Heterogeneity of Low Density Lipoprotein Responses to Fish-Oil Supplementation in Hypertriglyceridemic Subjects

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Previous studies have demonstrated a variable effect of fish oil on low density lipoprotein (LDL) cholesterol and apolipoprotein (apo) B levels, particularly in hypertriglyceridemic subjects. Since heterogeneity of LDL composition and metabolism in hypertriglyceridemic subjects is well described, the present study was undertaken to determine if the response of LDL to dietary fish-oil supplementation is dependent upon pretreatment differences in LDL composition. A single-blind, crossover design was used with 18 hypertriglyceridemic subjects, who were given supplements of a safflower-oil placebo or a fish-oil concentrate (4.0 g omega-3 fatty acids; [F4 dose]) for 1 month. Sixteen subjects then received an additional month of fish-oil supplementation at a higher dose (7.5 g omega-3 fatty acids [F7.5 dose]). The initial LDL cholesterol/apo B ratio, an index of LDL composition, was correlated positively with changes in LDL apo B levels (F4.0 dose; r=0.41, p=0.06; F7.5 dose: r=0.51, p=0.03) and negatively with changes in LDL cholesterol concentrations (F4.0 dose: r=−0.51, p=0.01; F7.5 dose: r=−0.50, p=0.02). Twelve subjects with LDL cholesterol/apo B ratios above 1.4 had large increases in LDL apo B (51% at both doses, p<0.05) but much smaller changes in LDL cholesterol levels during fish-oil treatment. Six subjects with LDL cholesterol/apo B ratios below 1.4 showed a trend toward increased LDL cholesterol (12% increase from baseline at F4 dose, 10% increase from baseline at F7.5 dose, p>0.05) but not in LDL apo B levels during fish-oil therapy. These data suggest that LDL responses to fish oil may be linked to underlying differences in LDL composition and, presumably, to differences in LDL metabolic behavior. (Arteriosclerosis 9:345–354, May/June 1989)

There is general agreement that hypertriglyceridemic subjects treated with fish oil show consistent reductions in plasma and very low density lipoprotein (VLDL) triglyceride levels. However, low density lipoprotein (LDL) responses during fish-oil supplementation are much more variable, and it has been reported that LDL concentrations increased, remained unchanged, or decreased. Although the reason for this variability has not been established, the aberration of lipoprotein metabolism in unclassified hypertriglyceridemic subjects may differ markedly, and the lack of consistency of LDL responses to fish oil may reflect this heterogeneity.

LDL concentration and composition are dependent upon both the source of LDL (i.e., whether derived from VLDL or synthesized directly) and the composition of the VLDL precursor pool. Familial hypertriglyceridemia (FHTG), for example, is characterized by overproduction of VLDL triglyceride relative to apolipoprotein (apo) B.

LDL apo B and cholesterol levels are not usually elevated, and the ratio of LDL cholesterol to apo B is either normal or slightly increased. LDL is derived primarily, if not exclusively, from the delipidation of VLDL. Familial combined hyperlipidemia (FCHL), on the other hand, is associated with increased VLDL and LDL apo B turnover. The concentration of LDL apo B is increased, and the ratio of LDL cholesterol to apo B is decreased due to the presence of a cholesterol-depleted, dense LDL subpopulation. Increased conversion of VLDL to LDL, as well as direct hepatic synthesis of LDL, may contribute to the increase in LDL concentration and alterations in LDL composition. Individuals with noninsulin-dependent diabetes mellitus (NIDDM) have overproduction of VLDL apo B, VLDL triglyceride, or both. LDL metabolism is also heterogeneous. In hypertriglyceridemic diabetic subjects, a polydisperse LDL, reflecting a spectrum of dense and buoyant LDL subspecies, has also been described.

Fish oil appears to exert its primary effect on VLDL metabolism by inhibiting VLDL triglyceride and apo B synthesis. Therefore, the LDL response to fish oil in a particular subject may depend upon both the efficiency of the delipidation pathway to form LDL and the relative importance of de novo LDL synthesis. In normal subjects, where VLDL is efficiently converted to LDL via the delipidation pathway, fish-oil diets decrease LDL apo B concentrations due to a reduction in LDL apo B synthesis. However, in hypertriglyceridemic subjects, the relative...
contribution of these pathways toward LDL synthesis varies according to the underlying disorder of lipoprotein metabolism; for example, the efficiency of VLDL conversion to LDL is decreased in FHTG, while de novo synthesis of LDL is enhanced in FCHL and in some subjects with NIDDM.8,15,20 Four subjects with FCHL have been shown to have distinctly different LDL responses to fish oil when compared to either normal subjects28 or to subjects with familial hypercholesterolemia, lending support for the presence of an association between the underlying disturbance of LDL metabolism and the response of LDL to fish oil.

An additional potential cause for the variability of the LDL response to fish oil observed in published studies is the wide range of dose administered, varying between 3 and 20 g omega-3 fatty acids. The magnitude of plasma triglyceride reductions obtained with fish oil are proportional to the dose given.5,27 and, thus, the degree of inhibition of VLDL triglyceride and apo B synthesis is also likely to be dose related. Therefore, the dose of fish oil may also affect the concentration, composition, and metabolism of LDL, particularly in subjects who derive most of their LDL from VLDL.

The purpose of this study was to determine whether the heterogeneity of the LDL response to fish-oil supplementation in hypertriglyceridemic subjects is associated with the underlying lipoprotein disorder, pretreatment differences in LDL composition, or both. In addition, the effects of two tolerable doses of fish oil were evaluated.

Methods

Subjects

Eighteen outpatients with hypertriglyceridemia (fasting plasma triglyceride greater than the 90th percentile for age and sex) participated in a single-blind, crossover comparison of the effects of fish oil versus safflower oil (placebo). Patients with renal insufficiency, liver disease, uncompensated hypothyroidism, or poorly controlled diabetes (fasting plasma glucose >240 mg/dl) were excluded. Patients using lipid-lowering agents were also excluded, but those taking medication that incidentally affects lipoprotein metabolism, such as antidiabetic, antihypertensive, or antianginal drugs, were included if their medication during the course of the study was unchanged.

Lipoprotein phenotypes were assigned by comparing the lipid values from our patients with reference lipid values obtained from the Lipid Research Clinics Prevalence Study.29 Patients were defined as Type IV if their basal plasma triglyceride levels were above, and LDL cholesterol levels below, the 90th percentile for age and sex. Patients were classified as Type IIB if both basal plasma triglyceride and LDL cholesterol values were above the 90th percentile.

The clinical characteristics of the patients are provided in Table 1. Of the 18 patients, eight had NIDDM and seven had hypertriglyceridemia. Of these, five had Phenotype IV and three had Phenotype IIB. These patients reported no family members with hyperlipidemia or early coronary artery disease (CAD); screening of 11 first-degree relatives of four patients revealed no hyperlipidemia. The mean age (± SE) was 52±5 years, body mass index was 26.7±1.4, plasma cholesterol was 238±19 mg/dl, and plasma triglycerides were 328±72 mg/dl. Antidiabetic therapy included sulfonylureas in five and insulin in three patients; fasting plasma glucose was 143±4 mg/dl, with a range of 102 to 233 mg/dl. Three patients were hypertensive and one was under treatment for CAD. Antihyperten-
sive therapy included angiotensin converting enzyme inhibitors and diuretics in two patients. One patient was taking nitrates and calcium channel blockers for angina.

Of the non-diabetic patients, four were considered to have FCHL as determined by family screening. Three had Phenotype IIIB, and one had Phenotype IV. The mean age was 48 ± 4 years, body mass index was 26.1 ± 2.2, plasma cholesterol was 251 ± 5 mg/dl, and plasma triglyceride was 400 ± 122 mg/dl. These patients were otherwise healthy except for one hypertensive patient who was receiving diuretic therapy.

Three patients were diagnosed with FHTG; all affected family members had hypertriglyceridemia only. This group contained three men (mean age, 55 ± 1 years; body mass index, 30.1 ± 1.1; plasma cholesterol, 238 ± 21 mg/dl; plasma triglycerides, 523 ± 169 mg/dl). These subjects had no concurrent medical illnesses and were not taking medication. Family screening was incomplete in the remaining three patients, and the underlying lipoprotein disorder could not be identified. The mean age of these unclassified patients was 60 ± 3 years, body mass index was 26.1 ± 2.4, plasma cholesterol was 263 ± 35, and plasma triglycerides were 367 ± 91.

The LDL cholesterol/apo B ratio was determined in 12 normal controls of similar ages (49 ± 3 years) as the study subjects. These individuals had no underlying illnesses and no family history of hyperlipidemia or premature CAD. Their mean LDL cholesterol/apo B ratio was 1.40 ± 0.17. Based upon this mean value, we defined a pretreatment LDL cholesterol/apo B ratio of greater than 1.4 as "high," and an initial pretreatment ratio of less than 1.4 as "low."

**Study Design**

The patients were randomized to receive a 1-month dietary supplementation with either 12 g daily safflower oil (SO) or fish oil and were then crossed over to the alternate treatment after a 1-month washout period (Figure 1). The daily dose of fish oil (MaxEPA, R.P. Scherer Corporation, Troy, MI) contained 4 g omega-3 fatty acids (2.6 g eicosapentaenoic acid, 1.4 g docosahexaenoic acid, as analyzed by the manufacturer) provided in 12 capsules (F4.0 dose). Each capsule also contained 9 calories and 6 mg cholesterol. After completing the oil supplement in the crossover arm, 16 patients received 7.5 g omega-3 fatty acids (5 g eicosapentaenoic acid, 2.5 g docosahexaenoic acid) in 15 capsules (Omega-500, Omegapharma, Ltd., St. Louis, MO) for an additional month (F7.5 dose). This preparation contained 9 calories and 1 mg cholesterol per capsule (F7.5 dose). Two patients did not receive the F7.5 dose of fish oil due to excessive flatulence and bloating. Except for mild flatulence andatractions reported by some patients, both doses were otherwise well tolerated.

Patients continued their usual diet in addition to taking the oil capsules throughout the course of the study. Patients were seen at 2-week intervals when capsule counts were obtained to check patient compliance. To ensure that the diet remained constant during the study, 3-day diet histories were performed by a nutritionist at each visit and were analyzed by a computer program (Nutritionist III Software, Silverton, OR). Patients were instructed to avoid eating fish during the course of the study and to abstain from alcoholic beverages for 3 days before each blood test. Blood was drawn in a fasting state for determination of lipid levels and lipoprotein composition before and at the conclusion of each period of supplementation.

The study was approved by the Clinical Research Center and the Medical College of Wisconsin human research committee, and informed consent was obtained from patients before the initiation of the study.

**Procedures**

Lipoprotein separation was achieved by sequential ultracentrifugation by using standard techniques. Following an overnight fast, 40 cc of venous blood was obtained using EDTA (1 mg/dl) as the anticoagulant. The plasma was separated, and preparative ultracentrifugation procedures were initiated within 6 hours of blood collection to quantitatively isolate the lipoprotein fractions. Chylomicrons were separated and removed by centrifugation at 8000 rpm for 30 minutes, and the plasma density was then adjusted to d = 1.0065 kg/l by addition of NaCl. VLDL was isolated by flotation at 37 000 rpm for 19 hours by using a 40.3 T fixed-angle rotor. To separate LDL and HDL, the density of the infranatant was adjusted to 1.063 and was spun at 35 000 rpm for 26 hours. For simplicity, this fraction is subsequently referred to as LDL. High density lipoprotein (HDL) cholesterol was determined from whole plasma after precipitation of apo B-containing lipoproteins with sodium phosphotungstate.

Triglyceride and cholesterol levels in plasma and lipoprotein fractions were determined by using an enzymatic assay (reagents obtained from Boehringer Corporation, Indianapolis, IN). Apo A-1 and apo B determinations in whole plasma and separated lipoprotein fractions were performed by electroimmunoassay by using polyclonal antibodies as described previously. Three patients (one each with FHTG, NIDDM, and an unclassified lipoprotein disorder) did not have apo B assays performed.

**Statistical Analysis**

Analysis of variance for repeated measures of LDL cholesterol, LDL apo B, LDL cholesterol/apo B ratio, body weight, and VLDL triglyceride/apo B ratio was used to
evaluate the effect of fish-oil dose on these variables compared with both baseline levels and safflower-oil treatment. The Pearson correlation coefficient was used to evaluate correlations between the initial LDL cholesterol/apo B ratio and treatment-induced changes in LDL cholesterol, LDL apo B levels, and the LDL cholesterol/apo B ratio. This statistic was also used to assess the associations between treatment-induced changes in LDL cholesterol and apo B and changes in body weight, plasma glucose, and VLDL triglyceride levels.

Results

The basal diet of study patients, shown in Table 2, consisted of 17% protein, 45% carbohydrate, and 33% fat (13% saturated, 13% monounsaturated, 7% polyunsaturated). The percent polyunsaturated fat intake was slightly, but significantly, increased during the SO and F7.5 periods, and the percent saturated fat intake was significantly elevated during the F4.0 period compared with SO treatment. Variability in dietary cholesterol intake was noted, but differences between treatment periods were not significant. Otherwise, the diet remained reasonably constant during the study. Mild increases in body weight were observed during both safflower- and fish-oil supplementation (Table 3). During fish-oil treatment, these increases were significant when compared to baseline (p<0.02), but not when compared to post-SO treatment values (F4 dose: 84.2±3.1 vs. 83.5±3.1 kg, p>0.05; F7.5 dose: 85.2±3.4 kg vs. 84.3±3.3 kg, p>0.05).

Plasma triglyceride levels were significantly decreased at both doses of fish-oil supplementation, but were unchanged during the SO treatment period (Table 3). Total cholesterol levels were not affected during therapy with either fish or safflower oil. HDL cholesterol concentrations were unchanged at the SO and F4 treatment, but showed a trend toward increased levels during fish-oil therapy at the F7.5 dose (p=0.08). Apo A-1 levels were unchanged from pre-supplementation levels during all treatment periods.

At the F4 dose of fish-oil supplementation, 15 of 18 patients had a decrease in VLDL triglyceride levels, for a mean reduction of 33% compared with pretreatment values. At the F7.5 dose, all 16 patients showed VLDL triglyceride reductions for a mean decrease of 57%. These reductions in plasma triglyceride levels that were achieved with fish-oil treatment were significant when compared with either pre-supplementation or SO treatment levels (Table 4). Similar decreases were observed in VLDL cholesterol levels during fish-oil treatment; however, reductions in VLDL apo B concentrations were smaller.

Both LDL cholesterol and LDL apo B levels increased during fish-oil supplementation (Table 5). At the F4 dose, 12 of 18 patients showed increases in LDL cholesterol concentrations, resulting in a mean 9% increase (Figure 2). At the F7.5 dose, 13 of 16 patients showed

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### Table 2. Characteristics of Diet as Determined by 3-Day Dietary Recall during Safflower- and Fish-Oil Supplementation

| Diet nutrients          | Presudy | SO     | F4     | F7.5    |
|-------------------------|---------|--------|--------|---------|
| Calories (kilocalories) | 2251 (199) | 2340 (181) | 2484 (23) | 2410 (181) |
| Protein (% total calories) | 17 (1) | 16 (1) | 16 (1) | 19 (1)   |
| CHO (% total calories)  | 46 (2)  | 47 (2) | 46 (2) | 41 (2)   |
| Saturated fat (% total calories) | 13 (1) | 12 (1) | 13 (1)† | 12 (1)   |
| Monounsaturated fat (% total calories) | 13 (1) | 12 (1) | 13 (1) | 14 (1)   |
| Polyunsaturated fat (% total calories) | 7 (1)  | 9 (1)† | 7 (1) | 10 (1)†  |
| Cholesterol (mg)        | 349 (59) | 316 (51) | 404 (40) | 420 (53) |

Values include nutrients contained in the oil supplements, SE in parentheses.

SO=safflower oil (12 g), F4=fish oil (4 g omega-3 fatty acid dose), F7.5=fish oil (7.5 g omega-3 fatty acid dose), CHO=dietary carbohydrate.

*p<0.05 vs. pre-study value. †p<0.05 vs. SO dose.

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### Table 3. Effect of Fish-Oil Supplementation on Body Weight and Plasma Triglyceride, Cholesterol, HDL Cholesterol, and Apo A-1 Concentrations

| Dose      | Presupplement | Postsupplement |
|-----------|---------------|----------------|
| F4 dose   |               |                |
| (n=18)    |               |                |
| Total TG  | 361 (55)      | 242 (34)*      |
| Total CH  | 262 (18)      | 246 (10)       |
| HDL CH    | 29.9 (3.3)    | 30.7 (2.3)     |
| Apo A-1   | 117 (9)       | 117 (9)        |
| Body wt (kg) | 82.9 (3.3)   | 84.2 (3.1)*    |
| F7.5 dose |               |                |
| (n=18)    |               |                |
| Total TG  | 348 (51)      | 162 (17)*      |
| Total CH  | 248 (11)      | 234 (15)       |
| HDL CH    | 30.8 (3.7)    | 36.6 (2.3)     |
| Apo A-1   | 124 (7)       | 128 (8)        |
| Body wt (kg) | 83.6 (3.2)   | 85.2 (3.4)*    |
| SO dose   |               |                |
| (n=18)    |               |                |
| Total TG  | 326 (48)      | 307 (45)       |
| Total CH  | 240 (11)      | 233 (9)        |
| HDL CH    | 30.6 (2.1)    | 33.3 (3.1)     |
| Apo A-1   | 122 (6)       | 129 (8)        |
| Body wt (kg) | 82.8 (3.1)   | 83.5 (3.1)     |

All values are mg/dl except where indicated, SE in parentheses.

HDL=high density lipoprotein, Apo B=apoB lipoprotein B, TG=triglyceride, CH=cholesterol, SO=safflower oil (12 g), F4=fish oil (4 g omega-3 fatty acid dose), F7.5=fish oil (7.5 g omega-3 fatty acid dose).

*p<0.05 vs. presupplement (baseline) value.
Table 4. Effect of Fish-Oil Supplementation on VLDL Composition

| Dose   | N    | Presupplement | Postsupplement |
|--------|------|---------------|----------------|
| F4 dose |      |               |                |
| VLDL TG| 18   | 275 (55)      | 184 (35)*†     |
| VLDL CH| 18   | 73 (23)       | 52 (14)        |
| VLDL apo B| 15 | 18 (3)       | 15 (5)         |
| F7.5 dose |      |               |                |
| VLDL TG| 16   | 260 (50)      | 112 (17)*‡     |
| VLDL CH| 16   | 53 (10)       | 25 (4)*‡       |
| VLDL apo B| 13 | 15 (4)     | 9 (3)          |
| SO dose |      |               |                |
| VLDL TG| 18   | 242 (45)      | 252 (49)       |
| VLDL CH| 17   | 59 (10)       | 55 (10)        |
| VLDL apo B| 11 | 16 (4)     | 21 (5)         |

All values are mg/dl, SE in parentheses. 
*P<0.05 vs. presupplement (baseline) value, †P<0.05 vs. postsupplement F4 dose, ‡P<0.05 vs. postsupplement SO dose.
VLDL = very low density lipoprotein, TG = triglyceride, CH = cholesterol, apo B = apolipoprotein B, SO = safflower oil (12 g), F4 = fish oil (4.0 g omega-3 fatty acid dose), F7.5 = fish oil (7.5 g omega-3 fatty acid dose).

Table 5. Effect of Fish-Oil Supplementation on LDL Composition

| Dose   | N | Presupplement | Postsupplement |
|--------|---|---------------|----------------|
| F4 dose |   |               |                |
| LDL TG | 18| 41 (6)        | 34 (4)         |
| LDL CH | 18| 138 (10)      | 147 (10)†      |
| LDL apo B | 15 | 92 (10) | 115 (7)*        |
| LDL CH:apo B| 15 | 1.7 (0.2) | 1.3 (0.1)*     |
| F7.5 dose |   |               |                |
| LDL TG | 16| 43 (7)        | 27 (3)         |
| LDL CH | 16| 143 (9)       | 157 (12)†      |
| LDL apo B | 14 | 91 (11) | 106 (9)        |
| LDL CH:apo B| 14 | 1.7 (0.2) | 1.5 (0.1)     |
| SO dose |   |               |                |
| LDL TG | 18| 43 (8)        | 34 (6)         |
| LDL CH | 17| 138 (12)      | 138 (9)        |
| LDL apo B | 11 | 105 (11) | 108 (7)        |
| LDL CH:apo B| 11 | 1.4 (0.1)| 1.3 (0.1)     |

All values are mg/dl, SE in parentheses.

LDL = low density lipoprotein, TG = triglyceride, CH = cholesterol, apo B = apolipoprotein B, SO = safflower oil (12 g), F4 = fish oil (4.0 g omega-3 fatty acid dose), F7.5 = fish oil (7.5 g omega-3 fatty acid dose).

*P<0.05 vs. presupplement value, †P<0.05 vs. postsupplement F4 dose, ‡P<0.05 vs. postsupplement SO dose.

Determinations in LDL cholesterol, producing a similar overall increase. Even larger increases in LDL apo B concentrations were observed, particularly at the F4 dose (28% increase at F4 vs. baseline, p<0.001), as 13 of 15 patients who had apo B levels measured at this dose showed increases (Figure 3). At the F7.5 dose, 9 of 13 evaluable patients showed rises in LDL apo B levels from baseline, for a mean increase of 15%.

Individual LDL responses to fish-oil supplementation varied according to the pretreatment density of LDL, as determined by the LDL cholesterol/apo B ratio (Figures 2, 3, and 4, Table 6). Six patients had ratios below the mean level obtained from normal individuals (1.40±0.17), suggesting the predominance of a particularly dense LDL subspecies. Four patients had FCHL, and two had NIDDM. Among the 12 patients with LDL cholesterol/apo B ratios above 1.4, six had NIDDM, three had FHTG, and three could not be accurately classified. Of the patients with low initial ratios, increases in LDL cholesterol after fish-oil supplementation were noted in five of six patients at the F4 dose, and in five of five evaluable patients at the F7.5 dose (mean increases of 12% and 11%, respectively, Figure 2, Table 6). In contrast, increases in LDL cholesterol were less consistent in the patients with higher initial LDL ratios (greater than 1.4), occurring in 7 of 12 patients at the F4 dose and in 8 of 11 patients at the F7.5 dose (mean increases of 3% and 9%, respectively).

LDL apo B responses to fish-oil supplementation did not always correspond to the observed changes in LDL cholesterol (Figure 3). Patients with low initial LDL ratios had inconsistent LDL apo B responses to fish oil, as four of six patients showed increased LDL apo B levels at the F4 dose, while two of five had increases at the F7.5 dose (mean changes of 2% and -19%, respectively). In contrast, LDL apo B levels were consistently elevated by fish oil in the patients with high initial LDL ratios, as all patients in this category showed LDL apo B elevations at both F4 and F7.5 doses (mean increases of 50% and 52%, respectively). The difference in the LDL apo B response to fish-oil supplementation between patients with high ratios versus patients with low ratios was significant (Table 6). During SO treatment, changes in LDL cholesterol and apo B levels were smaller and were not associated with differences in pretreatment LDL density.

Differences in VLDL composition were observed between the two groups. Patients with low initial LDL ratios had lower initial VLDL triglyceride/apo B ratios when compared with patients with high initial ratios (21.1±13 vs. 46.1±21, p<0.05). In addition, the effect of fish oil on VLDL composition was different between these groups. During fish-oil therapy, the VLDL triglyceride/apo B ratios increased in patients with low pretreatment ratios and decreased in patients with high initial ratios. However, due to the marked variability in VLDL triglyceride and apo B measurements, these differences were not significant.

LDL composition was also differently affected by fish-oil treatment in patients with high pretreatment LDL cholesterol/apo B ratios compared to patients with low ratios (Figure 4). Patients with high initial ratios showed marked decreases in this ratio with fish-oil treatment (percent decreases of 33% and 32%, respectively, at the F4 and F7.5 doses), whereas patients with lower initial ratios showed either unchanged or increased ratios (percent increases of 7% and 34%, respectively, at F4 and F7.5 doses).

Among all subjects, the pretreatment LDL cholesterol/apo B ratio was positively correlated with changes in LDL apo B during the F4 and F7.5 doses of fish-oil supplementation (r=0.41, p=0.06 and r=0.51, p=0.03, respectively), but not during SO supplementation (r=-0.08, p=0.41). On the other hand, significant negative correla-
Figure 2. The effect of fish-oil therapy at 4 g (F4) and 7.5 g (F7.5) omega-3 fatty acids on low density lipoprotein (LDL) cholesterol levels (mg/dl). A. Subjects with LDL cholesterol/apo B ratio less than 1.4. B. Subjects with LDL cholesterol/apo B ratio greater than 1.4. ● = noninsulin-dependent diabetes, ○ = familial combined hyperlipidemia, ■ = unclassified lipoprotein disorder.

Figure 3. The effect of fish-oil therapy at 4.0 g (F4) and 7.5 g (F7.5) omega-3 fatty acids on low density lipoprotein (LDL) apolipoprotein B (apo B) levels (mg/dl). A. Subjects with LDL cholesterol/apo B ratios less than 1.4. B. Subjects with LDL cholesterol/apo B ratios greater than 1.4. ● = noninsulin-dependent diabetes, ○ = familial combined hyperlipidemia, ■ = unclassified lipoprotein disorder.

Discussion

Fish-oil supplementation induced a consistent dose-dependent reduction in plasma and VLDL triglyceride levels in our patients. However, although an overall effect to increase LDL cholesterol and apo B levels was observed, the individual LDL responses to supplementation demonstrated a large degree of variability. This variability could be accounted for, in part, by classifying patients according to their initial LDL cholesterol/apo B ratio. Subjects with low ratios, suggesting a predominance of a particularly dense LDL subspecies, showed no increase in LDL apo B levels, but did have elevations in LDL cholesterol concent-

ions were observed between the initial LDL cholesterol/ apo B ratio and changes in LDL cholesterol during the F4 and F7.5 fish-oil doses ($r = -0.54, p = 0.01$ and $r = -0.50, p = 0.02$, respectively), but not during SO treatment ($r = -0.31, p = 0.11$). The initial LDL cholesterol/apo B ratio was negatively correlated with the fish-oil induced changes in this ratio ($r = -0.89, p < 0.001$ at the F4 dose; $r = 0.89, p < 0.001$ at the F7.5 dose).

During fish-oil treatment, changes in LDL cholesterol, LDL apo B, or the LDL cholesterol/VLDL apo B ratio did not correlate with changes in the following; plasma levels of VLDL triglyceride or VLDL apo B, the VLDL triglyceride/ apo B ratio, fasting plasma glucose, or body weight.
trations. Subjects with high initial LDL ratios, suggesting the predominance of more buoyant LDL particles, had marked increases in LDL apo B concentrations, yet smaller increases in LDL cholesterol levels, when challenged with fish oil. Since LDL apo B levels are directly proportional to LDL particle number,23 fish-oil treatment induced an absolute increase in LDL particles in these subjects. In addition, the LDL cholesterol/apo B ratio decreased, suggesting that LDL composition was also altered in these subjects during fish-oil treatment.

The magnitude of the dose-dependent reductions that occurred in VLDL triglyceride, VLDL cholesterol, or VLDL apo B levels did not correlate with the observed changes in LDL cholesterol or apo B concentrations, regardless of whether the initial LDL ratio was high or low. On the other hand, the baseline LDL cholesterol/apo B ratio correlated with fish-oil induced changes in both LDL composition and LDL apo B and cholesterol levels. These preliminary data suggest that, whereas the fish-oil induced effects on LDL are dose dependent, the effects on LDL are more closely linked to underlying differences in LDL composition and hence to differences in LDL metabolic behavior.

The dense, apo B-enriched LDL subspecies shows an altered kinetic behavior with increased turnover.25 A decreased cholesterol/apo B ratio, suggesting the presence of this dense LDL subspecies, has also been associated with an increased incidence of CAD,36,37 perhaps due to distinctive kinetic properties.34 LDL apo B overproduction, the dense LDL subspecies, and increased CAD risk are all characteristics of abnormal lipoprotein metab-

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**Table 6. Effect of Baseline LDL Cholesterol-Apo B Ratio on Fish-Oil Induced Changes in LDL and VLDL Composition**

|                  | N  | Baseline | Mean difference from baseline |             |
|------------------|----|----------|------------------------------|-------------|
|                  |    |          | F4.0                         | F7.5        | SO          |
| LDL cholesterol  |    |          |                              |             |
| (mg/dl)          |    |          |                              |             |
| LDL ratio<1.4    | 6  | 147 (15) | +18 (8)†                     | +15 (6)     | +6 (11)     |
| LDL ratio>1.4    | 12 | 132 (13) | +4 (9)                       | +13 (13)†   | −4 (8)      |
| LDL apo B (mg/dl)|    |          |                              |             |
| LDL ratio<1.4    | 6  | 125 (17) | +3 (8)†                      | −24 (12)‡   | +5 (9)      |
| LDL ratio>1.4    | 9  | 71 (9)   | +36 (5)*                     | +37 (10)*   | +4 (13)     |
| LDL cholesterol:apo B ratio (SE) |    |          |                              |             |
| LDL ratio<1.4    | 6  | 1.2 (0.02) | +0.1 (0.1)*                 | +0.4 (0.1)* | 0 (0.1)     |
| LDL ratio>1.4    | 9  | 2.1 (0.02) | −0.7 (0.1)*                 | −0.7 (0.2)* | −0.3 (0.2)  |
| VLDL TG:apo B ratio (SE) |    |          |                              |             |
| LDL ratio<1.4    | 6  | 32 (30)  | −11 (25)                     | +14 (25)    | 0.0 (3)     |
| LDL ratio>1.4    | 9  | 46 (21)  | −11 (25)                     | −15 (25)    | −12 (35)    |

The values include SE in parentheses.

LDL = low density lipoprotein, apo B = apolipoprotein B, VLDL = very low density lipoprotein, SO = safflower oil (12 g); F4 = fish oil (4.0 g omega-3 fatty acid dose), F7.5 = fish oil (7.5 g omega-3 fatty acid dose), LDL = low density lipoprotein.

†p<0.05 vs. presupplement (baseline) value, ‡p<0.05 vs. postsupplement SO dose, €p<0.05 between groups (subjects with LDL ratio <1.4 vs. subjects with LDL ratio >1.4), unpaired t test.
olism, which are found in FCHL and in some subjects with NIDDM.\textsuperscript{12,14,19,20} Of our six patients with a predominance of these dense LDL particles, FCHL was identified in four and NIDDM in two patients. Therefore, the classification of patients on the basis of low LDL ratios may identify those patients with underlying lipoprotein disorders that are associated with increased LDL turnover. Since full family screening was not possible in patients with NIDDM, concurrent lipoprotein disorders may have been present; thus, the two patients with low LDL ratios and NIDDM may also have had FCHL. Failor et al.\textsuperscript{24} have shown that LDL apo B concentrations in four patients with FCHL were not significantly changed during fish-oil treatment, whereas decreases were observed in normal subjects. Further work will be required to assess whether the association between low pretreatment LDL ratios and the fish-oil induced LDL responses observed in this study are the result of increased LDL turnover in general or are due to abnormalities in lipoprotein composition and metabolism specific to FCHL.

Although FCHL and FHTG are associated with specific abnormalities of lipoprotein composition and metabolism, NIDDM is characterized by marked heterogeneity. A triglyceride-enriched VLDL, overproduction of VLDL triglyceride and apo B, and decreased VLDL catabolism via the delipidation pathway have all been described.\textsuperscript{8,23} Compared with normal controls, LDL production and catabolic rates may be increased, decreased, or unchanged and are linked to the degree of glycemic control.\textsuperscript{18,20} In this study, the LDL response to fish oil in the diabetic subjects was also heterogeneous. However, the changes in LDL apo B and cholesterol concentrations and alterations in LDL composition observed in response to fish oil appeared to depend upon the pretreatment LDL cholesterol/apo B ratio and were independent of glycemic control, suggesting that the underlying composition and kinetic behavior of LDL are more important determinants of the LDL response than the diabetic state per se.

The mechanism behind the paradoxical effect of fish oil on raising LDL apo B levels specifically in patients without a predominance of dense LDL, despite lowering VLDL concentrations, is unknown. Alterations in VLDL composition affect LDL production, as suggested by evidence that synthesis of dense, triglyceride-depleted VLDL is converted to LDL much more efficiently than is triglyceride-rich VLDL of lower density.\textsuperscript{13,28} In this study, the VLDL triglyceride/apo B ratio was decreased during fish-oil administration in patients with high initial LDL ratios. This suggests the formation of a smaller, triglyceride-depleted VLDL particle during fish-oil treatment, a finding consistent with previous reports.\textsuperscript{2,40} Therefore, the increased production of this dense VLDL may have served to enhance the conversion of VLDL to LDL, thereby increasing LDL synthesis via the delipidation pathway. A second possibility is that LDL clearance may have been decreased. Omega-3 fatty acids have been shown to decrease hepatic LDL receptor activity in rats\textsuperscript{41} and in miniature pigs\textsuperscript{42} and to reduce the specific binding of LDL to HepG2 cells.\textsuperscript{43} Although LDL apo B clearance was unchanged during fish-oil treatment in normal subjects,\textsuperscript{44} the LDL receptor number or the affinity of this receptor for LDL may decrease in some dyslipidemic individuals treated with fish oil. A third potential mechanism is that fish oil encouraged the direct synthesis of increased amounts of dense LDL in these patients, resulting in increased concentrations of smaller LDL particles. To our knowledge, the effect of fish oil on de novo LDL synthesis in normal or hyperlipoproteinemic patients has not been studied.

On the other hand, LDL apo B concentrations were not increased by fish-oil treatment in those patients with initial low LDL cholesterol/apo B ratios, most of whom had FCHL. In these subjects, the VLDL triglyceride/apo B ratios were initially low and were unchanged during fish-oil supplementation. Therefore, these patients may have initially had a predominance of triglyceride-depleted VLDL particles whose composition and catabolism via the delipidation pathway were not affected by fish-oil treatment. Changes in LDL apo B levels would thus be expected to move closely parallel changes in VLDL concentrations and may explain why paradoxical increases in LDL apo B levels were not observed in these patients. However, if this mechanism were correct, both a significant decrease in LDL apo B levels and an inverse correlation between changes in the VLDL triglyceride/apo B ratio and LDL apo B levels would be anticipated, neither of which were observed in this study. An alternative possibility is significant de novo synthesis of LDL, which may represent a mechanism whereby the VLDL and LDL responses to fish oil remained independent, particularly if a large fraction of LDL were derived from this pathway. Direct synthesis of LDL has been described in both FCHL\textsuperscript{14,15} and NIDDM\textsuperscript{20} and, therefore, may have been operative in these patients. Further study will be required to determine which of these mechanisms is correct.

Several limitations of this study require mention. Because we used a fixed-angle rotor rather than cumulatively flotation to isolate lipoprotein fractions, separation may have been incomplete.\textsuperscript{13,44} Since intermediate density lipoprotein (IDL) is enriched in cholesterol and apo B compared to VLDL, incomplete separation may have artificially elevated VLDL cholesterol and apo B levels and contributed to the marked variability observed in the VLDL apo B triglyceride ratio. Inclusion of some VLDL in the LDL fraction would be more likely to affect LDL triglyceride levels than cholesterol or apo B and should not alter inferences about LDL composition based upon the ratio of cholesterol and apo B levels. An additional limitation of this study is that the LDL cholesterol/apo B ratio was assumed to indicate the presence of the dense LDL subspecies. Since this ratio reflects an average composition of all LDL particles, the presence of dense LDL may have gone undetected if sufficient particles of more buoyant density were present. Direct quantification of LDL subtypes by gradient gel electrophoresis,\textsuperscript{45} for example, would provide more precise information on the response of particular LDL subspecies to fish-oil treatment. Finally, since concentrations of IDL are frequently elevated in hypertriglyceridemia, our conclusions are limited by the inclusion of IDL (1.006 to 1.019) in the "LDL" fraction (1.005 to 1.053). The specific effect of fish oil on IDL in patients with hypertriglyceridemia is not known, although one report has suggested that IDL triglyceride,
and to a much smaller extent, cholesterol and apo B levels, may decrease. In our study, although plasma and VLDL triglyceride levels were similar in patients with LDL ratios above and below 1.4 (plasma triglyceride levels of 399±65 and 353±83 mg/dl, respectively), IDL concentrations may not have been equivalent in these two groups. In addition, differences in LDL composition, metabolic behavior, or both may have affected the LDL response to fish oil, thereby contributing, in part, to the different effects observed in the LDL plus LDL fraction. Future studies will require the complete separation of all lipoprotein fractions to better define the specific effect of fish oil on LDL apo B and cholesterol levels independent of IDL.

Our data suggest that the effect of fish oil on LDL concentration and composition depend upon the underlying features of LDL metabolism before treatment. The association between LDL metabolic behavior and LDL composition provides a means to estimate the response of LDL to fish oil by using the LDL cholesterol/apo B ratio. However, the full characterization of LDL metabolism and evaluation of the LDL precursor pool should allow a more complete assessment of the effect that fish oil has on LDL concentration and composition. This will entail detailed characterization of the VLDL and LDL kinetic behavior before and after fishoil supplementation. Further studies are therefore necessary to extend our findings before the efficacy of fish oil as a treatment for hypertriglyceridemia can be fully evaluated.

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Index Terms: fish oil • omega-3 fatty acids • low density lipoproteins • hypertriglyceridemia • type IV hyperlipidemia