Lipoproteins in Familial Dysbetalipoproteinemia
Variation of Serum Cholesterol Level Associated With VLDL Concentration

Shui-Ping Zhao, Augustinus H.M. Smelt, Jan A. Gevers Leuven, Arnoud M.J.M. van den Maagdenberg, Arnold van der Laarse, and Ferdinand M. van 't Hooft

Patients with familial dysbetalipoproteinemia (FD) associated with the apo E2/2 phenotype exhibit a marked interindividual variability in serum cholesterol and triglyceride concentrations. It has been proposed that this variability is due to a combination of the apo E2/2 phenotype and additional genetic factors implicated in diseases like familial hypercholesterolemia, familial combined hyperlipoproteinemia, and familial hypertriglyceridemia. To further explore the nature of this variability, the lipoprotein profiles of 17 patients with FD associated with the apo E2/2 phenotype were analyzed by a density-gradient ultracentrifugation technique and by 2-16% polyacrylamide gel electrophoresis. It was found that all patients with FD were characterized by 1) markedly increased cholesterol concentrations of large very low density lipoprotein (VLDL) (VLDL₁) (2.98±0.38 versus 0.08±0.03 mmol/L), small VLDL (VLDL₂) (4.68±1.93 versus 0.27±0.13 mmol/L), and intermediate density lipoprotein (IDL) (2.25±0.72 versus 0.39±0.16 mmol/L); 2) decreased low density lipoprotein (LDL) cholesterol level (1.84±0.54 versus 3.36±0.53 mmol/L); and 3) altered composition (enrichment by cholesteryl ester) of VLDL₁ and VLDL₂ compared with normolipidemic control subjects. The cholesterol levels of IDL and LDL showed minor interindividual variabilities and were not correlated with serum cholesterol and triglyceride levels. The compositions of VLDL₁ and VLDL₂ were independent of the concentrations of lipids in serum. However, the cholesterol concentrations of VLDL₁ and VLDL₂ showed considerable interindividual variabilities and were positively correlated with the serum cholesterol concentration (r=0.84 and r=0.95, respectively, both p<0.001). It is concluded that the marked interindividual variability in the serum cholesterol and triglyceride concentrations in patients with FD is mainly due to variations in the concentrations of VLDL₁ and VLDL₂. This suggests that a combination of the apo E2/2 phenotype and genetic abnormalities in the metabolism of triglyceride-rich lipoproteins may be required for the expression of FD. (Arteriosclerosis and Thrombosis 1993;13:316–323)

KEY WORDS • familial dysbetalipoproteinemia • VLDL • lipoproteins • density-gradient ultracentrifugation • LDL • IDL • apo E • cholesterol

Familial dysbetalipoproteinemia (FD), or type III hyperlipoproteinemia, is a genetic disorder of lipoprotein metabolism associated with elevations of both serum cholesterol and triglyceride concentrations. Patients with this disorder have an increased risk for the premature development of coronary heart disease and peripheral vascular disease, which may be related to the accumulation of lipoproteins of abnormal composition in the plasma. The primary genetic defect associated with FD is the occurrence of a variant form of apolipoprotein (apo) E that does not bind normally to either the apo B,E (low density lipoprotein [LDL]) receptors or to the putative remnant (apo E) lipoprotein receptors, thus leading to an impaired clearance of apo E-containing lipoproteins by the liver. By isoelectric focusing, three common genetic variants of apo E, designated E2, E3, and E4 according to their mobility on isoelectric focusing, can be recognized. Most patients with FD are homozygous for apo E2 (Arg₁₅₈→Cys). In Caucasian populations the apo E2/2 phenotype occurs with a frequency of 1%, whereas the frequency of FD is about 1:2,500. Thus, FD will develop in only a small percentage of the individuals with the apo E2/2 phenotype. This indicates that in general, FD is a multifactorial disease, i.e., additional factors, either genetic or environmental, are required for its expression.

Individuals with FD show a marked interindividual variation in the serum concentrations of cholesterol and triglyceride. However, the apo E2 (Arg₁₅₈→Cys) from hypocholesterolemic, normocholesterolemic, and hypercholesterolemic subjects all showed equally defective binding to the apo B,E (LDL) receptors in vitro. Utermann et al proposed that a combination of the apo E2/2 phenotype and additional genetic factors, implicated in diseases like familial hypercholesterolemia, familial combined hyperlipoproteinemia, or familial.
TABLE 1. Characteristics of 20 Patients With Familial Dysbetalipoproteinemia

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/gender</th>
<th>BMI (kg/m²)</th>
<th>Xa/XSP</th>
<th>CAD/PVD</th>
<th>C (mmol/L)</th>
<th>TG (mmol/L)</th>
<th>VLDL C/TGt</th>
<th>Apo E PT</th>
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<td>+/-</td>
<td>+/-</td>
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<td>7.96</td>
<td>1.30</td>
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<td>E2/2</td>
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<td>+/-</td>
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<tr>
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</table>

FD associated with rare apo E variants

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/gender</th>
<th>BMI (kg/m²)</th>
<th>Xa/XSP</th>
<th>CAD/PVD</th>
<th>C (mmol/L)</th>
<th>TG (mmol/L)</th>
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<td>+/-</td>
<td>7.75</td>
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<td>1.21</td>
<td>E3/2</td>
<td>E3/3*</td>
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<td>+/-</td>
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<td>E3/3*</td>
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<tr>
<td>20</td>
<td>49/M</td>
<td>21.8</td>
<td>+/-</td>
<td>+/-</td>
<td>9.23</td>
<td>5.88</td>
<td>1.14</td>
<td>E3/3</td>
<td>E3-L/3</td>
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</table>

BMI, body mass index; Xa, xanthomas; XSP, xanthoma striata palmaris; CAD, coronary artery disease; PVD, peripheral vascular disease; C, cholesterol; TG, triglyceride; VLDL, very low density lipoprotein; apo, apolipoprotein; PT, phenotyping; GT, genotyping; FD, familial dysbetalipoproteinemia; E3-L, E3-Leiden.

*With apo E2(Lys146—>Gln), as was discovered by allele-specific oligonucleotide probe for the E2(Lys146—>Gln) mutation.

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Ival hypertriglyceridemia, determines the expression of FD. Such a diversity of additional genetic factors would explain both the marked interindividual variability in the cholesterol and triglyceride concentrations in sera of patients with the disorder, as well as the relatively low frequency of the development of FD in individuals with the apo E2/2 phenotype. In addition, this hypothesis predicts that the lipoprotein profiles of patients with FD exhibit characteristic interindividual differences related to the nature of the additional genetic factor (or factors). The aim of the study was to elucidate which combination of lipoprotein abnormalities is associated with the presence of apo E2 (Arg158—>Cys) homozygosity and to determine which lipoprotein class (or classes) is responsible for the interindividual variability in the serum cholesterol and triglyceride concentrations in patients with FD.

Methods

Subjects

During routine screening of the apo E phenotype of all patients referred to the Lipid Clinic of the Leiden University Hospital in the period between July 1989 and December 1990, 17 hyperlipoproteinemic individuals with the apo E2/2 phenotype were discovered (Table 1). The sera of these patients were characterized by the presence of β-very low density lipoprotein (β-VLDL) as determined by agarose electrophoresis and by elevated VLDL concentration (VLDL cholesterol >1.0 mmol/L) as analyzed by routine ultracentrifugation. The VLDL cholesterol/triglyceride ratio was markedly increased in all patients (Table 1). All patients therefore fulfilled the "classic" biochemical criteria for FD. The nature of the apo E2/2 phenotype was analyzed in more detail. Cysteamine treatment18 of the serum sample before apo E phenotyping led to a "normal" modification of the apo E molecule (e.g., conversion of the apo E2/2 phenotype to the apo E4/4 phenotype). In agreement with this observation it was found that all patients were homozygous for apo E2 (Arg158—>Cys) as determined by DNA analysis.19 It thus appeared that all 17 patients were homozygous for apo E2 (Arg158—>Cys), the most common form associated with FD.

Three patients (patients No. 18, 19, and 20 in Table 1) showed only partial modification with cysteamine during apo E phenotyping. Using both allele-specific restriction endonuclease genotyping19,20 and variant-specific oligonucleotide probes21,22 it was found that two patients (patients No. 18 and 19 in Table 1) were heterozygous for a common APOE3 allele and APOE2 (Lys146—>Gln) allele, and one patient (patient No. 20 in Table 1) was heterozygous for a common APOE3 allele and an APOE3-Leiden allele (7-amino acid insertion in 120—126). All patients were advised to eat a low-fat diet (30% of total calories) with a saturated: monounsaturated: polyunsaturated fat ratio of 1:1:1 and less than 300 mg cholesterol per day. None of the patients reported a consumption of more than 15 g alcohol per day. None appeared to have renal, thyroid, or liver disease, as assessed by physical examination and routine laboratory tests. One patient (patient No. 1 in Table 1) had mild...
diabetes, but the level of blood glucose was well controlled by diet. All other patients had normal fasting blood glucose levels. None of the patients took lipid-lowering drugs or other drugs that might interfere with lipoprotein metabolism.

Ten normolipidemic individuals (six men and four women, average age 47.3 years) served as control subjects in this study. Normolipidemia was defined as a serum cholesterol concentration <6.1 mmol/L and serum triglyceride concentration <2.0 mmol/L. Five of the normolipidemic control subjects had the apo E3/3 phenotype, three the apo E3/2 phenotype, and two the apo E4/3 phenotype.

Density-Gradient Ultracentrifugation

Blood samples were taken in the morning after more than 12 hours of fasting. Serum was obtained by centrifugation at 1,000 g for 10 minutes at room temperature less than 4 hours after sampling. The separation of lipoproteins was started the day of blood collection using a recently developed two-step density-gradient ultracentrifugation technique (unpublished observations from our laboratory). In the first step high density lipoprotein (HDL), LDL, intermediate density lipoprotein (IDL), and VLDL were separated. In the second step VLDL was further separated into two subfractions, VLDL1 (large VLDL) and VLDL2 (small VLDL). The gradient of the first step consisted of 2 mL serum sample (adjusted to d=1.210 g/mL by adding solid potassium bromide) on the bottom of a polyallomer tube (14 mL, Kontron AG, Zurich, Switzerland), overlaid by 5 mL of d=1.030 g/mL and 5 mL of d=1.006 g/mL solutions. The gradient was centrifuged immediately at 274,400 g for 19 hours at 15°C in a TST swinging-bucket rotor using a Centrifkon T-2070 ultracentrifuge (Kontron AG). In the second step the gradient consisted of 2 mL VLDL solution (obtained from routine ultracentrifugation and adjusted to d=1.210 g/mL by adding solid potassium bromide) on the bottom of the tube, overlaid by 2 mL of d=1.100 g/mL, 4 mL of d=1.040 g/mL, and 4 mL of d=1.006 g/mL solutions. This gradient was ultracentrifuged at 210,900 g for 2 hours at 15°C in the same rotor and ultracentrifuge used in the first step.

The gradient was fractionated using a specially designed fractionator connected to a micropump and a fraction collector (LKB, Bromma, Sweden).

Chemical Analysis

The cholesterol concentrations and the compositions of VLDL1, VLDL2, IDL, and LDL were determined in pooled gradient fractions. VLDL1 was recovered in fractions 21–23 and VLDL2 in fractions 11–20 in the second-step ultracentrifugation. LDL was obtained in fractions 12–18 and LDL in fractions 6–11 in the first-step ultracentrifugation. The total cholesterol, free cholesterol, triglyceride, and phospholipid concentrations were determined enzymatically using test kits (Boehringer, Mannheim, FRG). Esterified cholesterol was calculated as the difference between total cholesterol and free cholesterol. The mass of cholesteryl ester (CE) was estimated as 1.67 × esterified cholesterol. Total protein was determined by a modification of the Lowry procedure with bovine serum albumin as a standard. The total lipoprotein mass (in milligrams per deciliter) was calculated as the sum of masses of free cholesterol, cholesterol ester, triglyceride, phospholipid, and total protein. HDL cholesterol concentration was measured in the d=1.006 g/mL infranatant obtained by routine ultracentrifugation after precipitation of IDL and LDL by phosphotungstic acid and MgCl2.

Gradient Gel Electrophoresis

The lipoprotein profiles in the sera of 20 patients with FD were analyzed by gradient gel electrophoresis. The electrophoresis was performed as described by McNamara et al23 using 2–16% nondenaturing polyacrylamide gradient gels (Pharmacia LKB, Uppsala, Sweden). The gels were stained with Sudan black B. Stained gels were scanned with an LKB 2202 Ultrascan laser densitometer (LKB, Paramus, N.J.).

Apolipoprotein E Phenotyping and Genotyping

The apo E phenotype was determined by isoelectric focusing of delipidated plasma samples before and after cyanate treatment followed by immunoblotting as described by Havekes et al.18 For apo E genotyping, genomic DNA was isolated from leukocytes by standard methods.26 The 5' part of exon 4 of the human APOE gene, encoding for amino acids 61–174, was amplified by the polymerase chain reaction (PCR) using the primers 402 (nucleotides 3,555–3,574, coding strand) and 401 (nucleotides 3,932–3,913, noncoding strand) as described earlier by van den Maagdenberg et al.22 For allele-specific restriction endonuclease genotyping as described first by Hixson and Vernier,19 15 μL PCR product was digested with 7.5 units restriction enzyme Hha I for 16 hours according to recommendations of the supplier (Pharmacia). Thereafter, the digested material was separated on a 10% neutral polyacrylamide gel for 3 hours at 10 V/cm, stained with 0.1 mg/mL ethidium bromide, and photographed.20 For hybridization with variant-specific oligonucleotide probes for the APOE2 (Lys→Gln) mutation21 and the APOE3-Leiden mutation,25 5 μL PCR product was separated on a 2% agarose gel by electrophoresis, and the gel was stained with ethidium bromide and photographed. Thereafter the DNA was transferred to a membrane (Biotrace, Gelman Sciences Inc., Ann Arbor, Mich.) and hybridized with adenosine 5'-[α-32P]triphosphate–labeled synthetic allele-specific oligonucleotide probes.21,22

Statistical Analysis

Results were expressed as mean±SD. All statistical analyses were performed with SPSS/PC+ software (SPSS Inc., Chicago, Ill.). Differences between mean values were evaluated statistically by Student's t test. Values of p<0.05 were considered to represent statistical significance.

Results

Characteristics of Subjects

Seventeen patients with FD associated with the apo E2/2 phenotype were studied (Table 1). They were given detailed dietary instructions. During a 3–12-month follow-up period all patients adhered to these instructions. Three or more measurements of serum cholesterol and triglyceride concentrations (on average, 4.9 measurements) were made per patient during the follow-up period. The mean coefficient of variation for
serum cholesterol concentrations in multiple determinations per patient was 10±4% and for serum triglyceride concentrations 17±5%, which compared well with corresponding values of 7% (serum cholesterol) and 22% (serum triglyceride) reported for normolipidemic individuals analyzed over a 6-month period.27,28 This indicates that patients with FD analyzed in the present study did not exhibit an unusual within-patient variability in their serum cholesterol and triglyceride concentrations, although considerable inter-patient differences were observed (Table 1).

Separation of Lipoproteins

The sera of all the patients with FD and of 10 normolipidemic individuals were analyzed by a density-gradient ultracentrifugation technique and by non-denaturing gradient polyacrylamide (2–16%) gel electrophoresis. Typical lipoprotein profiles of a patient with FD (patient No. 16 in Table 1) and of a normolipidemic individual analyzed by density-gradient ultracentrifugation are shown in Figure 1. Four distinct lipoprotein fractions (VLDL1, VLDL2, IDL, and LDL) were present in the sera of all patients with FD. A distinct IDL fraction was absent in the sera of normolipidemic individuals. As illustrated in Figure 2, four distinct lipoprotein fractions were also observed in the sera of all patients with FD analyzed by gradient gel electrophoresis. These lipoprotein fractions corresponded to VLDL1, VLDL2, IDL, and LDL fractions isolated by density-gradient ultracentrifugation. Note that a distinct IDL fraction was not detectable in the sera of normolipidemic individuals, as analyzed by density-gradient ultracentrifugation (Figure 1) and gradient gel electrophoresis (Figure 2).

The average cholesterol concentrations of VLDL1, VLDL2, IDL, LDL, and HDL in the sera of 17 patients with FD were compared with those of 10 normolipidemic subjects (Table 2). The cholesterol concentrations of VLDL1, VLDL2, and IDL were markedly higher in patients with FD than in normolipidemic subjects, and the LDL cholesterol concentration was considerably lower in FD patients than in normolipidemic individuals. As indicated in Figure 1, the IDL fraction in a normolipidemic individual contains substantial quantities of particles at the lower end of the LDL spectrum. This

### Table 2. Concentrations of Total Cholesterol and Lipoprotein Cholesterol in Sera of Patients With Familial Dysbetalipoproteinemia and in Sera of Normolipidemic Subjects

<table>
<thead>
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<th>Parameters (mmol/L)</th>
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<th>Controls (n=10)</th>
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<tr>
<td>HDL cholesterol</td>
<td>0.95±0.25</td>
<td>1.18±0.18</td>
<td>&lt;0.05</td>
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<tr>
<td>LDL cholesterol*</td>
<td>1.84±0.54</td>
<td>3.36±0.53</td>
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<td>IDL cholesterol*</td>
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<td>VLDL2 cholesterol*</td>
<td>2.98±3.08</td>
<td>0.08±0.03</td>
<td>&lt;0.01</td>
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</tbody>
</table>

FD, familial dysbetalipoproteinemia; HDL, high density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; VLDL1, very low density lipoprotein; VLDL2, large VLDL.

*Measured in pooled fractions isolated by density-gradient ultracentrifugation and corrected for cholesterol recovery (0.93 for FD, 0.99 for controls).
suggests that the IDL concentration in normolipidemic individuals is slightly overestimated.

**Interindividual Variability of Lipoproteins**

An important aspect of this study was the analysis of the interindividual differences in the lipoprotein (sub)fractions in the sera of patients with FD. In Figure 3, the cholesterol profiles of three patients with serum cholesterol concentrations of 8.25, 12.29, and 25.25 mmol/L are shown. Only minor interindividual differences in IDL and LDL cholesterol concentrations were observed. As illustrated in Figure 3, the marked differences in the serum cholesterol concentrations of patients with FD were mainly due to differences in the cholesterol concentrations of VLDL₁ and VLDL₂, a phenomenon that was observed in all patients with FD.

The relations between the serum cholesterol concentrations and the cholesterol concentrations of VLDL₁, VLDL₂, IDL, LDL, and HDL in the sera of the 17 patients with FD were analyzed statistically. The cholesterol concentrations of VLDL₁ (Figure 4A) and VLDL₂ (Figure 4B) correlated positively with the serum cholesterol concentration \((r=0.87\) and \(r=0.92\), respectively, both \(p<0.001\)); the cholesterol concentrations of IDL (Figure 4C), LDL (Figure 4D), and HDL (not shown) were not correlated with serum cholesterol concentration. The relations between the cholesterol concentrations of the lipoprotein (sub)fractions and serum triglyceride concentration were also analyzed. The cholesterol concentrations of VLDL₁ and VLDL₂ correlated positively to the serum triglyceride concentration \((r=0.95\) and \(r=0.72\), respectively, both \(p<0.001\)); the cholesterol concentrations of HDL and LDL correlated negatively to the serum triglyceride concentration (both \(r=-0.51\), \(p<0.05\)). The IDL cholesterol concentration was not correlated with the serum triglyceride concentration.

Table 3 presents the relations between the cholesterol concentrations of five lipoprotein (sub)fractions. As expected, VLDL₁ cholesterol concentration correlated strongly with VLDL₂ cholesterol concentration \((r=0.73, p<0.001)\). Moreover, IDL cholesterol concentration correlated positively with LDL cholesterol and HDL cholesterol concentrations \((r=0.68\) and \(r=0.62\), respectively, both \(p<0.01\)). No correlation was found in the cholesterol concentration of VLDL₃ and VLDL₄. In the sera of 17 patients with familial dysbetalipoproteinemia, the relations between the cholesterol concentrations of VLDL₁, VLDL₂, IDL, LDL, and HDL were analyzed statistically. The cholesterol concentrations of VLDL₁ and VLDL₂ correlated positively with the serum cholesterol concentration \((r=0.87\) and \(r=0.92\), respectively, both \(p<0.001\)); the cholesterol concentrations of IDL (Figure 4C), LDL (Figure 4D), and HDL (not shown) were not correlated with serum cholesterol concentration. The relations between the cholesterol concentrations of the lipoprotein (sub)fractions and serum triglyceride concentration were also analyzed. The cholesterol concentrations of VLDL₁ and VLDL₂ correlated positively to the serum triglyceride concentration \((r=0.95\) and \(r=0.72\), respectively, both \(p<0.001\)); the cholesterol concentrations of HDL and LDL correlated negatively to the serum triglyceride concentration (both \(r=-0.51\), \(p<0.05\)). The IDL cholesterol concentration was not correlated with the serum triglyceride concentration.

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Rare Apolipoprotein E Variants with apo E3-Leiden (patient No. 20) had an LDL cholesterol concentration of 2.59 mmol/L. In these patients the apo E2/2 phenotype and patients with rare apo E variants did not differ markedly.

**Discussion**

In the present study the lipoprotein profiles of 17 patients with FD associated with the apo E2/2 phenotype were analyzed in detail. The relative contribution of the different lipoprotein (sub)classes to the marked interindividual variability in the concentrations of lipids in the sera of patients with FD was evaluated. This evaluation might contribute to a better understanding of the factors that influence the development of hyperlipoproteinemia associated with the apo E2/2 phenotype. Since several reports suggest that the expression of FD in individual patients is dependent on factors such as diet, alcohol consumption, body weight, and endocrinological disturbances,1-3 considerable efforts were made to minimize the effects of these factors in the patient population studied in this report. During a 3-12-month follow-up period the serum lipoprotein concentrations remained quite constant. However, considerable interindividual differences as to the serum lipid levels were noted (Table 1), with cholesterol concentrations ranging from 7 to 25 mmol/L and triglyceride concentrations ranging from 3 to 28 mmol/L.

In agreement with observations published earlier,15,15,20 it was found that all patients with FD were characterized by markedly increased IDL concentrations compared with normolipidemic individuals (Table 2). However, the IDL cholesterol concentration in the patients with FD was not correlated to the serum cholesterol and triglyceride concentrations. Moreover, the IDL concentration in FD patients showed only minor interindividual variability.

Several studies have provided evidence that, on average, the LDL cholesterol concentration in patients with FD is not increased compared with the LDL cholesterol concentration in normolipidemic individuals.1-3 However, a rather large interindividual variability of the LDL cholesterol concentration in patients with FD has been observed.17,30,31 Indeed, in some patients with FD, markedly increased serum LDL concentrations were reported,17 suggesting that the apo E2/2 phenotype was superimposed by familial hypercholesterolemia or familial combined hyperlipoproteinemia. However, in the present study no significant interindividual variability of the LDL cholesterol concentration was found, and none of the patients had a distinctly elevated LDL cholesterol concentration.

### Table 3. Correlations Between Cholesterol Concentrations of Five Lipoprotein Classes in Sera of 17 Patients With Familial Dysbetalipoproteinemia

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>VLDL₁, C</th>
<th>VLDL₁, C</th>
<th>IDL, C</th>
<th>LDLC, C</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL₁, C</td>
<td>0.732 *</td>
<td></td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>IDL, C</td>
<td>-0.339 NS</td>
<td>0.186 NS</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>LDL, C</td>
<td>-0.434 NS</td>
<td>-0.146 NS</td>
<td>0.677 *</td>
<td>...</td>
</tr>
<tr>
<td>HDL, C</td>
<td>-0.415 NS</td>
<td>-0.094 NS</td>
<td>0.618 t</td>
<td>0.463 NS</td>
</tr>
</tbody>
</table>

VLDL₁, large very low density lipoprotein; C, cholesterol; VLDL₁, small VLDL; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; NS, not significant.

**Table 4. Composition of Isolated Lipoproteins in Sera of Patients With Familial Dysbetalipoproteinemia (n=17) and in Sera of Normolipidemic Control Subjects (n=10) Expressed in Percentages of Lipoprotein Mass (Mean±SD)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls</th>
<th>FD</th>
<th>Controls</th>
<th>FD</th>
<th>Controls</th>
<th>FD</th>
<th>Controls</th>
<th>FD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free cholesterol</td>
<td>6.3±0.6</td>
<td>6.9±2.4</td>
<td>6.2±0.6</td>
<td>9.3±1.6*</td>
<td>10.5±3.7</td>
<td>10.9±2.1</td>
<td>10.3±0.7</td>
<td>9.1±1.6</td>
</tr>
<tr>
<td>Cholesterol ester</td>
<td>7.4±1.2</td>
<td>24.6±6.5†</td>
<td>14.4±1.7</td>
<td>32.8±5.7†</td>
<td>33.8±4.6</td>
<td>36.8±5.1</td>
<td>43.1±1.9</td>
<td>34.2±4.7*</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>64.8±2.9</td>
<td>47.3±5.7†</td>
<td>49.6±4.7</td>
<td>29.8±5.7†</td>
<td>18.7±2.2</td>
<td>15.7±2.7†</td>
<td>3.9±0.3</td>
<td>11.3±2.4‡</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>13.4±1.6</td>
<td>14.8±2.2</td>
<td>18.7±1.5</td>
<td>18.2±2.2</td>
<td>18.1±1.4</td>
<td>19.7±3.9</td>
<td>19.7±1.1</td>
<td>20.2±2.7</td>
</tr>
<tr>
<td>Protein</td>
<td>8.1±1.5</td>
<td>6.4±1.4</td>
<td>11.1±1.4</td>
<td>9.7±2.6</td>
<td>18.9±2.8</td>
<td>16.9±2.9</td>
<td>23.0±2.3</td>
<td>25.2±3.6</td>
</tr>
</tbody>
</table>

VLDL₁, large very low density lipoprotein; VLDL₂, small VLDL; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; FD, familial dysbetalipoproteinemia.

All lipoproteins were isolated by density-gradient ultracentrifugation.

†p<0.05; †p<0.01; ‡p<0.001.
concentration (Table 2). It should be noted that in the present study, LDL was isolated by density-gradient ultracentrifugation and not by sequential ultracentrifugation. In preliminary studies we found that approximately 70% of the IDL isolated by density-gradient ultracentrifugation was recovered in the density interval of 1.006–1.019 g/mL when subjected to sequential ultracentrifugation, and the remaining 30% was recovered in the d=1.019–1.063 g/mL fraction. Thus, if the LDL of patients with FD is isolated by sequential ultracentrifugation, the concentration of LDL is overestimated.

Serum VLDL from patients with FD and from normolipidemic individuals was separated into two subfractions (VLDL, and VLDL2). In comparison to the VLDL subfractions from normolipidemic individuals, the VLDL subfractions from FD patients were enriched in CEs (Table 4). Interestingly, the chemical composition of VLDL1 and VLDL2 was not related to their concentrations in serum (Figure 5).

As shown in Figure 4 the differences in the cholesterol concentrations of VLDL1 and VLDL2 were mainly responsible for the observed interindividual variability in the concentration of cholesterol in the sera of FD patients. This suggests that the additional (genetic) factors that have been considered to be required for the expression of this disorder in individuals with the apo E2/2 phenotype play a role in the regulation of VLDL concentration. This hypothesis is supported by data from an extensive family study of patients with FD reported by Stuyt et al. They observed that many family members were characterized by a mild-to-moderate increase in the plasma triglyceride concentration, whereas only a few family members had an increased plasma cholesterol concentration. A similar occurrence of hypertriglyceridemia in family members of patients with FD was noted by Hazzard et al. These observations suggest that a combination of the apo E2/2 phenotype and a genetic abnormality in the metabolism of the triglyceride-rich lipoproteins is required for the expression of FD. It is possible that several different genetic abnormalities in the metabolism of the triglyceride-rich lipoproteins have the capacity to generate the expression of FD in individuals with the apo E2/2 phenotype. The variability in the expression of FD could therefore be due to differences in the effects of specific genetic abnormalities in the metabolism of the triglyceride-rich lipoproteins in individuals with the apo E2/2 phenotype.

Despite marked interindividual differences in the concentration of lipids and lipoproteins in the sera of FD patients, several features in the lipoprotein profiles were consistently observed in all patients: a depressed LDL cholesterol concentration, a markedly elevated IDL cholesterol concentration, and an altered composition (enrichment by CE) of both VLDL1 and VLDL2. In an extensive analysis of normolipidemic individuals and of a large number of patients with various types of primary and secondary hyperlipoproteinemias, we have not found a single individual with this combination of abnormalities, except for FD patients. Thus, it appears that the combined presence of a low LDL cholesterol concentration, a high IDL cholesterol concentration, and an altered VLDL composition is a unique feature of FD patients with the apo E2/2 phenotype. In preliminary studies we found that the lipoprotein profiles of individuals with the apo E2/2 phenotype and serum cholesterol levels below 7.0 mmol/L were characterized by the same combination of abnormalities. This indicates that both normolipidemic and hyperlipidemic individuals with the apo E2/2 phenotype are character-
ized by a similar combination of lipoprotein abnormalities, suggesting that apo E2/2 is a primary factor in the pathophysiology of these abnormalities. Since experimental studies indicate that apo E2 (Arg158→Cys) has a defective interaction with the apo B,E (LDL) receptor,10 it is tempting to speculate that impaired receptor binding in individuals with the apo E (Arg158→Cys) homozygosity is responsible for the observed combination of lipoprotein abnormalities. In an attempt to explore this hypothesis the lipoprotein profiles of patients with rare apo E mutants, one patient with apo E3-Leiden and two patients with apo E2 (Lys146→Gln), were analyzed (Figure 6). In the patients with apo E2 (Lys146→Gln), the LDL cholesterol concentration was higher and the IDL cholesterol concentration lower than in the patients with the apo E2/2 phenotype. In the patient with apo E3-Leiden, the cholesterol concentrations of LDL and IDL were intermediate between those in patients with the apo E2/2 phenotype and in patients with apo E2 (Lys146→Gln). Since it was demonstrated that the LDL receptor binding activities of apo E2 (Arg158→Cys), E3-Leiden, and E2 (Lys146→Gln) were 1%, 25%, and 40%, respectively, of the binding of apo E3,10 these observations suggest that the apo B,E (LDL) receptor binding activity of apo E is related to the concentrations of LDL and IDL. More detailed studies are required to test the hypothesis that the apo B,E (LDL) receptor binding activity of apo E is directly linked to the regulation of the concentrations of LDL and IDL in plasma.

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References

Lipoproteins in familial dysbetalipoproteinemia. Variation of serum cholesterol level associated with VLDL concentration.

S P Zhao, A H Smelt, J A Leuven, A M van den Maagdenberg, A van der Laarse and F M van 't Hooft

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