Small, Dense LDL and Elevated Apolipoprotein B Are the Common Characteristics for the Three Major Lipid Phenotypes of Familial Combined Hyperlipidemia

A.F. Ayyobi, S.H. McGladdery, M.J. McNeely, M.A. Austin, A.G. Motulsky, J.D. Brunzell

Objective—Familial combined hyperlipidemia (FCHL) is associated with variable lipid and lipoprotein phenotypes arbitrarily defined as type IIa, IIb, and IV based on plasma total cholesterol and triglyceride levels. This study sought to characterize consistent lipoprotein and lipid abnormalities across the 3 lipoprotein phenotypes in 62 patients with documented FCHL (IIa [n = 14], IIb [n = 19], and IV [n = 29]) and 44 healthy individuals.

Methods and Results—The lipoprotein cholesterol distribution was determined over 38 fractions obtained by density gradient ultracentrifugation. As expected, FCHL patients with hypertriglyceridemia (IIb and IV) had higher cholesterol levels in VLDL than IIa, whereas IIa showed higher cholesterol in the big, buoyant LDL and in HDL. LDL cholesterol was higher in IIb than IV; most of the increase in LDL cholesterol was associated with big, buoyant LDL rather than small, dense LDL (sdLDL). The differences in lipoproteins between phenotypes were attributable to changes in VLDL and big, buoyant LDL levels. Comparison of the FCHL patients with healthy individuals showed a significant elevation in plasma apolipoprotein B levels and sdLDL in all 3 FCHL phenotypes.

Conclusions—Although triglyceride and cholesterol levels are variable by lipoprotein phenotype, sdLDL and elevated plasma apolipoprotein B levels are consistent characteristics of FCHL shared by the 3 different lipoprotein phenotypes.

(Arterioscler Thromb Vasc Biol. 2003;23:●●●●●.)

Key Words: PLEASE ▪ SUPPLY ▪ KEY ▪ WORDS ▪ XXXX

Familial combined hyperlipidemia (FCHL) is associated with increased risk of premature cardiovascular disease (CAD).1,2 FCHL was originally described in families of survivors of myocardial infarction by the presence of hypertriglyceridemia, hypercholesterolemia, or both in the affected family members.3 FCHL also is characterized by an increase in apolipoprotein (apo) B and increased number of small, dense LDL (sdLDL) particles compared with healthy subjects.4–6 Although sdLDL is commonly attributed to the presence of hypertriglyceridemia, we have previously shown that the absolute mass of sdLDL persists after treatment with gemfibrozil and correction of the hypertriglyceridemia.5 The VLDL apo B secretion rate has been shown to be augmented in patients with FCHL, whereas it remains normal in other genetic forms of hypertriglyceridemia compared with healthy controls.7–9 FCHL was originally described as a monogenic trait; however, the inheritance of the FCHL-associated lipid phenotypes have been shown to be more complex.10 Segregation analyses have provided evidence for a gene for elevation of apo B levels11–12 and another gene for the presence of sdLDL.13–14 Although no specific major gene has yet been isolated for FCHL, work by Purnell et al15 has provided the physiological evidence for at least 2 independent defects, one for increased apo B production and another for insulin resistance with sdLDL and hypertriglyceridemia, contributing to the pathogenesis of FCHL. Considering the variable lipoprotein phenotype in FCHL, the question remains whether there are any consistent abnormalities shared among all 3 FCHL phenotypes.

FCHL phenotype variability at the lipoprotein level has previously been described in detail.6 It was shown that a single individual over a 6-year period undergoing no drug therapy can present with all 3 phenotypes at any given time, suggesting that environmental factors can strongly influence the variability in the phenotype whereas there is a genetic underlying cause for this disease. The lipoprotein phenotypic heterogeneity of FCHL has made the diagnosis of FCHL difficult. Demonstration of elevated plasma apo B levels16 and sdLDL has been shown to improve the diagnosis of FCHL.17 During a 20-year follow-up of FCHL subjects, elevated apo B was more persistent than elevated total cholesterol (TC) or triglyceride (TG).16 Of note, men with...
premature CAD and elevated apo B were shown to have either FCHL, familial hypercholesterolemia (FH), or elevated lipoprotein a [Lp(a)] levels (also Zambon, unpublished data). Therefore, before diagnosing FCHL by elevated apo B levels, the presence of FH and elevated Lp(a) levels must be excluded. There is little information on the influence of the distinct but extreme phenotypes of FCHL on plasma cholesterol distribution. Thus, we hypothesized that all lipoprotein phenotypes in FCHL, despite great TC and TG variability, share fundamental characteristics in the cholesterol distribution over all density gradient fractions. This may assist determining the best diagnostic characteristics and therapeutic approaches in the future.

We studied 62 individuals diagnosed with FCHL from the Seattle families initially identified and recruited in the 1970s and followed-up between 1994 and 1997. We compared the results from these individuals with those of a healthy, well-characterized normal cohort.

Methods

Patients

Inclusion/Exclusion Criteria

Sixty-two men and women diagnosed with FCHL based on criteria previously described were selected from 27 families that participated in the Genetic Epidemiology of Hypertriglyceridemia study. Individuals older than 70 years of age or taking lipid-lowering medications were excluded from the study (exclusions from groups IIa, IIb, and IV were n = 8, n = 1, and n = 13, respectively). Patients were stratified into lipid phenotypes using age- and sex-specific Lipid Research Clinic reference values. Lipid phenotype type IIA was defined as total cholesterol ≥95th percentile, IV as triglyceride ≥95th percentile, and IIb as both total cholesterol and triglycerides ≥90th percentile. Four individuals were taking hormone replacement therapy at the time of study (IIa n = 2 and IV n = 2). Analysis excluding 4 women undergoing hormone replacement therapy did not alter any significant findings and yielded similar results. Forty-four age- and sex-matched controls were selected from a cohort of 72 well-characterized healthy individuals matched for age and sex.

Body Mass Index

For FCHL subjects, self-reported height and weight were used to calculate BMI (kg/m²). For control subjects, height and weight were determined at the time of plasma sample collection.

Lipids/Lipoproteins

Plasma total cholesterol, TG, HDL cholesterol (HDL-C), LDL-C, HDL3-C, and apo B were determined by standard methodologies at the Northwest Lipid Research Laboratory. LDL-C was calculated by Friedewald’s formula. HDL-C and HDL3-C were determined after plasma precipitation with dextran sulfate and magnesium chloride.

LDL Relative Flotation Rate Determination

A discontinuous salt density gradient was created in an ultracentrifuge tube using a modification of a previous method. Samples were centrifuged at 65,000 rpm for 70 minutes (total ω = 1.95 × 10⁵) at 10°C in a Beckman VTi 65.1 vertical rotor. Thirty-eight 0.45-mL fractions were then collected from the bottom of the centrifuge tube, and cholesterol was measured in each fraction. The relative flotation rate, which characterizes LDL peak buoyancy, was obtained by dividing the fraction number containing the LDL-C peak by the total number of fractions collected. The coefficient of variation of the relative flotation rate value obtained by replicate analysis was 3.6%.

Statistical Analysis

Comparisons of continuous variables across groups, using the controls as the reference group, were conducted with linear regression, using a robust estimate of variance (sandwich estimator) that relaxed assumptions of independence for individuals from the same kindred. The distribution of plasma triglyceride levels was skewed, so the natural logarithm of triglycerides was used in the linear regression analysis. The distribution of men and women across groups was compared using the χ² test. The mean distribution of plasma lipoprotein cholesterol for each phenotype was compared with the other 2 phenotypes or the normal cohort. Results of these comparisons are presented in a difference plot, which includes the mean and 95% CI for the differences in each fraction (error bars). The differences in the cholesterol content of individual fractions were considered significant if the CI did not cross zero.

Results

FCHL patients were selected based on fasting plasma TC and TG levels. By definition, LDL-C was elevated in the patients with hypercholesterolemic phenotypes (IIa and IIb). HDL-C was observed to be significantly lower in the hypertriglyceridemic phenotypes (IIb and IV). Plasma apo B levels, however, were elevated in all 3 FCHL phenotypes, despite the variability observed in the plasma TC and TG levels (Table 1).

Total cholesterol, LDL-C, HDL-C, HDL₃-C, and HDL₄-C levels were significantly higher in the type IIA patients compared with those presenting with hypertriglyceridemia (types IIb and IV). Plasma TG levels were significantly lower in type IIA compared with type IIb and IV phenotypes (Table 1). Peak LDL density was significantly lower and hence more buoyant in type IIA compared with the hypertriglyceridemic individuals. Plasma apo B levels were significantly lower in patients with type IV lipoprotein phenotype than those with elevated cholesterol.

The mean lipoprotein cholesterol distribution profile obtained from each group was compared with the others by plotting the difference curve for 2 populations (Figure 1). IIA lipoprotein phenotype shows a characteristic increase in LDL cholesterol that corresponds with the more buoyant particles compared with types IIb and IV (Figures 1A and 1B). The hypertriglyceridemic type IIb and IV lipoprotein profiles showed significantly higher cholesterol levels in the VLDL density region compared with IIA phenotype. In fact, the IIb and IV groups seem to have a similar cholesterol distribution throughout the 38 fractions with the exception of 3 LDL fractions, which were elevated in the type IIb phenotype (Figure 1C).

To establish the common lipoprotein distribution abnormalities in FCHL, all 3 groups were compared with an age- and sex-matched control group (Figure 2). Comparison of the IIA phenotype with this group of healthy individuals (normal) showed significantly higher relative cholesterol content of fractions corresponding with the small and dense LDL particles. The relative cholesterol content of the big buoyant LDL and HDL fractions was significantly lower than the controls. Both type IIb and IV phenotypes (hypertriglyceridemic) showed significantly elevated cholesterol content of VLDL and sLDL fractions. All of the big, buoyant LDL and HDL cholesterol content was lower than in the control individuals. The most common features in
the lipoprotein cholesterol distribution were elevated relative cholesterol content in the fractions corresponding with sdLDL and a significant decrease in HDL fractions. The relative HDL findings were consistent with the absolute

**TABLE 1. Mean Characteristics by Lipoprotein Phenotype**

<table>
<thead>
<tr>
<th>Lipoprotein Phenotype</th>
<th>Controls, n=44</th>
<th>Ila, n=14</th>
<th>IIb, n=19</th>
<th>IV, n=29</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>40.2±7.2</td>
<td>42.8±16.3</td>
<td>39.6±11.8</td>
<td>44.9±14.6</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, n=42</td>
<td>15 (34)</td>
<td>7 (50)</td>
<td>8 (42)</td>
<td>12 (41)</td>
</tr>
<tr>
<td>Female, n=64</td>
<td>29 (66)</td>
<td>7 (50)</td>
<td>11 (58)</td>
<td>17 (59)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.0±4.3</td>
<td>26.8±5.9*</td>
<td>29.6±5.5*</td>
<td>28.9±5.4*</td>
</tr>
<tr>
<td><strong>Lipids and lipoproteins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC, mg/dL</td>
<td>183±30</td>
<td>284±36*</td>
<td>266±30*</td>
<td>209±34†</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>110±27</td>
<td>201±36*</td>
<td>153±31†</td>
<td>104±32†</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>57±13</td>
<td>57±12</td>
<td>42±13†</td>
<td>36±14†</td>
</tr>
<tr>
<td>HDL2-C, mg/dL</td>
<td>...</td>
<td>9±5</td>
<td>5±2†</td>
<td>5±4†</td>
</tr>
<tr>
<td>HDL3-C, mg/dL</td>
<td>...</td>
<td>48±8</td>
<td>37±11†</td>
<td>32±11†</td>
</tr>
<tr>
<td>TG, mg/dL</td>
<td>79±54</td>
<td>128±50*</td>
<td>382±209†</td>
<td>355±142†</td>
</tr>
<tr>
<td>Apo B, mg/dL</td>
<td>92±23</td>
<td>147±26*</td>
<td>140±18*</td>
<td>112±21†</td>
</tr>
<tr>
<td>LDL peak density (Rf by DGUC)</td>
<td>0.315±0.020</td>
<td>0.288±0.026*</td>
<td>0.251±0.022†</td>
<td>0.239±0.038†</td>
</tr>
</tbody>
</table>

DCG indicates density gradient ultracentrifugation; Rf, relative flotation rate. Statistics for triglycerides are based on natural logarithm.

*Significantly different than the control group (P<0.05); lipid and lipoprotein comparisons adjusted for age, sex, and BMI.
†IIb or IV group significantly different than the Ila group (P<0.05); lipid and lipoprotein comparisons adjusted for age, sex, and BMI.

**Figure 1. Density gradient difference plot.** The mean distribution of plasma lipoprotein cholesterol for each phenotypes was compared with the other 2 phenotypes (lower panels). Results of these comparisons are presented in a difference plot that includes the 95% CIs for the differences in each fraction (error bars). The differences in the cholesterol content of individual fractions were considered significant if the CI did not cross zero. A, Mean IIb profile was subtracted from the Ila profile. Fractions above the zero line are elevated in Ila, and those below the zero line are elevated in IIb. B, Comparison between Ila and IV phenotypes. C, Comparison between the 2 hypertriglyceridemic phenotypes IIb and IV. Fractions 1–6, 7–9, 10–20, 21–29, and 30–38 represent the approximate boundaries of HDL, sdLDL, LDL, IDL, and VLDL, respectively.
HDL-C levels in types IIb and IV, whereas HDL-C level in type IIa was the same as that of healthy individuals.

Age and sex distribution among the phenotypes were not significantly different. The difference in BMI was not significant among the 3 groups, although patients with hypertriglyceridemia tended to have a higher BMI.

Discussion

In this study, the association between various FCHL lipid phenotypes and plasma lipoprotein cholesterol distribution was investigated. Independent of lipid phenotype, FCHL subjects showed a persistent elevation of plasma apo B levels and small dense LDL particles compared with control subjects, despite the variability in plasma lipoprotein levels and distribution.

The lipoprotein cholesterol distribution profiles and biochemical analyses of the FCHL phenotypes clearly showed that the hypertriglyceridemic (IIb and IV) phenotypes preferentially distribute the plasma cholesterol into the VLDL and sdLDL fractions. In contrast, the relative cholesterol distribution in the hypercholesterolemic (type IIa) phenotype was similar to that of healthy individuals in the larger and more buoyant apo B-containing lipoproteins but with a significant enrichment of the sdLDL fractions. Although both relative and absolute HDL-C level was reduced in types IIb and IV, the reduction in relative HDL content of IIa lipoprotein fractions, despite normal absolute plasma levels, is attributed to the abnormally elevated TC levels (Figure 2 and Table 1).

We have previously described an inverse linear relationship between VLDL TG content and LDL cholesterol in FCHL patients. This observation may help explain the underlying processes influencing the lipoprotein cholesterol distribution in FCHL. One can hypothesize that the redistribution of apo B and plasma cholesterol is a key process in development of various phenotypes, given the elevated plasma TC and apo B levels in all FCHL phenotypes. The plasma apo B and cholesterol in the VLDL particles, when in abundance, is associated with significantly lower cholesterol levels in the bigger and more buoyant LDL particles. However, this effect is reversible by reducing plasma TG levels, which in turn may result in redistribution of the apo B and TC from the VLDL particles to the LDL particles. In fact, we have previously shown that significant reductions in TG with gemfibrozil in patients with FCHL resulted in redistribution of apo B and cholesterol from the VLDL particles to the big, buoyant LDL particles. Although this may seem to increase the relative peak LDL size and decrease the relative peak LDL density, the absolute mass of the sdLDL component of the lipoprotein profile remained increased. Thus, the FCHL
phenotype can be influenced by various environmental factors such as diet and exercise, which also can alter plasma TG levels. Accordingly, the BMI of hypertriglyceremic patients was significantly higher than that of the healthy individuals. Although BMI is a less exact measure of adiposity, it is conceivable that the increase in BMI and perhaps central adiposity strongly influence the FCHL phenotype. Elevated TG levels in FCHL may also be modulated by genetic factors such as the Finnish 1q21-q23 FCHL gene or half-normal lipoprotein lipase (LPL) activity. This is consistent with previous findings providing physiological evidence for the separate, but additive, genetic factors responsible for the development of the lipid phenotype in FCHL. There is increasing evidence suggesting a strong link between increasing intra-abdominal fat, insulin resistance, and lipid abnormalities such as increased apo B, elevated TG, a predominance of sdLDL, and reduction in HDL. All of the aforementioned abnormalities are also observed in FCHL. Based on this evidence, it is conceivable that the combination of the underlying genetic or environmental factors responsible for the “metabolic syndrome” together with inherited susceptibility to elevated apo B is the basis of development of FCHL (Figure 3).

The highly variable nature of FCHL, which is also associated with an increased risk of CAD, has made it difficult to identify and treat this disorder appropriately. Thus, we also studied the common features of FCHL in comparison with an age- and sex-matched cohort of healthy control subjects. All 3 phenotypes showed a distinct increase in plasma sdLDL, as well as a consistent reduction in the relative cholesterol distribution in the HDL fractions, independent of individual lipid abnormality. Furthermore, there was an increase in plasma apo B levels, although the magnitude of the increase for type IV was less than for types IIa and IIb. The apparent discordance in apo B levels may be attributed to 2 previously described mechanisms. Although highly speculative, one potential mechanism may involve rapid turnover of VLDL particles, which contain a major pool of the plasma apo B in patients with type IV lipid profile compared with LDL particles. More likely, however, is that individuals in FCHL families who had the defect causing hypertriglyceridemia but did not inherit the defect causing elevated plasma apo B levels were included in the type IV phenotype. This is supported by previous work by Hokanson et al demonstrating that plasma apo B levels remain elevated independent of drug-induced alterations in plasma TG levels.

The complexity of current standards for diagnosis of FCHL has recently received much attention. Measuring apo B levels in the presence of sdLDL seems to be a better diagnostic tool than the classical lipid analyses. Furthermore, a recent report from the Third Workshop on FCHL proposed to redefine the condition as a hypertriglyceridemic hyper-apo B disorder. Although this finding is in agreement with our results in the patients with hypertriglyceridemia (types IIb and IV), a significant number of individuals with type IIa lipid phenotype in this study (7 of 14 or 50% of individuals with type IIa) would be excluded with this new definition because they only exhibited hypercholesterolemia (type IIa) and elevated apo B levels. We have previously shown that an individual patient with FCHL can present with the entire spectrum of FCHL phenotypes over a 6-year follow-up period. We have also reported that correction of hypertriglyceridemia in FCHL patients has little or no effect on the mass of sdLDL particles, additionally suggesting that the presence of hypertriglyceridemia in FCHL may represent sensitivity to the environmental influences on the lipid phenotype of the individual patient. A recent follow-up study of 32 FCHL families has also shown a significant association between BMI and the severity of hypertriglyceridemia. In addition, patients with half-normal LPL activity levels and FCHL have been shown to have higher TG levels compared with those with FCHL but normal levels of LPL. Table 2 summarizes the major lipid/lipoprotein abnormalities that are associated with elevated apo B or sdLDL. A snapshot review of the previous work in light of the findings presented here suggests that sdLDL concurrent with elevated apo B levels [with normal levels of Lp(a)] represent a phenotype characteristic to FCHL independent of the macrocomposition of plasma lipoproteins.

The primary objective of this study was limited to the investigation of potential physiological pathways responsible for FCHL rather than determination/validation of diagnostic parameters for FCHL. Therefore, elevated apo B and sdLDL are not being proposed as diagnostic features for FHCL. This limitation was imposed by the small cohort size and limited spectrum of dyslipidemia. Furthermore, approximately one third of the eligible IIa and IV patients were excluded because they were taking lipid-lowering medications, which may bias the patient selection, because the patients with most severe cases were likely to be taking lipid-lowering medications.

In summary, we studied various FCHL phenotypes in 62 patients with FCHL in an attempt to provide a better understanding of the underlying biochemical and biophysical changes responsible for FCHL. The variability in the phenotype seems to be regulated by differential distribution of apo B in either VLDL or buoyant LDL fractions. Apo B levels...
were elevated in FCHL patients. Although some of the plasma apolipoprotein B exists as sdLDL, the remainder is found in VLDL, which seems to be in equilibrium with big, buoyant LDL in plasma. Day to day caloric variation can determine TG levels and distribution of apo between VLDL and big, buoyant LDL. 

sdLDL is always present, independent of FCHL lipid phenotype. Therefore, the sdLDL is the most prominent characteristic shared among the 3 FCHL phenotypes and is independent of the classical plasma lipoprotein levels. A second common related feature of FCHL seems to be significant reductions in the relative cholesterol content of HDL particles.

### Acknowledgments
A.F.A. was supported by an American Diabetes Association mentor-based fellowship. This study was partly funded by NIH-HL30086, NIH-HL49513, and NIH-RR37, where the studies were performed.

### References

### Table 2. Apo B and sdLDL in Various Dyslipidemias

<table>
<thead>
<tr>
<th>Lipid/Lipoprotein Disorder</th>
<th>Elevated Apo B</th>
<th>sdLDL</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCCHL (types IIA, IIb, and IV)</td>
<td>Yes</td>
<td>Yes</td>
<td>Current study</td>
</tr>
<tr>
<td>Lp (a)</td>
<td>Yes</td>
<td>Yes</td>
<td>Nakajima et al</td>
</tr>
<tr>
<td>FHTG</td>
<td>No*</td>
<td>Yes</td>
<td>Brunzell et al</td>
</tr>
<tr>
<td>HL deficiency</td>
<td>No</td>
<td>No</td>
<td>Zambon et al</td>
</tr>
<tr>
<td>LPL deficiency</td>
<td>No</td>
<td>No</td>
<td>Zambon et al</td>
</tr>
<tr>
<td>Elevated hepatic lipase</td>
<td>No</td>
<td>Yes</td>
<td>Zambon et al</td>
</tr>
<tr>
<td>Type III dyslipidemia</td>
<td>No</td>
<td>No</td>
<td>Chait et al</td>
</tr>
</tbody>
</table>

*apo B levels were in the normal range, although compared with controls, there was a small but significant increase.

FHTG indicates familial hypertriglyceridemia.


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Arterioscler Thromb Vasc Biol. published online May 15, 2003;
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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