Subclasses of Low-Density Lipoprotein and Very Low-Density Lipoprotein in Familial Combined Hyperlipidemia: Relationship to Multiple Lipoprotein Phenotype


Objective—The present study addresses the presence of distinct metabolic phenotypes in familial combined hyperlipidemia (FCHL) in relation to small dense low-density lipoprotein (sd LDL) and very low-density lipoprotein (VLDL) subclasses.

Methods and Results—Hyperlipidemic FCHL relatives (n=72) were analyzed for LDL size by gradient gel electrophoresis. Pattern B LDL (sd LDL, particle size <258 Å) and pattern A LDL (buoyant LDL, particle size ≥258 Å) were defined. Analyses showed bimodal distribution of LDL size associated with distinct phenotypes. Subjects with predominantly large, buoyant LDL showed a hypercholesterolemic phenotype and the highest apo B levels. Subjects with predominantly sd LDL showed a hypertriglyceridemic, low high-density lipoprotein (HDL) cholesterol phenotype, with moderately elevated apoB, total cholesterol level, and LDL cholesterol level. Subjects with both buoyant LDL and sd LDL (pattern AB, n=7) showed an intermediate phenotype, with high normal plasma triglycerides. VLDL subfraction analysis showed that the sd LDL phenotype was associated with a 10-times higher number of VLDL1 particles of relatively lower apo AI and apo E content, as well as smaller VLDL2 particles, in combination with increased plasma insulin concentration in comparison to pattern A.

Conclusions—The present observations underscore the importance of the VLDL triglyceride metabolic pathway in FCHL as an important determinant of the phenotypic heterogeneity of the disorder. (Arterioscler Thromb Vasc Biol. 2004; 24:1-7.)

Key Words: sd LDL ■ apolipoprotein B ■ triglycerides ■ insulin resistance ■ VLDL

Familial combined hyperlipidemia (FCHL) is a metabolic disease, delineated as a genetic disorder of lipid metabolism almost 3 decades ago. It is associated with a 2- to 5-fold increased risk of premature coronary artery disease. Despite recent progress, the genetic and metabolic backgrounds of FCHL have not been elucidated in detail. Subjects with FCHL present with a complex phenotype whose expression is influenced by genetic, metabolic, and environmental factors. Affected FCHL relatives are viscerally obese, hyperinsulinemic, insulin-resistant, and can show a number of abnormalities in lipid metabolism: hypercholesterolemia and/or hypertriglyceridemia, elevated apolipoprotein B (apoB) levels, small dense low-density lipoprotein (sd LDL), and decreased plasma high-density lipoprotein (HDL) cholesterol concentrations.

Very low-density lipoprotein (VLDL) and LDL consist of distinct, physicochemically heterogenic subclasses. A practical characterization of the LDL profile divides it into two major phenotypes: pattern A, characterized by a preponderance of large, buoyant particles, with peak particle diameter ≥258 Å, and pattern B, characterized by predominance of sd LDL particles, with peak particle diameter <258 Å. In the population, sd LDL phenotype and the concurrent metabolic abnormalities (relative hypertriglyceridemia and low HDL cholesterol) have been designated the atherogenic lipoprotein phenotype, consistent with its association with an increased risk of coronary artery disease. Furthermore, pattern B LDL has been recognized as a feature of the metabolic syndrome and is characteristic for insulin-resistant states, such as type 2 diabetes mellitus. It has been reported that presence of sd LDL is an inherent component of the dyslipidemia in FCHL and shares genetic determinants with the expression of FCHL. The aim of the present study was to investigate in detail the sd LDL phenomenon in FCHL. Kinetic studies have shown a
metabolic relationship between hepatic VLDL1 production and the appearance of sd LDL in plasma.\textsuperscript{8,16,17} It has been shown that FCHL subjects exhibit a higher production rate of VLDL–apoB than controls,\textsuperscript{18} but no distinction has been made so far between the VLDL1 or VLDL2 subclasses overproduced. We examined whether specific metabolic phenotypes are associated with pattern A or pattern B LDL in hyperlipidemic FCHL relatives, whether a relationship exists with the phenomenon of multiple lipoprotein phenotypes,\textsuperscript{1} and if specific VLDL subclasses are involved. Therefore, VLDL1 and VLDL2 subclasses have been analyzed with regard to lipid and apolipoprotein composition in carriers of pattern A, B, and AB LDL subspecies. This is the first study, to our knowledge, that addresses this issue in FCHL.

**Methods**

**Subjects**

Hyperlipidemic FCHL relatives (n = 72; 36 men and 36 women) were recruited at the Lipid Clinic of the Maastricht University Hospital. FCHL families (n = 27) were ascertained as previously described.\textsuperscript{4} Briefly, FCHL probands had a primary hyperlipidemia with varying phenotypic expression, including fasting plasma cholesterol >6.5 mmol/L and/or fasting plasma triglyceride (TG) concentration >2.3 mmol/L and a positive family history of premature coronary artery disease, ie, before the age of 60. In addition, FCHL probands had no tendon xanthomas, no apo E2/E2 genotype, and normal thyroid-stimulating hormone concentrations. Obesity (body mass index \(>30 \text{ kg/m}^2\)) or diabetes was an exclusion criterion for the ascertainment of a FCHL proband. The hyperlipidemic FCHL subjects, who were included in the present study, had been ascertained as an affected relative in a FCHL family, which contained at least one other first-degree relative with a different lipoprotein phenotype.\textsuperscript{1} In the present study, 38 subjects exhibited Fredrickson IIa lipoprotein phenotype, 17 with type IIb and 17 with type IV. The Human Investigation Review Committee of the Academic Hospital Maastricht approved the study protocol and all subjects gave informed consent.

Subjects were studied after an overnight fast (12 to 14 hours) and at least 3 days without alcohol consumption. Any lipid-lowering medication was stopped for 2 weeks before blood samples were collected. Venous blood was collected in pre-cooled tubes containing EDTA (1 mg/mL); anthropometric measurements, calculation of waist-to-hip ratio and body mass index, and measurements of fasting plasma concentrations of lipids, lipoproteins, and insulin were performed as described.\textsuperscript{4} Analyses of LDL subclass distributions, calculation of LDL peak particle diameter, and assignment of qualitative LDL subclass pattern were performed by means of non-denaturating gradient gel electrophoresis in the Lawrence Berkeley National Laboratory, University of California, Berkeley, as described elsewhere.\textsuperscript{8,14} VLDL1 and VLDL2 subfractions were separated by density gradient ultracentrifugation as described by Zhao et al.\textsuperscript{19} with minor modifications, which represent ultracentrifugation at 160,000 \(g\) for 2.5 hours at 4°C in a SW40 Ti rotor. Collection of fractions started from the top of the tube, where the upper 1.5 mL represents VLDL1 and the lower 5 mL represents VLDL2. In the VLDL1 and VLDL2 subfractions, concentrations of cholesterol and TG were determined in triplicate by standard laboratory techniques, apoAI, apoAII, apoB, apoCII, apoCIII, and apoE in duplicate by commercial immunoassay Human Apolipoprotein Lincoplex Kit (Cat; APO-62K; Linco Research, Mo).

**Statistical Analyses**

A t test was used to analyze differences between the groups. Log transformed values of TGs, body mass index, and insulin were used in the analyses, because these variables did not follow the normal distribution. Kolmogorov-Smirnov statistics was used to test normality of LDL size distribution. Pearson correlation coefficient (\(r\)) was used to describe relation between plasma and VLDL subclass TGs in univariate analysis. Mann-Whitney test was used in the VLDL subclass analysis because of the sample size (n = 15). In all analyses, the statistical package SSPS 11.0 (SSPS Inc) was used.

**Results**

**Distribution of LDL Size and Associated Metabolic Phenotypes in Hyperlipidemic FCHL Subjects**

The histogram of LDL particle size in hyperlipidemic FCHL subjects showed a clear, bimodal distribution (\(P=0.001\)) (Figure 1). The mean diameter of pattern A LDL was 268.1 Å, and the mean of pattern B LDL was 250.9 Å. Seven subjects showed an intermediate LDL phenotype (pattern AB), with average LDL particle diameter of 261.5 Å, indicating the presence of large, buoyant LDL and sd LDL.

Potential differences in metabolic phenotypes between carriers of pattern A versus B LDL were evaluated. The hyperlipidemic carriers of pattern B LDL (n = 31) showed significantly higher TGs (TG = 2.8 mmol/L versus...
1.5 mmol/L in carriers of pattern A LDL, \( P<0.001 \), but, remarkably, significantly lower total, LDL, and HDL cholesterol, apoB, and apoA1 in comparison with pattern A LDL carriers (Table 1). Of the pattern B LDL carriers, 51.6% (16 of 31) had total cholesterol \(<6.5 \text{ mmol/L}\). Thus, pattern B LDL-associated phenotype is consistent with either Fredrickson IIa phenotype and 1 subject exhibited IIb.

### Lipid Profile of VLDL1 and VLDL2 in Patterns A, AB, or B

Because there is only one apoB molecule per VLDL particle, the concentration of apoB (mmol/L) provides information about the number of VLDL particles in plasma. Pattern B subjects showed a 10-fold higher concentration of apoB in both VLDL subfractions than did pattern A subjects (Table 2); therefore, they had \( \approx 10 \)-times more VLDL1 and VLDL2 particles in plasma, despite the fact that their mean plasma apoB concentration was slightly lower (Table 1). Pattern AB subjects showed the most striking increase in their VLDL2 particle number, which was statistically significant when compared with pattern A subjects.

In pattern B subjects, VLDL1 subfraction had a 4-fold higher TG content than the corresponding VLDL2 subfraction (Table 2). By comparison, in carriers of pattern A LDL, this ratio was 0.95 \( (P<0.001) \). Also, VLDL1 in pattern B subjects contained 73% of all VLDL TGs and 65% of VLDL cholesterol. By contrast, VLDL1 in pattern A subjects contained approximately half of all VLDL TG (ie, 48%) and only 37% of VLDL cholesterol. With regard to particle lipid composition, pattern A and pattern B subjects showed variations in VLDL1 particle TG and cholesterol content, but on the average there was no statistical difference in lipid composition of the VLDL1 particles between the 3 groups (Table 2). Therefore, the higher TG content of the VLDL1 subfrac-tions with VLDL1 TG approached statistical significance \( (r=0.47; \ P=0.08) \). Therefore, the largest contribution to hypertriglyceridemia in pattern B carriers (and probably in pattern AB carriers) comes from VLDL1 TG (Figure 2A).

### Relationship Between Plasma and VLDL Subfraction TGs

Subsequently, the lipid composition of VLDL1 and VLDL2 subclasses were analyzed in typical carriers of pattern A \( (n=15) \), pattern B \( (n=15) \), or AB LDL \( (n=6) \).

In subjects with pattern B LDL, a statistically significant relationship was found between plasma TGs and VLDL1 TG \( (r=0.61; \ P=0.015) \), but not VLDL2. Subjects with pattern AB \( (n=6) \) showed a similar relationship between plasma TGs and VLDL1 TG, as observed in subjects with pattern B, although it did not reach statistical significance \( (r=0.70; \ P=0.12) \). In contrast, in subjects with pattern A LDL, a statistically significant relationship was found between plasma TGs and VLDL2 TG \( (r=0.52, \ P=0.047) \). The relationship with VLDL1 TG approached statistical significance \( (r=0.47; \ P=0.08) \). Therefore, the largest contribution to hypertriglyceridemia in pattern B carriers (and probably in pattern AB carriers) comes from VLDL1 TG (Figure 2A). In pattern A, VLDL2 TG and, to a lesser extent, VLDL1 TG contribute to plasma TG concentrations. Of note, there was a statistically significant positive relationship between VLDL1 TG and plasma insulin levels in pattern B subjects \( (r=0.64, \ P=0.01) \), but not in pattern A (Figure 2B).
tion in pattern B subjects was caused by a higher VLDL1 particle number, but not to the presence of TG-richer VLDL1 (larger) particles. Interestingly, pattern A subjects seemed to have TG-enriched and cholesterol-enriched VLDL2, ie, relatively larger particles, compared with pattern B and pattern AB subjects. Pattern B and pattern AB carriers showed remarkable similarity in VLDL2 lipid content, ie, reflecting relatively smaller VLDL2 particles.

Apolipoprotein Profiles of VLDL1 and VLDL2
Concentrations of apoAI, apoAII, apoCII, apoCIII, apoE, and apoB were measured in VLDL1 and VLDL2 subfractions in representative subjects of the FCHL patients described in Table 2. Figure 3 represents the apolipoprotein content of the VLDL1 particles in pattern A (n=6), B (n=5), or AB (n=5) subjects, expressed as percent of total apolipoproteins (set at 100%) calculated per apoB (ie, per VLDL1 particle). Of note, VLDL1 particles of subjects with pattern A exhibited significantly higher apoAI content in comparison to subjects with pattern B and AB, and significantly higher apoE than pattern B. A similar tendency was observed for VLDL2 particles (please see http://atvb.ahajournals.org), although it did not reach statistical significance. Of note, the apoE genotype of the subjects studied was analyzed and there was no explanation of the present results by the distribution of alleles.

Discussion
The present findings showed that hyperlipidemic relatives from well-defined FCHL families exhibited a bimodal distribution of LDL size, which in turn was associated with two metabolically distinct phenotypes. Carriers of sd LDL showed a hypertriglyceridemic, low HDL cholesterol phenotype,9 quite similar to the atherogenic lipoprotein phenotype, with moderately elevated plasma concentrations of apoB, total cholesterol, and LDL cholesterol. Therefore, carriers of sd LDL, also named pattern B LDL in this study, expressed hypertriglyceridemia either as Fredrickson IIb phenotype, considered classical for FCHL, or as hypertriglyceridemia per se (type IV). Carriers of large and buoyant LDL particles, named pattern A LDL in this study, were characterized by a hypercholesterolemic phenotype with high apoB and high LDL cholesterol in combination with near-normal plasma TG concentrations. Thus, pattern A LDL is associated with Fredrickson type IIa. The existence of distinct metabolic phenotypes appears to be related to VLDL particle metabolism. This was reflected by a 10-fold higher number of VLDL1 particles, with reduced apoAI and apoE content and, in addition, smaller size of VLDL2 in plasma of FCHL pattern B subjects compared with pattern A. The present findings are consistent with the original observation of bimodality of plasma TG in FCHL1 and offer further insight.
of the pathophysiology behind the multiple lipoprotein phenotypes in FCHL.

A mechanism that is relevant to VLDL metabolism in insulin resistance and that appears to be important in FCHL, as well, is hepatic secretion of heterogeneous VLDL subspecies. It has been shown that FCHL subjects exhibit a 2.7-fold overproduction of VLDL apoB. However, no distinction has been made thus far between the VLDL subclasses overproduced and their heterogeneous catabolism. A novel finding in the present study is the 10-fold increased number of VLDL1 particles in pattern B subjects compared with pattern A subjects. Moreover, the VLDL1 TG content was the main contributor to the hypertriglyceridemia in pattern B FCHL subjects. In addition, pattern B subjects showed higher plasma insulin concentrations, a surrogate marker of insulin resistance, and a statistically significant

| Table 2. Lipoprotein Profiles of VLDL1 and VLDL2 in Hyperlipidemic FCHL Subjects |
|---------------------------------------------|-----------------|----------------|-----------------|--|
| N                                         | Pattern A LDL   | Pattern B LDL  | P Value         | Pattern AB     |
| VLDL1                                      |                 |                |                 |                |
| ApoB‡, nmol/L                              | 0.4 ± 0.3       | 4.6 ± 3.8      | 0.04            | 1.0 ± 0.5      |
| TG per apoB‡, mmol/mg                      | 0.7 ± 0.3       | 1.2 ± 1.6      | NS              | 0.6 ± 0.3      |
| Total TG (mmol/L)                          | 0.19 ± 0.09     | 1.10 ± 0.59    | P<0.001         | 0.30 ± 0.10†   |
| % Total VLDL TG                            | 47.9 ± 6.6      | 73.1 ± 14.3    | 52.6 ± 16.1     |                |
| Chol per apoB mmol/mg                      | 0.2 ± 0.1       | 0.56 ± 0.70    | NS              | 0.30 ± 0.50    |
| Total cholesterol (mmol/L)                 | 0.06 (0.03–0.08)| 0.43 (0.22–0.67)| P<0.001         | 0.15 ± 0.04§   |
| % Total VLDL-C                             | 37.0 ± 12.5     | 65.1 ± 18.0    | 40.3 ± 16.0     |                |
| VLDL2                                      |                 |                |                 |                |
| ApoB‡, nmol/L                              | 1.2 ± 1.4       | 11.4 ± 1.8     | 0.004           | 8.1 ± 2.3|| |
| TG per apoB‡, mmol/mg                      | 0.7 ± 0.9       | 0.09 ± 0.01    | 0.055           | 0.08 ± 0.01†   |
| Total TG (mmol/L)                          | 0.21 ± 0.11     | 0.35 ± 0.16    | 0.045           | 0.27 ± 0.10    |
| % Total VLDL TG                            | 52.1 ± 6.6      | 26.9 ± 14.3    | 47.4 ± 16.1     |                |
| Chol per apoB mmol/mg                      | 0.3 ± 0.1       | 0.07 ± 0.02    | 0.095           | 0.07 ± 0.01    |
| Total cholesterol (mmol/L)                 | 0.11 ± 0.10     | 0.25 ± 0.12    | 0.003           | 0.25 ± 0.11†   |
| % Total VLDL-C                             | 63.0 ± 12.5     | 34.9 ± 18.0    | 59.7 ± 16.0     |                |
| VLDL1 TG/VLDL2 TG                          | 0.95 ± 0.26     | 4.06 ± 2.91    | P<0.001         | 1.13 (0.57–1.98)* |
| VLDL1-C/VLDL2-C                            | 0.65 ± 0.35     | 2.20 (0.95–4.67)| P<0.001         | 0.64 (0.35–1.14)* |

Normally distributed variables are mean ± SD. Skewed variables are median (interquartile range).
VLDL TG indicates triglycerides in VLDL; VLDL-C, cholesterol in VLDL; NS, not significant; Chol, cholesterol.
Pattern B vs pattern AB: $P<0.001$, $\hat{P}<0.01$, $*P<0.05$.
Pattern A vs pattern AB: $\hat{P}<0.01$, $\hat{P}<0.05$.
‡Pattern A, n = 6; pattern B, n = 5; pattern AB, n = 5; in these analyses.

Figure 3. Apolipoprotein profile of VLDL1 particles in hyperlipidemic FCHL carriers of pattern A, AB, or B LDL subspecies. $*P<0.05$ pattern B versus pattern A, $\hat{P}<0.05$ pattern AB versus A. Data are expressed as percent of total apolipoproteins (mol) per apoB (mol).
positive relationship between the latter and VLDL1 TG, whereas such a relationship was not found in pattern A subjects. Increased VLDL1 secretion in insulin resistance has been formally demonstrated by stable isotope methodology in patients with type 2 diabetes mellitus. Such a kinetic study has not been performed in FCHL to date, but altogether it is plausible that a similar mechanism is operational in hypertriglyceridemic FCHL subjects, as well. VLDL2 secretion, in contrast to VLDL1, is not regulated by insulin but is dependent, at least in part, on cholesterol availability in the liver. VLDL2 is preferentially metabolized to large, buoyant LDL. Accordingly, a relatively increased production rate of VLDL2 apoB or direct synthesis of LDL apoB can lead to the phenotypic expression of isolated hypercholesterolemia with normal LDL size in pattern A subjects. Therefore, we suggest that differences in liver insulin sensitivity in FCHL cause differences in secretion of VLDL1 and VLDL2 that can explain, at least in part, the present findings. Moreover, a similar mechanism can underlie the change of lipid phenotype observed in FCHL subjects.

The present observations underscore the biological importance of the VLDL TG metabolic pathway in FCHL, especially when put in the perspective of reported linkage and association studies. In FCHL, linkage and association have been described with several genes encoding for apolipoproteins that are part of VLDL lipoproteins: the apoAI-CIII-AIV-AV gene cluster, apoAII gene, and plasma concentrations of apoCIII, apoCII, and apoB. Moreover, linkage between apoAI-CIII-AIV-AV gene cluster and the presence of sd LDL has been reported. The effect of different apolipoproteins on VLDL catabolism is well known. For instance, apoE affects the hepatic uptake of VLDL subfractions; apoE, apoCII, and apoCIII affect lipolysis; and, finally, VLDL apolipoproteins modulate lipid and protein exchange with other lipoproteins. In the present study, apoAI and apoE were less abundant on VLDL particles in pattern B FCHL subjects (Figure 3) than in pattern A. Therefore, our data suggest that changes in VLDL apolipoprotein content may be relevant for VLDL metabolism by lipolysis and hepatic clearance, which can contribute to the accumulation of VLDL1 (and VLDL2) lipoproteins observed in plasma in FCHL subjects with sd LDL. Furthermore, it has been shown that accumulation of VLDL1 in plasma and modifier genes that affect VLDL1 catabolism, such as CETP, hepatic lipase, and lipoprotein lipase, affect sd LDL frequency in FCHL.

The cross-sectional design of this study prevents a definitive conclusion on the metabolic pathways involved in the phenotypic expression of FCHL. It is worth mentioning, however, that our present findings are consistent with a recent publication by Ayyobi et al., which associates the difference in lipoprotein phenotypes in FCHL with changes in VLDL and large, buoyant LDL levels. This study and the present observations reflect long-term adaptation changes in FCHL and are therefore difficult to interpret in a simple manner. Further insight in the relative contribution of VLDL subclass secretion and catabolism to the FCHL phenotype will require stable isotope studies.

In summary, a novel finding in the present study is that hyperlipidemic FCHL subjects showed bimodal distribution of LDL size, and each peak of LDL subclasses corresponded to a distinct phenotype. Subjects with predominance of large, buoyant LDL showed a hypercholesterolemic phenotype (Fredrickson type IIa) and the highest apoB levels. Subjects with predominance of sd LDL presented with a hypertriglyceridemic, low HDL cholesterol phenotype, moderately elevated apoB levels, total cholesterol, and LDL cholesterol (type IIb and IV); in addition, these were characterized by a 10-times-higher number of VLDL particles of lower apoAI and apoE content (VLDL1) and smaller size (VLDL2) in plasma, compared with pattern A. The present observations underscore the importance of the VLDL TG metabolic pathway in FCHL as an important determinant of the phenotypic heterogeneity of the disorder.

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References


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