Plasma Triglyceride and LDL Heterogeneity in Familial Combined Hyperlipidemia

John E. Hokanson, Melissa A. Austin, Alberto Zambon, and John D. Brunzell

Familial combined hyperlipidemia (FCHL) is a genetic disorder characterized by increases in plasma cholesterol and/or triglyceride, elevated apolipoprotein B, and heterogeneous low density lipoprotein (LDL). To examine the relation between plasma triglyceride concentrations and LDL heterogeneity, 13 hypertriglyceridemic FCHL patients with a predominance of small LDL (LDL subclass phenotype B) were treated with gemfibrozil. The distribution of LDL was determined using nondenaturing gradient gel electrophoresis and nonequilibrium density gradient ultracentrifugation. Mean plasma triglyceride levels decreased 55% (p<0.01) after 3 months of treatment. Mean LDL peak particle size remained small (247±4 versus 249±5 Å), and the correlation between change in plasma triglyceride concentrations and a change in LDL peak particle size was not significant. Individual changes in LDL flotation rate (Rf) were, however, inversely correlated with changes in triglyceride concentration (R=0.60, p<0.05). Although mean LDL Rf increased during treatment (p<0.005) due to an increase in buoyant LDL, dense LDL remained elevated compared with that of a control population. Thus in FCHL patients, small, dense LDL persists despite decreases in plasma triglyceride concentrations. (Arteriosclerosis and Thrombosis 1993;13:427–434)

KEY WORDS • triglycerides • LDL • familial combined hyperlipidemia

Low density lipoprotein (LDL) cholesterol levels are a major risk factor for coronary disease.1-4 LDL, however, has long been recognized as a heterogeneous class of particles, varying in flotation rate, density, and size.5-7 A predominance of small LDL (LDL subclass phenotype B) has been shown to be associated with increased risk of myocardial infarction and an atherogenic lipoprotein profile.8,9 In cross-sectional studies, LDL subclass phenotype B is strongly associated with an increase in plasma triglyceride concentrations among patients with myocardial infarction,8 in normal individuals,10 and in families with familial combined hyperlipidemia (FCHL).11 In a subset of the Framingham cohort, small LDL particles were also shown to be associated with high plasma triglyceride concentrations.12

LDL flotation rate has also been shown to be associated with plasma triglyceride concentrations in FCHL, familial hypertriglyceridermia, familial hypercholesterolemia, and normolipidemic subjects.13 In the same report, Failor et al13 showed that when plasma triglyceride concentrations were perturbed in individuals with FCHL, LDL flotation rate varied in relation to triglyceride. This relation paralleled a linear regression model for the same two variables between individuals in a cross-sectional analysis of the entire population.

FCHL is a relatively common form of hyperlipidemia among families of survivors of myocardial infarctions14 and among families with primary hypertriglyceridermia.15 Although the lipoprotein phenotype varies in FCHL, with very low density lipoprotein (VLDL) levels, LDL levels, or both intermittently elevated within an individual, plasma apolipoprotein B levels usually remain elevated.16 LDL particles in FCHL families appear to be heterogeneous, with an increased frequency of small, dense LDL particles.17 In FCHL, a common major gene and a significant multifactorial inheritance component apparently influence the inheritance of LDL subclass phenotype B.11 Although LDL subclass phenotypes are associated with significant differences in apolipoprotein B concentrations, the increase in apolipoprotein B concentrations in FCHL families cannot be fully attributed to the putative LDL subclass phenotype B gene.11 The overall apolipoprotein B distribution in FCHL family members may be due to a distinct subset of individuals with phenotype B who are susceptible to even higher elevations of apolipoprotein B.18 Therefore, an additional gene or genes might be present in FCHL, which also contributes to apolipoprotein B levels.

The current prospective intervention study investigated the relation of changes in LDL size and density with changes in plasma triglyceride concentrations within individual FCHL patients. Specifically, this study addressed the following hypotheses: 1) Does LDL subclass phenotype B persist within an FCHL patient...
Despite a decrease in plasma triglyceride concentrations, or does the LDL subclass phenotype change in concert with changes in plasma triglyceride concentrations? and 2) Do the flotation properties of LDL and other apolipoprotein B-containing lipoproteins change when plasma triglyceride concentrations are reduced?

Methods

Study Subjects

Patients for the study were selected from two sources. First, 27 individuals from previously studied FCHL families were screened for inclusion into the study group. The inclusion criteria were 1) plasma triglyceride concentrations above the Lipid Research Clinics Prevalence Study age- and gender-specific 90th percentile levels, 2) LDL subclass phenotype B as determined by gel electrophoresis (GGE), and 3) the absence of any drugs known to affect plasma lipid concentrations. Of the 11 patients who were eligible for the study, three subjects declined participation and one subject did not complete the treatment protocol due to a femur fracture, resulting in seven patients for whom complete data were available. The second group consisted of patients from the Lipid Clinic at the University of Washington Medical Center with a family history and lipid abnormalities consistent with FCHL and elevated apolipoprotein B levels. These patients were screened for inclusion into this study using the same criteria. Six patients from this group were studied, for a total of 13 patients.

An age- and gender-matched control population was selected from 72 individuals not taking lipid-altering drugs who were recruited through advertisements at the University of Washington. The first individual sampled who matched for gender and the closest age (in years) to a patient was chosen as the control subject.

The present study was approved by the Human Subjects Review Committee of the University of Washington, and informed consent was obtained from all subjects.

Protocol

An initial baseline blood sample was collected from all eligible patients and the control population. FCHL patients then completed a medical history, received a thorough physical examination, a medical history, and a medication history. All patients then completed a medical history, received a medical history, and an informed consent was obtained from all subjects.

Non-denaturing Gradient Gel Electrophoresis

To estimate LDL size and to determine LDL subclass phenotypes, 6 μL of plasma was electrophoresed after being diluted 4:1 (vol/vol) with a 50% sucrose/0.01% sodium dodecyl sulfate (SDS)/Na2EDTA (0.003 M) buffer at pH 8.3. Gels were stained with oil red O and scanned at 490 nm. Coomassie Blue was used to stain the following standards of known diameter for calibration purposes: ferritin (12.2 nm), thyroglobulin (17.0 nm), thyroglobulin dimer (23.6 nm; Pharmacia), and carboxyethylated latex beads (38.0 nm; Dow Chemical). A standard curve was calculated relating diameter and migration distance by using a quadratic equation least-squares best fit, and the diameters of the major LDL peaks in sample lanes were estimated from that equation. Krauss and Burke have shown this method to be more sensitive than either flotation rate or equilibrium density ultracentrifugation at discriminating individual subpopulations of LDL. Reproducibility of peak particle diameter by this assay on a single subject's plasma frozen at –70°C for up to 11 months and electrophoresed 87 consecutive times in our laboratory showed a coefficient of variation of 2.5%.

LDL subclass phenotypes A, B, or I were determined in a blinded fashion by three independent observers using previously developed criteria. LDL subclass phenotype A generally has the major peak diameter of greater than 255 Å, with skewing to the right due to minor peaks of smaller diameter. Scans of major peak particle diameter less than or equal to 255 Å and with skewing of the curve to the left, due to minor peaks of larger diameter, were classified as LDL subclass phenotype B. Phenotype 1 (intermediate) consists of one broad major peak or two distinct peaks, with a peak particle diameter between 255 and 260 Å. When there was not complete agreement between observers, the sample was electrophoresed again by following the same procedure.

Nonequilibrium Density Gradient Ultracentrifugation (DGUC)

As previously described, this technique is a modification of single vertical-spin density gradient ultracentrifugation, which is designed to optimize the resolution of apolipoprotein B-containing lipoproteins. A discontinuous salt gradient was formed in a Sorvall TV-865B tube (DuPont, Wilmington, Del.) by pipetting 2 mL of plasma adjusted to a density of 1.08 g/mL and a final volume of 5 mL under 12 mL of a 1.06 g/mL NaCl solution. Samples were centrifuged at 65,000 rpm for 90 minutes at 10°C. Thirty-eight fractions (0.45 mL/tube) were collected from the bottom of the centrifuge tube (flow rate of 1.7 mL/min). LDL buoyancy (Rf) was calculated as the LDL peak fraction divided by the total number of fractions collected. The intra-assay coefficient of variation of the values for nine pairs of samples was 0.2%.

Lipid and Apolipoprotein Analysis

VLDL was isolated by using standard preparative ultracentrifugation, and high density lipoprotein (HDL) was separated from LDL using dextran sulfate. Plasma total, VLDL, LDL, and HDL cholesterol fractions were determined by enzymatic methods (Boehringer Mannheim, Indianapolis, Ind.). Plasma total, VLDL, LDL, and HDL triglycerides were measured using an enzymatic kit (Sigma Chemical Co., St. Louis, Mo.). Apolipoprotein B was analyzed by immunonephelometry (Behring Diagnostic, Sommerville, N.J.).
months, mean LDL cholesterol concentration remained 17%. After 3 month of therapy, there was a significant increase in the treatment: 59% and 52%, respectively, after 1 month and 61% and 55% after 3 months of treatment. After 1 and the reduction persisted after 3 months of treatment plasma triglyceride concentration was reduced by 53%, than that of the control population (Table 1).

LDL. The LDL in the study group was also less buoyant 95th percentile of a normal population. 28 Based on the mean apolipoprotein B level was also greater than the triglyceride concentration before treatment was elevated. 28

Statistical Analysis

Two-tailed, paired, Student’s t test was used to compare mean values at 1 and 3 months of treatment to baseline mean values. Pearson correlation coefficients were calculated for assessing associations between changes in plasma triglyceride concentrations and changes in LDL peak particle size and LDL Rf. A probability value of 0.05 or less was used for statistical significance.26

Group DGUC profiles were compared by calculating the mean and 95% confidence intervals of the difference between baseline and 3 months of treatment for each fraction.27

Results

The study subjects consisted of eight men and five women with FCHL with a mean age of 52 years (Table 1). As expected from the selection criteria, mean plasma triglyceride concentration before treatment was elevated. Mean apolipoprotein B level was also greater than the 95th percentile of a normal population.28 Based on the inclusion criteria, all of the patients had LDL subclass phenotype B, and as a result, the group overall had small LDL. The LDL in the study group was also less buoyant than that of the control population (Table 1).

After 1 month of treatment with gemfibrozil, mean plasma triglyceride concentration was reduced by 53%, and the reduction persisted after 3 months of treatment (Table 2). Both mean VLDL triglyceride and VLDL cholesterol concentrations decreased significantly during treatment: 59% and 52%, respectively, after 1 month and 61% and 55% after 3 months of treatment. After 1 month of therapy, there was a significant increase in the mean LDL cholesterol concentration of 17%. After 3 months, mean LDL cholesterol concentration remained elevated from baseline although not significantly so (p=0.17). HDL cholesterol concentration increased 14%, a small but statistically significant change that persisted for the group throughout the treatment protocol. This was due to an increase in the concentration of HDL1 cholesterol (31±7 mg/dL at baseline versus 36±6 mg/dL after 3 months of treatment, p<0.005), while HDL2 cholesterol concentration was not significantly different (4.2±2 mg/dL versus 5.0±2.4 mg/dL).

Change in mean LDL peak particle diameter with treatment was not statistically significant; the value changed less than 1% between baseline and 3 months of treatment (Table 2). There was, however, a statistically significant overall increase in the buoyancy of LDL after 1 month of gemfibrozil therapy, which was further increased at 3 months of therapy.

Individual responses due to gemfibrozil show a strong trend toward a decrease in plasma triglyceride after 1 month, with only moderate subsequent changes (Figure 1A). Although the magnitude of the HDL cholesterol changes was not as great as triglyceride (Figure 1B), an increase in HDL was observed at both 1 and 3 months of gemfibrozil treatment.

Individual LDL cholesterol (Figure 1C) and apolipoprotein B (Figure 1D) responses were variable during the course of gemfibrozil treatment. Apolipoprotein B concentrations paralleled the changes in LDL cholesterol, with the individual expressing the maximum increase in LDL (100 mg/dL) also having the largest increase in apolipoprotein B (54 mg/dL), and the individual with the largest decrease in LDL (70 mg/dL) also showing the greatest decrease in apolipoprotein B (Table 3). The correlation between changes in LDL cholesterol and changes in apolipoprotein B concentrations after 3 months of gemfibrozil therapy was statistically significant (R=0.82, p<0.001). Overall, nine individuals had an increase in LDL cholesterol concentration after 3 months

### Table 1. Baseline Characteristics of FCHL Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Gender</th>
<th>Plasma triglyceride (mg/dL)</th>
<th>Cholesterol (mg/dL)</th>
<th>ApoB (mg/dL)</th>
<th>LDL peak diameter (A)</th>
<th>LDL genotype</th>
<th>LDL Rf</th>
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</thead>
<tbody>
<tr>
<td>G.A.</td>
<td>42</td>
<td>M</td>
<td>588</td>
<td>240</td>
<td>123</td>
<td>92</td>
<td>25</td>
<td>B</td>
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<tr>
<td>E.R.</td>
<td>64</td>
<td>F</td>
<td>382</td>
<td>195</td>
<td>66</td>
<td>87</td>
<td>42</td>
<td>B</td>
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<tr>
<td>L.H.</td>
<td>32</td>
<td>F</td>
<td>192</td>
<td>206</td>
<td>37</td>
<td>132</td>
<td>37</td>
<td>B</td>
</tr>
<tr>
<td>E.C.</td>
<td>72</td>
<td>F</td>
<td>230</td>
<td>274</td>
<td>39</td>
<td>196</td>
<td>39</td>
<td>B</td>
</tr>
<tr>
<td>A.C.</td>
<td>74</td>
<td>M</td>
<td>334</td>
<td>188</td>
<td>64</td>
<td>96</td>
<td>28</td>
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<tr>
<td>R.C.</td>
<td>39</td>
<td>M</td>
<td>872</td>
<td>255</td>
<td>169</td>
<td>63</td>
<td>22</td>
<td>B</td>
</tr>
<tr>
<td>D.Hu.</td>
<td>50</td>
<td>F</td>
<td>267</td>
<td>252</td>
<td>52</td>
<td>153</td>
<td>48</td>
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<tr>
<td>D.Hl.</td>
<td>40</td>
<td>M</td>
<td>355</td>
<td>307</td>
<td>90</td>
<td>184</td>
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<td>B</td>
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<tr>
<td>T.M.</td>
<td>61</td>
<td>M</td>
<td>573</td>
<td>261</td>
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<td>133</td>
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<tr>
<td>A.G.</td>
<td>63</td>
<td>M</td>
<td>363</td>
<td>257</td>
<td>80</td>
<td>138</td>
<td>39</td>
<td>B</td>
</tr>
<tr>
<td>J.A.</td>
<td>32</td>
<td>M</td>
<td>208</td>
<td>262</td>
<td>41</td>
<td>177</td>
<td>44</td>
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<tr>
<td>F.L.</td>
<td>53</td>
<td>M</td>
<td>229</td>
<td>245</td>
<td>53</td>
<td>161</td>
<td>31</td>
<td>B</td>
</tr>
<tr>
<td>R.D.</td>
<td>53</td>
<td>F</td>
<td>258</td>
<td>247</td>
<td>42</td>
<td>161</td>
<td>44</td>
<td>B</td>
</tr>
</tbody>
</table>

Mean 51.9* 373.2 245.3 73.4 136.4 35.5 147.3 247.5† 0.244†

SD 14.3 196.4 32.7 38.8 41.2 8.1 31.6 4.2 0.031

FCHL, familial combined hyperlipidemia; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; apo, apolipoprotein; Rf, LDL flotation rate.

*Not significantly different than 1:1 age- and gender-matched control subjects, n=13 (46.2±8.4).
†p<0.001 compared with mean value for matched control subjects (259.5±9.9).

p<0.001 compared with mean value for matched control subjects (0.317±0.017).
TABLE 2. Mean Lipid and Apolipoprotein B Concentrations and LDL Physical Properties at Baseline and at 1 and 3 Months of Treatment With Gemfibrozil

<table>
<thead>
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<th></th>
<th>Baseline</th>
<th>1 Month</th>
<th>3 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride*</td>
<td>Plasma</td>
<td>373 ± 196</td>
<td>177 ± 76†</td>
</tr>
<tr>
<td></td>
<td>VLDL</td>
<td>316 ± 199</td>
<td>129 ± 77†</td>
</tr>
<tr>
<td></td>
<td>LDL</td>
<td>39 ± 13</td>
<td>31 ± 9</td>
</tr>
<tr>
<td></td>
<td>HDL</td>
<td>14 ± 4</td>
<td>10 ± 4‡</td>
</tr>
<tr>
<td>Cholesterol*</td>
<td>Plasma</td>
<td>245 ± 33</td>
<td>235 ± 43</td>
</tr>
<tr>
<td></td>
<td>VLDL</td>
<td>73 ± 39</td>
<td>35 ± 15‡</td>
</tr>
<tr>
<td></td>
<td>LDL</td>
<td>136 ± 41</td>
<td>159 ± 39†</td>
</tr>
<tr>
<td></td>
<td>HDL</td>
<td>36 ± 8</td>
<td>42 ± 8‡</td>
</tr>
<tr>
<td>Apolipoprotein B*</td>
<td>LDL</td>
<td>147 ± 32</td>
<td>140 ± 29</td>
</tr>
<tr>
<td></td>
<td>LDL</td>
<td>247 ± 4</td>
<td>251 ± 7</td>
</tr>
</tbody>
</table>
| LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; Rf, LDL flotation rate. All data are expressed as mean ± SD.

*Concentrations in milligrams per deciliter.

Statistical significance compared with baseline values was determined by paired, two-tailed, Student's t test: †p < 0.05; *p < 0.01; ‡p < 0.005.

of therapy while four individuals had a decrease in LDL cholesterol concentration during the same treatment.

The correlation between the change in plasma triglyceride and the change in LDL peak particle diameter was not statistically significant (Figure 2A). LDL subclass phenotype B persisted in all but two FCHL patients after 3 months of treatment, and in both cases LDL converted to an intermediate phenotype (Figure 2B). In one of these patients, LDL had transiently converted to phenotype A at 1 month of treatment. However, there was a statistically significant correlation between change in triglyceride and overall change

FIGURE 1. Line plots of plasma triglyceride (panel A), high density lipoprotein (HDL) cholesterol (panel B), low density lipoprotein (LDL) cholesterol (panel C), and apolipoprotein (apo) B (panel D) for 13 familial combined hyperlipidemia patients at baseline, at 1 month, and at 3 months of gemfibrozil treatment. All values are in milligrams per deciliter. Significance levels compared with mean baseline values by paired Student's t test: *p < 0.05; **p < 0.01; ***p < 0.005.
in LDL Rf ($R=0.60$, $p<0.05$; Figure 2C). Of the 13 patients studied, all but one individual had an increase in LDL Rf (Figure 2D).

An example of densitometric scans of gradient gels (Figure 3A) and DGUC profiles (Figure 3B) at baseline and at 3 months of treatment shows the characteristic
Figure 3. Densitometric scans of gradient gel electrophoresis (panel A) and density gradient ultracentrifugation lipoprotein cholesterol profiles (panel B), untreated (dashed line) and after 3 months of treatment with gemfibrozil (solid line) for patient L.H. The y axis (optical density) was adjusted so that the major peak of low density lipoprotein (LDL) was matched in amplitude. The major peak particle diameter was 248 Å at baseline and 246 Å on therapy (within the variation of the assay) (panel A). Cholesterol profiles are expressed as concentration (milligrams per deciliter) in each fraction. Baseline LDL flotation rate (Rf) was 0.243 and on therapy was 0.316 (panel B). VLDL, very low density lipoprotein.

Response to gemfibrozil therapy. Although the peak particle diameter changed very little, there was an increase in the concentration of the larger-diameter subfractions of LDL relative to the major peak. Despite these changes, the LDL of this patient was still clearly a phenotype B. The DGUC profile (Figure 3B) indicates an overall increase in LDL buoyancy during treatment. Among all subjects, nine patients showed an increase in the concentration of larger-diameter subfractions of LDL relative to the major peak of LDL. All of these patients had an increase in LDL Rf based on DGUC. Three patients had an increase in the diameter of the major peak of LDL only, with one patient showing no increase in larger-diameter LDL particles.

The distribution of the mean values and 95% confidence intervals of plasma lipoprotein cholesterol by DGUC is shown for patients at baseline (Figure 4A), after 3 months of gemfibrozil therapy (Figure 4B), and for the control population (Figure 4C). A decrease in mean VLDL cholesterol distribution and an increase in the total LDL cholesterol distribution can be seen in the treated group compared with baseline. These changes in the distribution of cholesterol across the density gradient during treatment can best be visualized by calculating the mean difference in the percentage of total plasma cholesterol and the 95% confidence limits for each fraction (Figure 4D): this plot shows that the increase in LDL cholesterol was specific to buoyant LDL and was accompanied by a decrease in the less buoyant LDL and VLDL.

The difference between the baseline DGUC distribution of these patients with the matched control population shows the characteristic lipid profile of FCHL with an increase in less buoyant LDL, intermediate density lipoprotein (IDL), and VLDL and a corresponding decrease in buoyant LDL and HDL (Figure 4E). After treatment, the distribution of VLDL and LDL among these patients appears more similar to that of the control group (Figure 4F). However, there was still a statistically significant increase in less buoyant LDL and a decrease in HDL among the treated FCHL patients.

Discussion

In this study, none of the 13 FCHL patients with LDL subclass phenotype B converted to an LDL subclass phenotype A with 3 months of gemfibrozil treatment, whereas two patients converted to an intermediate phenotype. This result was obtained despite substantial reductions in plasma triglyceride concentrations. Because the dichotomous classification of LDL subclass phenotype may have limited the ability to detect an association between LDL heterogeneity and plasma triglyceride concentrations, LDL peak particle diameter was also evaluated as a continuous variable. The correlation between change in plasma triglyceride concentration and change in LDL peak particle diameter with gemfibrozil treatment was not statistically significant. Therefore, despite the strong association between plasma triglyceride concentrations and LDL peak particle diameter described in previous cross-sectional studies, changes in plasma triglyceride concentrations do not substantially alter LDL peak particle diameter within these individual FCHL patients. These results suggest that plasma triglyceride concentrations do not control LDL subclass phenotype B expression.
in FCHL. Despite the persistence of LDL subclass phenotype B, there were changes in the overall size distribution of LDL in these patients. An increase in the concentration of the larger-diameter subfractions of LDL relative to the major peak of LDL was seen in most patients.

The changes in LDL cholesterol concentrations with treatment reported in this study were also not distributed evenly throughout the flotation gradient of LDL (Figure 4D). Although specific subpopulations of LDL are not clearly discriminated in DGUC flotation profiles, cholesterol in the less buoyant fractions of LDL decreased slightly, while the mean distribution of cholesterol increased significantly in the more buoyant fractions of LDL. The distribution of cholesterol across the DGUC gradient in these FCHL patients treated with gemfibrozil compared with the control population (Figure 4F) shows that the less buoyant fractions of LDL remained elevated. Therefore, despite the increase in large, buoyant LDL and the consequent increase in R, the small, dense LDL fraction persisted during gemfibrozil treatment.

Combining the LDL size distribution and flotation data, large, buoyant LDL cholesterol is increased, possibly due to an increase in the production of this fraction.
of LDL or an increase in the cholesteryl ester content of LDL with a subsequent increase in the buoyancy of LDL. This is consistent with the significant increase in the mean DGUC profile between fractions 11–17 (Figure 4D) and the relative increase in concentration of LDL of larger diameter than the major peak in nine of 13 patients during treatment. Also, in accordance with the persistence of LDL subclass phenotype B, the particles in fractions 9 and 10 of the DGUC profile remained elevated in these FCHL patients.

The two FCHL subjects in this study who changed from LDL subclass phenotype B to an intermediate phenotype could represent phenocopies. That is, in these individuals, LDL subclass phenotype may be controlled by factors that relate to plasma triglyceride concentrations and are unrelated to the proposed single gene controlling LDL subclass phenotype B.9,11

The baseline lipid parameters in this study group, in particular the high triglyceride and low HDL cholesterol concentrations, would predict a high risk of cardiac events that can be favorably altered with gemfibrozil treatment.29 It is possible that the changes in triglyceride and HDL cholesterol may be offset by the increase in cholesterol in buoyant LDL and the persistence of small, dense LDL.

Based on these results, small, dense LDL appears to be a persistent trait in hypertriglyceridemic FCHL patients with LDL subclass phenotype B, despite a lowering of plasma triglyceride concentrations with gemfibrozil. Therefore, in FCHL, LDL subclass phenotype B may occur as separate processes.

Acknowledgments

The authors wish to thank Carrie Nelson for technical expertise in GGE and DGUC and Kody Wallace for assistance in the recruitment of patients.

References

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doi: 10.1161/01.ATV.13.3.427

*Arteriosclerosis, Thrombosis, and Vascular Biology* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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