Effect of n−3 fatty acids on the composition and binding properties of lipoproteins in hypertriglyceridemic patients[1–3]

Hsiu-Ching Hsu, Yuan-Teh Lee, and Ming-Fong Chen

ABSTRACT

Background: Treatment of hyperlipidemic patients with fish oil results in an increase in plasma LDL cholesterol despite a marked decrease in the LDL precursor, VLDL.

Objective: We studied the relation between VLDL composition and LDL concentrations.

Design: Fourteen hypertriglyceridemic patients were treated with encapsulated fish oil (containing 1.45 g eicosapentaenoic acid and 1.55 g docosahexaenoic acid/d) for 4 wk. Venous blood samples were collected before and after treatment. Eleven normolipidemic subjects served as a control group.

Results: Fish oil effectively lowered plasma lipid and apolipoprotein (apo) E concentrations in the hypertriglyceridemic patients, whereas apo B concentrations increased. The lipid and apolipoprotein content of VLDL decreased, whereas LDL cholesterol and LDL apo B increased. Fractionation of VLDL by heparin-affinity chromatography showed that before treatment hypertriglyceridemic patients had more VLDL in the 0.05-mol NaCl/L subfraction and less in the 0.20-mol/L subfraction than did control subjects ($P < 0.05$), whereas the subfraction distribution pattern was normalized after fish-oil treatment. Nevertheless, plasma concentrations of the 0.05-mol NaCl/L subfraction were decreased and those of the 0.20-mol/L subfraction were increased in hypertriglyceridemic patients after fish-oil treatment ($P < 0.05$). Fish-oil treatment both enhanced VLDL binding and lowered LDL binding to fibroblasts.

Conclusion: Treatment of hypertriglyceridemic patients with fish oil caused differential effects on VLDL subfractions and decreased LDL binding to fibroblast receptors, which may have contributed to the paradoxical increase in LDL-cholesterol concentrations. *Am J Clin Nutr* 2000;71:28–35.

KEY WORDS Fish oil, VLDL, LDL, hypertriglyceridemia, fibroblast, apolipoprotein B, humans

INTRODUCTION

Epidemiologic studies have indicated that populations who consume large amounts of n−3 polyunsaturated fatty acid (PUFA)–enriched fish oil have a low incidence of atherosclerotic disease (1, 2). Dietary fish-oil almost invariably lowers plasma triacylglycerol concentrations; however, the effects of fish oil on plasma cholesterol concentrations have been inconsistent (3–7). An increase in LDL-cholesterol concentrations was observed in response to dietary fish-oil supplements in subjects with hyperlipidemia and hypertriglyceridemia (5–7). The mechanisms by which n−3 PUFAs reduce plasma triacylglycerol concentrations have been shown to be due to a reduction in the synthesis and secretion of VLDL, the LDL precursor (8, 9). However, the reasons for the paradoxical increase in LDL-cholesterol concentrations despite a reduced VLDL content are less well understood. Several mechanisms have been proposed, including the preferential conversion of the synthesized VLDL particles to LDL (10) or reduced LDL receptor activity (11).

Human VLDL consists of a heterogeneous population of particles differing in size, physical characteristics, chemical composition, cellular interaction, and metabolic fate (12). There is a poorly understood relation between VLDL composition and LDL concentrations that may play a role in the increase in LDL seen with fish-oil feeding. The goals of this study were *1*) to compare the distribution and composition of VLDL particles in healthy persons with that in patients with hypertriglyceridemia, *2*) to determine how n−3 PUFA supplementation alters the distribution of VLDL subfractions, *3*) to evaluate the role of altered VLDL subfractions in determining LDL concentration and composition, and *4*) to examine whether altered VLDL and LDL composition affects the interaction of these particles with fibroblasts.

SUBJECTS AND METHODS

Subjects

Fourteen patients (11 men and 3 women) with Fredrickson phenotype IV hypertriglyceridemia (13) aged 49–60 y ($\bar{x} \pm$ SD: 54 ± 5 y) were recruited for this study from the outpatient department of the National Taiwan University Hospital. Each patient was following the American Heart Association Step I diet and

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had undergone repeated measurements of plasma lipids for ≥1 y; fasting plasma triacylglycerol concentrations ranged from 3.63 to 9.77 mmol/L. None of the patients had hypertension or diabetes mellitus and none were smokers. Patients were excluded if they had secondary hypertriglyceridemia; coronary artery disease (defined as a demonstrated narrowing of >50% of the angiographic luminal diameter); relevant valvular, myocardial, or hypertensive cardiovascular disease; or stroke. Patients with a history of drug or alcohol abuse, women taking oral contraceptive agents or who were pregnant or lactating, and patients with concomitant conditions or who were taking medications that might interfere with the evaluation of the results, such as diuretics, sex hormones, or thyroid medications, were also excluded. All patients were free of liver, renal, and other major organ diseases and were required to have discontinued any treatment with lipid-lowering agents ≥8 wk before the study. The patients' body weights remained constant throughout the study period. Eleven healthy, normolipidemic subjects (8 men and 3 women) aged 49–59 y ($\bar{x}$ ± SD: 54 ± 4 y) with body heights and weights similar to those of the patients were enrolled as the control group. The study protocol was approved by the Human Subjects Review Committee of the National Taiwan University Hospital.

Study protocol

After the hypertriglyceridemic patients gave their informed consent, they were told to discontinue treatment with any lipid-lowering agents ≥8 wk before the study began. At the end of this 8-wk run-in period, patients were given 10 capsules of encapsulated fish oil/d containing a total of 3 g n−3 PUFAs [1.45 g eicosapentaenoic acid (EPA) and 1.55 g docosahexaenoic acid (DHA)]; the 10 capsules were given in 3 divided doses for 4 wk. The fish oil (TAMA Biochemical Co Ltd, Tokyo) consisted of triacylglycerol (1 g/capsule) with little cholesterol (0.5 mg/g) and was stabilized with vitamin E (1.1 mg/g). The percentages of fatty acids by weight were as follows: 10:0, 0.63%; 12:0, 1.08%; 14:0, 10.2%; 16:0, 10.6%; 18:0, 3.28%; 14:1n−5, 2.62%; 16:1n−7, 12.02%; 18:1n−9, 6.09%; 18:2n−6, 3.52%; 18:3n−6, 2.85%; 20:4n−6, 2.97%; 20:5n−3, 21.35%; and 22:6n−3, 22.65%. Patients were required to continue following the American Heart Association Step I diet but maintained the traditional Chinese composition of their diets (≈30% of energy from fat with a ratio of polyunsaturated to saturated fatty acids of ≈1.0–1.2) throughout the study. Venous blood samples were collected before and after 4 wk of treatment to analyze the lipid profile, isolate lipoproteins, analyze the chemical composition and subfraction distributions of VLDL particles, and to test the competitive binding of VLDL and LDL to fibroblast receptors. Results were compared with those in the control group.

Blood sample collection

After subjects had fasted overnight for ≥12 h, venous blood samples were collected into tubes containing EDTA (1 g/L). Plasma was obtained by centrifuging the blood at 4°C for 15 min at 300 × *g*. Fresh plasma samples were used immediately for lipid profile analysis and lipoprotein preparation. VLDL (density < 1.006 kg/L), LDL (density = 1.019–1.063 kg/L), and HDL (density = 1.063–1.21 kg/L) fractions were isolated by sequential ultracentrifugation with a 50.4 Ti angle rotor (Beckman, Fullerton, CA) (14). A portion of the isolated lipoproteins was used to analyze the lipid profile. The remainder was dialyzed at 4°C for 24 h against 3 changes of ≥1000 volumes of vacuum-degassed 10-mmol phosphate-buffered saline/L, pH 7.4, to remove the EDTA for further assays. The protein concentrations of the lipoprotein fractions were measured with Folin phenol reagent according to the Lowry method (15) with bovine serum albumin as the standard. Ten percent (wt:vol) sucrose was added to the prepared LDL fraction as a cryopreservative to maintain the characteristics of the freshly prepared lipoproteins (16); LDL was then stored at −80°C until analyzed. All frozen LDL samples from the same patient were analyzed simultaneously at the end of the study for competitive binding to fibroblast receptors.

Assessment of lipid profile

Plasma triacylglycerol, cholesterol, and phospholipid concentrations were assayed enzymatically with commercial kits (Merck Chemical Co, Darmstadt, Germany, and Boehringer Mannheim, Mannheim, Germany) in an Epose 5060 autoanalyzer (Eppendorf Corp, Hamburg, Germany). The lipid in the lipoprotein was extracted with Folch's solution (chloroform:methanol, 2:1 by vol); cholesteryl ester, triacylglycerol, free cholesterol, and phospholipid were separated by microthin-layer chromatography on silica-coated quartz rods in a developing solution (hexane:ether:formic acid, 60:5:0.5, by vol) with flame-ionization detection (17) in a thin-layer chromatography autodetector (Iatron Laboratories Inc, Tokyo).

The fatty acid pattern was analyzed by the method of Miwa and Yamamoto (18). Fatty acids were derivatized with 2-nitrophenylhydrazine hydrochloride in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride by using margaric acid (17:0) as the internal standard and were separated by reversed-phase HPLC with methanol:water (85:15) as the eluent at a flow-rate of 1.2 mL/min. Absorbance was monitored at 230 nm. Apolipoprotein (apo) A-I, B, C-II, C-III, and E concentrations were measured by the antigen-antibody reacted immunoassay (19) with commercial kits (Sigma Chemical Co, St Louis, and Daiichi Pure Chemicals Co, Tokyo). Turbidimetric absorbance was monitored at 340 nm in an Epose 5060 autoanalyzer.

Separation of VLDL subfractions

VLDL subfractions were separated by a modification of the method of Huff and Telford (20); the modification included isolating 5 instead of 2 VLDL subfractions by elution with buffers of increasing ionic strength. Namely, heparin-Sepharose (Affi-Gel Heparin; Bio-Rad, Richmond, CA), packed in a 1.5 × 20 cm column, was saturated with bovine serum albumin and then equilibrated with 2 mmol phosphate buffer/L, pH 7.4, containing 0.05 mol NaCl/L and 0.01% (wt:vol) sodium azide. VLDLs, isolated by ultracentrifugation, were dialyzed against equilibration buffer and then 2–3-mg samples (based on protein content) were applied to the column at 4°C and left for 2 h. Elution was performed with 2 mmol phosphate buffer/L, pH 7.4, containing 0.05, 0.12, 0.20, 0.38, or 3.00 mol NaCl/L, at a flow rate of 30 mL/h. Each step was continued until absorbance at 280 nm returned to baseline. The pooled subfractions were collected and concentrated on an RCF-Conflict centrifugal concentrator (Bio-Molecular Dynamics, Beaverton, OR). The recovery of VLDL protein ranged from 84% to 95%.

Labeling of LDL

LDL from control subjects was radiolabeled by the ^{125}I monochloride method in 1 mol glycine NaOH buffer/L at pH 10.0. The atom-to-mole ratio of iodine to protein was kept <1.0 (21). After iodination, the LDL was dialyzed against Eagle's minimum essential medium (MEM) for 4 h and then stabilized by the addition of 1% human serum albumin (wt:vol) and dialyzed

overnight against culture medium. The stabilized [^{125}I]LDL was stored at 4°C and used within 10 d. The specific activity ranged from 200 to 400 cpm/ng LDL protein.

Culture of fibroblast cells

Human foreskin was obtained from healthy, normolipidemic subjects undergoing circumcision and was refrigerated in sterile, chilled MEM until processed. The skin was trimmed with fine scissors to remove fat, cut into fine pieces, and digested with collagenase (0.1% wt:vol) at 37°C. Free cells were collected in tubes containing chilled MEM every 15 min for 2 h. The cells were then centrifuged at 4°C for 20 min at 300 × *g*. The cell pellets were dispersed, washed twice with MEM, and cultured in a 75-cm^2 flask in 10 mL MEM containing 10% (by vol) fetal calf serum, 100 × 10^3 IU penicillin/L, and 100 g streptomycin/L at 37°C in a 5% CO_2 incubator (22).

Competition between [^{125}I]LDL and lipoproteins for binding to fibroblasts

Confluent fibroblast cells from control subjects were trypsinized and seeded at a concentration of 2.5 × 10^5 cells per dish. After 5–7 d in culture when the cells reached confluence, the medium was removed and the cells were washed twice with medium. Then 10% lipoprotein-deficient human serum was added to the medium in place of fetal calf serum for an additional 48 h. The cells were then chilled to 4°C by replacing the medium with chilled medium and stored in a cold room for 30 min. [^{125}I]LDL (10–15 mg/L) and cold lipoprotein (VLDL subfraction or unlabeled LDL at the indicated concentrations) were added to the medium and the cells were incubated at 4°C for 3 h. The medium was then removed and the cells were washed 6 times with medium. Finally, the cell monolayer was solubilized in 1 mL of 0.1 mol NaOH/L and a portion was used to determine the amount of [^{125}I]LDL bound to the cells. Another portion was used for protein determination. The bound ^{125}I was counted in a gamma counter. Values obtained with cells incubated in the absence of cold lipoproteins were taken as the 100% control, and the data were expressed as a percentage of the control (23).

Statistics

All data are expressed as means ± SDs. Differences in the same hypertriglyceridemic patient before and after 4 wk of treatment with n−3 PUFAs were evaluated by paired *t* tests. Differences between control subjects and hypertriglyceridemic patients before and after n−3 PUFA treatment were evaluated by unpaired *t* tests. Differences in the surface-to-core ratio and chemical composition of the VLDL subfractions were assessed by analysis of variance and a Scheffe test was used to identify differences among the individual means. Statistical analyses were performed with SAS (version 6.011; SAS Institute Inc, Cary, NC). $P < 0.05$ was considered statistically significant.

RESULTS

Plasma lipid profile

As shown in **Table 1**, plasma concentrations of total triacylglycerol, VLDL triacylglycerol, and LDL triacylglycerol decreased significantly in hypertriglyceridemic patients after 4 wk of n−3 PUFA treatment. Plasma concentrations of total cholesterol and VLDL cholesterol also decreased, whereas concentrations of LDL and HDL cholesterol increased. Plasma phospholipid and apo E

TABLE 1
Plasma lipid profile of control subjects and of hypertriglyceridemic (HTG) patients before and after 4 wk of supplementation with fish oil[1]

Lipid profile	Control subjects (n = 11)	HTG patients (n = 14)	
		Before	After
Triacylglycerol (mmol/L)			
Total	1.50 ± 0.29	5.22 ± 1.68[2]	2.52 ± 0.59[2,3]
VLDL	0.77 ± 0.11	3.78 ± 1.59[2]	1.69 ± 0.23[2,3]
LDL	0.45 ± 0.09	0.63 ± 0.10[2]	0.41 ± 0.06[3]
HDL	0.20 ± 0.04	0.18 ± 0.07	0.17 ± 0.09
Cholesterol (mmol/L)			
Total	5.40 ± 0.49	6.33 ± 0.67[2]	5.79 ± 0.54[3]
VLDL	0.54 ± 0.20	1.52 ± 0.23[2]	0.69 ± 0.18[3]
LDL	2.89 ± 0.54	3.33 ± 0.49[2]	3.51 ± 0.25[2,3]
HDL	1.47 ± 0.23	1.03 ± 0.20[2]	1.16 ± 0.15[2,3]
Phospholipid (mmol/L)	2.49 ± 0.24	3.66 ± 0.28[2]	3.20 ± 0.16[2,3]
Apolipoprotein A-I (μmol/L)	39.28 ± 6.75	37.14 ± 12.50	38.92 ± 7.50
Apolipoprotein B (μmol/L)			
Total	1.76 ± 0.34	2.05 ± 0.43	2.27 ± 0.50[2,3]
VLDL	0.32 ± 0.11	0.65 ± 0.22[2]	0.51 ± 0.25[2,3]
LDL	1.27 ± 0.22	1.17 ± 0.18	1.36 ± 0.32[3]
Apolipoprotein C-II (μmol/L)			
Total	5.39 ± 1.34	6.74 ± 2.02	6.51 ± 2.24
VLDL	3.48± 1.23	4.72 ± 1.23[2]	3.59 ± 1.01[3]
Apolipoprotein E (μmol/L)			
Total	1.55 ± 0.20	2.02 ± 0.47[2]	1.82 ± 0.47[2,3]
VLDL	0.73 ± 0.11	1.34 ± 0.35[2]	1.02 ± 0.23[3]

[1] $\bar{x}$ ± SD.
[2] Significantly different from control subjects, $P < 0.05$ (unpaired *t* test).
[3] Significantly different from before treatment, $P < 0.05$ (paired *t* test).

TABLE 2
Chemical composition of LDL as a percentage of apolipoprotein (apo) B content in control subjects and hypertriglyceridemic (HTG) patients before and after 4 wk of supplementation with fish oil[1]

	Control subjects (n = 11)	HTG patients (n = 14)	
		Before	After
	% of apo B		
Triacylglycerol	54 ± 10	89 ± 21[2]	50 ± 19[3]
Cholesteryl ester	144 ± 43	151 ± 42	147 ± 29
Free cholesterol	2 ± 6	39 ± 12	36 ± 10
Phospholipid	89 ± 19	83 ± 16	79 ± 17

[1] $\bar{x}$ ± SD.
[2] Significantly different from control subjects, $P < 0.05$ (unpaired t test).
[3] Significantly different from before treatment, $P < 0.05$ (paired t test).

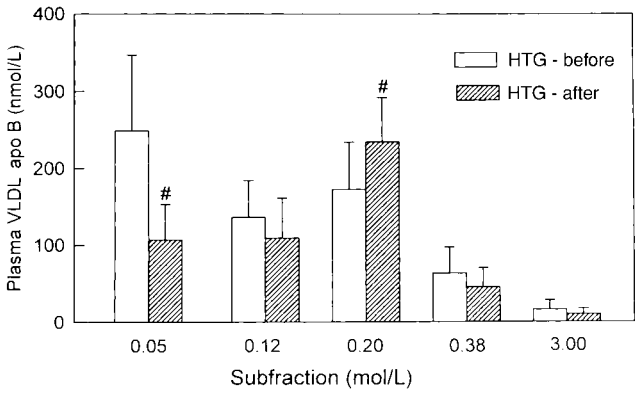

FIGURE 1. Concentrations of plasma VLDL subfractions eluted from a heparin-affinity column by 0.05, 0.12, 0.20, 0.38, and 3.00 mol NaCl/L in hypertriglyceridemic (HTG) patients (n = 14) before and after 4 wk of supplementation with fish oil. Apo, apolipoprotein. [#]Significantly different from before treatment, $P < 0.05$ (paired t test).

concentrations decreased, whereas plasma concentrations of apo A-I and apo C-II did not change significantly. Consistent with the increase in LDL cholesterol, plasma apo B and LDL apo B concentrations increased significantly. However, concentrations of apo B, apo C-II, and apo E in VLDL decreased significantly.

The analysis of the chemical composition of the LDL particles (**Table 2**) showed that cholesterol and phospholipid contents, expressed as a weight percentage of apo B, did not change significantly with n−3 PUFA treatment, whereas the triacylglycerol content decreased significantly from 89 ± 21% to 50 ± 19%. As a percentage of total fatty acids, plasma concentrations of EPA (0.81 ± 0.02% compared with 4.11 ± 0.07%) and DHA (3.63 ± 0.07% compared with 5.55 ± 0.09%) increased significantly with treatment in hypertriglyceridemic patients. Plasma fatty acid concentrations decreased significantly with treatment (16.58 ± 4.52 mmol/L before treatment compared with 12.07 ± 2.55 mmol/L after).

Distribution and chemical composition of VLDL subfractions

As shown in **Table 3**, before n−3 PUFA treatment, there was more VLDL in the 0.05-mol NaCl/L subfraction in hypertriglyceridemic patients than in control subjects, whereas there was less VLDL in the 0.20-mol NaCl/L subfraction. After n−3 PUFA treatment, the subfraction distribution pattern of VLDL was not significantly different between hypertriglyceridemic patients and control subjects. Nevertheless, in hypertriglyceridemic patients after fish-oil treatment, the amount of VLDL in the 0.05-mol NaCl/L subfraction had decreased remarkably compared with before treatment, the amount in the 0.20-mol NaCl/L had increased, and the amounts in the other subfractions had decreased but not significantly so (**Figure 1**).

VLDL subfractions eluted at the same salt concentration in control subjects and in hypertriglyceridemic patients (before or after n−3 PUFA treatment) did not differ significantly in their percentage chemical composition. In general, the higher the salt concentration, the more protein, phospholipid, and cholesteryl ester and less triacylglycerol that was eluted. As shown in **Table 4**, the subfractions eluted at a salt concentration >0.20 mol/L had a higher surface-to-core ratio than did the subfractions eluted at lower salt concentrations. Subfractions eluted at higher salt concentrations contained a higher percentage of protein than did subfractions eluted at lower salt concentrations. When expressed as a percentage of total apolipoproteins, there was less apo B and more apo E in the subfractions eluted at higher salt concentrations, whereas apo C-II and C-III were distributed fairly equally between subfractions (**Table 5**).

TABLE 3
Distribution pattern based on apolipoprotein (apo) B content of VLDL eluted from heparin-Sepharose in control subjects and in hypertiglyceridemic (HTG) patients before and after 4 wk of supplementation with fish oil[1]

VLDL subfraction	Control subjects (n = 11)	HTG patients (n = 14)	
		Before	After
	% of apo B		
0.05 mol/L	19.2 ± 13.3[a]	38.4 ± 15.1[a,2]	21.0 ± 10.1[a,3]
0.12 mol/L	21.5 ± 9.3[a]	20.7 ± 7.1[b]	23.8 ± 12.8[a]
0.20 mol/L	49.2 ± 19.0[b]	28.1 ± 17.3[a,b,2]	45.8 ± 11.3[b,3]
0.38 mol/L	7.7 ± 2.2[c]	9.7 ± 4.8[c]	7.6 ± 2.5[c]
3.00 mol/L	2.3 ± 0.6[d]	2.8 ± 1.5[d]	1.8 ± 0.8[d]

[1] $\bar{x}$ ± SD. Means within a column with different superscript letters are significantly different, $P < 0.05$ (ANOVA and Scheffe's test).
[2] Significantly different from control subjects, $P < 0.05$ (unpaired t test).
[3] Significantly different from before treatment, $P < 0.05$ (paired t test).

TABLE 4
Surface-to-core ratios of VLDL subfractions from control subjects and hypertriglyceridemic (HTG) patients before and after 4 wk of supplementation with fish oil[1]

VLDL subfraction	Control subjects (n = 11)	HTG patients (n = 14)	
		Before	After
0.05 mol/L	0.27 ± 0.06[a]	0.21 ± 0.10[a]	0.24 ± 0.09[a]
0.12 mol/L	0.38 ± 0.06[b]	0.32 ± 0.09[a]	0.37 ± 0.06[b]
0.20 mol/L	0.57 ± 0.06[c]	0.52 ± 0.08[b]	0.52 ± 0.02[c]
0.38 mol/L	0.51 ± 0.08[c]	0.48 ± 0.06[b]	0.50 ± 0.04[c]
3.00 mol/L	0.57 ± 0.02[c]	0.55 ± 0.03[b]	0.57 ± 0.02[c]

[1] $\bar{x}$ ± SD. Surface-to-core ratio = phospholipid + protein + free cholesterol/triacylglycerol + cholesteryl ester. Means within a column with different superscript letters are significantly different, $P < 0.05$ (ANOVA and Scheffe's test).

TABLE 5
Protein percentage and apolipoprotein (apo) composition of VLDL subfractions from control subjects and hypertriglyceridemic (HTG) patients before and after 4 wk of supplementation with fish oil[1]

Subfraction	Percentage chemical composition: protein[2]	Percentage of total apolipoproteins			
		Apo B[2]	Apo E[2]	Apo C-II	Apo C-III
0.05 mol/L	%			%	
Control	4.8 ± 1.6[a]	52 ± 17[a]	18 ± 6[a]	15 ± 3[a]	16 ± 7[a]
HTG before	4.2 ± 1.2[a]	55 ± 16[a]	14 ± 5[a]	14 ± 5[a]	17 ± 8[a]
HTG after	4.6 ± 2.0[a]	52 ± 15[a]	19 ± 9[a]	13 ± 4[a]	16 ± 7[a]
0.12 mol/L					
Control	6.8 ± 1.0[a]	64 ± 12[a,b]	13 ± 8[a]	11 ± 2[a]	12 ± 6[a]
HTG before	5.9 ± 1.2[a]	56 ± 18[a,b]	18 ± 6[a]	12 ± 4[a]	13 ± 7[a]
HTG after	6.0 ± 1.1[a]	61 ± 14[a,b]	16 ± 7[a]	10 ± 3[a]	13 ± 6[a]
0.20 mol/L					
Control	11.2 ± 1.6[b]	68 ± [a,b]	12 ± 9[a]	8 ± 6[a,b]	12 ± 6[a]
HTG before	10.1 ± 1.8[b]	62 ± 18[a,b]	14 ± 7[a]	11 ± 7[a]	12 ± 5[a]
HTG after	10.3 ± 1.6[b]	69 ± 14[a,b]	11 ± 8[a]	8 ± 4[a,b]	11 ± 5[a,b]
0.38 mol/L					
Control	9.2 ± 1.1[b]	43 ± 16[a,c]	25 ± 9[a,b]	15 ± 7[a]	17 ± 5[a]
HTG before	8.3 ± 2.0[b]	45 ± 17[a,c]	22 ± 14[a,b]	16 ± 6[a]	16 ± 4[a]
HTG after	8.1 ± 1.6[b]	44 ± 18[a,c]	25 ± 8[a,b]	14 ± 7[a]	17 ± 5[a]
3.00 mol/L					
Control	10.9 ± 1.8[b]	21 ± 10[c,d]	34 ± 10[b,c]	20 ± 8[a,c]	24 ± 9[a]
HTG before	12.6 ± 1.6[b]	24 ± 8[c,d]	36 ± 8[b,c]	19 ± 9[a]	21 ± 6[a]
HTG after	11.7 ± 1.7[b]	21 ± 9[c,d]	33 ± 11[b,c]	22 ± 6[a,c]	25 ± 8[a,c]

[1] $\bar{x}$ ±SD; n = 11 control subjects, 14 HTG patients. Means within a column with different superscript letters are significantly different, $P < 0.05$ (ANOVA and Scheffe's test).
[2] Significant difference among subfractions, $P < 0.05$.

Competition between [^{125}I]LDL and unlabeled lipoprotein for binding to fibroblasts

In the competition study with VLDL, more [^{125}I]LDL bound to fibroblast receptors in hypertriglyceridemic patients before n−3 PUFA treatment than in control subjects (**Figure 2**). After n−3 PUFA treatment, binding of [^{125}I]LDL in hypertriglyceridemic patients decreased but was not significantly different from that in control subjects, indicating that the VLDL from hypertriglyceridemic patients had a lower binding affinity for fibroblasts than did the VLDL from control subjects and that n−3 PUFA treatment increased VLDL binding to fibroblast receptors. VLDL subfractions eluted at higher salt concentrations had a lower percentage of [^{125}I]LDL binding than did subfractions eluted at lower salt concentrations (**Figure 3**), indicating that the subfractions eluted at the higher salt concentrations had better fibroblast receptor binding. In the competition study with unlabeled LDL, the percentage of [^{125}I]LDL binding in hypertriglyceridemic patients increased after n−3 PUFA treatment (**Figure 4**), suggesting that n−3 PUFA treatment resulted in lower LDL binding to fibroblasts.

DISCUSSION

We showed that treatment of hypertriglyceridemic patients with n−3 PUFAs had a differential effect on the subfractions of the heterogeneous VLDL particles and decreased LDL binding to fibroblast receptors. These results may explain the observed paradoxical increase in plasma LDL-cholesterol concentrations with n−3 PUFA treatment even when precursor VLDL concentrations are decreased.

Although the present study was not a double-blind, placebo-controlled trial, the marked increase in plasma EPA and DHA as a percentage of fatty acids in hypertriglyceridemic patients treated with n−3 PUFAs strongly indicates good adherence of the subjects to the study protocol. In accordance with the inhibitory effect on triacylglycerol synthesis and the increase in the fractional catabolic rate of triacylglycerol-rich lipoproteins caused by n−3 PUFAs (5, 24), plasma concentrations of total triacylglycerol, VLDL triacylglycerol, VLDL apo B, VLDL apo C-II, VLDL apo E, and LDL triacylglycerol decreased significantly in hypertriglyceridemic patients with n−3 PUFA treatment. Although plasma VLDL-cholesterol concentrations in the patients decreased, LDL-cholesterol concentrations increased significantly. Plasma apo B is derived from

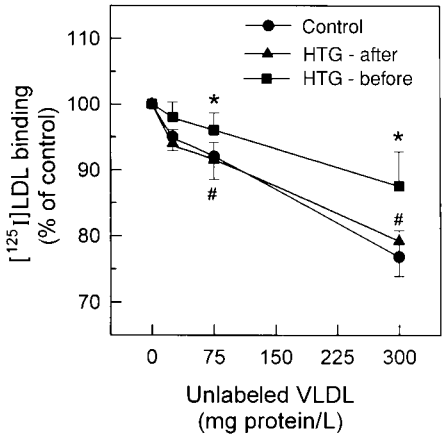

FIGURE 2. Competition for binding to fibroblasts between [^{125}I]LDL and increasing concentrations of unlabeled VLDL from control (n = 11) and hypertriglyceridemic (HTG; n = 14) subjects before and after 4 wk of supplementation with fish oil. *Significantly different from control group, $P < 0.05$ (unpaired t test). #Significantly different from before treatment, $P < 0.05$ (paired t test).

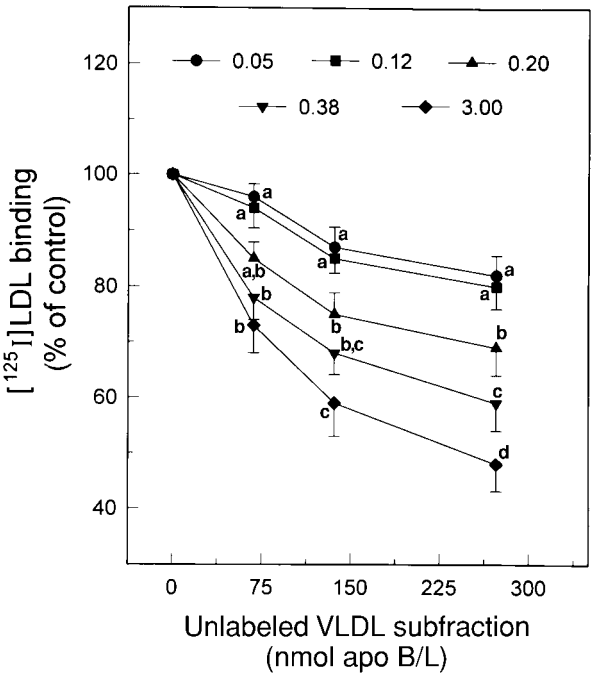

FIGURE 3. Competition for fibroblast binding between [^{125}I]LDL and increasing concentrations of unlabeled VLDL subfractions (n = 14) eluted from a heparin-affinity column by 0.05, 0.12, 0.20, 0.38, and 3.00 mol NaCl/L. Apo, apolipoprotein. Means within a concentration with different superscript letters are significantly different, $P < 0.05$ (ANOVA and Scheffe's test).

VLDL and LDL particles; however, the observed increase in apo B concentrations did not seem to be in proportion to the marked decrease in VLDL concentrations. The magnitude of the plasma LDL-cholesterol increase was consistent with the increase in LDL apo B. Thus, the cholesterol content of the LDL particles did not change significantly when the measurement was made as a percentage of apo B, implying that the increase in apo B concentrations was due to the increase in LDL particles or to the altered chemical composition of VLDL.

It has been reported that in familial combined hyperlipidemia, fish oil induces changes predominantly in VLDL and LDL particle number, but not in lipoprotein composition (25) . This finding is in contrast with our present results in hypertriglyceridemic patients with Fredrickson phenotype IV. In the present study, the triacylglycerol content of LDL particles decreased after n−3 PUFA treatment when measured as a percentage of apo B. The increased relative proportion of VLDL in the 0.20-mol/L subfraction (which had a higher protein content relative to the 0.05-mol/L subfraction) and decreased proportion in the 0.05-mol/L subfraction (which had a lower protein content relative to the 0.20-mol/L subfraction) represented a normalization of the distribution of VLDL subfractions after treatment. Nevertheless, the plasma concentrations of VLDL particles with lower surface-to-core ratios were decreased (the 0.05-mol/L subfraction) and concentrations of particles with higher surface-to-core ratios were increased (the 0.12-mol/L subfraction).

The amount or conformation of apo B and apo E associated with VLDL particles influences the partitioning of VLDL into hepatic removal or LDL conversion pathways. Thus, these apolipoproteins affect VLDL catabolism and LDL production (26, 27). The liver secretes a variety of VLDL particles with different affinities for heparin and with distinguishable metabolic pathways. The lower-affinity fractions tend to be rapidly removed by the liver whereas the higher-affinity factions are preferentially converted to LDL (28). Cardin et al (29, 30) and Weisgraber et al (31, 32) identified the important heparin-binding regions on apo B and apo E; these are located in the area near the LDL receptor recognition site. The apo E–enriched VLDL subfraction shows stronger binding to human skin fibroblasts than the apo E–poor VLDL subfraction (33, 34). The present study showed an increase in the apo E– and apo B–enriched VLDL subfraction (0.20-mol/L subfraction) after n−3 PUFA treatment; this subfraction would be more competitive with LDL for receptors than the apo E– and apo B–poor VLDL subfraction, resulting in more LDL being retained in plasma.

After n−3 PUFA treatment, the LDL of hypertriglyceridemic patients had reduced triacylglycerol contents and reduced binding to fibroblasts. Altering the core lipids of LDL leads to changes in the conformation of LDL and the interaction of LDL with fibroblasts (27, 35). Lindsey et al (36) reported that LDL from humans fed fish oil was enriched in n−3 PUFAs. These authors also reported reduced LDL receptor activity in HepG2 cells treated with n−3 PUFAs. These observations might in part explain the elevated plasma LDL concentrations of the hypertriglyceridemic patients in the present study. In a study in swine, although plasma LDL was elevated, the development of atherosclerotic lesions was inhibited by dietary supplementation with fish oil (37, 38). Therefore, more studies are required to characterize LDL and to clarify the link between plasma LDL concentrations and the progress of atherosclerosis.

Other procedures, such as cumulative floating ultracentrifugation or gel filtration chromatography, have been reported to be as useful as heparin-affinity chromatography in VLDL

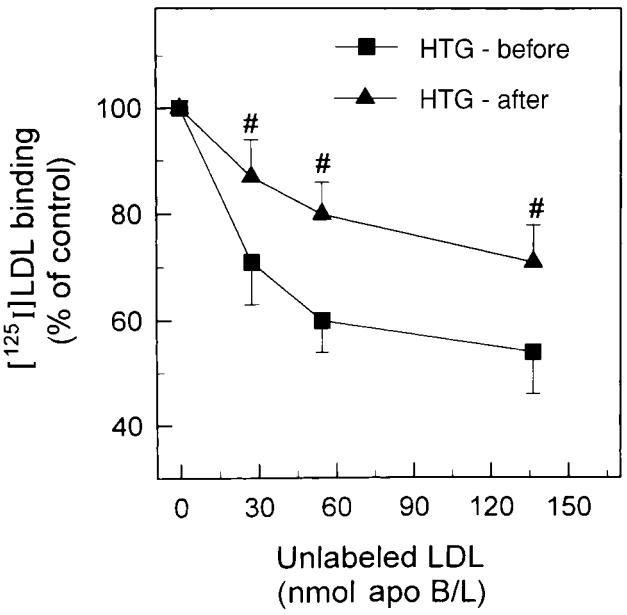

FIGURE 4. Competition for fibroblast binding between [^{125}I]LDL and increasing concentrations of unlabeled LDL from hypertriglyceridemic (HTG; n = 14) patients before and after 4 wk of supplementation with fish oil. #Significantly different from before treatment, $P < 0.05$ (paired t test).

subfraction analysis (39–41). It would therefore be worthwhile to compare different methods for studying the effect of n−3 PUFAs on lipoprotein composition and metabolism. In addition, it may be worthwhile to delineate those LDL subfractions—and their compositional characteristics—that may be altered by n−3 PUFA treatment.

The treatment of hypertriglyceridemic patients with Fredrickson phenotype IV with a daily dose of 3 g n−3 PUFAs for 4 wk resulted in a lowering of plasma VLDL concentrations, a redistribution of VLDL particles, an increase in LDL concentrations, and a decrease in LDL binding to fibroblast receptors. Longer clinical trials are required to clarify whether these observations are persistent or transitory.

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REFERENCES

1. Bang HO, Dyerberg J, Nielsew AB. Plasma lipids and lipoprotein pattern in Greenlandic west-coast Eskimos. Lancet 1971;1: 1143–6.
2. Kromann N, Green A. Epidemiological studies in the Upernavik district, Greenland. Incidence of some chronic disease. Acta Med Scand 1980;208:401–6.
3. Nestel PJ. Polyunsaturated fatty acids (n−3, n−6). Am J Clin Nutr 1987;45(suppl):1161–7.
4. Harris WS. Fish oils and plasma lipid lipoprotein metabolism in humans. J Lipid Res 1989;30:785–807.
5. Harris WS, Connor WE, Illingworth DR, Rothrock DW, Foster DM. Effect of fish oil on VLDL triacylglycerol kinetics in humans. J Lipid Res 1990;31:1549–58.
6. Sullivan DR, Sanders TAB, Trayner IM, Thompson GR. Paradoxical elevation of LDL apolipoprotein B concentrations in hypertriglyceridaemic patients and normal subjects ingesting fish oil. Atherosclerosis 1986;61:129–34.
7. Harris WS, Dujovne CA, Zuker M, Johnson B. Effects of a low saturated fat, low cholesterol fish oil supplement in hypertriglyceridemic subjects: a placebo controlled trial. Ann Intern Med 1988;109:465–70.
8. Nestel PJ, Connor WE, Reardon MF, Connor S, Wong S, Boston R. Suppression by diets rich in fish oil of very-low-density lipoprotein production in man. J Clin Invest 1984;74:82–9.
9. Sanders TAB, Sullivan DR, Reeve J, Thompson GR. Triglyceride lowering effect of marine polyunsaturates in patients with hypertriglyceridemia. Arteriosclerosis 1985;5:459–65.
10. Sanders TAB. Influence of moderate intakes of fish oil on blood lipids. In: Land WEM, ed. Polyunsaturated fatty acids and eicosanoids. Champaign, IL: American Oil Chemists Society, 1987:70–86.
11. Roach PD, Kambouris AM, Trimble RP, Nestel PJ. The effects of dietary fish oil on hepatic high density and low density lipoprotein receptor activities in the rat. FEBS Lett 1987;1:159–62.
12. Evans AJ, Wolf BM, Strong WLP, Huff MW. Reduced lipolysis of large apo-poor very-low-density-lipoprotein subfractions from type IV hypertriglyceridemic subjects in vitro and in vivo. Metabolism 1993;42:105–15.
13. Fredrickson DS, Lee RS. A system for phenotyping hyperlipoproteinemia. Circulation 1965;31:321–7.
14. Redgrave TR, Carlson LA. Changes in plasma very low density and low density lipoprotein content, composition, and size after a fatty meal in normo- and hypertriglyceridemic man. J Lipid Res 1979;20:217–22.
15. Lowry OH, Rosebrough NJ, Karr AL, Randall RJ. Protein measurement with Folin reagent. J Biol Chem 1951;193:265–75.
16. Rumsey SC, Stucchi AF, Nicolosi RJ, Goldensberg HN, Ramakrishnan R, Deckelbaum RJ. Human plasma LDL cryopreserved with sucrose maintains in vitro kinetics indistinguishable from freshly isolated human LDL. J Lipid Res 1994;35:1592–8.
17. Vandamme D, Blaton V, Peeters H. Screening of plasma lipids by thin-layer chromatography with flame ionization detection on chromarods. J Chromatogr 1978;145:151–4.
18. Miwa H, Yamamoto M. Improved method of determination of biologically important C10:0-C22:6 fatty acids as their 2-nitrophenylhydrazide by reversed-phase high-performance liquid chromatography. J Chromatogr 1986;351:275–82.
19. Rifai N, King ME. Immunoturbidimetric assays of apolipoproteins A-I, A-II and B in serum. Clin Chem 1986;32:957–60.
20. Huff MW, Telford DE. Characterization and metabolic fate of two very low density lipoprotein subfractions separated by heparin-Sepharose chromatography. Biochim Biophys Acta 1984;796:251–61.
21. Bilheimer DW, Eisenberg S, Levy RI. The metabolism of very low density lipoproteins: preliminary in vitro and in vivo observation. Biochim Biophys Acta 1972;260:212–8.
22. Goldstein JL, Brown MS. Binding and degradation of low density lipoprotein by cultured human fibroblasts. J Biol Chem 1974; 249:5153–62.
23. Goldstein JL, Brown MS. Binding and degradation of low density lipoproteins by cultured human fibroblasts. Comparison of cells from a normal subject and from a patient with homozygous familial hypercholesterolemia. J Biol Chem 1988;263:16842–8.
24. van Vlijmen BJ, Mensink RP, van't Hof HB, Offerman RF, Hofker MH, Havekes LM. Effect of dietary fish oil on serum lipids and VLDL kinetics in hyperlipidemic apolipoprotein E*3-Leiden transgenic mice. J Lipid Res 1998;39:1181–8.
25. Tato F, Keller C, Wolfram G. Effects of fish oil concentrate on lipoproteins and apolipoproteins in familial combined hyperlipidemia. Clin Investig 1993;71:314–8.
26. Yang CY, Gu ZW, Valentinova N, et al. Human very low density lipoprotein structure: interaction of the C apolipoproteins with apolipoprotein B-100. J Lipid Res 1993;34:1311–21.
27. Kinoshita M, Krul ES, Schonfeld G. Modification of the core lipids of low density lipoproteins produces selective alterations in the expression of apoB-100 epitopes. J Lipid Res 1990;31:701–8.
28. Gomez CD, Saez GT, Lasuncion MA, Herrera E. Different hydrolytic efficiencies of adipose tissue lipoprotein lipase on very low density lipoprotein subfraction separated by heparin-Sepharose chromatography. Biochim Biophys Acta 1993;1167:70–1.
29. Cardin AD, Randall CJ, Hirose N, Jackson RL. Physical-chemical interaction of heparin and plasma low density lipoproteins. Biochemistry 1987;26:5513–8.
30. Cardin AD, Hirose N, Blankenship DT, et al. Binding of high reactive heparin to human apolipoprotein E: identification of two heparin-binding domains. Biochem Biophys Res Commun 1986;134:783–9.
31. Weisgraber KH, Rall SC. Human apolipoprotein B-100 heparin binding sites. J Biol Chem 1987;262:1097–103.
32. Weisgraber KH, Rall SCJ, Mahley RW, Milne RW, Marcel YL, Sparrow JT. Human apolipoprotein E. Determination of the heparin binding sites of apolipoprotein E3. J Biol Chem 1986;261:2068–76.
33. Fielding PE, Fielding CJ. An apoE-free very low density lipoprotein enriched in phosphatidylethanolamine in human plasma. J Biol Chem 1986;261:5233–6.
34. Trezzi E, Calvi C, Roma P, Catapano AL. Subfraction of human very low density lipoproteins by heparin-Sepharose affinity chromatography. J Lipid Res 1983;24:790–5.
35. Keidar SAC, Goldberg K, Cook J, Bateman J, Schonfeld G. High carbohydrate fat-free diet modulates epitope expression of LDL-apoB-100 and interaction of LDL with human fibroblasts. J Lipid Res 1989;30:1331–9.
36. Lindsey S, Pronczuk A, Hayes KC. Low density lipoprotein from humans supplemented with n−3 fatty acids depresses both LDL receptor activity and LDLr mRNA abundance in HepG2 cells. J Lipid Res 1992;33:647–58.
37. Kim DN, Ho HT, Lawrence DA, Schmee J, Thomas WA. Modification of lipoprotein patterns and retardation of atherogenesis by fish oil supplement to a hyperlipidemic diet for swine. Atherosclerosis 1989;76:35–54.

38. Thomas WA, Kim DN, Schmee J. Retardation of atherogenesis and other effects of a fish oil additive to a hyperlipidemic diet for swine. Ann N Y Acad Sci 1990;598:308–23.
39. Patti L, Swinburn B, Riccardi G, Rivellese AA, Howard BV. Alterations in very low density lipoprotein subfractions in normotriglyceridemic non-insulin-dependent diabetics. Atherosclerosis 1991;91: 15–23.
40. Lossow WJ, Lindgren FT, Murchio JC, Stevens GR, Jensen LC. Particle size and protein content of six fractions of the Sf > 20 plasma lipoproteins isolated by density gradient centrifugation. J Lipid Res 1969;10:68–75.
41. Musliner TA, Giotas C, Krauss RM. Presence of multiple subpopulations of lipoproteins of intermediate density in normal subjects. Arteriosclerosis 1986;6:79–87.

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