Plasma Lipoproteins in Familial Dysbetalipoproteinemia Associated With Apolipoproteins E2(Arg158→Cys), E3-Leiden, and E2(Lys146→Gln), and Effects of Treatment With Simvastatin


Abstract
Using a density-gradient ultracentrifugation technique, we analyzed in detail the plasma lipoprotein profiles of 18 patients with familial dysbetalipoproteinemia (FD) who had apolipoprotein (apo) E2(Arg158→Cys) homozygosity (the E2-158 variant, n=6), apoE3-Leiden heterozygosity (the E3-Leiden variant, n=6), or apoE2(Lys146→Gln) heterozygosity (the E2-146 variant, n=6), with average plasma cholesterol concentrations of 8.99±1.34 mmol/L, 9.29±1.55 mmol/L, and 8.46±1.10 mmol/L, respectively. No significant differences in sex, age, body mass index, dietary habits, and standard laboratory tests between the three groups were observed. The lipoprotein profiles of all FD patients were characterized by higher concentrations of very-low-density lipoprotein (VLDL), LDL, and intermediate-density lipoprotein (IDL) and a higher cholesteryl ester content of VLDL1 and VLDL2 than in 6 normolipidemic control subjects with an average plasma cholesterol concentration of 5.90±0.53 mmol/L. Major differences between the plasma lipoprotein profiles of patients with the E2-158 variant, the E3-Leiden variant, and the E2-146 variant and the normolipidemic control subjects were in IDL cholesterol concentration (1.70±0.26, 1.50±0.26, 1.05±0.36, and 0.47±0.14 mmol/L, respectively), LDL cholesterol concentration (1.83±0.50, 3.09±0.32, 3.79±0.76, and 3.77±0.56 mmol/L, respectively), and the molar ratio of IDL cholesterol to LDL cholesterol (0.98±0.28, 0.48±0.04, 0.28±0.09, and 0.12±0.03, respectively). After 10 weeks of simvastatin treatment the concentrations of plasma cholesterol, VLDL2 cholesterol, IDL cholesterol, and LDL cholesterol in 3 patients with the E2-158 variant fell significantly, by 46%, 56%, 53%, and 48%, respectively; they also fell in 3 patients with the E3-Leiden variant, by 48%, 54%, 57%, and 52%, respectively, and in 3 patients with the E2-146 variant, by 38%, 55%, 46%, and 35%, respectively. Simvastatin therapy lowered plasma activity of cholesteryl ester transfer protein but had no significant effect on plasma activity of lecithin:cholesterol acyltransferase. It is concluded that patients with FD due to various apoE variants have different lipoprotein profiles, mainly with regard to IDL and LDL levels, although they have a number of similar features of dysbetalipoproteinemia. Simvastatin therapy effectively reduced the plasma concentrations of total cholesterol, VLDL2 cholesterol, IDL cholesterol, and LDL cholesterol in the three groups of patients studied. It is proposed that apoE-dependent defects of the conversion of IDL to LDL may be an important mechanism in the pathophysiology of FD. (Arterioscler Thromb. 1994;14:1705-1716.)

Key Words • very-low-density lipoprotein • low-density lipoprotein • intermediate-density lipoprotein • familial dysbetalipoproteinemia • simvastatin • apolipoprotein E

Human apolipoprotein (apo) E, a protein of 299 amino acid residues, is a constituent of several plasma lipoproteins, including chylomicrons, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and a subclass of high-density lipoprotein (HDL), HDL1. Three common alleles of apoE at a single gene locus on chromosome 19 code for apoE2, apoE3, and apoE4, which can be distinguished by isoelectric focusing. Three homozygous phenotypes (apoE2/2, apoE3/3, and apoE4/4) and three heterozygous phenotypes (apoE3/2, apoE4/3, and apoE4/2) arise from the expression of any two of the three alleles. Amino acid substitutions account for the differences between apoE4, apoE3, and apoE2. ApoE4 differs from apoE3 in that in apoE4 arginine is substituted for the cysteine normally occurring at amino acid residue 112. The most common form of apoE2 designated apoE2(Arg158→Cys), differs from apoE3 at residue 158, where cysteine is substituted for the normally occurring arginine. Several in vitro studies have provided evidence

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From the Departments of Cardiology (S.-P.Z., T.F.F.P.V., A. Van der L., F.M.Van 't H.), Internal Medicine (A.H.M.S.), and Human Genetics (A.M.J.M. Van den M., R.R.F.), Medical Faculty, University of Leiden, Department of Biochemistry I (A.V.T.), Cardiovascular Research Institute COEUR, Erasmus University, Rotterdam, and Gausius Laboratory (J.A.G.L., L.M.H.), IVVO-TNO, Leiden, Netherlands; Cardiovascular Research Laboratory (S.-P.Z.), Second Affiliated Hospital, Hunan Medical University, Changsha, People's Republic of China; and King Gustaf V Research Institute (F.M.Van 't H.), Karolinska Hospital, Stockholm, Sweden.

Correspondence to A. Van der Laarse, PhD, Department of Cardiology, Building 1, CS-P24, University Hospital, Rijnsburgerweg 10, 2333 AA Leiden, Netherlands. © 1994 American Heart Association, Inc.
that apoE2(Arg158→Cys), the E2-158 variant, has a binding affinity to the low-density lipoprotein (LDL) receptor less than 2% that of apoE3 and apoE4. This phenomenon is considered to be of primary importance in the pathophysiology of familial dysbeta-lipoproteinemia (FD), or type III hyperlipoproteinemia, a lipoprotein disorder associated with high plasma cholesterol and triglyceride (TG) concentrations. More than 90% of patients with FD are homozygous for apoE2(Arg158→Cys). However, in the population the majority of individuals homozygous for the E2-158 variant are normocholesterolemic, suggesting that additional endogenous or exogenous factors influence the expression of the disorder. In addition to the E2-158 variant, a number of rare apoE variants have been identified in patients with FD, including apoE-Leiden with a 7-amino acid insertion (the E3-Leiden variant and the E2-146 variant). In vitro LDL receptor-binding studies demonstrated that the E3-Leiden variant had 25% of normal binding activity and the E2-146 variant had 40%, providing further evidence that defective apoE binding might be of primary importance in the pathophysiology of FD.

In previous studies we observed that the lipoprotein profiles of both normocholesterolemic and hypercholesterolemic individuals with the E2-158 variant are characterized by markedly lower LDL cholesterol concentrations, considerably higher cholesteryl ester (CE) content of the VLDL subfractions, and variably higher cholesterol concentrations in VLDL1, VLDL2, and IDL than normolipidemic control subjects. We proposed that the lipoprotein abnormalities in the plasma of individuals with the E2-158 variant were related to an impaired conversion of IDL to LDL, a hypothesis supported by in vivo studies. It has been suggested that the binding of IDL to a membrane receptor may be an essential step in the conversion of IDL to LDL, and apoE might be involved in this process. However, little is known about the regulation of the conversion of IDL to LDL, nor is there a suitable in vitro model to test the hypothesis that the binding activities of apoE and its variants influence the conversion. In the present study the plasma lipoprotein profiles of patients with three different apoE variants (the E2-158 variant, the E3-Leiden variant, and the E2-146 variant) with depressed LDL receptor-binding activities in vitro (discussed above) were compared with the plasma lipoprotein profiles of normolipidemic individuals to search for any relation between apoE receptor-binding activity and the plasma concentrations of IDL and LDL. Furthermore, we investigated whether simvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor that has been reported to reduce plasma concentrations of lipids and lipoproteins in patients with FD associated with the E2-158 variant, was effective in improving the plasma lipoprotein profiles in these three groups of patients.

Methods

Subjects

All subjects in this study visited the Lipid Clinic of the Leiden University Hospital between July 1989 and September 1992. During the study period, 7 hypercholesterolemic patients with the E3-Leiden variant, 7 with the E2-146 variant, and 6 with the E2-158 variant were evaluated. Two patients (1 with the E3-Leiden variant and 1 with the E2-146 variant) were excluded from the study because their lipid and lipoprotein levels varied widely over time, presumably because of irregular dietary habits. All other patients had minimal intraindividual variability in lipoprotein levels under optimal dietary conditions (see below), as evaluated by repeated lipid analysis over an interval of at least 2 months. The ages of the patients ranged from 23 to 72 years, with the average age of the patients with the E2-158 variant, the E3-Leiden variant, and the E2-146 variant being 51.0±8.1, 50.6±12.3, and 52.3±18.4 years, respectively. None of the patients appeared to have renal, thyroid, or liver disease, as assessed by physical examination and routine laboratory tests. All patients had normal fasting blood glucose levels. None took lipid-lowering drugs or any other drugs that might interfere with the lipoprotein metabolism for at least 6 weeks before blood samples were taken for the analysis of the lipoprotein profile (except for 9 patients who received simvastatin therapy; see below).

All patients with the E2-158 variant, as determined during routine screening of the apoE phenotype of patients with hypercholesterolemia, were referred to the Lipid Clinic. None of these patients were related. In the group of patients with the E3-Leiden variant, patient No. 12 was a newly identified proband, probably related to the large family described by De Knijff et al; the other patients (No. 7 through No. 11) were diagnosed during a family study of this proband. Patients No. 14 to No. 16 were newly identified with the E2-146 variant. Patients No. 14 and No. 16 were members of the family of patient No. 18. Patients No. 15 and No. 17 were members of a previously described family with the E2-146 variant. Table 1 lists the clinical characteristics of the study subjects.

All patients were analyzed using a standardized protocol. This analysis included a careful clinical examination; several routine laboratory tests; repeated analysis of fasting cholesterol, TG, and HDL cholesterol concentrations; agarose electrophoresis; and routine plasma ultracentrifugation. The diet of all patients was evaluated by a qualified dietitian using a 24-hour dietary history. Patients who adhered to a low-fat diet (<30% of total calories) with a fatty acid intake (saturated: monounsaturated:polyunsaturated) ratio of 1:1:1, cholesterol intake less than 300 mg/day, and alcohol consumption less than 15 g/day, were given specific dietary instructions. Patients who did not adhere to these guidelines were given individualized instruction in writing and sent to the dietitian for further explanation of the instructions. The effects of the dietary advice were evaluated again 2 to 3 months later by repeated biochemical measurements (see above) and reevaluation of the dietary history. All data presented in this study were obtained under standardized dietary conditions.

After the baseline period, 9 patients—3 with the E2-158 variant, 3 with the E3-Leiden variant, and 3 with the E2-146 variant—were treated with simvastatin (Zocor, Merck Sharp & Dohme) at a dose of 20 mg daily. Blood samples were taken at baseline and at the end of 10 weeks of treatment. Treated patients reported good compliance, and none experienced any side effects. No changes in routine laboratory test results, dietary habits, or body weight were observed. Patients who did not adhere to these guidelines were given individualized instruction in writing and sent to the dietitian for further explanation of the instructions.

Six normolipidemic individuals (3 men and 3 women, aged 58.1±9.6 years [mean±SD]) served as control subjects in this study. Normolipidemia was defined by plasma cholesterol concentration <6.5 mmol/L and plasma TG concentration <2.0 mmol/L. One of the normolipidemic control subjects had the apoE4/2 phenotype, four had the apoE3/3 phenotype, and one had the apoE3/2 phenotype. All clinical characteristics of the normolipidemic subjects were analyzed in the Lipid Clinic in the same way as those of the hyperlipoproteinemic patients.

Separation of Lipoproteins

Blood samples were taken in the morning after a fast of more than 12 hours. Plasma was obtained by centrifugation at
TABLE 1. Clinical Characteristics of Patients With Familial Dysbetalipoproteinemia (FD) Having ApoE2(Arg158→Cys) Homozygosity (E2-158 Variant), ApoE3-Leiden Heterozygosity (E3-Leiden Variant) and ApoE2(Lys146→Gln) Heterozygosity (E2-146 Variant)

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/ Sex</th>
<th>BMI, kg/m²</th>
<th>Total C, mmol/L</th>
<th>Total TG, mmol/L</th>
<th>VLDL-C, mmol/L</th>
<th>LDL-C, mmol/L</th>
<th>HDL-C, mmol/L</th>
<th>ApoE Phenotype</th>
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<tr>
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<td></td>
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<tr>
<td>1</td>
<td>55/F</td>
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<td>7.75</td>
<td>2.97</td>
<td>3.64</td>
<td>3.24</td>
<td>0.87</td>
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</tr>
<tr>
<td>2</td>
<td>48/M</td>
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<td>7.80</td>
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<td>3.51</td>
<td>3.45</td>
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<td>E2/2</td>
</tr>
<tr>
<td>3</td>
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<td>4.28</td>
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<td></td>
</tr>
<tr>
<td>7</td>
<td>36/F</td>
<td>22.6</td>
<td>7.09</td>
<td>2.51</td>
<td>2.28</td>
<td>3.75</td>
<td>1.05</td>
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<tr>
<td>8</td>
<td>39/F</td>
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<td>8.39</td>
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<td>5.13</td>
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<td>11</td>
<td>66/M</td>
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<td>5.00</td>
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<td>68/F</td>
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<td>5.36</td>
<td>5.33</td>
<td>1.05</td>
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</tr>
<tr>
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<td>66/F</td>
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<td>7.13</td>
<td>3.87</td>
<td>2.21</td>
<td>3.87</td>
<td>1.05</td>
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<td>3.50</td>
<td>4.10</td>
<td>0.70</td>
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</tr>
<tr>
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<td>8.42</td>
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<td>2.30</td>
<td>5.01</td>
<td>1.11</td>
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<tr>
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<td>2.49</td>
<td>2.49</td>
<td>5.37</td>
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<tr>
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<td>2.75</td>
<td>6.50</td>
<td>1.07</td>
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</tr>
</tbody>
</table>

Apo indicates apolipoprotein; BMI, body mass index; C, cholesterol; TG, triglyceride; VLDL-C, very-low-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; and HDL-C, high-density lipoprotein cholesterol.

*Including intermediate-density lipoprotein.

1000g 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 modification of the two-step density-gradient ultracentrifugation technique. In the first step HDL, LDL, IDL, and VLDL were separated. In the second step VLDL was separated further into two subfractions, VLDL1 (large VLDL) and VLDL2 (small VLDL). The cholesterol concentrations and the composition of both gradients. The cholesterol concentrations and the injection of both gradients. The cholesterol concentrations and the cholesterol concentrations and the cholesterol concentrations in pooled gradient fractions. IDL was obtained in fractions 13 through 18 and LDL in fractions 6 through 12 of the first-step ultracentrifugation. VLDL1 was recovered in fractions 21 through 23 and VLDL2 in fractions 11 through 20 of the second-step ultracentrifugation. The total cholesterol (TC), free cholesterol (FC), TG, and phospholipid (PL) concentrations were determined enzymatically using test kits (Boehringer). EC was calculated as the difference between TC and FC. The mass of CE (in milligrams) was estimated as 1.67×esterified cholesterol. Total protein (P) was determined by a modification of the Lowry procedure with bovine serum albumin as a standard. The total lipoprotein mass (in milligrams) was calculated as the sum of masses of FC, CE, TG, PL, and P. HDL-cholesterol concentration was measured in the d=1.006 kg/L infranatant obtained by routine ultracentrifugation after precipitation of IDL and LDL by phosphotungstic acid and MgCl₂.

**Lecithin: Cholesterol Acyltransferase and Cholesteryl Ester Transfer Protein Assays**

Plasma lecithin: cholesterol acyltransferase (LCAT) activity was determined using excess exogenous substrate, containing [3H]cholesterol, as described. Incubations lasted 6 hours at 37°C in a total volume of 0.145 mL. The reaction was stopped by addition of 0.30 mL cold methanol. The lipids were extracted twice with 0.4 mL hexane. FC and CE were separated using disposable silica columns. [3H]Cholesteryl esters were eluted with 3.0 mL hexane:diethylether (6:1, vol/vol).
Plasma CE transfer protein (CETP) activity was measured in the supernatant fraction of each plasma sample after precipitation of endogenous apoB-containing lipoproteins by phosphotungstic acid and MgCl₂. The exchange of CEs between [³⁵S]cholesterol ester-labeled LDL and unlabeld LDL was measured during a 16-hour incubation. After incubation, LDL was precipitated by Mn²⁺ ions, following the method of Morton and Zilversmit, and the radioactivity of HDL was determined.

The activities of LCAT and CETP were measured in plasma stored at −80°C. The measured activities were proportional to the amount of plasma used in the incubations. All assays were performed in duplicate. The within-day coefficients of variation were 4.5% for LCAT and 2.7% for CETP. The measured activities reflect the activity of the enzyme and transfer protein (measured under optimal conditions) and are independent of endogenous plasma lipoproteins. The activities were related to the activity in a human plasma pool, and expressed in arbitrary units (AU) as percent of the activity in the plasma pool.

ApoE Phenotyping and Genotyping

ApoE phenotype was determined by isoelectric focusing of delipidated plasma samples before and after cysteamine treatment followed by immunoblotting, as described by Havekes et al. For apoE genotyping, genomic DNA was isolated from leukocytes by standard methods. The 5' part of exon 4 of the human apolipoprotein E (APOE) gene, encoding amino acids 61 through 174, was amplified by polymerase chain reaction (PCR) using the primers 402 (nucleotides 3555 through 3574, coding strand) and 401 (nucleotides 3932 through 3913, noncoding strand), as described by Van den Maagdenberg et al. For allele-specific restriction endonuclease genotyping of the common polymorphisms at codons 112 and 158, 15 μL of PCR product was digested with 7.5 U restriction enzyme HhaI for 16 hours, according to recommendations of the supplier (Pharmacia) and as described first by Hixson and Vernier. Thereafter, the digested material was separated on a 10% neutral polyacrylamide gel for 3 hours at 10 V/cm and stained with 0.1 g/L ethidium bromide. For APOE*2(Lys146>Gln) allele detection, PCR was done using a site-directed mutagenic amplification primer. Primer 3012 (nucleotides 3848 through 3867, a noncoding strand) containing a G→T nucleotide mismatch at position 3851 and primer 398 (nucleotides 3678 through 3697, a coding strand) were used in a PCR. The reaction mix contained 0.5 μg genomic DNA, 0.5 mmol/L MgCl₂, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), 0.2 mmol/L deoxynucleoside triphosphates, 200 μg/mL bovine serum albumin, 50 pmol of each primer, 0.1 U Taq polymerase (Boehringer Mannheim), and 10% dimethylsulfoxide in a total volume of 50 μL. The PCR was done for 32 cycles (1 minute at 95°C, 30 seconds at 55°C, and 90 seconds at 72°C) after an initial denaturation of 4 minutes. Fifteen microliters of PCR products was digested with PvuII according to the recommendations of the supplier (Pharmacia), and fragments were separated on a 7.5% polyacrylamide gel.

Statistical Analysis

Results were expressed as mean±SD. Differences between groups in mean concentrations of lipids, lipoproteins, apolipoproteins, and other characteristics were analyzed using one-way ANOVA followed by a Scheffé multiple-comparison test. Because the distributions of plasma TG, VLDL1 cholesterol, and VLDL2 cholesterol concentrations were highly skewed, their logarithmically transformed concentrations were used for statistical comparison. All statistical analyses were performed with SPSS/PC+ software (SPSS Inc). A value of P<.05 was considered statistically significant.

Results

Patients with FD were identified by routine analysis of the apoE phenotype using isoelectric focusing of delipidated plasma samples before and after cysteamine treatment. All patients with the E2-158 variant had the apoE2/2 phenotype and a complete modification (into E4/4) with cysteamine. Patients with the E3-Leiden variant and the E2-146 variant had an incomplete modification of apoE3 and apoE2, respectively. Further analysis of the APOE gene by allele-specific restriction endonuclease genotyping with the restriction enzyme HhaI, PvuII, or both revealed that all patients with the E2-158 variant were homozygous for the APOE*2(Arg158→Cys) allele, whereas all patients with the E3-Leiden variant and the E2-146 variant were heterozygous for the APOE*3-Leiden and APOE*2(Lys146→Gln) allele, respectively, as illustrated in Fig 1.

All patients whose laboratory results were analyzed in the present study had plasma cholesterol concentrations between 7 and 12 mmol/L under optimal dietary conditions (Table 1), and no differences were observed between the average plasma cholesterol concentrations of the three groups of patients (Table 2). None of the patients were substantially overweight, and the average body mass index for the patients with the E2-158 variant, the E3-Leiden variant, and the E2-146 variant was 27.3±2.6, 25.6±2.8, and 24.4±2.2 kg/m², respectively. None of the patients had clinical or biochemical signs of secondary hyperlipoproteinemia, and among the three groups no differences in various routine laboratory tests were observed. None of the patients reported unusual dietary habits, and no differences in various dietary parameters were found between the three groups (data not shown). Thus, the three groups of patients were comparable with regard to several clinical and biochemical parameters.
The plasma lipoprotein profiles of all subjects were analyzed by routine ultracentrifugation (Table 1) and by density-gradient ultracentrifugation. Typical plasma lipoprotein profiles from the three groups of patients with FD analyzed by density-gradient ultracentrifugation are shown in Fig 2 and compared with the lipoprotein profile of a normolipidemic control subject. The average plasma cholesterol concentrations of VLDL1, VLDL2, IDL, LDL, and HDL in the three groups of patients with FD were compared with those of 6 normolipidemic subjects (Table 2). The lipoprotein composition of all patients with FD was characterized by high cholesterol concentrations of VLDL1, VLDL2, and IDL compared with normolipidemic control subjects. However, there were marked differences in IDL cholesterol and LDL cholesterol concentrations between FD patients with different apoE variants. The patients with the E2-158 variant had a substantially lower LDL cholesterol concentration and a markedly higher IDL cholesterol concentration than patients with the other two variants. In contrast, the patients with the E2-146 variant had a normal LDL cholesterol concentration and a moderately higher IDL cholesterol concentration in the patients with the E2-158 variant were between those of the patients with the E2-158 variant and those with the E2-146 variant.

Several lipoprotein parameters were evaluated to find one that could be used to discriminate between the four groups (the three with FD and the group of normolipidemic subjects) (Table 3). As illustrated in Fig 3, the molar ratio of IDL cholesterol to LDL cholesterol appeared to be a sensitive parameter in distinguishing between the groups. The IDL/LDL ratio had a relatively broad distribution (from 0.55 to 1.44) in the patients with the E2-158 variant, whereas this ratio was quite invariable in the patients with the E3-Leiden variant (from 0.44 to 0.54), in those with the E2-146 variant (from 0.21 to 0.41), and in normolipidemic subjects (0.09 to 0.17). There was no overlap of the IDL/LDL ratio between the four groups. Any pair of means was significantly different from the others at the P<0.05 level.

After 10 weeks of simvastatin therapy, the plasma lipoprotein profiles in the 9 treated patients with FD (3 per variant) had changed considerably compared with baseline (Fig 4). As illustrated in Fig 5, the plasma concentrations of TC, VLDL2 cholesterol, IDL cholesterol, and LDL cholesterol fell considerably in each patient with FD, irrespective of the apoE variant. The effects of simvastatin treatment on the mean concentrations of plasma lipids and lipoproteins are summarized in Table 4. Overall, simvastatin treatment was associated with decreases in concentrations of plasma chole-

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**Table 2. Cholesterol Concentrations of Lipoproteins in Plasma of Patients With Familial Dysbeta1ipoproteinemia Having ApoE2(Arg158→Cys) Homozygosity (E2-158 Variant), ApoE3-Leiden Heterozygosity (E3-Leiden Variant), and ApoE2(Lys146→Gln) Heterozygosity (E2-146 Variant), and in Plasma of Normolipidemic Control Subjects**

<table>
<thead>
<tr>
<th>Lipoproteins, mmol/L</th>
<th>E2-158 Variant (n=6)</th>
<th>E3-Leiden Variant (n=6)</th>
<th>E2-146 Variant (n=6)</th>
<th>Control Subjects (n=6)</th>
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</thead>
<tbody>
<tr>
<td>Plasma-C</td>
<td>8.99±1.33*</td>
<td>9.29±1.54*</td>
<td>8.46±1.10*</td>
<td>5.90±0.53</td>
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<tr>
<td>VLDL1-C</td>
<td>1.22±0.39*</td>
<td>0.84±0.40*</td>
<td>0.66±0.35*</td>
<td>0.08±0.03</td>
</tr>
<tr>
<td>VLDL2-C</td>
<td>3.36±0.89†</td>
<td>2.82±0.75†</td>
<td>1.99±0.20†</td>
<td>0.39±0.12</td>
</tr>
<tr>
<td>IDL-C</td>
<td>1.70±0.26†</td>
<td>1.50±0.26†</td>
<td>1.05±0.36*</td>
<td>0.47±0.14</td>
</tr>
<tr>
<td>LDL-C</td>
<td>1.83±0.50†</td>
<td>3.09±0.32†</td>
<td>3.79±0.76</td>
<td>3.77±0.56</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.88±0.13*</td>
<td>1.04±0.03</td>
<td>0.96±0.16*</td>
<td>1.19±0.23</td>
</tr>
</tbody>
</table>

Apo indicates apolipoprotein; C, cholesterol; VLDL1, large very-low-density lipoprotein; VLDL2, small VLDL; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; and HDL, high-density lipoprotein. Values are mean±SD.

*P<0.05 compared with normolipidemic control subjects; †P<0.05 compared with control subjects and patients with E2-146; and ‡P<0.05 compared with control subjects, patients with E2-146, and patients with E3-Leiden.
TABLE 3. Composition of Lipoproteins in Plasma of Patients With Familial Dysbetaproteinemia Having ApoE2(Arg158—Cys) Homozygosity (E2-158 Variant), ApoE3-Leiden Heterozygosity (E3-Leiden Variant), and ApoE2(Lys146—Gln) Heterozygosity (E2-146 Variant), and in Plasma of Normolipidemic Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Free Cholesterol</th>
<th>Cholesterol Ester</th>
<th>Triglyceride</th>
<th>Phospholipid</th>
<th>Protein</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>E2-158</td>
<td>E3-Leiden</td>
<td>E2-146</td>
<td>Control</td>
<td></td>
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<tr>
<td>VLDL1</td>
<td>6.5±1.4</td>
<td>9.1±1.8*</td>
<td>8.0±1.8</td>
<td>5.8±2.0</td>
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</tr>
<tr>
<td></td>
<td>19.4±5.4*</td>
<td>17.2±4.0*</td>
<td>16.8±4.3*</td>
<td>8.2±4.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>55.4±5.7*</td>
<td>55.7±5.7*</td>
<td>55.8±5.6*</td>
<td>65.2±5.1</td>
<td>12.4±4.3</td>
</tr>
<tr>
<td></td>
<td>13.2±1.7</td>
<td>11.7±1.6</td>
<td>11.8±3.6</td>
<td>12.4±4.3</td>
<td>8.4±2.0</td>
</tr>
<tr>
<td>VLDL2</td>
<td>9.5±1.0</td>
<td>10.6±1.3</td>
<td>9.9±1.3</td>
<td>7.3±2.0</td>
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<tr>
<td></td>
<td>31.9±3.8†</td>
<td>27.6±1.8*</td>
<td>23.0±4.2*</td>
<td>16.7±4.9</td>
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<tr>
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<td>23.6±4.9*</td>
<td>32.2±1.4†</td>
<td>39.6±5.4*</td>
<td>48.1±5.7</td>
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<tr>
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<td>29.3±2.9†</td>
<td>17.1±2.3</td>
<td>15.1±2.9</td>
<td>16.0±3.3</td>
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</tr>
<tr>
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<td>17.8±1.7</td>
<td>12.6±2.3</td>
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<tr>
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<td>40.8±2.5</td>
<td>37.7±5.1</td>
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<tr>
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<td>11.4±1.5*</td>
<td>15.0±5.1</td>
<td>15.8±3.8</td>
<td>18.1±1.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>19.3±1.0</td>
<td>18.3±1.9</td>
<td>17.1±2.8</td>
<td>18.2±2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16.7±2.0</td>
<td>17.8±2.6</td>
<td>20.8±3.3</td>
<td>19.3±2.8</td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td>10.5±2.0</td>
<td>10.4±1.3</td>
<td>9.5±0.8</td>
<td>9.2±0.7</td>
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</tr>
<tr>
<td></td>
<td>38.7±2.5</td>
<td>41.7±2.9</td>
<td>41.5±1.4</td>
<td>42.1±2.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.2±1.4‡</td>
<td>5.0±0.6</td>
<td>5.7±0.8</td>
<td>5.1±1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18.9±1.2</td>
<td>19.8±1.6</td>
<td>19.1±3.0</td>
<td>19.6±0.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24.9±2.8</td>
<td>23.1±2.2</td>
<td>24.3±4.4</td>
<td>24.0±2.7</td>
<td></td>
</tr>
</tbody>
</table>

Apo indicates apolipoprotein; VLDL1, large very-low-density lipoprotein; VLDL2, small VLDL; IDL, intermediate-density lipoprotein; and LDL, low-density lipoprotein. Values are expressed as wt/wt and given as mean±SD.

*p<.05 compared with normolipidemic control subjects; †p<.05 compared with control subjects and patients with E2-146; and *p<.05 compared with control subjects, patients with E3-Leiden, and patients with E2-146.

In the three groups of patients. The concentrations of plasma TG and VLDL1 cholesterol tended to decrease in the three groups taking simvastatin, but these changes did not reach statistical significance except for the plasma TG concentration of patients with the E2-146 variant.

The compositions of the lipoprotein subfractions in the three groups of patients after simvastatin therapy were compared with those measured at baseline (Table 5). During simvastatin treatment, no significant changes in the compositions of VLDL1, IDL, and LDL were observed, and only minor changes in the composition of VLDL2 were found. However, the CE content tended to be lower and the TG content tended to be higher in all apoB-containing lipoprotein subfractions of the three groups of patients during simvastatin therapy, although only the change in TG content of VLDL2 of the patients with the E2-158 variant reached statistical significance.

The activities of CETP and LCAT in the plasma of 7 patients with FD (E2-158 variant, n=3; E3-Leiden variant, n=2; and E2-146 variant, n=2) were determined. At baseline, the activity of CETP in the plasma of patients with the E2-158 variant (145±27 AU) was significantly higher than that in normolipidemic control subjects (77±15 AU), whereas the activity of CETP in the plasma of patients with the E3-Leiden variant and the E2-146 variant did not differ significantly from that

![Fig 3. Scatterplot of molar ratio of IDL cholesterol to LDL cholesterol in plasma of patients with familial dysbeta lipoproteinemia associated with apolipoprotein (apo) E2(Arg158—Cys) homozgyosity (E2-158), apoE3-Leiden heterozygosity (E3-L), and apoE2(Lys146—Gln) heterozygosity (E2-146), and in plasma of normolipidemic subjects (control). Closed triangles indicate individual values; open triangles, mean±SD.](image-url)
Zhao et al

Lipoprotein Profiles of ApoE Variant

1711

FIG 4. Graphs of cholesterol profiles of patients with familial dysbetalipoproteinemia having apolipoprotein (apo) E2(Arg158→Cys) homozygosity (E2-158, top), apoE3-Leiden heterozygosity (E3-Leiden, middle), and apoE2(Lys146→Gln) heterozygosity (E2-146, bottom), at baseline (closed triangles) and during simvastatin therapy (open triangles), and of a normolipidemic control subject (circles) after the first-step density-gradient ultracentrifugation (left side) and the second-step density-gradient ultracentrifugation (right side). Horizontal bars indicate the fractions pooled for each lipoprotein class. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; IDL, intermediate-density lipoprotein; VLDL1, large VLDL; VLDL2, small very-low-density lipoprotein.

Discussion

In this study we analyzed in detail the lipoprotein profiles of hypercholesterolemic patients with FD associated with the E2-158 variant, the E3-Leiden variant, and the E2-146 variant, and those of a control group of normolipidemic subjects. To facilitate a comparison of the lipoprotein profiles of the three groups of FD patients with different apoE variants, only patients with a plasma cholesterol concentration of approximately 9 mmol/L (range, 7 through 12 mmol/L) were evaluated. The three groups of patients with FD were comparable with regard to age, sex, and several clinical and biochemical characteristics.

The main aim of the present study was to compare the plasma lipoprotein profiles of FD patients with the E2-158 variant, the E3-Leiden variant, and the E2-146 variant. This comparison might provide an indication of the role of these apoE variants in the pathophysiology of FD. In agreement with previous reports,14-17,19 we observed that the lipoprotein profiles of the patients with the three variants were characterized by markedly higher VLDL concentrations and altered compositions (CE enrichment) of the VLDL subfractions compared in normolipidemic control subjects. The activity of CETP was lower in all patients during simvastatin therapy (Fig 7). Overall, the activity of CETP in the plasma of the patients with FD decreased significantly from 116±37 AU at baseline to 85±28 AU after simvastatin therapy. No differences in the plasma activity of LCAT were observed between the patients (106±22 AU) and normolipidemic control subjects (101±6 AU) or between the patients before (106±22 AU) and during (93±16 AU) simvastatin therapy (Fig 7).

FIG 5. Graphs of changes induced by 10 weeks of simvastatin treatment in cholesterol level in plasma, a subtraction of very-low-density lipoprotein (VLDL2) cholesterol, intermediate-density lipoprotein (IDL) cholesterol, and low-density lipoprotein (LDL) cholesterol in patients with familial dysbetalipoproteinemia associated with apolipoprotein (apo) E2(Arg158→Cys) homozygosity (circles), apoE3-Leiden heterozygosity (triangles), and apoE2(Lys146→Gln) heterozygosity (squares). Before (closed symbols) and during (open symbols), before and at the end of simvastatin treatment.

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