Apolipoprotein composition of HDL in cholesteryl ester transfer protein deficiency

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Abstract **Our purpose was to compare HDL subpopulations, as determined by nondenaturing two-dimensional gel electrophoresis followed by immunoblotting for apolipoprotein A-I (apoA-I), apoA-II, apoA-IV, apoCs, and apoE in heterozygous, compound heterozygous, and homozygous subjects for cholesteryl ester transfer protein (CETP) deficiency and controls. Heterozygotes, compound heterozygotes, and homozygotes had CETP masses that were 30, 63, and more than 90% lower and HDL-cholesterol values that were 64, 168, and 203% higher than those in controls, respectively. Heterozygotes had ~50% lower preβ-1 and more than 2-fold higher levels of α-1 and preα-1 particles than controls. Three of the five heterozygotes' α-1 particles also contained apoA-II, which was not seen in controls. Compound heterozygotes and homozygotes had very large particles not observed in controls and heterozygotes. These particles contained apoA-I, apoA-II, apoCs, and apoE. However, these subjects did not have decreased preβ-1 levels. Our data indicate that CETP deficiency results in the formation of very large HDL particles containing all of the major HDL apolipoproteins except for apoA-IV. We hypothesize that the HDL subpopulation profile of heterozygous CETP-deficient patients, especially those with high levels of α-1 containing apoA-I but no apoA-II, represent an improved anti-atherogenic state, although this might not be the case for compound heterozygotes and homozygotes with very large, undifferentiated HDL particles.**—Asztalos, B. F., K. V. Horvath, K. Kajinami, C. Nartsupha, C. E. Cox, M. Batista, E. J. Schaefer, A. Inazu, and H. Mabuchi. **Apolipoprotein composition of HDL in cholesteryl ester transfer protein deficiency.** ***J. Lipid Res.*** **2004.** 45: **448–455.**

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Cholesteryl ester transfer protein (CETP) is a 74 kDa hydrophobic glycoprotein bound mainly to HDL. CETP catalyzes the equilibrium of nonpolar lipids [triglyceride (TG) and cholesteryl ester (CE)] between plasma lipoprotein particles (1, 2). The major role of CETP is a net transfer of CE from HDL to TG-rich lipoprotein (TRL) and of LDL and TG from TRL to LDL and HDL. CETP mRNA is expressed in several tissues, but the majority of circulating CETP originates from the liver (3).

CETP plays a key role in HDL metabolism (4). It regulates total plasma HDL-cholesterol (HDL-C) level and also facilitates the remodeling of HDL particles (5). A high CETP concentration correlates with a low HDL-C level, a strong risk factor for coronary artery disease (6). On the other hand, Asian subjects with CETP deficiency have markedly increased HDL-C (3- to 6-fold) and apoA-I concentrations (7–9). CETP deficiency-induced increase in HDL-C level is mainly found in the large HDL2 subclass. Moreover, the average HDL size of CETP-deficient subjects is significantly increased and enriched in cholesterol (10). These large HDL particles have been reported to be less effective in promoting cholesterol efflux from lipid-loaded macrophages than HDL particles of control subjects (11).

Several mutations of the CETP gene have been identified as causes of CETP deficiency and increased HDL-C levels. These include a G-to-A substitution within intron 14 at the donor splice site (Int14A), a mutation that is present in up to 2% of the total Japanese population and in as many as 27% of people in the Omagari area of Japan, as well as a missense mutation in exon 15 (D422G) present in up to 7% of the Japanese population (12–16). Inazu et al. (9) reported that heterozygous CETP deficiency caused by the Int14A mutation was potentially anti-atherogenic and might be associated with an increased life span. Hirano et al. (16) found no correlation between CETP deficiency and longevity. They published a U-shaped correlation curve between HDL-C and the incidence of ischemic electrocardiogram changes indicating

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that both low and high HDL-C levels can be atherogenic. Hirano et al. (17) and Kakko et al. (18) speculated that alcohol intake might modify the atherogenicity of some CETP polymorphisms, because it decreases CETP levels. Recently, Barter et al. (19) summarized these finding in a review article.

In the present study, we investigated the size and apolipoprotein composition of HDL, separated by two-dimensional nondenaturing gel electrophoresis (2dE), from the plasma of 9 homozygous, 4 compound heterozygous, and 5 heterozygous subjects with CETP deficiency and compared them with those of 50 age- and gender-matched controls.

METHODS

Study population

Plasma samples were obtained from subjects with genetic CETP deficiency and compared with those of healthy controls. We studied nine subjects (three males and six females) homozygous for the mutation involving intron 14 (Int14A), resulting in the null phenotype, four compound heterozygotes (two males and two females) with the same Int14A plus one missense mutation of D442G in exon 15, resulting in a defective phenotype with a potentially dominant-negative effect, and five heterozygotes (three males and two females), two with the Int14A and three with the D442G mutation. The control group was composed of 25 healthy male and 25 healthy female subjects of similar age. **Table 1** contains data about age, gender, and biochemical parameters of the subjects studied. None of the subjects were on medication known to alter lipid levels. All participants of the study gave written informed consent. The study protocol was approved by Kanazawa University.

Blood was collected from subjects after an overnight fast into EDTA tubes and centrifuged at low speed at 4°C for 20 min to obtain plasma. Plasma was divided into aliquots and stored at −80°C. To further protect lipoprotein particles from degradation, plasma aliquots were thawed fast (1–2 min) in a 37°C water bath and put on ice until use. Samples were stored and handled the same way for control and CETP-deficient subjects. We did not notice any signs of sample degradation attributable to sample handling or storage in this study. It is worth noting here that the 2dE, immunoblot, image analysis method is sensitive and indicative for degraded samples because degraded plasma samples show a distinct pattern visible on the two-dimensional electrophoretogram.

Lipid, apolipoprotein, and CETP measurements

Total cholesterol, HDL-C, and TG were measured by automated enzymatic assays using a Hitachi 911 analyzer and kits from Roche Diagnostics (Indianapolis, IN). LDL-C was calculated by the Friedewald formula. Phospholipids and free cholesterol (FC) were measured by automated enzymatic assays using kits from Wako Diagnostics (Richmond, VA). CE was calculated by subtracting FC from total cholesterol. ApoA-I and apoA-II were assayed immunoturbidimetrically using Wako kits. Lipoprotein A-I (LpA-I) was measured by rocket electrophoresis, using cross-electrophoresis kits from Sebia (Norcross, GA). LpA-I:A-II was calculated by subtracting LpA-I from plasma total apoA-I. CETP mass was measured by ELISA kits from Wako. ApoA-IV, apoC-I, apoC-II, apoC-III, and apoE were determined by dot-blot analyses, and their concentrations were expressed as percentile of controls. Between-run coefficients of variation for all assays were less than 10%.

HDL subpopulation analysis

Two-dimensional nondenaturing agarose-polyacrylamide gel electrophoresis and image analysis to determine the apoA-I-containing HDL subpopulations were carried out on plasma previously stored at −80°C as described (20, 21). Briefly, in the first dimension, HDL particles were separated on an agarose gel according to charge. This separation identified three fractions, referred to as preβ, α, and preα particles, on the basis of mobility relative to albumin. Low-endosmosity 0.7% agarose was cast into 3 mm thick vertical glass cassettes and electrophoresed in a Pharmacia GE 2/4 recirculating apparatus. Four microliters of plasma per sample channel was electrophoresed at constant voltage (250 V) and temperature (10°C) in Tris-tricine buffer until the endogenous albumin, stained with bromophenol blue, moved

TABLE 1. Characteristics of subjects

Variables	Controls	Heterozygotes	Compound Heterozygotes	Homozygotes
Male-female	25:25	3:2	2:2	3:6
Age (years)	56 ± 8	35 ± 18[a]	52 ± 7[a]	57 ± 7.1[a]
Cholesteryl ester transfer protein (μg/ml)	1.24 ± 0.4	0.87 ± 0.2[a]	0.46 ± 0.2[a,b]	<0.1[a,b]
Total cholesterol	195 ± 36	180 ± 22	261 ± 29[a,b]	280 ± 35[a,b]
FC	55 ± 10	49 ± 6	69 ± 7[a,b]	77 ± 11[a,b]
EC	140 ± 27	131 ± 16	193 ± 23[a,b]	203 ± 24[a,b]
FC/EC	0.39 ± 0.03	0.37 ± 0.00	0.36 ± 0.02	0.38 ± 0.02
Phospholipids	211 ± 33	218 ± 30	307 ± 61[a,b]	337 ± 35[a,b]
LDL-cholesterol	120 ± 31	77 ± 13[a]	102 ± 29[b]	102 ± 25[b]
HDL-cholesterol	52 ± 14	85 ± 26[a]	139 ± 22[a,b]	157 ± 29[a,b]
Triglyceride	120 ± 70	88 ± 40	101 ± 52	104 ± 37
apoA-I	144 ± 29	154 ± 26	224 ± 41[a,b]	252 ± 25[a,b]
LpA-I	56 ± 18	63 ± 25	86 ± 10[a]	89 ± 18[a,b]
LpA-I:A-II	88 ± 18	92 ± 6	138 ± 33[a,b]	163 ± 28[a,b]
apoA-II	35 ± 5	35 ± 2	42 ± 10[a,b]	40 ± 6[a,b]

Data are presented in mg/dl unless indicated otherwise. Values are means ± SD. apoA-I, apolipoprotein A-I; EC, esterified cholesterol; FC, free cholesterol; LpA-I, lipoprotein A-I.

[a] $P < 0.05$ (compared with controls).

[b] $P < 0.05$ (compared with heterozygotes).

3.5 cm from the origin. Individual strips were cut out and transferred to the top of nondenaturing concave-gradient (3–35%) polyacrylamide gels, and particles were further separated according to size. Electrophoresis was performed to completion in Tris-borate buffer at constant voltage (250 V) and temperature (10°C) for 24 h. Gels were electrotransferred to nitrocellulose membranes at constant voltage (30 V) and temperature (10°C) for 24 h in Tris-glycine buffer.

Before immunoblotting, membranes were fixed with 0.03% glutaraldehyde and the free protein binding capacity was blocked by incubating membranes in PBS containing 0.05% Tween (PBST) and 5% nonfat dry milk. ApoA-I was immunolocalized by incubation with PBST containing 5% milk and monospecific goat anti-human apoA-I antibody for 7 h followed by incubation with ^{125}I-labeled secondary antibody [Fa(b)^2 fragment of rabbit anti-goat IgG] overnight. ApoA-II was immunolocalized with fluorochrome (Cy-5)-labeled first antibody [F(ab′)$_2$ fragment of goat anti-human apoA-II] added to the incubation medium together with the secondary antibody for apoA-I. Separate membranes were immunoprobed for apoA-IV, apoC-I, apoC-II, apoC-III, and apoE as described above with primary antibodies specific for these apolipoproteins and ^{125}I-labeled secondary antibody. Signals were quantitatively determined by image analysis using a FluoroImager in X-ray mode for the ^{125}I label and in red-fluorescent mode for the Cy5 label.

The subpopulations were determined both as percentage distribution and concentration relative to apoA-I. Preβ-1 and preβ-2 particles did not overlap with any other HDL particles, so they were easily delineated. Designation of the α-mobility HDL subpopulations was based on the integration of α-migrating HDL. We delineated each peak area shown on the integration curve for the α-mobility particles. Preα-mobility particles had similar sizes as their α-mobility counterparts and were clearly separated from each other. Finally, all apoA-I-containing HDL subpopulations were encircled (we pooled the two preβ-1 particles into one subpopulation and also the three preβ-2 particles into one subpopulation), and signals were measured in each area and used to calculate the percentage distribution of the apoA-I-containing HDL subpopulations. Data were expressed as pixels linearly correlated with the disintegrations per minute of the ^{125}I bound to the antigen-antibody complex (22). The apoA-I concentrations of the subpopulations were calculated by multiplying percentiles of subfractions by plasma total apoA-I concentrations. We had a coefficient of variation of less than 10% for all of the α-mobility subpopulations and less than 15% for the rest of the particles.

Statistical analysis

ANOVA was used to test the hypothesis of no difference between data obtained from control and affected subjects. $P < 0.05$ was considered significant. For analysis, nonnormally distributed data were logarithmically transformed.

RESULTS

Table 1 shows the plasma biochemical parameters of the four groups: homozygotes (HOs), compound heterozygotes (CHs), heterozygotes (HEs), and controls. Subjects with either mutation had significantly lower levels of CETP mass: HOs, <0.1 μg/ml; CHs, 0.46 μg/ml; and HEs, 0.87 μg/ml, compared with controls (1.24 μg/ml). The nine HOs had increased total cholesterol levels (44%) but normal free-to-esterified cholesterol ratios. They had slightly decreased LDL-C (−14%) and TG (−13%) levels, whereas their mean HDL-C level was 3-fold higher than the mean control value. HOs had 75% higher apoA-I and 13% higher apoA-II concentrations than controls. The differences between CHs and controls were very similar to the differences described for HOs and controls. Compared with controls, HEs had 35% and 27% lower LDL-C and TG, respectively, and 64% higher HDL-C, with no notable differences for apoA-I and apoA-II. In all affected groups, the LpA-I fraction was increased to approximately the same extent as that of LpA-I:A-II.

The apoA-I-containing HDL subpopulations are expressed both as percentage distribution and as absolute concentrations (mg/dl) in **Tables 2** and **3**. We detected two extra, large α-mobility particles in HO and CH subjects with mean sizes of 12.9 nm (α-L) and 16.6 nm (α-VL) (**Fig. 1**). Adjacent to the α-L and α-VL particles, there were preα-mobility particles with similar sizes. These very large α and preα particles were not present in any of the control or HE subjects. In HOs, the percentiles of the α-L, preα-L, α-VL and preα-VL were 24.7, 4.6, 18.9, and 1.4%, respectively. The normal-sized α-1 was 17% lower, whereas the normal-sized preα-1 was 23% higher, than these percentiles in controls. The percentiles of α-2, α-3, and the adjacent preα-2 and preα-3 were 72, 75, 69, and 73% lower, respectively, in HOs than in controls. Moreover, a new, small particle, similar in size to preβ-1 HDL but with faster mobility, was detected in HO subjects. We designated this particle as fast preβ-1 (preβ-1F). Among the CHs, only one subject had α-VL particles, but all of them had α-L particles and their major HDL particle was the regular-sized α-1. HEs had no HDL fraction that was not present in controls. They had more than 2-fold higher percentiles of α-1 and preα-1 than did controls. The percentiles of α-2, α-3, and the adjacent preα-2 and preα-3 particles, and the smallest particle, preβ-1, were markedly lower by 15, 43, 29, 35, and 43%, respectively, in HEs compared with controls.

Absolute concentrations were calculated by multiplying plasma apoA-I concentrations with the percentiles of HDL subspecies (Table 3). The increased total apoA-I concentrations (75, 56, and 7% in the HO, CH, and HE groups compared with controls) further enhanced the differences among affected and control subjects in terms of the large HDL particles. ApoA-I concentrations in HDL particles with similar or larger sizes than normal-sized α-1 (≥11.2 nm) were 29.1 mg/dl in controls, 69.3 mg/dl in HEs, 162.8 mg/dl in CHs, and 171.1 mg/dl in HOs. On the other hand, concentrations of the smaller particles (α-2, α-3, preα-2, and preα-3) were significantly lower in all affected groups than in the control group. Preβ-1 level was low (8.7 mg/dl) among HEs, whereas it was increased in the CH (19.7 mg/dl) and in the HO (30.1 mg/dl) groups compared with controls (15.1 mg/dl).

We also compared plasma total apoA-II, apoA-IV, apoC-I, apoC-II, apoC-III, and apoE concentrations and their distribution and mass within the HDL subpopulations, as determined by immunoblotting for these apolipoproteins, among the four groups. Compared with controls, apoA-II was slightly increased in CH (18%) and HO (13%) sub-

TABLE 2. Percentage distribution of the apoA-I-containing HDL subpopulations

Variables	Modal Diameter	Controls	Heterozygotes	Compound Heterozygotes	Homozygotes
	nm		%		
preβ-1_F	5.9				1.3 ± 0.7[a,b]
preβ-1	5.6	10.6 ± 3.4	5.8 ± 1.6[a]	8.6 ± 4.0	12.0 ± 3.3[b]
preβ-2	12.8	1.3 ± 0.9	1.4 ± 0.5	1.0 ± 0.2	0.8 ± 0.4
α-VL	16.6			15.8 ± 0.0[a,b]	18.9 ± 6.3[a,b]
α-L	12.9			16.3 ± 4.6[a,b]	24.7 ± 7.7[a,b]
α-1	11.2	15.5 ± 4.3	34.0 ± 9.0[a]	29.1 ± 9.1[a]	12.9 ± 3.5[b]
α-2	9.2	33.6 ± 5.1	28.3 ± 5.5[a]	15.7 ± 8.4[a,b]	9.3 ± 1.6[a,b]
α-3	7.6	27.4 ± 6.0	15.7 ± 3.5[a]	11.5 ± 4.6[a]	6.7 ± 1.1[a,b]
preα-VL	16.0			1.3 ± 0.0[a,b]	1.4 ± 1.1[a,b]
preα-L	11.9			5.5 ± 1.8[a,b]	4.6 ± 1.7[a,b]
preα-1	11.0	4.2 ± 1.9	9.7 ± 1.5[a]	4.6 ± 0.6[b]	5.4 ± 1.4
preα-2	9.4	4.9 ± 1.7	3.5 ± 0.4	2.1 ± 0.9[a,b]	1.5 ± 0.4[a,b]
preα-3	7.7	2.6 ± 1.3	1.7 ± 0.5	1.2 ± 0.7[a]	0.7 ± 0.3[a,b]

Values are means ± SD. F, fast; L, large; VL, very large.
[a] $P < 0.05$ (compared with controls).
[b] $P < 0.05$ (compared with heterozygotes).

jects and was similar in HEs. ApoA-II was present in the α-2 and α-3 subpopulations of controls, whereas, in affected subjects, apoA-II was present in all α-mobility HDL subpopulations (**Table 4**, **Fig. 2**). In HOs, apoA-II was also present in the preβ-1F particle. In affected subjects, the apoA-I/A-II ratio increased in all LpA-I:A-II subpopulations compared with controls as a result of a disproportionate increase of total apoA-I to total apoA-II and of a disproportionate displacement of these proteins into the larger sized particles (**Table 5**).

When we determined the apoA-IV profile, we found that neither the concentration nor the percentage distribution and localization of the apoA-IV-containing particles was different between affected and control subjects.

Plasma apoC-I concentration was higher by 13% in HE, 23% in CH, and 34% in HO subjects compared with controls. In control subjects, apoC-I was present in the three α-mobility HDL particles, with large individual variability in the percentage distributions. In HE and CH subjects, apoC-I was present predominantly in the α-1 subpopulation, whereas in HOs, 95% of apoC-I was in the three large α-mobility particles (α-1, α-L, and α-VL).

ApoC-II concentration in plasma was lower by 23% and 10% in HE and CH subjects, respectively, whereas it was 18% higher in HO subjects than in controls. The distribution of apoC-II in controls and affected subjects was very similar to that of apoC-I in these subjects.

Plasma apoC-III concentration was 13% lower in HEs and was similar in CHs compared with controls. Despite a 50% higher apoC-III level in three of the HO subjects, the mean concentration of apoC-III in the nine HOs was only 11% higher than that in controls. In control subjects, apoC-III was present in the α-2 and α-3 particles and in a small preβ-mobility particle. ApoC-III distribution in HE and control subjects was similar; however, in HOs and CHs, the majority of apoC-III was in the three largest α-mobility particles, unlike in controls. In the HO and CH groups, some apoC-III was also detected in a small α-mobility particle (similar in size to the preβ-mobility apoC-III in controls).

ApoE concentration was increased by 16, 38, and 43% in HE, CH, and HO subjects, respectively, compared with the control group. ApoE did not comigrate with apoA-I in control subjects, although it comigrated with apoA-I in the three largest α-mobility particles (α-1, α-L, and α-VL) of all affected subjects but most markedly in HOs (**Fig. 3**).

DISCUSSION

CETP plays an important role in HDL metabolism by facilitating the transfer of CE to apoB-containing lipoproteins in partial exchange for TG. Significant interest in CETP has been generated by the description of Japanese subjects heterozygous and homozygous for CETP deficiency with very high levels of HDL-C. It has previously been shown that CETP deficiency is associated with marked increases in large HDL or HDL2 and that HOs have marked apoE enrichment in this fraction (7, 9, 23).

TABLE 3. apoA-I concentrations in HDL subpopulations

Variables	Controls	Heterozygotes	Compound Heterozygotes	Homozygotes
		mg/dl		
preβ-1_F				3.2 ± 1.8[a,b]
preβ-1	15.1 ± 5.6	8.7 ± 1.8[a]	19.7 ± 11.7[b]	30.1 ± 8.7[a,b]
preβ-2	1.9 ± 1.2	2.1 ± 0.6	2.3 ± 0.6	2.1 ± 1.0
α-VL			38.3 ± 0.0[a,b]	47.9 ± 16.5[a,b]
α-L			35.5 ± 6.3[a,b]	62.3 ± 21.3[a,b]
α-1	23.0 ± 9.9	54.1 ± 20.4[a]	63.8 ± 20.1[a]	32.3 ± 8.4[b]
α-2	48.6 ± 13.5	42.7 ± 5.6[a]	36.0 ± 24.4	23.3 ± 4.4[a,b]
α-3	38.5 ± 7.8	23.4 ± 1.6[a]	26.3 ± 14.4[a]	16.8 ± 2.8[a,b]
preα-VL			3.1 ± 0.0[a,b]	3.1 ± 2.2[a,b]
preα-L			12.0 ± 3.3[a,b]	11.8 ± 5.3[a,b]
preα-1	6.1 ± 3.2	15.2 ± 4.4[a]	10.1 ± 0.8[a]	13.7 ± 4.7[a]
preα-2	7.1 ± 3.0	5.4 ± 1.1	4.8 ± 3.0	3.7 ± 1.2[a,b]
preα-3	3.7 ± 1.6	2.6 ± 0.5	2.8 ± 2.2	1.9 ± 1.0[a]

Values are means ± SD.
[a] $P < 0.05$ (compared with controls).
[b] $P < 0.05$ (compared with heterozygotes).

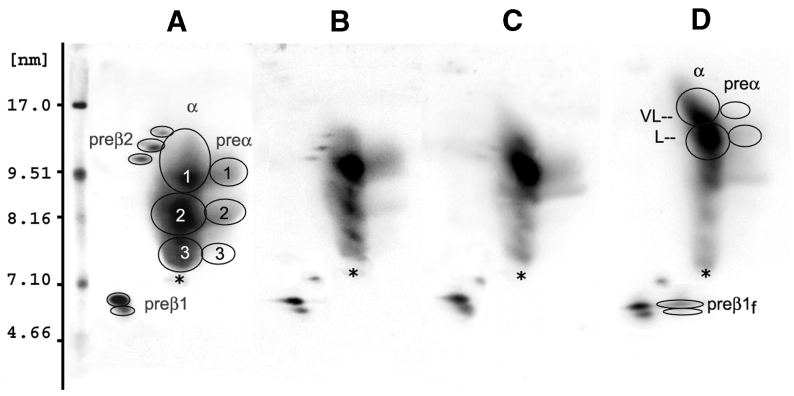

Fig. 1. The apolipoprotein A-I (apoA-I)-containing HDL subpopulations of a male control subject (A), a male heterozygous (B), a male compound heterozygous (C), and a male homozygous (D) cholesteryl ester transfer protein (CETP)-deficient subject. Bands representing Pharmacia high molecular weight standards can be seen at left. Asterisks represent the positions of endogenous albumin marking the α-mobility front. Notice the eight regular apoA-I-containing HDL subpopulations (preβ-1, preβ-2, α-1, α-2, α-3, preα-1, preα-2, and preα-3) in panel A and the extra subpopulations [fast preβ-1 (preβ-1F), very large (VL) α and preα, and large (L) α and preα] in homozygotes in panel D. Note that the figure allows for the quantitative comparison of the apoA-I-containing HDL subpopulations within each subject and for qualitative comparison among the representative subjects.

It has also been reported that statin treatment not only modestly increases HDL-C but also causes a significant increase in large HDL particles (24, 25), attributable, at least in part, to decreased CETP activity (26). Pharmaceutical companies have had great interest in developing small molecules to increase HDL-C and decrease coronary heart disease (CHD) risk. For this reason, a number of companies have developed CETP inhibitors. One of these agents has been reported to decrease diet-induced atherosclerosis in animals and to significantly increase HDL-C in these animals as well as in humans (27, 28).

The 2dE method followed by immunoblotting and image analysis allows for the precise delineation of specific HDL subspecies and provides metabolic information as it separates intermediates (lipid-poor, discoidal, and spheroidal fractions) representing the main stages of HDL synthesis. Using this methodology, we have reported the presence of 12 apoA-I-containing HDL subspecies in human plasma (20). The largest α-mobility HDL particle, α-1, resembles HDL-2 separated by ultracentrifugation (29). In control subjects, only α-2 and α-3 HDL particles contain apoA-II, the rest are apoA-I-only particles (21). In patients with defective cellular cholesterol efflux with mutations in the ATP-binding cassette transporter A1 gene (Tangier disease), HOs not only have a marked decrease in HDL but they only have preβ-1 HDL, whereas HEs have markedly reduced α-1 and α-2 HDL particles (30). Homozygous Tangier patients have an ~3-fold increased risk for CHD, whereas for HEs, this risk is increased ~1.5-fold.

We have documented that patients with CHD have marked decreases in α-1 and preα-1 HDL and that statins can increase both α-1 and preα-1 HDL levels significantly (24, 25, 29). More recently, we have reported that simvastatin-niacin treatment in the HDL Atherosclerosis Treatment Study markedly increased both α-1 and preα-1 HDL and that subjects with the greatest increases in α-1 had no progression of coronary artery stenosis, in contrast to other subjects (31). Based on these data, we believe that the large LpA-I α-1 is an anti-atherogenic HDL particle.

In the present study, we document the presence of novel apoA-I-containing particles in patients with CETP deficiency. We confirm previous observations that subjects with heterozygous CETP deficiency have increased HDL-C level and size. In these subjects, a 30% decrease in CETP mass resulted in a marked alteration in the HDL particle distribution. Most notably, there was an increase in the large α-1 and preα-1 particles and a decrease in the small preβ-1 particle. The unique finding was that three of the five HEs had apoA-II in α-1 HDL, which was an LpA-I particle in controls.

As expected, the magnitude of the HDL alterations observed in HEs was significantly amplified in CHs and HOs, with 63% lower and no detectable CETP masses, respectively. These subjects not only had marked increases in α-1 HDL but also the appearance of larger than normal α- and preα-mobility HDL particles not observed in controls and HEs. The α-mobility particles contained apoA-II besides apoA-I but, possibly even more significantly, contained

TABLE 4. apo-A-II concentrations in HDL subpopulations

Variables	Modal Diameter	Controls	Heterozygotes	Compound Heterozygotes	Homozygotes
	nm		*mg/dl*		
preβ-1_F	5.9				$0.8 \pm 0.4^{a,b}$
α-VL	17.6				$6.4 \pm 2.6^{a,b}$
α-L	13.9			$10.7^{a,b}$	$7.9 \pm 2.2^{a,b}$
α-1	11.2		6.9 ± 4.2	$13.0 \pm 4.7^{a,b}$	11.2 ± 2.6^{a}
α-2	9.2	24.4 ± 6.1	18.5 ± 3.6	16.3 ± 10^{a}	$8.3 \pm 2.3^{a,b}$
α-3	7.6	12.1 ± 2.4	9.3 ± 4.4	9.9 ± 6.5	$5.3 \pm 1.9^{a,b}$

Values are means ± SD.
[a] $P < 0.05$ (compared with controls).
[b] $P < 0.05$ (compared with heterozygotes).

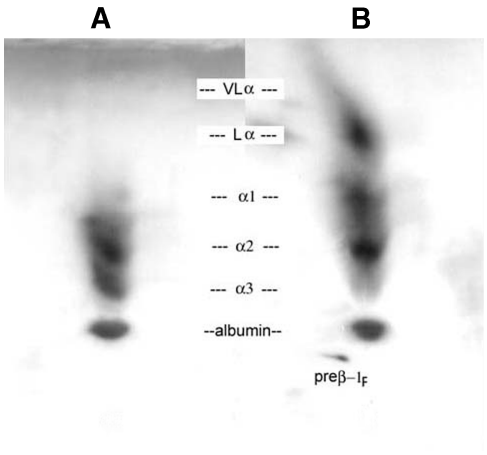

Fig. 2. ApoA-II-containing HDL subpopulations of a control subject (A) and a homozygous CETP-deficient subject (B). The homozygous subject has apoA-II in the preβ-1F and in the three largest α-mobility particles.

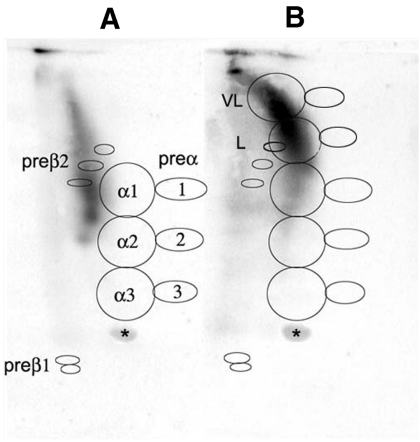

Fig. 3. ApoE-containing HDL particles of a control subject (A) and a homozygous CETP-deficient subject (B). The ovals indicate the positions of the apoA-I-containing HDL particles of the same subjects after concomitant immunoprobing of the membranes for apoA-I. The asterisks mark the positions of the endogenous albumin.

other apolipoproteins, specifically apoE, apoC-III, apoC-II, and apoC-I in order of decreasing abundance. It has to be noted that comigration of different apolipoproteins generally does not mean that these proteins are in the same particle. However, comigration of different apolipoproteins in a two-dimensional system significantly increases the probability of their being in the same particle.

Our data are in agreement with earlier observations that subjects homozygous for CETP deficiency have HDL particles containing apoA-I, apoA-II, and apoE and also with reports that apoE resides mainly in the large HDL particles (8, 32, 33). It has been reported that HDL particles enriched in apoA-II and apoE have a decreased capacity for efflux of cholesterol from macrophages (11) and that the plasma residence time of apoE is ~10 times longer if apoE is present in LpE:A-II:A-I particles (34).

It is difficult to speculate about the interaction of these undifferentiated large particles with the HDL-modifying enzymes and CETP, in light of the fact that all three C apolipoproteins are present in these particles. ApoC-I and apoC-III inhibit lipoprotein lipase (LPL) activity, whereas apoC-II activates LPL activity. Both apoC-II and apoC-III reduce hepatic lipase (HL) activity, whereas apoC-I has no effect on it. On the other hand, apoC-I activates LCAT, whereas the other two apoCs inhibit LCAT. ApoC-I reduces and apoC-III increases CE transfer by CETP. Recently, Jong, Hofker, and Havakes (35) summarized the influence of apoCs on these factors. It would be interesting to know how these large lipid-rich particles (α-1, α-L, and α-VL) influence the distribution of apoC-III between the HDL and TRL fractions, because non-HDL apoC-III is a CHD risk factor. The precise role of these particles in promoting cholesterol efflux remains to be determined.

In contrast to HEs, CHs and HOs had significantly increased concentrations of preβ-1 particles. We assume that the observed increase in preβ-1 level was not attributable to lower LCAT activity because the plasma FC/CE ratios of CETP-deficient subjects were not different from those of controls. The increased preβ-1 level was not the result of higher HL activity either, because HL produces small α-mobility particles besides preβ-1, but those particles were significantly lower in CETP-deficient subjects. We postulate that the higher than normal preβ-1 levels in HO subjects were the result of increased phospholipid transfer protein (PLTP) activity, based on reports that PLTP produces preβ-1 and large HDL-2-like particles (36).

Our data strongly indicate that CETP plays a significant role in modulating HDL metabolism affecting HDL-C level and the distribution, size, and apolipoprotein composition of HDL particles. Our studies suggest that CETP deficiency not only causes the appearance of very large HDL particles but also causes the apoA-I in these particles to associate initially with apoA-II and then with all of the other HDL apolipoproteins except apoA-IV. Consequently, CETP is essential for the formation of heterogeneous HDL not only in size but also in apolipoprotein composition.

In agreement with others, we postulate that HDL particles with altered apolipoprotein composition in HOs and CHs may not be as efficient as regular HDL particles in promoting reverse cholesterol transport. We believe that

TABLE 5. Ratios of apoA-I and A-II in HDL subpopulations

Variables	Controls	Heterozygotes	Compound Heterozygotes	Homozygotes
Preβ				3.2 ± 2.4[a,b]
α-VL				12.2 ± 7.6[a,b]
α-L			5.6[a,b]	5.9 ± 2.0[a,b]
α-1		9.7 ± 6.2	3.0 ± 1.0[a,b]	3.1 ± 1.4[a,b]
α-2	2.0 ± 0.6	2.4 ± 0.8	2.3 ± 0.9	3.0 ± 0.8[a]
α-3	3.1 ± 0.6	3.2 ± 1.9	4.2 ± 4.1	4.0 ± 3.1

Values are means ± SD.
[a] $P < 0.05$ (compared with controls).
[b] $P < 0.05$ (compared with heterozygotes).

the LpA-I:A-II HDL particles have lesser capability to promote scavenger receptor class B type I-mediated cholesterol uptake in the liver than LpA-I HDL. This issue was recently reviewed by Fidge (37) and debated by deBeer et al. (38).

On the other hand, we assume that the significantly increased apoA-I but not apoA-II in α-1 HDL particles in HEs, along with the significantly decreased α-3 and preβ-1 levels, mark an effective reverse cholesterol transport. In addition, HE subjects have very low LDL-C levels, probably attributable to decreased lipid exchange between LDL and HDL, resulting in small CE-poor but TG-rich LDL particles. In complete CETP deficiency in humans, the cholesteryl esters of VLDL and its catabolic product, LDL, originate predominantly from intracellular ACAT (39).

Considering this information, we assume that the HDL subpopulation profile of heterozygous CETP-deficient patients, especially those with high LpA-I α-1 levels, represents an improved anti-atherogenic state, although this might not be the case for CHs and HOs with very large, undifferentiated HDL particles.

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