Structural and functional differences of subspecies of apoA-I-containing lipoprotein in patients with plasma cholesteryl ester transfer protein deficiency

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Abstract ApoA-I-containing lipoproteins exist in plasma in two main forms: one contains only apoA-I (LpA-I) while the other contains both apoA-I and apoA-II (LpA-I/A-II). We characterized structural and functional changes of these lipoproteins in six patients with cholesteryl ester transfer protein (CETP) deficiency. In these patients, the amount of LpA-I and LpA-I/A-II had increased significantly. Sixty-five percent of plasma apoA-I was associated with LpA-I/A-II, which indicated that LpA-I/A-II was predominant. The chemical composition of both LpA-I and LpA-I/A-II was characterized by increased ratios of neutral to polar lipid, compared with findings in normal subjects. Particle sizes of these lipoproteins shifted to larger diameter ranges, as compared to the size seen in normal subjects. Incubation of patients' LpA-I and LpA-I/A-II with CETP markedly corrected the chemical and physical abnormalities in these lipoproteins. Cholesterol-reducing capacities of these lipoproteins from macrophage foam cells were significantly lower than in normal controls. Cholesterol esterification rates in LpA-I, LpA-I/A-II, and plasma were significantly lower in patients than in normal controls. We propose that the structure and function of LpA-I and LpA-I/A-II are severely affected in the presence of CETP deficiency.—**Ohta, T., R. Nakamura, K. Takata, Y. Saito, S. Yamashita, S. Horiuchi, and I. Matsuda.** Structural and functional differences of subspecies of apoA-I-containing lipoprotein in patients with plasma cholesteryl ester transfer protein deficiency. *J. Lipid Res.* 1995. **36:** 696–704.

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Plasma levels of high density lipoproteins (HDL) are inversely correlated with the risk of coronary heart disease (1, 2). Although the precise mechanism involved in this inverse correlation remains unknown, it has been postulated that HDL plays a role in "reverse cholesterol transport," i.e., the transport of cholesterol from peripheral cells to the liver for excretion (3, 4). In this transport model, HDL removes excess free cholesterol (FC) from peripheral tissues and this FC is esterified on HDL by the action of lecithin:cholesterol acyltransferase (LCAT) (5). LCAT accelerates HDL-mediated cellular cholesterol efflux (6, 7). Cholesteryl ester (CE) is then transferred to the liver by two known pathways: *i)* uptake of HDL particles by the liver (8, 9), and *ii)* uptake of HDL-CE by LDL-receptor in the liver via transfer of HDL-CE to very low density lipoprotein (VLDL) and/or low density lipoprotein (LDL), in a process mediated by cholesteryl ester transfer protein (CETP) (3, 10). In humans, the latter is a major pathway for HDL-CE delivery to the liver (3). Thus, plasma CETP appears to be very important in maintaining reverse cholesterol transport in humans. According to studies on patients with CETP deficiency and on transgenic mice (11–14), low plasma CETP activity is associated with high plasma HDL levels, whereas high plasma CETP activity is associated with low plasma HDL levels. In addition, homozygotes for CETP deficiency seem to be protected from atherosclerotic coronary heart disease (11, 12). Taken together, these results suggest that CETP-mediated HDL-CE transfer to the liver may be atherogenic in nature. However, the relation of CETP to other key steps in reverse cholesterol transport, such as HDL-mediated cholesterol efflux from cells and the cholesterol esterification rate in HDL, remains to be elucidated.

Abbreviations: CETP, cholesteryl ester transfer protein; LpA-I, apoA-I containing lipoproteins with A-I only; LpA-I/A-II, apoA-I containing lipoproteins with A-I and A-II; HDL, high density lipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; LDP, lipoprotein-deficient plasma; TG, triglyceride; PL, phospholipid; FC, free cholesterol; CE, cholesteryl ester; FER, fractional esterification rate; MER, molar esterification rate; GGE, gradient gel electrophoresis.

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Lipoproteins containing apoA-I isolated by immunological methods probably better reflect the native state of these particles than does HDL isolated by ultracentrifugation (15, 16). These lipoproteins consist of two subspecies: one contains apoA-I but no apoA-II (LpA-I), and the other contains both apoA-I and apoA-II (LpA-I/A-II). Recent clinical and experimental studies have shown that LpA-I is more anti-atherogenic than LpA-I/A-II (17–20). These data seem to be consistent with our earlier in vitro findings that LpA-I can remove more cholesterol from macrophage foam cells than does LpA-I/A-II (7). In addition, plasma CETP and LCAT are mostly associated with apoA-I-containing lipoproteins (21–23). In the present study, as a first step in examining the relation of CETP to atherosclerosis, we isolated LpA-I and LpA-I/A-II from six patients with CETP deficiency and characterized structural and functional differences of these lipoproteins.

MATERIAL AND METHODS

Subjects and materials

Plasma was obtained from six unrelated hyperalphalipoproteinemic patients (two males and four females) with complete deficiency in CETP activity. None of the patients had clinical signs or symptoms of atherosclerotic coronary heart disease. As noted in work by other investigators (12), three patients were homozygous for a point mutation in the 5'-splice donor site of intron 14 of the gene for CETP. Gene analysis of the other three patients had not yet been done. Plasma from 40 normolipidemic subjects (20 males and 20 females) was used for comparative purposes. All chemicals used were of the best grade available and were obtained from commercial sources. [7-^{3}H]cholesterol and [^{14}C]cholesteryl oleate were purchased from DuPont New England Nuclear.

Isolation of LpA-I and LpA-I/A-II from plasma

LpA-I and LpA-I/A-II were isolated from plasma samples from patients and controls by a combination of anti-apoA-I and anti-apoA-II immunosorbent columns, as described previously (7, 24). Briefly, plasma was applied to an anti-apoA-I column. After washing extensively with 0.01 mol/l Tris, 0.5 mol/l NaCl, 1 mmol/l EDTA, pH 7.5 (buffer A), the column was eluted with 0.1 mol/l acetic acid and 1 mmol/l EDTA, (pH 3.0). Each effluent was immediately adjusted to pH 7.4 with 1.0 mol/l Tris solution and dialyzed against 0.15 mol/l NaCl and 1 mmol/l EDTA, pH 7.4 (buffer B). Finally, the sample was concentrated in buffer B using an ultrafiltration cell (Amicon Corp, Danvers, MA) equipped with a PM-10 membrane and applied to an anti-apoA-II column. The column was washed with buffer A to obtain LpA-I. The bound fraction was eluted from the column to obtain LpA-I/A-II. Both LpA-I and LpA-I/A-II were dialyzed and concentrated in buffer B. In this procedure, >90% of lipids and apolipoproteins applied were recovered in the unbound and bound fractions. When the unbound plasma fraction from the anti-apoA-I column was applied to an anti-apoA-II column, no bound fraction was obtained. Thus, we considered that all of the plasma apoA-II was associated with apoA-I as LpA-I/A-II.

Cholesterol efflux from macrophage foam cells

Experiments for cholesterol efflux from foam cells were carried out as described previously (7, 25). Briefly, peritoneal macrophages from Wistar rats were harvested in PBS and suspended in Dulbecco's modified minimal essential medium containing 3% BSA (medium A). This cell suspension was placed in a 22-mm plastic dish and the preparation was incubated for 4 h. Adherent macrophages were converted to foam cells by a 16-h incubation with 100 μg/ml of acetyl-LDL in 1.0 ml of medium A. These foam cells were subjected to efflux assays by incubation with medium A containing 100–400 μg protein of LpA-I or LpA-I/A-II. Parallel incubations without lipoproteins served as controls. The culture medium was removed 3, 6, 12, and 24 h after the onset of efflux experiments and the cellular lipids were then extracted directly from macrophage monolayers as described previously (7, 25). Unless otherwise specified, the data derived from these efflux assays were the mean of quintuplicate runs in five separate experiments. The mass of FC and CE was quantified by a modification of the enzymatic/fluorometric method of Heider and Boyett (26).

Fractional and molar esterification rates in plasma

[^{3}H]FC was incorporated onto polystyrene tissue culture wells (Corning) as follows: absolute ethanol (100 μl) containing 0.2 μCi [^{3}H]FC was placed in wells and the ethanol was evaporated under nitrogen. One hundred μl of plasma sample in 400 μl of phosphate buffer saline (PBS) was then added to each well and the [^{3}H]FC was equilibrated with FC in plasma by incubation at 4°C for 16 h. [^{3}H]FC-labeled plasma samples were then incubated at 37°C for 1 h. The enzyme reaction was stopped by immersing the sample tubes in an ice bath. The lipids in incubation samples were extracted with methanol–chloroform 2:1 (v/v), and the extract was then dried under nitrogen and dissolved in 60 μl isopropanol. Aliquots (20 μl) of lipid extracts were spotted in duplicate on a thin-layer chromatography (TLC) plate (Merck) and developed in n-hexane–diethyl ether–acetic acid–methanol 85:20:1:1 (v/v). Spots corresponding to FC and CE were cut out from the plate and the radioactivities were determined. The fractional esterification rate (FER) was expressed as the difference between the percentage of radioactive cholesterol esterified before and after incubation at

37°C and the molar esterification rate (MER) was calculated based on the specific activity (dpm/nmol FC) of each sample.

Cholesterol esterification in LpA-I and LpA-I/A-II

The esterification of cholesterol in LpA-I and LpA-I/A-II was determined as described above, except that incubation for [^{3}H]FC-labeled LpA-I and LpA-I/A-II was at 37°C for 16 h.

Cholesteryl ester transfer activity

The activity of CETP was assayed as described previously (11). Human plasma HDL_3 was radiolabeled with [^{14}C]cholesteryl oleate (27) and used as the donor lipoprotein. Unlabeled LDL was used as the acceptor for radiolabeled cholesteryl esters. Ten μl of plasma from each subject was used as the source of CETP. The assay was performed in a total volume of 500 μl containing 10 mM Tris-HCl, pH 7.4, and 150 mM NaCl for 3 h at 37°C. The reaction was terminated by incubation on ice and the LDL was precipitated using phosphotungstate-$MgCl_2$ (28, 29). Cholesteryl ester transfer activity was expressed as a percentage of the donor CE transferred to LDL.

Incubation of patients' LpA-I and LpA-I/A-II with CETP

Patients' LpA-I and LpA-I/A-II were incubated with lipoprotein-deficient plasma (CETP source) at 37°C for 16 h. Lipoprotein-deficient plasma (LDP) was isolated from fresh plasma (30). The supernatant recovered after ultracentrifugation at 150,000 *g* for 48 h at a density of 1.25 g/ml was subjected to a second 48 h period of centrifugation at d 1.25 g/ml. The upper one third of the infranatant after a second spin was recovered and added to the first d 1.25 g/ml infranatant. ApoA-I in LDP was removed by anti-apoA-I immunosorbent column. LDP was concentrated 2-fold and dialyzed against phosphate-buffered saline (PBS). Endogenous LCAT activity in LDP was inactivated by heating at 58°C for 20 min. CETP activity in LDP was not affected by this procedure. Patients' LpA-I and LpA-I/A-II isolated from 3 ml plasma were incubated with 5.0 ml of LDP and plasma fraction with <1.063 g/ml (isolated from 10 ml normal plasma). After incubation, LpA-I and LpA-I/A-II were isolated from incubation sample, as mentioned above.

Protein and lipid analysis

The apoA-I, apoA-II, and apoE concentrations of plasma, LpA-I and LpA-I/A-II were measured by radial immunodiffusion assay (31, 32). Total cholesterol (TC), FC, triglyceride (TG), and phospholipid (PL) concentrations of these samples were determined by enzymatic methods, using commercial kits. The concentrations of CE were calculated as (TC minus FC) times 1.62. HDL-cholesterol was measured by selective precipitation of LDL as described above (28, 29). The protein content of each fraction from the immunosorbent columns was determined by the method of Lowry et al. (33). Values of lipids and apolipoproteins in LpA-I and LpA-I/A-II were corrected, based on the % recoveries during the isolation.

Electrophoretic analysis

Slab gel electrophoresis was performed according to Weber and Osborn (34). The Stokes diameters of the lipoprotein particles were estimated by gradient polyacrylamide gel electrophoresis (GGE) on Pharmacia precast PAA 4/30 gels, according to the procedure specified by the manufacturer. Electrophoresis calibration kits for high- or low-molecular weight proteins (Pharmacia) were used. The Stokes diameters of these high-molecular weight proteins are: thyroglobulin, 17.0 nm; apoferritin, 12.2 nm; catalase, 10.4 nm; lactate dehydrogenase, 8.2 nm and bovine albumin, 7.5 nm.

Statistical evaluation

The unpaired *t*-test was used to statistically evaluate the data.

RESULTS

All plasma lipid and apolipoprotein concentrations in patients, except for TG, were significantly higher than those in controls (**Table 1**). CETP activity in all the patients was negligible. These data are consistent with the typical lipid and apolipoprotein pattern in patients with CETP deficiency reported earlier (11, 12).

TABLE 1. Plasma lipid and apolipoprotein concentrations in patients with CETP deficiency

Subjects	N	FC	CE	TG	HDL-C	A-I	A-II	E	CETA
					mg/dl				%
Patients	6	78 ± 9[a]	340 ± 19[a]	103 ± 42	157 ± 12[a]	242 ± 25[a]	45 ± 6[a]	17.4 ± 5.4[a]	0 ± 0[a]
Controls	40	51 ± 5	212 ± 28	69 ± 15	57 ± 7	135 ± 15	32 ± 5	4.3 ± 1.7	25 ± 4

N, number of subjects: FC, free cholesterol; CE, cholesteryl esters; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; CETA, cholesteryl ester transfer activity. Values are expressed as mean ± SD.

[a]Significantly different from controls ($P < 0.005$).

TABLE 2. Lipid and apolipoprotein concentrations of LpA-I and LpA-I/A-II in patients with CETP deficiency

Subjects	FC	CE	TG	PL	A-I	A-II	E
				mg/dl			
LpA-I							
Patients	15 ± 4[a]	78 ± 6[b]	9 ± 7	82 ± 12[b]	84 ± 22[b]	0	1.08 ± 0.54[a]
Controls[c]	9 ± 2	34 ± 6	8 ± 2	45 ± 7	55 ± 5	0	0.43 ± 0.10
LpA-I/A-II							
Patients	26 ± 7[b]	156 ± 32[b]	10 ± 3	155 ± 25[b]	154 ± 20[b]	45 ± 6[b]	5.50 ± 1.51[b]
Controls[c]	10 ± 2	49 ± 6	8 ± 2	66 ± 9	80 ± 9	32 ± 5	1.07 ± 0.25

FC, free cholesterol; CE, cholesteryl esters; TG, triglyceride; PL, phospholipid. Values are expressed as mean ± SD.
[a]Significantly different from controls, $P < 0.05$.
[b]Significantly different from controls, $P < 0.005$.
[c]Twenty males and 20 females.

Characterization of LpA-I and LpA-I/A-II

As shown in **Table 2,** the absolute concentrations of FC, CE, PL, apoA-I, and apoE in LpA-I of patients were significantly higher than those of controls ($P < 0.05$ or 0.005). A significant lower percentage of plasma apoA-I was located in LpA-I of the patients (35 ± 3%, mean ± SD) than in LpA-I of normal subjects (41 ± 5%) ($P < 0.05$). To clarify whether these differences were due to quantitative or qualitative changes in LpA-I particles, we determined the relative lipid and protein composition of LpA-I (**Table 3**). LpA-I particles in patients were rich in CE and poor in protein, compared to those in normal controls ($P < 0.005$). The proportion of core (CE + TG) to surface (FC + PL) lipid in patients was slightly higher than that in normal controls with no statistical significance.

As with LpA-I, all of the lipid, except TG, and apolipoprotein concentrations in LpA-I/A-II were significantly higher in patients than in controls (Table 2, $P < 0.005$). To clarify the qualitative changes, we determined the lipid and protein composition of LpA-I/A-II (Table 3). In patients, the percentages of CE and PL were increased and the percentage of protein was decreased, which indicated that LpA-I/A-II in patients contained lipid-rich particles, as compared to findings in normal controls. The proportion of core to surface lipid in patients was slightly higher than that in normal controls, with no statistical significance.

We next characterized the particle size of LpA-I and LpA-I/A-II by gradient gel electrophoresis (GGE). In GGE, normal LpA-I migrated as two distinct particles (11.1 nm and 8.8 nm in diameter) (**Fig. 1**). A few large particles were also detected. However, as shown in Fig. 1, LpA-I in CETP deficiency showed a different profile. In this case, particles 12.5 and 10.0 nm in diameter were predominant. With regard to LpA-I/A-II, LpA-I/A-II particles in normal subjects were between 8.0 and 10.4 nm in diameter (Fig. 1) while those in patients were mainly between 10.4 and 12.5 nm in diameter. These results indicate that LpA-I and LpA-I/A-II particles in CETP deficiency are increased in size, as compared to those in normal controls, and the extent of this increase is much greater in LpA-I/A-II than in LpA-I. The protein moiety of LpA-I and LpA-I/A-II in patients, as determined by SDS-polyacrylamide gel electrophoresis, was indistinguishable from findings in normal controls (data not shown).

TABLE 3. Percent composition of LpA-I and LpA-I/A-II in patients with CETP deficiency

Subjects	Protein	FC	CE	TG	PL	Ratio of Neutral to Polar Lipid
			%			
LpA-I						
Patients	44.4 ± 2.3[a]	4.5 ± 1.0	23.6 ± 1.5[a]	2.7 ± 1.9	24.8 ± 1.9	0.90 ± 0.11
Controls[b]	51.4 ± 4.0	4.6 ± 0.5	17.2 ± 1.7	4.0 ± 2.0	22.7 ± 2.3	0.78 ± 0.13
LpA-I/A-II						
Patients	42.8 ± 1.4[a]	4.3 ± 0.8	25.7 ± 0.9[a]	1.6 ± 0.9	25.5 ± 1.0[a]	0.92 ± 0.12
Controls[b]	54.9 ± 4.9	3.4 ± 0.3	16.6 ± 1.5	2.7 ± 1.3	22.4 ± 1.9	0.75 ± 0.10

FC, free cholesterol; CE, cholesteryl esters; TG, triglyceride; PL, phospholipid; neutral lipid, (CE + TG); polar lipid, (FC + PL). Values are expressed as mean ± SD.
[a]Significantly different from controls ($P < 0.005$).
[b]Twenty males and 20 females.

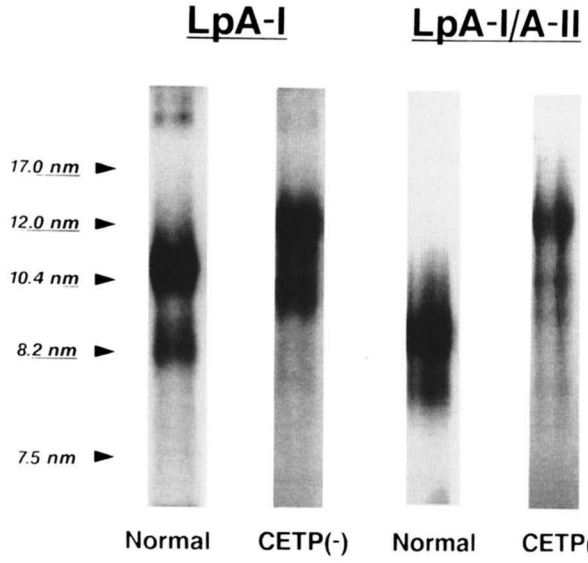

Fig. 1. Nondenaturing gradient polyacrylamide gel electrophoresis of LpA-I and LpA-I/A-II isolated from patients with CETP deficiency and from normal controls. Particle diameters of standards are inserted; Normal: normal control; CETP (−): patient with CETP deficiency.

Effect of LpA-I and LpA-I/A-II on cholesterol efflux from foam cells

In our assay system, cellular cholesterol reduction mediated by LpA-I or LpA-I/A-II was not observed before 6 h of incubation. Although cellular cholesterol reduction after 6 and 12 h of incubation was less than that at 24 h, the relative abilities of LpA-I and LpA-I/A-II from patients and those from controls were similar to those at 24 h. Thus, only data for 24 h of incubation are shown in **Fig. 2.** When macrophage foam cells were subjected to a 24-h incubation with 100–400 μg of normal LpA-I, cellular contents of CE and FC were reduced by 50% and 30%, respectively. As shown in Fig. 2, LpA-I in patients reduced CE and FC mass by only 20–30%. Incubation with 100–400 μg of normal LpA-I/A-II led to a reduction in CE mass (50%) and had no apparent effect on FC mass. Similar to LpA-I, the CE-reducing capacity of LpA-I/A-II in patients was weak (50% of normal). All these data indicate that apoA-I-containing lipoproteins in patients with CETP deficiency are poor acceptors for cellular cholesterol.

Endogenous cholesterol esterification by LpA-I, LpA-I/A-II and plasma

Cholesterol esterification by LCAT associated with HDL accelerates HDL-mediated cholesterol efflux from cells (6, 7). As shown in **Table 4,** in normal LpA-I and LpA-I/A-II, 28% and 7% of their own FC was esterified by the LCAT associated with these lipoproteins, respectively. More CE was produced in LpA-I than in LpA-I/A-II, in both patients and normal subjects. However, both percentage esterification and the mass of CE produced

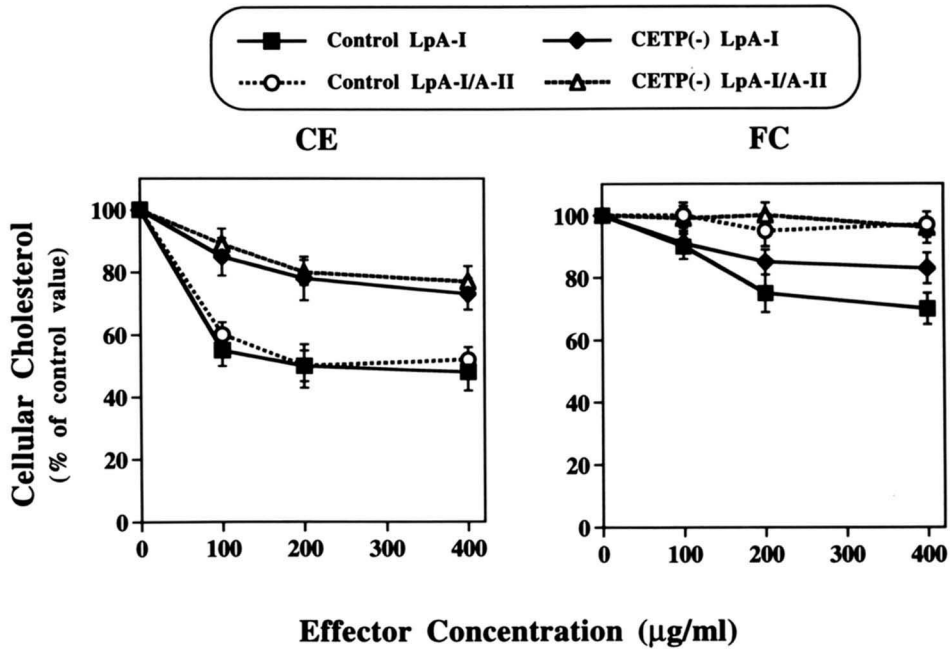

Fig. 2. Effect of LpA-I and LpA-I/A-II from normal controls and patients with CETP deficiency on CE and FC mass in macrophage foam cells. Macrophage foam cells were incubated for 24 h with varying concentrations of LpA-I and LpA-I/A-II from normal controls and patients with CETP deficiency. Control experiments were done without LpA-I and LpA-I/A-II (0 μg/ml). Cellular lipids were extracted and determined for FC and CE. Data are the mean ± SE of five experiments. Mean values of CE and FC for 100% were 150 and 120 nmol/mg cell protein, respectively.

TABLE 4. Endogenous cholesterol esterification rate by LpA-I and LpA-I/A-II in patients with CETP deficiency

Subjects	LpA-I		LpA-I/A-II	
	%	CE Generated	%	CE Generated
Patients	7.0 ± 2.8[a]	4.0 ± 0.5[a]	3.0 ± 1.1[a]	1.9 ± 0.3[a]
Controls[b]	28.2 ± 3.9	8.3 ± 1.5	7.2 ± 2.1	3.2 ± 0.4

% means percent esterification of [^{3}H]FC in LpA-I or LpA-I/A-II. CE (cholesteryl esters) is expressed as nmol/ 16 h/200 μg protein. Values are expressed as mean ± SD.
[a]Significantly different from controls ($P < 0.005$).
[b]Ten males and 10 females.

TABLE 5. Fractional and molar esterification rates in plasma in patients with CETP deficiency

Subjects	FER_{plasma}	MER_{plasma}
	%/h	μmol/h/l
Patients	1.0 ± 0.2[a]	22.3 ± 2.7[a]
Controls[b]	3.6 ± 0.5	48.1 ± 7.0

FER, fractional esterification rate; MER, molar esterification rate. Values are expressed as mean ± SD.
[a]Significantly different from controls ($P < 0.005$).
[b]Ten males and 10 females.

were much lower in patients than in the normal controls. As plasma concentrations of LpA-I and LpA-I/A-II are much higher in CETP-deficient patients than in normal controls, the absolute rate of CE formation in the patients' plasma may not differ too much from that in normal control plasma. To clarify CE production in the patients' plasma, we studied the fractional and molar esterification rates in plasma (FER_{plasma} and MER_{plasma}). As shown in **Table 5,** FER_{plasma} and MER_{plasma} were significantly lower in patients than in normal controls. These results indicate that in plasma, CE production in CETP-deficient patients is much lower than that in controls. As most plasma LCAT is associated with apoA-I-containing lipoproteins (21–23), these results suggest that in CETP-deficient patients, LCAT associated with LpA-I or LpA-I/A-II may use less FC from VLDL or LDL than in normal controls, possibly due to the failure to create an efficient diffusion gradient between apoA-I-containing lipoproteins and VLDL or LDL.

Characterization of patients' LpA-I and LpA-I/A-II after incubation with LDP (CETP source)

To create a plasma-like environment, LpA-I and LpA-I/A-II were incubated with LDP and plasma fraction with d < 1.063 g/ml (as CE acceptor). In the preliminary experiment, significant changes in particle size and chemical composition were not observed after incubation. Therefore, we did similar experiments using LCAT-inactivated LDP. As shown in **Table 6,** chemical composition of patients' LpA-I and LpA-I/A-II were markedly corrected, although the proportion of core to surface lipid was slightly higher than that in normal controls. Particle sizes of LpA-I and LpA-I/A-II were smaller than those of original particles but still larger than those of normal particles (predominant particle diameter: LpA-I, 9.0–11.5 nm and LpA-I/A-II, 9.5–11.7 nm).

DISCUSSION

The present studies demonstrate the following. *i)* The amount and particle sizes of both LpA-I and LpA-I/A-II increase significantly in patients with CETP deficiency and these changes were more prominent in LpA-I/A-II than in LpA-I. *ii)* Two-thirds of apoA-I-containing lipoproteins in patients are LpA-I/A-II. *iii)* The cholesterol-reducing capacities of patients' LpA-I and LpA-I/A-II from foam cells are significantly lower than those of controls' LpA-I and LpA-I/A-II. *iv)* Cholesterol esterification rates in LpA-I, LpA-I/A-II and plasma are significantly lower in patients than in normals.

Rubin et al. (19) and Schultz et al. (20) reported data on human apoA-I transgenic mice (AITg) and human

TABLE 6. Percent composition of LpA-I and LpA-I/A-II in patients with CETP deficiency after incubation with CETP

Subjects	Protein	FC	CE	TG	PL	Ratio of Neutral to Polar Lipid
			%			
LpA-I						
Patients	49.2 ± 5.0	4.3 ± 1.0	19.7 ± 3.5	5.0 ± 2.0	21.9 ± 3.1	0.94 ± 0.18
Controls	51.4 ± 4.0	4.6 ± 0.5	17.2 ± 1.7	4.0 ± 2.0	22.7 ± 2.3	0.78 ± 0.13
LpA-I/A-II						
Patients	55.2 ± 5.5	2.9 ± 1.0	18.5 ± 3.0	3.5 ± 1.8	20.0 ± 3.2	0.96 ± 0.17
Controls[a]	54.9 ± 4.9	3.4 ± 0.3	16.6 ± 1.5	2.7 ± 1.3	22.4 ± 1.9	0.75 ± 0.10

FC, free cholesterol; CE, cholesteryl esters; TG, triglyceride; PL, phospholipid; neutral lipid, (CE + TG); polar lipid, (FC + PL). Values are expressed as mean ± SD.
[a]Twenty males and 20 females.

apoA-I and apoA-II transgenic mice (AI,AIITg). Both strains were protected against atherosclerosis, but the protection in AITg was much greater than that in AI,AIITg. In AI,AIITg, particle sizes of LpA-I and LpA-I/A-II were very close to those seen in our patients. Furthermore, similar to our patients, LpA-I/A-II was predominant (88% of human apoA-I was associated with particles that also contained human apoA-II). Because mice have no plasma CETP (13), the changes in particle size and the predominance of LpA-I/A-II may be caused by the lack of CETP, as in the case of human CETP deficiency. If the data on these transgenic mice can be extrapolated to our patients, protection from atherosclerosis may be marginal in patients with CETP deficiency. Thus, we examined the function of increased LpA-I and LpA-I/A-II in these patients.

The anti-atherogenic function of HDL is currently attributed to its role in reverse cholesterol transport. The first step in reverse cholesterol transport is the removal of cellular cholesterol by apoA-I-containing lipoproteins (A-ILp) (3, 4, 35). In a previous study (7), we found that the interaction of LpA-I or LpA-I/A-II with macrophage foam cells induced a mass reduction in cholesterol in these cells, and the cholesterol-reducing capacity of LpA-I was greater than that of LpA-I/A-II. In patients with CETP deficiency, the cholesterol-reducing capacities of LpA-I and LpA-I/A-II were only one-half of the normal counterparts (Fig. 2). In addition, the particle diameters of patients' LpA-I and LpA-I/A-II were much larger than those in normal controls (Fig. 1). Based on studies of human arteries and lymph (36–38), only smaller LpA-I particles can enter the interstitium, and these are thought to be the most likely acceptors of FC from cell membranes. Thus, larger LpA-I and LpA-I/A-II particles may well be excluded from the interstitium. If so, increased concentrations of plasma LpA-I and LpA-I/A-II in CETP-deficient patients cannot compensate for the impaired cellular cholesterol-reducing capacities. Kinetic studies of apoA-I in patients with CETP deficiency showed that the rate of apoA-I production was normal and that elevated apoA-I and HDL levels are due to a decrease in the catabolic rate of apoA-I (39). Therefore, it is unlikely that the production of small LpA-I particles (which are rapidly enlarged after removing cellular cholesterol because of the lack of CETP) is increased in patients. Taken together, these results suggest that the first step of reverse cholesterol transport mediated by A-ILp is disturbed in patients with CETP deficiency. With respect to the mechanism involved in the impaired cholesterol-reducing capacity of LpA-I and LpA-I/A-II, LCAT activity is closely linked to the cholesterol-reducing capacity of LpA-I (7, 25). Upon complete inactivation of LCAT, the cholesterol-reducing capacity of LpA-I, but not that of LpA-I/A-II, is lost (7, 25). Thus, the lower cholesterol esterification in patients' LpA-I, which is discussed in the following section, can explain the impaired cholesterol-reducing capacity of patients' LpA-I. On the other hand, a higher ratio of FC to PL (patients: 0.17, controls: 0.15) and a greater particle size (patients: 10.4–12.5 nm, controls: 8.0–10.4 nm) seem to be related to the reduced cholesterol-reducing capacity of patients' LpA-I/A-II, as suggested in earlier reports (7, 25, 40).

The second step in reverse cholesterol transport is cholesterol esterification of FC from cells by LCAT associated with A-ILp (3, 4). Our present data clearly show that cholesterol esterification in patients' LpA-I and LpA-I/A-II is weakened significantly (Table 4). Although the plasma LCAT mass in these patients was not measured, plasma LCAT activity, measured using an exogenous substrate that correlated well with the LCAT mass (41, 42), was within normal ranges (T. Ohta, K. Takata, R. Nakamura, and I. Matsuda, unpublished data). Thus, there may be two possible causes for the lower cholesterol esterification in LpA-I and LpA-I/A-II. First, the plasma concentration of LpA-I and LpA-I/A-II is significantly increased. This finding and a normal LCAT mass in patients suggest that the LCAT mass associated with each particle of these lipoproteins decreases. Second, according to the current concept (30, 43), reactivity of LCAT with HDL particles decreases as a function of particle size (large particles are less reactive than small particles). Thus, it is rational to consider that the patients' large LpA-I and LpA-I/A-II particles are less reactive with LCAT than are those of normal controls. Both mechanisms may contribute to the lower esterification rate in patients' LpA-I, LpA-I/A-II and plasma.

In conclusion, the first and the second steps for reverse cholesterol transport mediated by A-ILp are disturbed in patients with CETP deficiency. However, these subjects appear to be protected from atherosclerotic coronary heart disease, although the protection may be marginal (11, 12). This raises three possibilities. First, reverse cholesterol transport that does not use A-ILp may function in patients with CETP deficiency (44, 45). Second, the third step for reverse cholesterol transport, i.e., direct uptake of A-ILp by the liver, may be accelerated in these patients (8, 9). Finally, an unknown protective mechanism other than reverse cholesterol transport may function in patients with CETP deficiency. Further studies are needed to clarify these problems.

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