baseline. In the multiple dose study, fish oil began lowering triglyceride levels by day 1, and continued doing so through day 14 (7.97 ± 3.06 to 4.57 ± 1.58, $P < 0.01$). LDL-C levels began rising immediately from 2.51 ± 0.87 mM/L at baseline to a plateau of 3.65 ± 0.59 ($P < 0.01$) after only 4 days. Both triglyceride and LDL-C levels returned toward normal in the washout period. We conclude that fish oil treatment altered plasma EPA levels very quickly, and produced measurable decreases in triglyceride levels which were preceded by increases in LDL-C levels.

Keywords: fish oil; plasma lipids; cholesterol; triglycerides; low density lipoproteins; eicosapentaenoic acid

Introduction

Fish oils rich in the n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are particularly effective in the treatment of hypertriglyceridemia.[1] Unfortunately, the exact mechanisms by which they affect plasma lipid and lipoprotein metabolism are still unclear. There is evidence that the hypotriglyceridemic effect may be transient,[2] and that potentially adverse effects on low density lipoprotein (LDL) cholesterol (C) may accompany the triglyceride lowering effect.[3,4] In spite of these uncertainties, evidence for a cardioprotective effect of n-3 fatty acids continues to accumulate.[5,6]

In the vast majority of published studies involving fish oil, the acute changes (those occurring within days) in plasma lipid levels have not been reported, and thus, the rate at which triglyceride and LDL-C levels change are unknown. Likewise, how these parameters correlate with changes in serum levels of EPA in the short term is unexplored. In this paper, we report the results of two separate studies, which, together, address the following three questions: (1) What is the effect of a single dose of fish oil on plasma triglyceride and EPA levels? (2) How does a 2-week regime of fish oil supplementation affect plasma triglyceride, LDL-C and HDL-C and EPA levels? (3) How rapidly do these lipids return to normal when fish oil is stopped? Understanding these relationships may help to clarify the mechanisms by which n-3 fatty acids impact human lipid metabolism.

Address reprint requests to Dr. William S. Harris, 3800 Cambridge Street, The University of Kansas Medical Center, Kansas City, KS 66103, USA.
The fish oil supplements were provided by Steve Blechman of Twin Labs, Inc., Ronkonkoma, NY, USA.
This study was supported by a grant from the NHLBI (HL40832). Dr. Mario I. Sztern was a recipient of an American Physician Fellowship grant.
Received May 30, 1990; accepted November 16, 1990.

Table 1 Principal fatty acids of the fish oil preparation*

Fatty Acid	Percent
14:0	8.0
16:0	16.8
18:0	2.2
16:1	8.4
18:1	8.6
18:2 n-6	1.3
18:3 n-3	1.0
18:4 n-3	3.3
20:5 n-3	22.6
22:5 n-3	2.1
22:6 n-3	11.3

* Twin Labs, Inc., Ronkonkoma, NY, USA.

Materials and methods

Single dose study

Fourteen normolipidemic volunteers (mean age 33) were divided randomly into two groups. The first group (5 females, 4 males) received 10–20 capsules of fish oil (1 capsule/4 kg body weight). Each 1-gram capsule (Dale Alexander's Omega-3 Oil, Twin Labs, Inc., Ronkonkoma, NY, USA) contained 198 mg of EPA plus DHA. The dose of n-3 FAs was about 50 mg/kg. The second group (3 females, 2 males) received the same number of placebo capsules containing safflower oil. The entire dose was consumed after an overnight fast as a bolus (within 5 minutes) before the subject's normal breakfast. Blood was drawn at baseline, 24, 48, and 72 hr post-dosing, always in the fasting state.

Multiple dose study

Five patients with primary, isolated hypertriglyceridemia (Type IV) were recruited (2 males, 3 females). Their mean age was 53; two were overweight, but none were obese. All patients were newly diagnosed, were not consuming low-fat diets or taking drugs known to affect lipid metabolism. Secondary hypertriglyceridemias were ruled out. Hypertriglyceridemic patients were selected for this study because one of our goals was to study the effects of fish oil on LDL-C levels, and these only change in patients fed fish oil, not in normals. The patients were instructed not to change their usual home diets throughout the study. A fixed dose of fish oil (12 capsules/day providing 2.4 g n-3 fatty acids) was given for 14 days. This intake was chosen because it was felt to represent a clinically feasible dose. Dosing was stopped after 14 days, and the rate at which blood lipids, EPA, and DHA returned towards normal was followed over the subsequent 10 days. (Unfortunately, a longer washout period was not logistically feasible with these pateints.) Blood for lipid profiles and plasma phospholipids fatty acid levels was drawn at baseline, then daily for the first 4 days, and then twice a week for the next 3 weeks. Informed consent was obtained from all participants, and the protocol was approved by the Human Subjects' Committee at the University of Kansas Medical Center.

Lipid analysis

Blood was drawn with minimal stasis into tubes containing 1 mg EDTA/ml and centrifuged at 1500*g* for 10 min to obtain plasma. The plasma phospholipid fatty acids (FAs) were determined as follows: The plasma lipids were extracted with chloroform:methanol (2:1), and the phospholipids were isolated by thin-layer chromatography on silica gel G with hexane:diethyl ether:chloroform:acetic acid (80:10:10:1, vol:vol). The phospholipid FAs were methylated with boron trifluoride and analyzed by gas chromatography (GC-9A, Shimadzu Instrument Company, Columbia, MD, USA) with a 30-m, 0.32mm-id, SP-2330 capillary column (Supelco, Inc., Bellefonte, PA, USA). The oven was set at 175° C for 2 min, then increased at 5° C/min to 210° C; the detector and injector were set at 250° C. The carrier gas was N_2. FAs are reported as percent of total FA methyl esters. The plasma was analyzed for cholesterol,[7] and triglycerides[8] by enzymatic methods on an ABA 200 bichromatic analyzer (Abbott Diagnostics, Irving, TX, USA). The plasma HDL-C levels were measured after the apolipoprotein B containing lipoproteins were precipitated with a mixture of heparin and manganese chloride.[9] The VLDL fraction ($d < 1.006$) was removed by aspiration after spinning 0.175 mL of plasma at 100,000 rpm for 1 hour in a Beckman TL-100 ultracentrifuge using a TLA-100 rotor.[10] LDL-C was determined by subtracting HDL-C from the cholesterol content of the $d > 1.006$ fraction.

Statistics

All the data are expressed as means ± SD. ANOVA with repeated measures and multiple comparisons was used. Differences were assessed by Duncan's test. A P value of <0.05 was considered significant.

Results

Single dose study

Triglycerides showed a significant decrease from 1.09 mmol/L to 0.91 mM/L (15%, $P < 0.01$) as early as 24 hr after the single dose of fish oil (*Figure 1*). Eight of the nine subjects showed this decrease at day 1. At 48 hr triglyceride levels were still significantly reduced (0.90 mmol/L; 16% below baseline; $P < 0.01$), but at 72 hr they had largely normalized, rising to 0.96 mmol/L (N.S.). The plasma triglyceride levels in the placebo group did not change significantly (*Figure 1*). EPA levels in plasma phospholipids followed the opposite course, rising rapidly within 24 hr to 440% of baseline, but thereafter decreasing to 347% of baseline by 48 hr and 247% at 72 hr (*Figure 2*). All of these changes from baseline were significant ($P < 0.01$), and all subjects experienced an increase in EPA levels. Mean DHA levels increased from 2.0% at baseline to 2.4% at 24

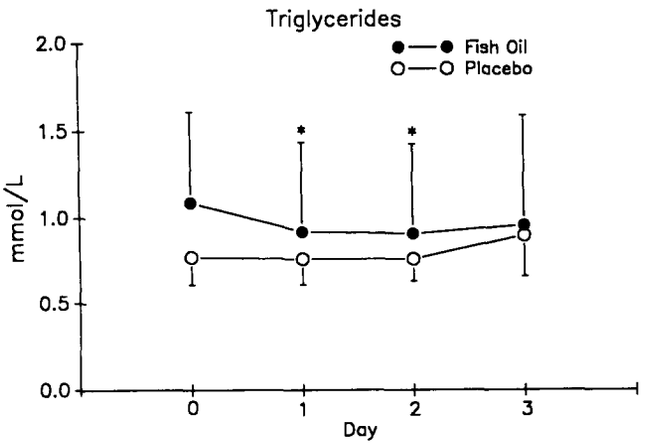

Figure 1 Acute effects of a single dose of fish oil (50 mg n-3 FAs/kg body wt) given to 9 normolipidemic volunteers. The decreases in triglycerides were significant at all 3 days post-dosage ($P < 0.01$), but by day 3, triglyceride levels were not significantly different from baseline. Placebo-treated subjects ($n = 5$) showed no changes in plasma triglyceride levels.

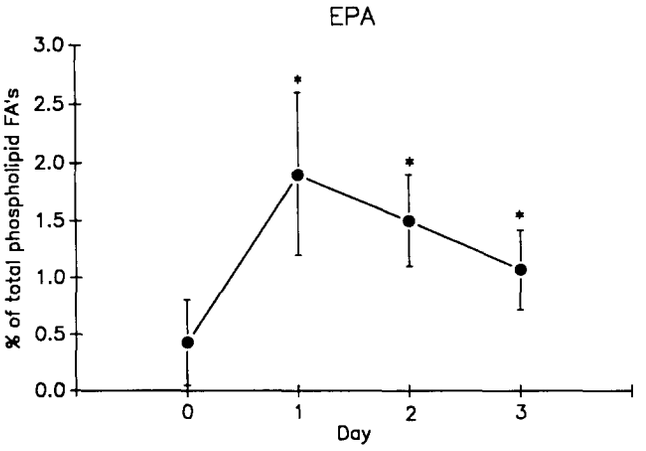

Figure 2 Acute effects of a single dose of fish oil (50 mg n-3 FAs/kg body wt) given to 9 normolipidemic volunteers. The increases in EPA were significant at all 3 days post-dosage ($P < 0.01$).

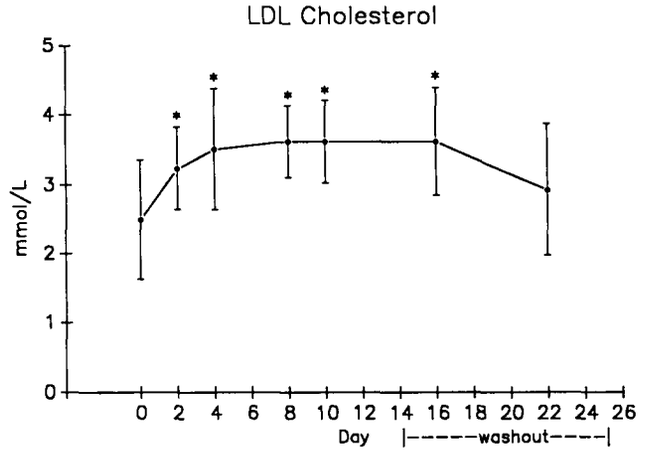

Figure 3 The short-term effects of fish oil supplements on plasma levels of LDL cholesterol. Hypertriglyceridemic patients ($n = 5$) were given 2.4 g of n-3 fatty acids per day between days 0 and 14 at which time supplementation was stopped. Values given as means ± SD. * = $p < 0.01$ versus day 0.

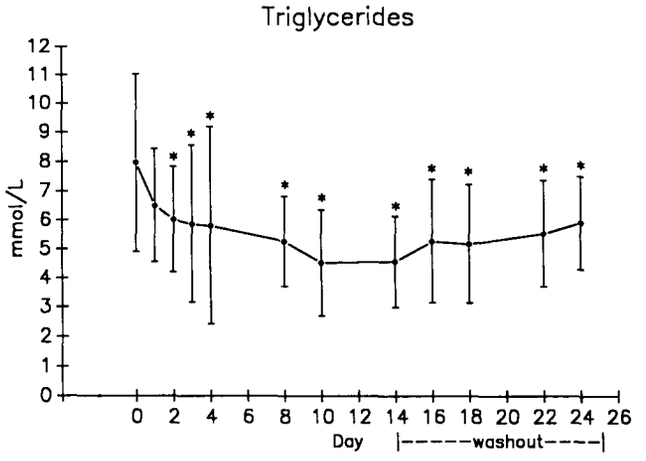

Figure 4 The short-term effects of fish oil supplements on plasma levels of triglycerides. Hypertriglyceridemic patients ($n = 5$) were given 2.4 g of n-3 fatty acids per day between days 0 and 14 at which time supplementation was stopped. Values given as means ± SD. * = $P < 0.01$ versus day 0.

hr (NS), and to 2.8% at both 48 and 72 hr ($P < 0.01$ versus baseline). These changes were less marked than those seen with EPA.

Multiple dose study

In the patient group, the changes in plasma levels of triglycerides, LDL-C, HDL-C, and phospholipid EPA and DHA were followed. Mean LDL-C levels at baseline were low (2.51 mmol/L) as is typical of type IV patients. After 48 hr, they had increased to 3.24 mmol/L (+29%, $P < 0.01$), with 4/5 of the patients increasing. LDL-C levels reached a plateau of 3.65 mmol/L (44% increase, $P < 0.01$) after 4 days which continued through day 16. LDL-C levels began to drop in the washout period, and reached 2.95 mmol/L after ten days of no treatment (N.S. versus baseline, *Figure 3*).

Mean triglyceride levels decreased from 7.97 mmol/L at baseline to 6.49 mmol/L at 24 hr (N.S.) and 6.02 mmol/L at 48 hr ($P < 0.01$). Triglyceride levels dropped in every patient by day 2. This statistically significant decrease continued through day 14 when values of 4.57 mmol/L were reached (43% below baseline, $P < 0.01$). Triglyceride levels appeared to be stabilizing after two weeks of treatment. During the 10-day washout period, triglyceride levels returned slowly towards baseline, but at day 24, remained significantly below baseline (5.91 mmol/L, $P < 0.01$, *Figure 4*). Mean HDL-C levels were 1.30, 1.37, 1.37, 1.30, 1.24, and 1.22 mmol/L on days 0, 2, 4, 14, 18, and 24, respectively. None of these changes was statistically significant.

EPA levels in plasma phospholipids increased consistently (from 0.7 to 5.1%, $P < 0.01$) during the

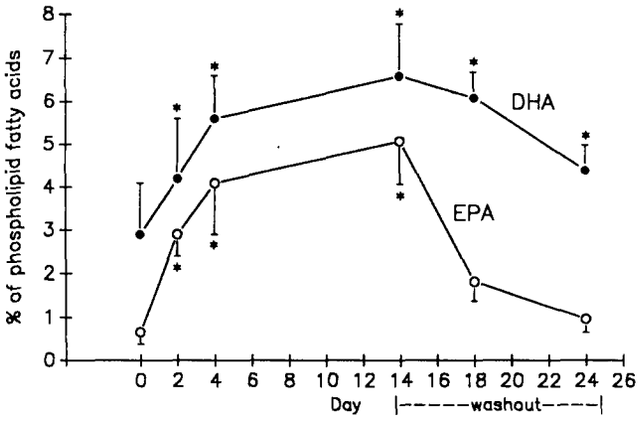

Figure 5 The short-term effects of fish oil supplements on plasma levels of phospholipid EPA and DHA levels. Hypertriglyceridemic patients (n = 5) were given 2.4 g of n-3 fatty acids per day between days 0 and 14 at which time supplementation was stopped. Values given as means ± SD. * = $P < 0.01$ versus day 0.

2-week treatment phase, and returned to baseline levels by the end of the washout period (1.0%, N.S. versus baseline; *Figure 5*). DHA levels similarly increased (from 2.9% at baseline to 6.6% at 14 days, $P < 0.01$), but unlike EPA did not return to baseline after a 10-day washout (*Figure 5*). EPA and DHA levels rose in all patients by day 2.

Discussion

The purpose of these studies was to determine how fish oil supplementation altered plasma lipid levels in the short-term, after both a single dose and multiple doses. In the single dose study, fish oil produced statistically significant decreases in plasma triglyceride and increased phospholipid EPA levels within 24 hr. DHA levels were clearly increased at 48 hr and remained elevated at 72 hr unlike EPA which had returned to baseline by this time. The changes in triglyceride levels persisted longer than the EPA, but not the DHA, changes. In the multiple dose study conducted in hypertriglyceridemic patients, fish oil had an immediate impact on EPA, triglyceride, and LDL-C levels. EPA levels increased by 700% and DHA 228% by day 14, at which time triglyceride levels had fallen by 43%. All of these parameters appeared to be stabilizing by 2 weeks. Perhaps the most interesting observation was the rapid change in LDL-C levels. Fish oil supplementation produced a significant increase in LDL-C by day 2 which had stabilized by day 4. Thus, long before n-3 FA and triglyceride levels had stabilized, LDL-C levels had risen markedly (by 44%) and appeared to be in a steady state. During the 10-day washout phase, triglyceride levels remained significantly depressed; however, LDL-C and EPA levels had returned nearly to baseline levels. Thus, LDL-C appeared to follow plasma n-3 FA levels more closely than did triglyceride levels. It would have been instructive to have followed triglyceride levels over a longer washout period, but we were unable to do so in this trial.

In a previous study, Harris et al.[11] examined the effect of fish oils on triglyceride levels in subjects with carbohydrate-induced hypertriglyceridemia. Fish oil blocked the development of the hypertriglyceridemia when fish oil and the high carbohydrate diet were initiated at the same time. Fish oil also reversed the hypertriglyceridemia when it was added after a week of carbohydrate induction. Pertinent to this discussion was the observation that it took only 72 hr for fish oil to reverse already elevated triglyceride levels, but it took 10–12 days for triglyceride levels to rise when fish oil was removed from the high carbohydrate diet. These observations support those made in the current study and suggest that tissue (perhaps hepatic) and not plasma EPA levels control plasma triglyceride levels.

It was anticipated that triglyceride levels would decrease due to reduced hepatic VLDL secretion since there is considerable evidence from cell culture,[12-14] hepatic perfusion,[15] and human kinetic studies[16-18] that n-3 fatty acids inhibit hepatic triglyceride synthesis. What was remarkable was the rapidity and extent of the effect arising from as little as one dose of fish oil.

The short-term effects of chronic dosing with fish oil on triglyceride levels were much the same as with the single dose. Stable triglyceride levels were reached between 10 and 14 days of therapy. During the washout phase, EPA levels in the plasma phospholipids returned to baseline well before triglyceride levels normalized, again suggesting that tissue EPA levels may be responsible for the hypotriglyceridemic effect, not plasma.

Endres et al.[19] recently reported that as long as 10 weeks after stopping fish oil supplementation, cytokine production by human mononuclear cells continued to be inhibited, and that production returned to normal only after 20 weeks had elapsed. This is clear evidence that, even if EPA levels in plasma return to baseline, there may be lingering, cellular effects of these fatty acids.

The rapid change in LDL cholesterol levels could have resulted from a variety of factors. Direct inhibition of LDL receptor activity, a change in the affinity LDL apolipoprotein B for its receptor, increased LDL synthesis from VLDL precursors, or direct production of LDL are all possibilities. Which mechanism was responsible cannot be determined from this experiment. There are data from cell culture studies suggesting that EPA can down-regulate the LDL receptor.[20] However, fish oil feeding in normotriglyceridemic subjects does not usually raise LDL cholesterol levels.[1] If EPA had a direct effect on cellular receptors, one would expect to see a rise in LDL in all subjects. Since hypertriglyceridemia appears to be a pre-requisite for LDL rising with fish oil treatment, it is more likely that changes in VLDL composition (and therefore, metabolism) are responsible for the increasing LDL levels in patients given EPA.

It is well known that hypertriglyceridemic VLDL is metabolically different than normal VLDL.[21] The former can compete with LDL for receptor-mediated removal,[22] can be toxic to endothelial cells,[23] and can

convert macrophages into foam cells.[24] Normal VLDL does not have these effects. In addition, if fish oil causes the liver to secrete smaller, denser VLDL particles, their conversion into LDL may be accelerated,[25] leading to higher plasma LDL levels. An enhancement of lipoprotein lipase activity does not appear to play a role in this phenomenon,[1,26] but further studies are needed to evaluate this possibility more carefully. The factors which influence LDL levels, and the effects of fish oils on lipoprotein metabolism are both complex, and further studies will be needed to clarify why LDL levels rise in hypertriglyceridemic patients given fish oil.

Acknowledgments

The authors are grateful to Beverly Fehner, M.T., and Sheryl Windsor, M.T., for excellent technical assistance, to Sherry Severson and Elaine Schwensen for preparation of the manuscript, and Carlos A. Dujovne M.D., for his continuing support.

References

1 Harris, W.S. (1989). Fish oils and plasma lipid and lipoprotein metabolslim in humans: a critical review. *J. Lipid Res.* **30,** 785–807

2 Schectman, G., Kaul, S., Cherayil, G.D., et al. (1989). Can the hypotriglyceridemic effect of fish oil concentrate be sustained? *Ann. Int. Med.* **110,** 346–352

3 Harris, W.S., Dujovne, C.A., Zucker, M.L., et al. (1988). Effects of a low saturated fat, low cholesterol fish oil supplement in hypertriglyceridemic patients. *Ann. Int. Med.* **109,** 465–470

4 Sullivan, D.R., Sanders, T.A.B., Trayner, I.M., et al. (1986). Paradoxical elevation of LDL apoprotein B levels in hypertriglyceridaemic patients and normal subjects ingesting fish oil. *Atherosclerosis* **61,** 129–134

5 Burr, M.L., Gilbert, J.F., Holliday, R.M., et al. (1989). Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). *Lancet* **1,** 757–761

6 Leaf, A. and Weber, P.C. (1988). Cardiovascular effects of n-3 fatty acids. *N. Engl. J. Med.* **318,** 549–557

7 Allain, C.C., Poon, L.S., Chan, C.S.G., et al. (1974). Enzymatic determination of total serum cholesterol. *Clin. Chem.* **20,** 470–475

8 Bucolo, D. and David H. (1973). Quantitative determination of serum triglycerides by the use of enzymes. *Clin. Chem.* **19,** 476–482

9 Warnick, G.R. and Albers, J.J. (1978). A comprehensive evaluation of the heparin-manganese precipitation procedure for estimating high density lipoprotein cholesterol. *J. Lipid Res.* **19,** 65–76

10 Bronzert, T.J. and Brewer, H.D., Jr. (1977). New micromethod for measuring cholesterol in plasma lipoprotein fractions. *Clin. Chem.* **23,** 2089–2098

11 Harris, W.S., Connor, W.E., Inkeles, B., et al. (1984). Omega-3 fatty acids prevent carbohydrate-induced hypertriglyceridemia. *Metabolism* **33,** 1016–1019

12 Wong, S., Reardon, M., and Nestel, P. (1985). Reduced triglyceride formation from long-chain polyenoic fatty acids in rat hepatocytes. *Metabolism* **34,** 900–905

13 Strum-Odin, R., Adkins-Finke, B., Blake, W.L., et al. (1987). Modification of fatty acid composition of membrane phospholipid in hepatocyte monolayer with n-3, n-6 and n-9 fatty acids and its relationship to triacylglycerol production. *Biochim. Biophys. Acta.* **921,** 378–391

14 Nossen, J.O., Rustan, A.C., Gloppestad, S.H., et al. (1986). Eicosapentanoic acid inhibits synthesis and secretion of triacylglycerols by cultured rat hepatocytes. *Biochim. Biophys. Acta.* **879,** 56–65

15 Wong, S.H., Nestel, P.J., Trimble, R.P., et al. (1984). The adaptive effects of dietary fish and safflower oil on lipid and lipoprotein metabolsim in perfused rat liver. *Biochim. Biophys. Acta.* **792,** 103–109

16 Nestel, P.J., Connor, W.E., Reardon, M.R., et al. (1984). Suppression by diets rich in fish oil of very low density lipoprotein production in man. *J. Clin. Invest.* **74,** 72–89

17 Sanders, T.A.B., Sullivan, D.R., Reeve, J., et al. (1985). Triglyceride-lowering effect of marine polyunsaturates in patients with hypertriglyceridemia. *Arteriosclerosis* **5,** 459–465

18 Harris, W.S., Connor, W.E., Illingworth, O.R., et al. Effects of Fish Oil on VLDL triglyceride kinetics in man. *J. Lipid Res.* (in press).

19 Endres, S., Ghorbani, R., Kelley, V., et al. (1989). The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *N. Eng. J. Med.* **320,** 265–271

20 Wong, S. and Nestel, P.J. (1987). Eicosapentaenoic acid inhibits the secretion of triacylglycerol and of apoprotein B and the binding of LDL in Hep G2 cells. *Atherosclerosis* **64,** 139–146

21 Gianturco, S.H., Packard, C.J., Shepherd, J., et al. (1980). Abnormal suppression of HMG-Co.A reductase activity in cultured human fibroblasts by hypertriglyceridemic VLDL subclasses. *Lipids* **15,** 456–463

22 Gianturco, S.H., Brown, F.B., Gotto, A.M., et al. (1982). Receptor-mediated uptake of hypertriglyceridemic VLDL by normal human fibroblasts. *J. Lipid Res.* **23,** 984–993

23 Gianturco, S.H., Eskin, S.G., Navarro, L.T., et al. (1980). Abnormal effects of hypertriacylglycerolemic VLDL on HMG-CoA reductase activity and viability of cultured bovine aortic endothelial cells. *Biochim. Biophys. Acta.* **618,** 143–152

24 Gianturco, S.H., Brown, S.A., Via, D.P., et al. (1986). The beta-VLDL receptor pathway of murine P338D_1 macrophages. *J. Lipid Res.* **27,** 412–420

25 Packard, C.J., Munro, A., Lorimer, A.R., et al. (1984). Metabolism of apolipoprotein B in large, triglyceride-rich very low density lipoproteins of normal and hypertriglyceridemic subjects. *J. Clin. Invest.* **74,** 2178–2192

26 Harris, W.S., Connor, W.E., Alam, N., and Illingworth, D.R. (1988). Reduction of postprandial triglyceridemia in humans by dietary n-3 fatty acids. *J. Lipid Res.* **49,** 1451–1460