Plasma Lipoprotein Profiles of Normocholesterolemic and Hypercholesterolemic Homozygotes for Apolipoprotein E2(Arg<sub>158</sub>→Cys) Compared

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We compared plasma lipoprotein profiles of 15 individuals with normcholesterolemic (plasma cholesterol 4.81 ± 0.90 mmol/L) familial dysbetalipoproteinemia (NFD) and 15 patients with hypercholesterolemic (plasma cholesterol 10.61 ± 2.32 mmol/L) familial dysbetalipoproteinemia (HFD), matched for age and sex. All subjects were homozygous for apoE2(Arg<sub>158</sub>→Cys). Compared with 15 normolipidemic controls (plasma cholesterol 5.47 ± 0.92 mmol/L), subjects with NFD and HFD had greater cholesterol concentrations of large very-low-density lipoprotein (VLDL1), small VLDL (VLDL2), and intermediate-density lipoprotein, each of which was correlated to their plasma total cholesterol concentration. VLDL1 and VLDL2 subfractions were enriched in cholesteryl ester, and plasma cholesteryl ester transfer protein activities were increased in both NFD and HFD; however, absolute changes were larger in HFD than in NFD. Concentrations of low-density lipoprotein cholesterol were lower in HFD (1.89 ± 0.48 mmol/L) and NFD (1.56 ± 0.36 mmol/L) than in normolipidemic controls (3.35 ± 0.73 mmol/L). We conclude that all subjects homozygous for apoE2(Arg<sub>158</sub>→Cys) show features of dysbetalipoproteinemia.

Indexing Terms: familial dysbetalipoproteinemia/lipoproteins/cholesteryl ester transfer protein/lecithin:cholesterol acyl transferase

Apolipoprotein (apo) E is a component of most of the major lipoprotein classes, including chylomicrons, very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), and a subclass of high-density lipoproteins (HDL) (1).9 ApoE serves as a specific high-affinity ligand for the low-density lipoprotein (LDL) receptor and the LDL receptor-related protein, initiating the cellular uptake and degradation of apoE-containing lipoprotein particles (1–3). There is also evidence that apoE is necessary for efficient conversion of VLDL to IDL, and finally to LDL (4). ApoE thus plays an important role in the metabolism of plasma lipoproteins, a concept reinforced by recent studies in transgenic mice, in which the apoE gene was either overexpressed (5, 6) or knocked out (7–9).

ApoE is a genetically polymorphic plasma protein of 299 amino acid residues (10). The three major alleles of apoE at a single gene locus, located on chromosome 19, code for three major isoforms of apoE—apoE2, E3, and E4—which differ slightly in amino acid content and can be distinguished by isoelectric focusing (11–13). Three homozygous phenotypes (apoE2/2, E3/3, and E4/4) and three heterozygous phenotypes (apoE3/2, E4/3, and E4/2) arise from the expression of any two of the three alleles (14). ApoE2 differs from apoE3, the most common form, by a substitution of cysteine for arginine at residue 158. Whereas apoE3 and apoE4 bind normally to the apoE (LDL) receptor, apoE2 is defective in interacting with this receptor (<2% of the receptor binding activity of apoE3) (15–18). The apoE2/2 phenotype is associated with familial dysbetalipoproteinemia (FD) (19–21). The defective apoE is the underlying genetic defect responsible for the lipoprotein abnormalities of FD; the normal catabolism of chylomicrons, VLDLs, and IDL is disrupted, resulting in the accumulation of these lipoproteins in plasma (1–3). However, most individuals who have the apoE2/2 phenotype are normocholesterolemic (2), with only 1–4% developing hypercholesterolemia, most probably because of additional genetic and (or) environmental factors (1–3, 20).

Utermann et al. (20) proposed that the development of hypercholesterolemia in FD requires the simultaneous but independent inheritance of other genetic defects that produce hyperlipoproteinemia, in addition to the apoE2/2 phenotype (20). This hypothesis is supported by our recent analysis of the lipoprotein profiles of a large number of hypercholesterolemic patients with FD (22). In that study we found that the expression of hypercholesterolemic FD (HFD) was largely dependent on the plasma VLDL-cholesterol concentrations, which suggests that a combination of the apoE2/2 phenotype and genetic abnormalities in the metabolism of VLDLs is required. However, to gain further insight into the physiological role of apoE and the mechanism of development of HFD, both normocholesterolemic FD (NFD) and HFD subjects homozygous for apoE2(Arg<sub>158</sub>→Cys) should be evaluated. Therefore, in this study we com-
pared the plasma lipoprotein profiles of 15 NFD individuals with those of 15 HFD individuals.

**Materials and Methods**

**Subjects**

All individuals described in this study were analyzed in the Lipid Clinic of the Leiden University Hospital between July 1989 and September 1992. During the study period we evaluated 15 normocholesterolemic individuals (plasma cholesterol concentration <6.5 mmol/L) and 28 hypercholesterolemic patients (plasma cholesterol concentration >7.0 mmol/L), all with the apoE2/2 phenotype, i.e., homozygous for apoE2(Arg158→Cys), as determined by apoE genotyping. The 15 subjects with NFD were matched for age and sex with 15 patients with HFD. We discovered 12 patients with HFD during routine apoE phenotype screening for the hypercholesterolemic patients referred to the Lipid Clinic; three other patients with HFD were identified during screening of the family members of these probands. Six men with NFD, originally identified in a study by Smit et al. (23), were reanalyzed for the present study. Nine more individuals with NFD were discovered during screening of family members of patients with HFD or subjects with NFD.

All individuals were investigated according to a standardized protocol: careful clinical examination; several routine laboratory tests; repeated analysis of the fasting plasma cholesterol, triglyceride, and HDL-cholesterol concentrations; agarose electrophoresis; and routine ultracentrifugation. The diet was evaluated by a qualified dietician, who obtained the 24-h dietary history. All subjects showed minimal intraindividual variability in lipoprotein concentrations, as evaluated by repeated lipid analysis over at least 2 months. None of the individuals appeared to have diabetes mellitus or renal, thyroid, or liver disease, as assessed by physical examination and routine laboratory tests. All subjects had normal fasting blood glucose concentrations. None of the patients with HFD took lipid-lowering drugs or other drugs that might interfere with lipoprotein metabolism for at least 6 weeks before blood samples were obtained for the present study.

Fifteen normolipidemic individuals served as controls in this study. Normolipidemia was defined by plasma cholesterol concentration <6.5 mmol/L and plasma triglyceride concentration <2.0 mmol/L. Four of the normolipidemic controls had the apoE4/3 phenotype, one had the apoE4/2 phenotype, seven had the apoE3/3 phenotype, and three had the apoE3/2 phenotype. These 15 normolipidemic individuals were analyzed by the same methods as the subjects with the apoE2/2 phenotype. The Ethical Committee of University Hospital Leiden gave approval for this study; informed consent was obtained from all subjects.

**Separation of Lipoproteins**

Blood samples were taken in the morning after >12 h of fasting. Plasma was obtained by centrifugation at 1000g for 10 min at room temperature <4 h after sampling. The separation of lipoproteins was started the day of blood collection with a two-step density gradient ultracentrifugation technique (22, 24). In the first step, HDL, LDL, IDL, and VLDL were separated. This gradient consisted of 2 mL of serum sample (adjusted to a density of 1.210 kg/L by adding solid KBr) on the bottom of a polyallomer tube (14 mL; Kontron, Zürich, Switzerland), overlayed by 6 mL of a 1.030 kg/L solution and 4 mL of a 1.006 kg/L solution. After preparation of the gradient, the sample was centrifuged immediately at 210 000g for 24 h at 15°C in a TST swinging-bucket rotor in a Centrkon T-2070 ultracentrifuge (Kontron).

In the second step, VLDL was further separated into two subfractions, VLDL1 (large VLDL) and VLDL2 (small VLDL). The gradient for this consisted of 2 mL of VLDL solution (obtained by routine ultracentrifugation and adjusted to a density of 1.210 kg/L by adding solid KBr) on the bottom of the tube, overlayed by 2 mL of a 1.100 kg/L solution, 4 mL of a 1.040 kg/L solution, and 4 mL of a 1.006 kg/L solution. This gradient was ultracentrifuged at 210 000g for 2 h at 15°C in the same rotor and ultracentrifuge as in the first step. The gradients were fractionated with a specially designed fractionator (25) connected to a micropump and a fraction collector (LKB, Bromma, Sweden).

**Chemical Analysis**

The cholesterol concentration was determined in each fraction of both gradients. The cholesterol concentrations and the compositions of VLDL1, VLDL2, IDL, and LDL were determined in pooled gradient fractions. IDL was obtained in fractions 13–18 and LDL in fractions 6–12 in the first-step ultracentrifugation. VLDL1 was recovered in fractions 21–23 and VLDL2 in fractions 11–20 in the second-step ultracentrifugation. The total cholesterol, free cholesterol, triglyceride, and phospholipid concentrations were determined enzymatically with test kits from Boehringer Mannheim (Mannheim, Germany). Esterified cholesterol was calculated as the difference between total cholesterol and free cholesterol. The mass of cholesteryl ester was estimated as 1.67 × esterified cholesterol. Total protein was determined by a modification of the Lowry procedure (26) with bovine serum albumin as a standard. The total lipoprotein mass (mg/dL) was calculated as the sum of masses of free cholesterol, cholesteryl ester, triglyceride, phospholipid, and protein. HDL-cholesterol concentration was measured in the 1.006 kg/L density infranatant fraction 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 with excess exogenous substrate containing [3H]cholesterol, as described (27). Incubations were for 6 h at 37°C in a total volume of 0.145 mL. The reaction was stopped by adding 0.30 mL of cold methanol. The lipids were extracted twice with 0.4 mL of hexane. Free and esterified cholesterol were sepa-
rated with disposable silica columns. [3H]Cholesteryl esters were eluted with 3.0 mL of hexane:diethylether (6:1 by vol) (28).

Plasma cholesteryl ester transfer protein (CETP) activity was measured in the supernatant fraction of each plasma after precipitation of endogenous apoB-containing lipoproteins with phosphotungstate/Mg²⁺ (29). The exchange of cholesteryl esters between [¹⁴C]cholesteryl ester–labeled LDL and unlabeled HDL was measured during a 16-h incubation. After incubation, the LDL was precipitated by Mn²⁺, according to Morton and Zilversmit (30), and the radioactivity of the HDL was determined.

The activities of LCAT and CETP were measured in plasma that had been stored at −80°C. The measured activities varied linearly with the amount of plasma used in the incubations. All assays were performed in duplicate. The within-day CVs were 4.5% for LCAT and 2.7% for CETP. The measured activities reflect the activity of the enzyme and transfer protein as such (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 as a percentage of the activity in the plasma pool (arbitrary units).

**ApoE Phenotyping and Genotyping**

The 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. (31). For apoE genotyping, genomic DNA was isolated from leukocytes by standard methods (32). The 5' part of exon 4 of the human apoE gene, coding for amino acids 61–174, was amplified by the polymerase chain reaction (PCR) with the use of primers 402 (nucleotides 3555–3574, coding strand) and 401 (nucleotides 3932–3913, noncoding strand), as described by Van den Maagdenberg et al. (33). For allele-specific restriction endonuclease genotyping as described by Hixson and Vernier (34), 15 µL of PCR product was digested with 7.5 U of restriction enzyme HhaI for 16 h according to recommendations of the supplier (Pharmacia, Uppsala, Sweden). Thereafter, the digested material was separated on a 10% neutral polyacrylamide gel for 3 h at 10 V/cm and stained with 0.1 g/L ethidium bromide (35, 36).

**Statistical Analysis**

Results were expressed as mean ± SD. Differences in mean concentrations of lipids, lipoproteins, apolipoproteins, and other variables between various groups were analyzed by one-way analysis of variance, followed by the Scheffé multiple comparison test. When the distributions of the plasma lipid and lipoprotein concentrations were highly skewed, we used their logarithmically transformed concentrations for statistical comparison. All statistical analyses were performed with SPSS/PC+™ software (SPSS, Chicago, IL). P < 0.05 was considered significant.

**Results**

The apoE2/2 phenotype in plasma of all subjects with HD (both HFD and NFD) and the apoE4/3, E4/2, E3/3, or E3/2 phenotype in plasma of the normolipidemic controls were modified completely by prior treatment of the plasma sample with cysteamine; i.e., the apoE4/3, E4/2, E3/3, E3/2, and E2/2 phenotypes were converted to the apoE4/4 phenotype. All subjects with FD were homozygous for apoE2(Arg118→Cys), and no additional rare apoE mutants were detected.

In light of evidence that various endogenous and exogenous factors may influence the plasma lipoprotein profiles in patients with HFD, we analyzed in detail several clinical and biochemical characteristics of these subjects. None of the patients was substantially overweight (body mass index >95th percentile), reported unusual dietary habits, or showed clinical or biochemical signs of concomitant disease. As shown in Table 1, no significant non-lipid-related differences were found between the 15 subjects with NFD and the 15 patients with HFD, matched for age and sex.

The plasma lipoproteins of all patients with HFD were characterized by an increased VLDL concentration (VLDL-cholesterol >1.0 mmol/L, as analyzed by routine ultracentrifugation), an increased molar ratio of VLDL-cholesterol to VLDL-triglyceride (>1.0), and (or) the presence of β-VLDL (as determined by agarose electrophoresis). Thus, these 15 patients fulfilled the "classical" biochemical criteria for HFD (type III hyperlipoproteinemia). The 15 subjects with NFD had plasma lipoprotein abnormalities qualitatively similar to those observed in patients with HFD: 8 subjects with NFD had a VLDL-cholesterol concentration >1.0 mmol/L, 11 subjects had a molar ratio of VLDL-cholesterol to VLDL-triglyceride >1.0, and β-VLDL was detectable in 12 subjects with NFD. All subjects with NFD displayed at least one feature of dysbetalipoproteinemia, e.g., the

<table>
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<th>Table 1. Clinical characteristics of HFD patients, NFD subjects, and normolipidemic controls.</th>
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<tr>
<td><strong>HFD</strong></td>
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<tr>
<td>Age, years</td>
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<tr>
<td>Gender, M/F</td>
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<tr>
<td>Body mass index, kg/m²</td>
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<tr>
<td>Diet*</td>
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<tr>
<td>Protein, %</td>
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<tr>
<td>Carbohydrate, %</td>
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<tr>
<td>Alcohol, %</td>
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<tr>
<td>Fat, %</td>
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<tr>
<td>Saturated, %</td>
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<tr>
<td>Monounsatd., %</td>
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<tr>
<td>Polyunsatd., %</td>
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<tr>
<td>Cholesterol, mg/dL</td>
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<tr>
<td>Glucose, mmol/L</td>
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<td>TSH, mIU/L</td>
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* Dietary components are expressed as percent of total calories, as determined by 24-h dietary history analysis.

n = 15 for each group. There were no significant differences between HFD and NFD groups.

TSH, thyroid-stimulating hormone.
presence of $\beta$-VLDL and (or) cholesterol-enriched VLDL.

The lipoprotein profiles in plasma of the patients with HFD, the subjects with NFD, and the normolipidemic controls were analyzed by density gradient ultracentrifugation. In the typical plasma lipoprotein profiles presented in Fig. 1, the patient with HFD and the subject with NFD had similar abnormalities in their lipoprotein profiles, but the absolute quantities of the abnormalities were less in the subject with NFD than in the patient with HFD.

The mean cholesterol concentrations of lipoprotein (sub)classes in plasma are presented in Table 2. The patients with HFD and the subjects with NFD had significantly higher cholesterol concentrations of VLDL1, VLDL2, and IDL, and significantly lower cholesterol concentrations of LDL than did the normolipidemic controls. The HDL-cholesterol concentration was reduced in patients with HFD, but subjects with NFD and normolipidemic controls had similar concentrations. The concentrations of plasma cholesterol, VLDL1-cholesterol, VLDL2-cholesterol, IDL-cholesterol, and LDL-cholesterol were significantly higher in HFD than in NFD, whereas the HDL-cholesterol concentration was significantly lower in HFD than in NFD.

To illustrate the impact of the lipoprotein abnormalities in subjects with FD, we calculated the contributions of each lipoprotein (VLDL1, VLDL2, IDL, LDL, and HDL) to the total cholesterol concentration in plasma (Fig. 2). In normolipidemic controls LDL was the major contributor (mean 61% of total cholesterol) to total cholesterol concentration, whereas VLDL1, VLDL2, and IDL (together 17% of total cholesterol) were only minor contributors. In NFD and HFD, however, 42% and 72%, respectively, of the total plasma cholesterol concentration was recovered in the VLDL1+VLDL2+IDL fractions.

The relations between the total cholesterol concentration and the cholesterol concentrations of lipoproteins (VLDL1, VLDL2, IDL, and LDL) in plasma of patients with HFD and subjects with NFD were statistically analyzed (Fig. 3). For reasons of clarity, the correlations are shown for HFD and NFD subjects in combination. The cholesterol concentrations of VLDL1 and VLDL2 were correlated to the total cholesterol concentration in plasma of patients with HFD (for VLDL1, $r = 0.708, P < 0.01$; for VLDL2, $r = 0.940, P < 0.001$) and subjects with NFD ($r = 0.691$ for VLDL1 and 0.730 for VLDL2, both $P < 0.01$). Also, the IDL-cholesterol concentration was correlated to total cholesterol concentration in plasma of patients with HFD ($r = 0.592, P < 0.05$) and subjects with NFD ($r = 0.825, P < 0.001$). However, the LDL-cholesterol concentrations in plasma of subjects with HFD or NFD were not correlated to total cholesterol concentration ($r = -0.136$ and 0.306, respectively).

The chemical composition of the lipoprotein fractions isolated by density gradient ultracentrifugation was analyzed. We found many differences in the composition of VLDL1, VLDL2, IDL, and LDL between patients with HFD, subjects with NFD, and normolipidemic controls (Table 3). However, in agreement with a previous report (22), the most significant differences in the composition

<p>| Table 2. Concentrations of lipids and lipoproteins in plasma of HFD patients, NFD subjects, and normolipidemic controls. |</p>
<table>
<thead>
<tr>
<th>Mean ± SD conc, mmol/L</th>
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<tr>
<td><strong>Plasma cholesterol</strong></td>
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<tr>
<td>10.61 ± 2.32*</td>
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<tr>
<td><strong>Plasma triglyceride</strong></td>
</tr>
<tr>
<td><strong>VLDL1-cholesterol</strong></td>
</tr>
<tr>
<td><strong>VLDL2-cholesterol</strong></td>
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<tr>
<td><strong>IDL-cholesterol</strong></td>
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<tr>
<td><strong>LDL-cholesterol</strong></td>
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<td><strong>HDL-cholesterol</strong></td>
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*P < 0.05 vs control and vs NFD. *P < 0.05 vs control. n = 15 for each group.

![Fig. 1. Plasma lipoprotein profiles of an HFD patient (●●), an NFD subject (○○), and a normolipidemic control (△△) after the first step (A) and the second step (B) of density gradient ultracentrifugation.](image-url)
of lipoprotein (sub)fractions were related to the cholesteryl ester and triglyceride content. The VLDL1, VLDL2, and IDL fractions of patients with HFD were depleted of triglyceride and enriched in cholesteryl esters, whereas the LDL fraction was enriched in triglyceride and depleted in cholesteryl esters, in comparison with corresponding lipoproteins in normolipidemic controls. The subjects with NFD also differed from normal in the composition of these lipoproteins, but in general to a lesser extent than in the patients with HFD.

The plasma activities of CETP and LCAT were also measured. Patients with HFD had a significantly higher mean activity of CETP than did subjects with NFD, whose activity was in turn higher than that in normolipidemic subjects (Table 4). The mean activity of LCAT in HFD was also significantly higher than that in normolipidemic subjects. In the plasma of 29 subjects with FD, CETP activity was correlated to the concentrations of VLDL1-cholesterol (r = 0.537, P < 0.01), plasma total cholesterol (r = 0.520, P < 0.01), VLDL2-cholesterol (r = 0.506, P < 0.01), IDL-cholesterol (r = 0.474, P < 0.01), and plasma total triglyceride (r = 0.413, P < 0.05). Multiple regression analysis showed that the VLDL1-cholesterol concentration was an independent variable determining the magnitude of the CETP activity. In addition, in these 29 subjects, CETP activity and LCAT activity were correlated (r = 0.452, P < 0.01). No relation was found between the plasma LCAT activity and the plasma lipid and lipoprotein concentrations.

**Discussion**

Factors such as age, sex, body weight, diet, and concomitant disease are thought to play an important role in the expression of HFD (2, 18), although the relative importance of these factors has not been analyzed in detail. In the present study, we compared the clinical and biochemical characteristics of sex- and age-matched patients with HFD and subjects with NFD. None of patients with HFD was substantially overweight, reported unusual dietary habits, or showed clinical and biochemical signs of concomitant disease. Moreover, no significant differences were observed between HFD patients and NFD subjects with regard to body mass index, dietary habits, and routine laboratory tests (Table 1). These observations further support the hypothesis proposed by Utermann et al. that additional genetic abnormalities are implicated in the expression of HFD (20).

An important aim of this study was to compare the plasma lipoprotein profiles of subjects with NFD and patients with HFD. Similarities in the plasma lipoprotein profiles of NFD and HFD subjects might provide additional insight into the pathophysiology of

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**Table 3. Relative composition (%) of plasma lipoproteins isolated by density gradient ultracentrifugation from HFD patients, NFD subjects, and normolipidemic controls.**

<table>
<thead>
<tr>
<th></th>
<th>VLDL1</th>
<th>VLDL2</th>
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<tr>
<td></td>
<td>HFD</td>
<td>NFD</td>
</tr>
<tr>
<td>FC</td>
<td>6.7 ± 1.2</td>
<td>7.9 ± 2.2*</td>
</tr>
<tr>
<td>CE</td>
<td>18.9 ± 4.9*</td>
<td>14.9 ± 3.7*</td>
</tr>
<tr>
<td>TG</td>
<td>54.1 ± 5.4*</td>
<td>54.4 ± 5.1*</td>
</tr>
<tr>
<td>PL</td>
<td>14.7 ± 3.0</td>
<td>13.4 ± 4.1</td>
</tr>
<tr>
<td>Prot.</td>
<td>5.3 ± 1.7*</td>
<td>9.5 ± 3.3</td>
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<tr>
<th></th>
<th>IDL</th>
<th>LDL</th>
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<tr>
<td></td>
<td>HFD</td>
<td>NFD</td>
</tr>
<tr>
<td>FC</td>
<td>11.3 ± 1.6*</td>
<td>10.0 ± 0.7</td>
</tr>
<tr>
<td>CE</td>
<td>40.0 ± 3.1*</td>
<td>37.0 ± 3.7*</td>
</tr>
<tr>
<td>TG</td>
<td>12.4 ± 2.4*</td>
<td>14.4 ± 2.4*</td>
</tr>
<tr>
<td>PL</td>
<td>19.8 ± 1.1*</td>
<td>18.0 ± 1.5</td>
</tr>
<tr>
<td>Prot.</td>
<td>16.8 ± 1.8*</td>
<td>20.6 ± 2.3</td>
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* P < 0.05 vs control; ** P < 0.05 vs NFD and control.

n = 15 for each group. Measures of free cholesterol (FC), cholesteryl esters (CE), triglyceride (TG), phospholipid (PL), and protein (Prot.) are expressed as a percentage of the total mass of each lipoprotein.

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apoE2(Arg158→Cys), whereas differences might indicate the role of the additional (genetic) factors in the expression of HFD. We found remarkable consistencies in the lipoprotein abnormalities in NFD and HFD, in comparison with normolipidemia. In agreement with a recent report from our laboratory (22), we found that the lipoprotein profile of all patients with HFD was characterized by a markedly decreased LDL-cholesterol concentration, a substantially increased cholesteryl ester content of the VLDL subfractions, and variably increased concentrations of VLDL1, VLDL2, and IDL. Moreover, in agreement with earlier observations of others (37, 38), we noted that plasma CETP and LCAT activities were greater in patients with HFD than in normolipidemic controls. Comparable abnormalities in the lipoprotein profiles were observed in all subjects with NFD. These observations confirm and extend reports of other laboratories (12, 18, 20, 39) that have suggested that the plasma lipoprotein profiles of subjects with NFD display several characteristics of dysbetaolipoproteinemia.

The differences in the NFD and HFD lipoprotein profiles were mainly quantitative. The concentrations of VLDL1, VLDL2, and IDL were moderately increased in subjects with NFD and markedly increased in patients with HFD. The cholesteryl ester content of these lipoprotein (sub)fractions was greater in HFD than in NFD, and the plasma activities of CETP and LCAT were higher in HFD than in NFD. Patients with HFD had lower HDL-cholesterol concentrations than did normolipidemic controls, but HDL-cholesterol concentrations in subjects with NFD and normolipidemic controls were not significantly different. Taken together, the magnitude of the lipoprotein abnormalities is greater in HFD than in NFD, except for LDL-cholesterol, which is markedly decreased in patients with HFD, but even more so in subjects with NFD (Table 2).

The results of the present study emphasize the importance of functionally normal apoE in the regulation of LDL concentrations, the chemical composition of the VLDL subfractions, and the metabolism of VLDL1, VLDL2, and IDL. Mahley et al. (1, 2) have proposed that the abnormalities of the lipoprotein profile of individuals homozygous for apoE2(Arg158→Cys) are due to an impairment of the clearance of the triglyceride-rich lipoproteins caused by a defective binding of apoE2 to heparin receptors, presumably leading to a decreased internalization and degradation of the apoE-containing lipoproteins. The impaired clearance favors prolonged lipolytic processing of triglyceride-rich lipoproteins, thus leading to accumulation of cholesterol-enriched lipoproteins of reduced size and β-mobility instead of normal pre-β-mobility (1–3). The data from our study appear to be in agreement with this hypothesis. However, the hypothesis does not readily explain the markedly lower LDL-cholesterol concentrations in patients with HFD and subjects with NFD than in normolipidemic controls. Although Mahley and Rall proposed that up-regulation of the apoB,E (LDL) receptor could account for the low LDL-cholesterol concentrations in FDB (2), in vivo studies provided evidence that LDL catabolism in individuals with apoE2(Arg158→Cys) homozygosity was not enhanced (4, 40). The metabolic studies thus suggested that a low LDL-cholesterol concentration in individuals homozygous for apoE2(Arg158→Cys) was due to an impaired conversion of IDL to LDL (4, 40).

In view of the markedly reduced LDL-cholesterol concentrations in subjects with NFD and patients with HFD, one may speculate that the impaired conversion of LDL precursors to LDL is of primary importance in the pathophysiology of apoE disorders. Assuming that the lipolytic cascade VLDL1→VLDL2→IDL→LDL operates in all individuals with apoE2(Arg158→Cys) homozygosity, one might expect that an impaired IDL-to-LDL conversion would lead to decreased LDL-cholesterol concentrations and increased concentrations of IDL, VLDL2, and VLDL1, the characteristic lipoprotein abnormalities observed in all subjects with FD irrespective of their plasma total cholesterol concentrations. Additional genetic factors presumably related to the metabolism of triglyceride-rich lipoproteins would further increase the concentrations of VLDL1, VLDL2, and IDL, thus leading to hypercholesterolemia in individuals with apoE2(Arg158→Cys) homozygosity.

The results of the metabolic studies conducted by Shepherd and coworkers (4, 41–43) and the data presented here are compatible with this hypothesis. Interestingly, earlier in vitro studies (44, 45) indicated that the presence of apoE2 impairs the remodeling of particles during lipolytic processing of VLDL to LDL, suggesting that properties of apoE other than receptor binding may be involved in the conversion of VLDL to LDL. Alternatively, binding of IDL to a membrane receptor may be an essential step in the conversion of IDL to LDL, so that a markedly lower binding affinity for apoE2(Arg158→Cys), compared with that of normal apoE3, prohibits adequate conversion. This view is supported by our recent observation that patients heterozygous for apoE3-Leiden and patients homozygous for apoE2(Lys146→Gln) exhibit marked differences in the IDL/LDL ratio as compared with patients homozygous for apoE2(Arg158→Cys) (22, 46). Unfortunately, little is known about the regulation of the conversion of IDL to LDL, and additional studies are required to establish the involvement of apoE in this process.

CETP mediates an important pathway for reverse cholesterol transport. The CETP activity and concentrations in plasma were greater in patients with FD than in...
normalolipidemic controls (37, 38). The enhanced transfer of cholesteryl esters from HDL to apoB-containing lipoproteins could make a major contribution to the VLDL-cholesteryl ester mass (37), suggesting that the increased cholesteryl ester content of VLDLs in FD is due to the increased CETP activity. Our study confirms and extends these observations. Patients with HFD had higher plasma activities of CETP and LCAT than did normalolipidemic controls. Thus an enhancement of cholesteryl esterification (mediated by LCAT) and subsequent transfer (mediated by CETP) of the cholesteryl ester to triglyceride-rich lipoproteins may account, at least in part, for the increased cholesteryl ester content of VLDL1 and VLDL2 in individuals with FD. Moreover, the CETP-mediated transfer of cholesteryl esters should be further enhanced by the increased availability of acceptor lipoproteins in subjects with greater VLDL concentrations (47). In addition, individuals with FD also have an increased concentration of apoE (38), which may stimulate transfer of cholesteryl esters between HDL and VLDL activity as mediated by enhancing the affinity of CETP for VLDL (48), thus leading to an increased cholesteryl ester content of VLDLs.

In summary, increased cholesterol concentrations of VLDL1, VLDL2, and IDL and a decreased LDL-cholesterol concentration were observed in all subjects with apoE2(Arg16→Cys) homozygosity, hypercholesterolemic or not. These abnormalities are considered to be attributable to homozygosity for apoE2, which hampers the conversion of IDL to LDL and may impair catabolism of VLDL. Only in the presence of other (genetic) factors, probably associated with overproduction of VLDLs, will individuals with NFD develop HFD.

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References