Gender effects on the distribution of the cholesteryl ester transfer protein in apolipoprotein A-I-defined lipoprotein subpopulations

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Abstract Two subpopulations of apolipoprotein A-I-containing lipoproteins, those containing only apoA-I (LpA-I) and those containing both apoA-I and apoA-II (LpA-I/A-II), were isolated by immunoaffinity chromatography of plasma from 44 subjects, comprising four groups (male or female, with or without hyperlipidemia). ApoA-I-defined particles (LpAs) were assessed for their content of cholesteryl ester transfer protein (CETP) and for their ability to act as substrates for CETP. Although plasma CETP concentration was similar in all groups, the plasma concentration of LpA-I-associated CETP was significantly higher in females than in males (1.56 ± 0.11 versus 0.93 ± 0.13 mg/l, $P < 0.05$). In females, the major fraction of CETP was found in LpA-I, whereas in normolipidemic males CETP was evenly distributed between LpA-I and LpA-I/A-II, and in hyperlipidemic males the majority of CETP was found in LpA-I/A-II. In all groups, the percentage of CETP in LpA-I was correlated with the concentration of apoA-I in LpA-I ($r = 0.64$, $P < 0.001$). Native gradient gel electrophoresis of isolated LpAs showed that CETP was broadly distributed within different sized particles. LpA-I and LpA-I/A-II showed similar efficiency of CETP-mediated cholesteryl ester exchange with LDL. In conclusion, even though LpA-I has a much higher apparent affinity for CETP than LpA-I/A-II, both LpAs can bind CETP and act as equivalent CETP substrates in vitro. Thus, in subjects with low levels of LpA-I (notably hyperlipidemic males), most of the plasma neutral lipid exchange will involve LpA-I/A-II. This could be a factor contributing to a general impairment in reverse cholesterol transport in subjects with low LpA-I.—**Moulin, P., M. C. Cheung, C. Bruce, S. Zhong, T. Cocke, H. Richardson, and A. R. Tall.** Gender effects on the distribution of the cholesteryl ester transfer protein in apolipoprotein A-I-defined lipoprotein subpopulations. *J. Lipid Res.* 1994. **35:** 793–802.

Supplementary key words apolipoprotein A-II • hyperlipidemia • high density lipoprotein • reverse cholesterol transport

The plasma high density lipoproteins form a heterogeneous group of particles (1). The investigation of subpopulations of lipoproteins defined by their apolipoprotein composition has provided novel insights into lipoprotein metabolism and atherogenesis. Recent studies suggest that some subpopulations of apoA-I-defined particles might have specialized functions in the promotion of reverse cholesterol transport and consequently might contribute more specifically to protection against atherosclerosis (2, 3). This metabolic pathway includes several successive steps, i.e., transport of free cholesterol between cell membrane and lipoproteins, esterification by lecithin:cholesterol acyl transferase (LCAT), then either transfer of esterified cholesterol by the cholesteryl ester transfer protein (CETP) and transport of the esterified cholesterol back to the liver or a more direct pathway involving uptake of HDL cholesterol and cholesteryl ester by the liver involving several different mechanisms (1, 4, 5). A reduction of cholesterol concentration and abnormalities of the size distribution of lipoproteins containing only apoA-I have been reported in patients with coronary heart disease (6, 7) or at risk of coronary heart disease (8, 9). It has been shown that cholesterol transport from cell membranes and its esterification preferentially involves prebeta-migrating species of LpA-I (6), and that in a mouse adipose cell line, cholesterol efflux is mediated by LpA-I but not LpA-I/A-II (7). In contrast, several investigators have shown that both LpA-I and LpA-I/A-II had similar abilities to remove cholesterol from human fibroblasts (10–12). The complex involvement of these subpopulations with the esterification and the transfer process of

Abbreviations: CETP, cholesteryl ester transfer protein; HDL, high density lipoproteins; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoproteins; LpA-I, lipoproteins containing apoA-I only; LpA-I/A-II, lipoproteins containing apoA-I and apoA-II; LpAs, apoA-I-defined particles; PL, phospholipid; RIA, radioimmunoassay; TC, total cholesterol; VLDL, very low density lipoproteins.

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cholesterol occurring in the plasma in vivo is still incompletely understood.

Studies in patients with CETP deficiency and in transgenic mice expressing the human CETP gene suggest that CETP is involved in the exchange of HDL cholesteryl ester with triglycerides of VLDL and with subsequent metabolism of the triglyceride-enriched HDL to smaller particles (13, 14). Recent studies in vitro suggest that CETP is involved in the conversion of alpha-migrating HDL into prebeta-migrating HDL (15). Cheung et al. (16) have previously shown in normolipidemic subjects that CETP and LCAT activities were present mainly in LpA-I. The purpose of the present study was to determine the distribution of CETP between LpA-I and LpA-I/A-II in subjects with various amounts of LpA-I. Upon finding an unexpectedly high proportion of CETP in LpA-I/A-II in hyperlipidemic males with low LpA-I, we decided to compare these apoA-I-defined particles as CETP substrates in vitro.

METHODS

Subjects

The study group comprised 17 normolipidemic and 9 hyperlipidemic females, and 4 normolipidemic and 14 hyperlipidemic males (**Table 1**). Amongst the hyperlipidemic females, 3 had Type IIa hypercholesterolemia (LDL cholesterol ≥ 1.6 g/l mean of two determinations within 10% of each other) and 6 had a mild type IIb hyperlipidemia (LDL cholesterol > 1.6 g/l and plasma triglycerides > 1.5 g/l < 3 g/l). Three hyperlipidemic males had a type IIa hypercholesterolemia and 11 had a mild type IIb hyperlipidemia. None of the subjects was taking any medication or had any disease known to interfere with lipid metabolism. Hyperlipidemic patients were consuming an AHA Phase I diet for 6–7 weeks prior to study. Blood samples were drawn on EDTA (1.5 mg/ml) after a 12-h fast. The study was approved by the University of Washington Institutional Review Board.

Analytical procedures

Lipids were measured in plasma and lipoprotein fractions by enzymatic methods using an Abott Spectrum Biochromatic Analyzer (Irving, TX) (17). Cholesteryl ester was determined by difference between total and free cholesterol. ApoA-I and apoA-II concentrations were measured by specific radioimmunodiffusion (18). HDL particle sizes were determined by nondenaturating gradient polyacrylamide gel electrophoresis (gPAGE) as previously described using precast 4–30% gels (Pharmacia LKB Biotechnology) (19). Proteins were stained with Coomassie Blue G-250 and gels were scanned with a laser densitometer and with LKB 2400 Gelscan software (7).

ApoA-I-defined HDL subpopulation

HDL particles containing both apoA-I and A-II (LpA-I/A-II) and those containing apoA-I but no A-II (LpA-I) were isolated at 4°C by an established immunoaffinity chromatography procedure (20, 21). Briefly, after adsorption of plasma samples with dextran sulfate cellulose to remove all apoB-containing lipoproteins, the apoB-free plasma was sequentially adsorbed with anti-A-II and anti-A-I Sepharose CL-4B to remove LpA-I/A-II and LpA-I, respectively. Nonadsorbed proteins were removed by washing with 0.01 M Tris-HCl buffer (pH 7.4), containing 0.15 M NaCl, 1 mM EDTA, and 0.05% sodium azide. Lipoproteins bound to dextran sulfate cellulose and the immunosorbents were eluted with 3 M NaSCN in 0.02 M sodium phosphate, pH 7.0, and immediately filtered though a column packed with Sephadex G-25 to remove the thiocyanate. Nonadsorbed proteins and adsorbed lipoproteins were concentrated at 4°C by Micro-Confilt concentrator (Spectrum, Houston, TX) for further analysis.

TABLE 1. Lipid and apolipoprotein concentrations in normolipidemic and hyperlipidemic subjects

	Females		Males		Males vs. Females[a]	Normo- vs. Hyperlipidemic[a]
	Normo-lipidemic	Hyper-lipidemic	Normo-lipidemic	Hyper-lipidemic		
n	17	9	4	14		
Plasma						
Total cholesterol (g/l)	1.72 ± 0.06	2.84 ± 0.10***	1.86 ± 0.16	2.64 ± 0.05***	ns	0.05
Triglycerides (g/l)	0.62 ± 0.05	1.43 ± 0.08***	0.68 ± 0.11	1.25 ± 0.14**	ns	ns
LDL cholesterol (g/l)	1.03 ± 0.05	1.95 ± 0.1***	1.09 ± 0.14	1.86 ± 0.05*	ns	0.05
ApoB (g/l)	0.84 ± 0.03	2.41 ± 0.67**	nd	1.70 ± 0.05 ns	nd	nd
HDL cholesterol (g/l)	0.63 ± 0.03	0.52 ± 0.05*	0.50 ± 0.02	0.47 ± 0.02 ns	0.03	ns
ApoA-I (g/l)	1.45 ± 0.05	1.43 ± 0.08 ns	1.33 ± 0.06	1.27 ± 0.04 ns	0.05	ns
ApoA-II (g/l)	0.32 ± 0.01	0.34 ± 0.02 ns	0.31 ± 0.02	0.33 ± 0.01 ns	ns	ns

Comparison by *t* test between two groups: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, not significant; nd, not done.
[a]Overall comparison between males and females, normo- and hyperlipidemic, by two-way ANOVA.

CETP measurements

CETP mass was measured by a solid phase competitive RIA, using the monoclonal antibody TP2 (22). The recovery of CETP in apoA-I-defined subpopulations, isolated by immunoaffinity chromatography, was 75 ± 7%, lower than the 95% recovery for apoA-I (20, 21). To determine whether CETP recovery was reduced as a result of loss of immunoreactivity, we performed four time-course incubations of different plasma samples adjusted to 3 M NaSCN (**Fig. 1**). The samples were extensively dialyzed at each time point (including t = 0) and CETP concentration was measured by RIA. A significant decrease of the CETP immunoreactivity occurred after 1 h (−20% ± 6%, $P < 0.05$ vs. t=0), the time needed to desorb the particles from the anti-A-I and anti-A-II immunosorbent. After 4 h of incubation, 45 ± 6% of the immunoreactivity was lost ($P < 0.01$ vs. t=0) (Fig. 1). Thus we conclude that the fairly low recovery (75 ± 7%) was due to a general loss of immunoreactivity and was unlikely to be due to the failure to recover a particular CETP-containing fraction. CETP concentrations in apoA-I-defined particles were corrected for recovery due to slight differences of CETP recovery between groups; however, there was no significant difference of recovery in normolipidemic compared with hyperlipidemic subjects or in females compared with males (as determined by two-way ANOVA). A small amount of CETP (2.7 ± 0.5%) was found in the dextran-bound fraction and in the "lipoprotein-free," i.e., apoA-I unbound fraction (1.4 ± 0.4%). The concentration of CETP in the dextran-bound fraction was not correlated with the plasma concentration of apoB-containing lipoproteins ($r = 0.22$ vs. triglycerides, $r = 0.18$ vs. LDL cholesterol, $r = 0.06$ vs. apoB, n = 16).

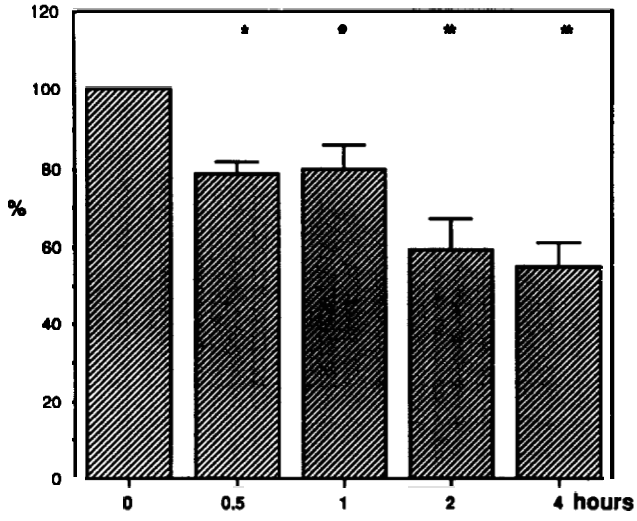

Fig. 1. Loss of CETP immunoreactivity during an incubation with 3 M NaSCN. Data are normalized for the initial concentration; each point is the mean of four different samples (except for time 2 h, n ½ 3) (see Methods). Significant differences versus time 0 are presented as $^{*}P < 0.05$ and $^{**}P < 0.01$ (paired t test).

CETP was detected in HDL subpopulations by gradient gel electrophoresis [native gradient PAGE 4–30% (Pharmacia)], electroblotted onto nitrocellulose (overnight, 4°C, 0.3 Amp limit) using a 25 mM Tris, 192 mM glycine, pH 8.3, buffer in a Trans-blot cell apparatus (Bio-Rad, Richmond, CA) and probed with the monoclonal antibody TP2 (directly radioiodinated, or detected by chemiluminescence using a secondary polyclonal antibody conjugated to horseradish peroxidase) as previously described (23, 24). Densitometric scanning of the gels and the autoradiograms was performed with a Molecular Dynamics laser densitometer.

CETP activity was determined by measuring the rate of transfer of radiolabeled CE from LDL to apoA-I-defined particles. LDL was labeled with [^{3}H]cholesteryl oleate according to Morton and Zilversmit (25), to a specific activity of 1500 cpm/μg CE. As the amounts of endogenous CETP in the apoA-I-defined particle pools were different, purified recombinant CETP was added to equalize the content of CETP in the isolated LpAs. The specific activity of purified recombinant CETP and CETP in fresh diluted plasma was determined by measuring the rate of labeled CE transfer from HDL to LDL and dividing the activity by the CETP mass measured by RIA. The specific activities of recombinant and plasma CETP were, respectively: 9.7 and 8.8 nmol/h • μg; these values are within the experimental error of each other. Incubations were performed at 37°C, for 0, 1, and 2 h; the LDL was precipitated by heparin-$MnCl_2$; and the radioactivity in the supernatant was measured (26). The counts transferred were less than 30% of the maximum transferable and plots of CE transferred versus time showed only negligible flattening of the curve. The slope for the first hour was used to calculate the rate of CE transfer for each concentration of LpAs.

Statistical analysis

Normality of the distributions was checked by a normality test. Differences were tested by paired or unpaired Student's t test, one-way or two-way ANOVA, when appropriate. Correlations were studied by linear regression analysis. All analyses were performed with the Statworks™ software; significance levels given are those for the two-tailed test. Data are presented as mean ± SEM.

RESULTS

Lipid and apolipoprotein concentrations in plasma lipoproteins and in LpA-I and LpA-I/A-II

Lipid and apolipoprotein concentrations are shown for plasma (Table 1) and for immunoaffinity-isolated HDL

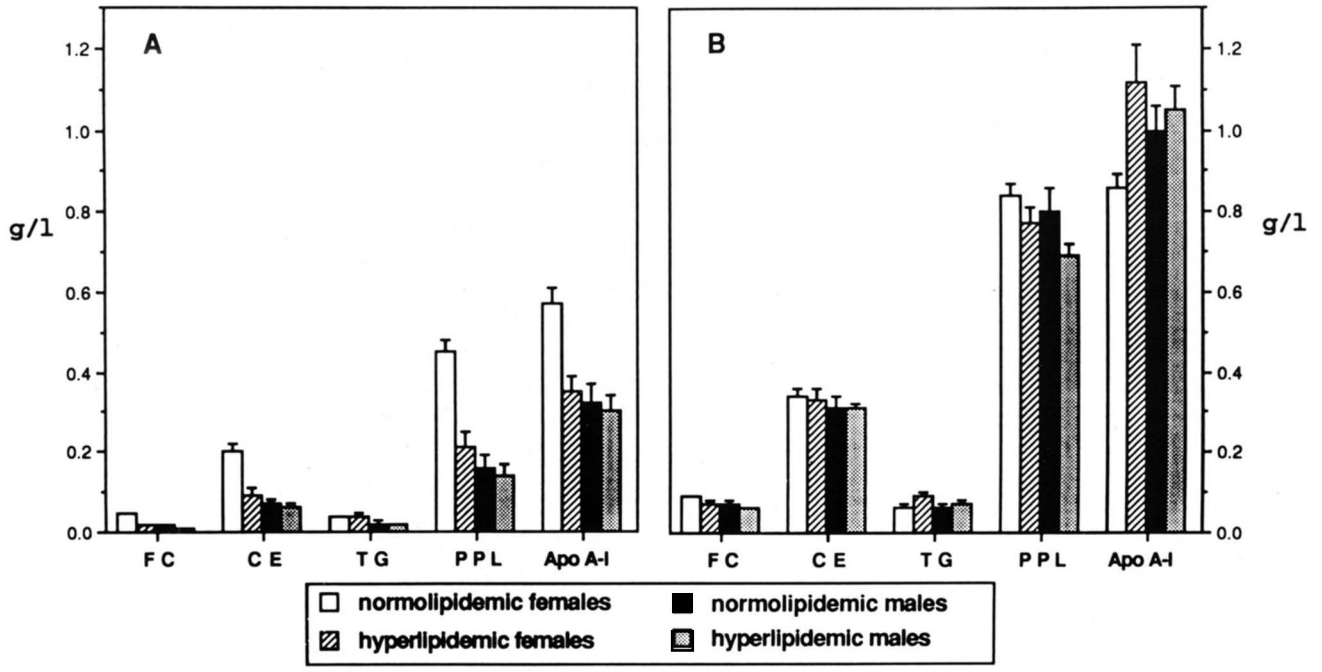

Fig. 2. Lipid and apoA-I concentrations in apoA-I-defined HDL subpopulations, shown for LpA-I (panel A) and LpA-I/A-II (panel B). FC, free cholesterol; CE, cholesteryl ester; TG, triglycerides; PL, phospholipids. In panel A, the differences are significant between normo- and hyperlipidemic subjects ($P < 0.01$) and between males and females ($P < 0.05$; overall comparison by two-way ANOVA); for normolipidemic females versus hyperlipidemic females, hyper- and normolipidemic males ($P < 0.01$; t test); for normo- or hyperlipidemic females versus normo- or hyperlipidemic males ($P < 0.05$; t test).

subpopulations (**Fig. 2**). Data are presented for both normolipidemic and hyperlipidemic males and females. HDL cholesterol concentration was reduced in males compared to females ($P < 0.03$) (Table 1). Hyperlipidemia resulted in significantly lower HDL cholesterol in females ($P < 0.05$), but not in males. The decrease in HDL cholesterol in males and in hyperlipidemic females was mainly due to a pronounced reduction of cholesteryl ester in LpA-I (Fig. 2A). Males and hyperlipidemic females also had decreases of phospholipid and apoA-I in LpA-I, compared to normolipidemic females (all $P < 0.001$). Lipid and apoA-I concentrations in LpA-I/A-II were similar in the different groups (Fig. 2B). The selective decrease of LpA-I in males and hyperlipidemic females was similar to previous reports (27, 28).

CETP distribution in LpA-I and LpA-I/A-II

Fig. 3 shows the plasma CETP concentration, as well as the proportion of CETP recovered in the immunoaffinity-isolated LpA subfractions and in the "dextran sulfate plus lipoprotein-free" fraction. Plasma CETP concentrations were similar in the four groups. Very little (< 4%) of the plasma CETP was recovered in the "dextran sulfate plus lipoprotein-free" fraction in any group. However, there were marked differences in the distribution of CETP between LpA-I and LpA-I/A-II. CETP concentration in LpA-I was significantly higher in females compared to males (1.56 ± 0.15 and 1.56 ± 0.17 mg/l for normo- and hyperlipidemic females, respectively, vs. 1.18 ± 0.24 and 0.85 ± 0.15 mg/l for normo- and hyperlipidemic males, respectively; $P = 0.004$ for gender effect by two-way ANOVA) (Fig. 3). Conversely, CETP concentration in LpA-I/A-II was lower in females compared to males

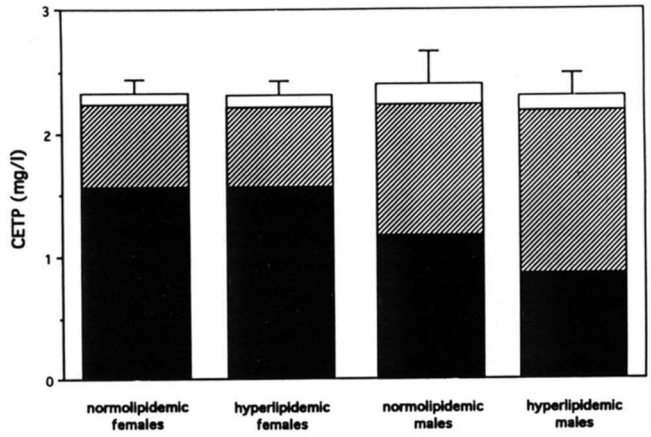

Fig. 3. CETP concentrations in plasma and apoA-I-defined lipoproteins. Concentrations of CETP are presented; the total height corresponds to total plasma CETP concentration. The subdivisions in each bar represent the relative distribution of the plasma pool of CETP in the LpA-I (■), LpA-I/A-II (□), and the "dextran sulfate plus apoA-I" unbound final fraction (Non LpA-bound, □). The differences in CETP concentration in LpA-I or LpA-I/A-II between males and females are significant ($P < 0.01$).

(0.69 ± 0.10 and 0.65 ± 0.15 mg/l for normo- and hyperlipidemic females vs. 1.06 ± 0.21 and 1.32 ± 0.24 mg/l for normo- and hyperlipidemic males, respectively, $P = 0.011$ for gender effect by two-way ANOVA) (Fig. 3). Analysis of the data by two-way ANOVA showed no significant effect of hyperlipidemia on the concentration of CETP in LpA-I and LpA-I/A-II ($P = 0.29$ and $P = 0.35$, respectively). Subset analysis according to type of hyperlipidemia also failed to reveal effects of hyperlipidemia. The percentage of CETP found in LpA-I was: female IIb, 75 ± 14%; female IIa, 87 ± 37%; male IIb, 40 ± 16%; male IIa, 39 ± 9%. The differences in concentration of CETP in LpA-I and LpA-I/A-II define marked differences in the distribution of CETP among apoA-I-defined particles. In normo- and hyperlipidemic females, the major fraction of CETP was found in LpA-I (66 ± 6%) and the minor fraction in LpA-I/A-II (30 ± 4%) ($P < 0.01$). By contrast, in normolipidemic males, the CETP distribution was similar in both types of particles, i.e., 48 ± 7% vs. 44 ± 6% (ns) and inverted in hyperlipidemic males 40 ± 8% in LpA-I vs. 55 ± 7% in LpA-I/A-II (ns).

There was a positive correlation between the relative amount of CETP in apoA-I-defined particles [(CETP in LpA-I/A-II)/(CETP in LpA-I)] and the relative distribution of cholesterol [(TC in LpA-I/A-II)/(TC in LpA-I)], phospholipid [(PL in LpA-I/A-II)/(PL in LpA-I)], and apoA-I [(apoA-I in LpA-I/A-II)/(apoA-I in LpA-I)] in these particles ($r = 0.45$, $P = 0.006$; $r = 0.47$; $P = 0.003$; $r = 0.38$, $P = 0.019$, respectively) indicating that the CETP distribution is determined by the mass ratio of the two classes of particles. The molar ratios, CETP/cholesterol, CETP/phospholipids, and CETP/apoA-I, were compared for each subclass of apoA-I-defined particles. The molar ratio of CETP to each of these constituents was increased 3- to 6-times in LpA-I compared to LpA-I/A-II, both in normo- or hyperlipidemic subjects and in males and females (not shown). The data are summarized in **Table 2** for all subjects. These results suggest that LpA-I has a much higher affinity for CETP than LpA-I/A-II.

In order to ascertain which compositional parameter was the major independent determinant of CETP distribution in apoA-I-defined particles, a multiple regression analysis was performed. All the parameters describing each subpopulation of particles, i.e., free and esterified cholesterol, triglycerides, phospholipids, and apoA-I concentrations in both LpA-I and LpA-I/A-II were introduced in the model. ApoA-I concentration in LpA-I was found to be the exclusive variable showing an independent significant correlation with either CETP concentration or CETP relative distribution in LpA-I. The correlation between apoA-I in LpA-I and percentage of plasma CETP in LpA-I is illustrated in **Fig. 4.**

To see whether CETP might influence the composition of HDL subclasses, CETP concentration in apoA-I-defined subpopulations was correlated with their compositional parameters. Based on earlier findings, an inverse correlation of CETP with lipid and apoA-I might be expected (13, 23). However, CETP concentration LpA-I was positively correlated with apoA-I; this relation was significant in each group of subjects and CETP in LpA-I was also significantly correlated with the lipid concentrations measured in LpA-I (not shown). CETP concentration in LpA-I/A-II was weakly related to the triglyceride concentration in those particles: $r = 0.31$, $P = 0.04$). No correlation was found between total plasma CETP and lipid or apoA-I concentrations in any subclass of apoA-I-defined particles (not shown).

We also studied correlations between CETP concentration in each subclass of particles and their size distribution as determined by native gradient gel electrophoresis. For LpA-I, significant negative correlations occurred between CETP concentration and the proportion of the larger subclass (11.2–17 nm), while the correlation was positive with the large intermediate subclass (9.2–11.2 nm) (**Table 3**). CETP was not correlated with the two smaller classes of LpA-I. In LpA-I/A-II, there was a negative correlation with the (9.2–11.2 nm) subclass and a positive correlation between CETP concentration and the distribution of the smaller particles (< 8.2 nm) (Table 3). These data were statistically significant only for the overall group.

TABLE 2. Molar ratio of CETP versus total cholesterol, phospholipid, and apolipoprotein A-I in apoA-I-defined HDL subpopulations

	CETP/TC			CETP/PL			CETP/ApoA-I		
	LpA-I	LpA-I/A-II		LpA-I	LpA-I/A-II		LpA-I	LpA-I/A-II	
				mmol/mol					
All subjects (n = 44)									
Mean	0.067	0.013	$P < 0.001$	0.058	0.013	$P < 0.001$	1.255	0.367	$P < 0.001$
SEM	0.008	0.002		0.006	0.002		0.091	0.043	

TC, total cholesterol; PL, phospholipids.

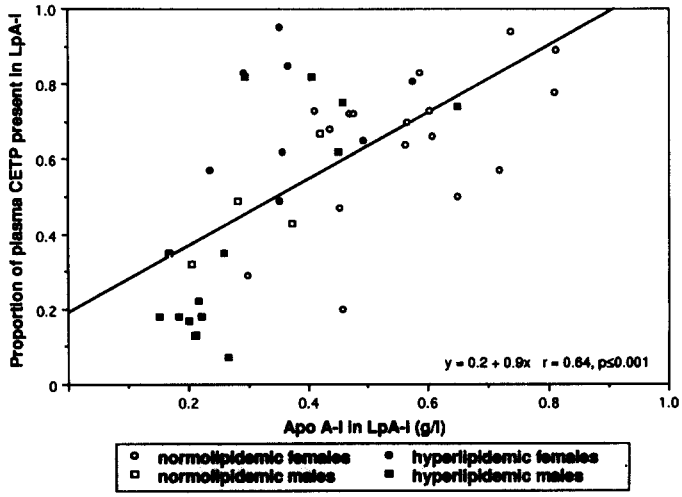

Fig. 4. Correlation between apoA-I concentration and the distribution of CETP within LpA-I. The proportion of CETP found in LpA-I is calculated as: concentration of CETP in LpA-I/concentration of plasma CETP.

Western blotting of CETP in isolated LpAs

To analyze the distribution of CETP within HDL subclasses, we performed nondenaturating gradient gel electrophoresis of apoA-I-defined particles detecting CETP by Western blot using the anti-CETP monoclonal antibody TP2. Densitometric scans of Coomassie-stained gels are shown in **Fig. 5A and C,** and scans of Western blots are shown in Fig. 5B and D. The size distribution of LpA-I and LpA-I/A-II was as previously reported (3, 20, 28) (Fig. 5A, 5C). The distribution of CETP was similar to that of total HDL protein, as determined by Coomassie staining. CETP was found to be broadly distributed within different sized particles of LpA-I or LpA-I/A-II, except for its absence in smaller LpA-I. Electrophoresis of plasma consistently resulted in isolation of CETP in a lower M_r region than obtained for immunoaffinity-isolated LpAs from the same sample (not shown).

LpA-I and LpA-I/A-II compared as CETP substrates

The ability of apoA-I-defined particles to serve as a substrate for CETP was assessed by measuring the transfer of CE from [^{3}H]CE-labeled LDL into apoA-I-defined particles. LpA-I and LpA-I/A-II have different amounts of CETP associated with them. Preliminary experiments showed that when labeled LDL and different apoA-I-defined particles with equal CE content were mixed, the CE transfer rate was proportional to the amount of endogenous CETP in the acceptor LpA particles. In order to better compare these particles as substrate for CETP, the differences in endogenous CETP content were equalized by the addition of exogenous pure recombinant CETP. It was verified that recombinant CETP and CETP in fresh plasma have identical specific activity (see Methods). Purified recombinant CETP readily associates with HDL at physiological concentrations (C. Bruce and

TABLE 3. Correlations between CETP concentration and the size distribution of apoA-I-defined HDL subpopulations

	Normolipidemic n = 18		Hyperlipidemic n = 23		All Combined n = 41	
	r	*P*	*r*	*P*	*r*	*P*
LpA-I						
CETP vs.						
% 17.0–11.2 nm	−0.54	0.02	−0.30	0.17	−0.48	0.00
% 11.2–9.2 nm	0.35	0.16	0.16	0.46	0.39	0.01
% 9.2–8.2 nm	0.05	0.83	0.03	0.88	0.00	0.98
% 8.2–7.0 nm	0.45	0.19	0.08	0.73	0.18	0.27
LpA-I/A-II						
CETP vs.						
% 17.0–11.2 nm	0.56	0.02	0.03	0.90	0.09	0.55
% 11.2–9.2 nm	−0.18	0.48	−0.30	0.16	−0.38	0.02
% 9.2–8.2 nm	0.15	0.53	0.01	0.94	0.04	0.79
% 8.2–7.0 nm	0.22	0.37	0.39	0.07	0.42	0.01

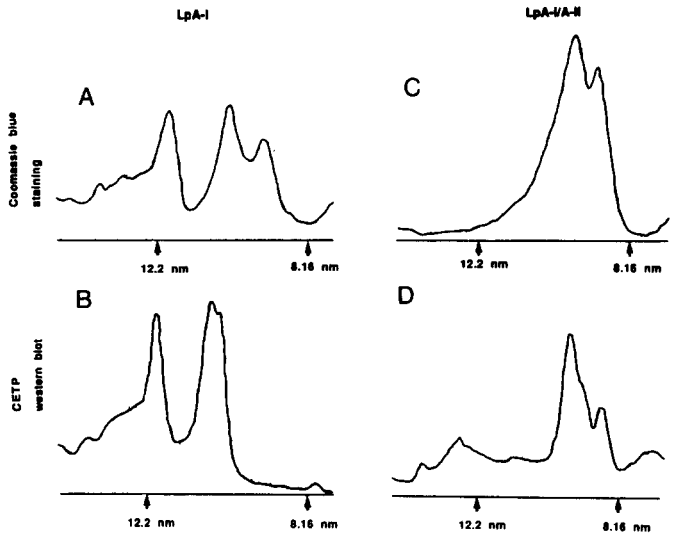

Fig. 5. Association of CETP with apoA-I-defined HDL subpopulations Panels A and C show a representative densitometric scan of Coomassie-stained lipoproteins isolated by immunoaffinity chromatography (A: LpA-I, C: LpA-I/A-II). Panels B and D show a representative densitometric scan of the autoradiogram corresponding to the Western blot of CETP for the same samples (B: LpA-I, D: LpA-I/A-II).

A. Tall, unpublished data). Under conditions of equal CETP and acceptor CE levels, the CE transfer rates from LDL to both subpopulation of apoA-I-defined particles were found to be similar at three different concentrations of acceptor particles (**Fig. 6**).

DISCUSSION

There were two important unanticipated findings in this study. First, the distribution of CETP between LpA-I and LpA-I/A-II was found to be different in males and females, with LpA-I being the major particle carrying CETP in females, while LpA-I/A-II played a much more prominent role in males. These differences appear to be driven by the higher concentrations of LpA-I in females. Second, both LpA-I and LpA-I/A-II were found to be equivalent substrates for CETP in vitro.

One of the goals of this study was to see whether CETP might influence LpA-I composition or particle size. However, the small variation of plasma CETP concentration observed in these subjects did not have a discernible effect on the concentration of the different components of apoA-I-defined particles. These data are consistent with earlier cross-sectional studies where CETP variation was not or was very weakly correlated with changes in HDL mass measurements (22, 29). Our study does not preclude the occurrence of subtle effects of CETP on the lipid or protein content of HDL subspecies of discrete size. In fact, there was an inverse correlation between CETP concentration and the percent of LpA-I found in the largest sized particles and a positive correlation with mid-size particle distribution (Table 3). These results suggesting an interconversion of larger HDL subclasses, as a result of CETP activity in vivo, are consistent with the observed accumulation of larger sized apoA-I-enriched particles in genetic CETP deficiency or in animals with impairment of CE transfer (13, 30). Although the correlations were not very strong (Table 3), the findings are of interest, as

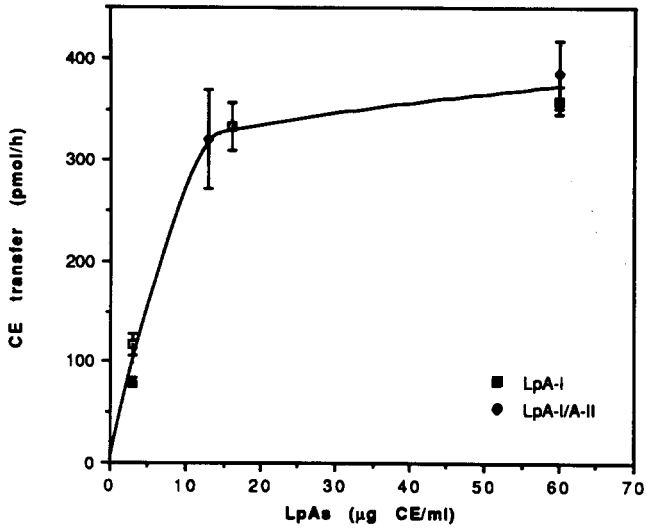

Fig. 6. Rate of CE transfer from radiolabeled LDL into different concentrations of apoA-I-defined particles. Varying amounts of LpA-I and LpA-I/A-II were incubated with [^{3}H]CE-labeled LDL (46 µg CE/ml), with exogenous CETP added to adjust the total CETP level to 70 ng in a final volume of 100 µl. The rate of incorporation of the radioactivity into apoA-I-defined particles was measured from the linear part of a CE transfer versus time plot (see Methods) and is plotted against the concentration of the acceptor particles (i.e., LpA-I or LpA-I/A-II).

subjects with smaller sized LpAs may be more susceptible to coronary artery disease (3).

In females, there was a preferential association of CETP with LpA-I (Fig. 3). These data are consistent with an earlier report where the majority of CETP activity was found to be associated with LpA-I in a small group of normolipidemic subjects (16). In contrast, hyperlipidemic males showed a preponderant distribution of CETP in LpA-I/A-II. The molar ratio of CETP to other constituents was markedly higher in LpA-I than LpA-I/A-II (Table 2), suggesting a higher affinity of LpA-I for CETP. As LpA-I concentration is more variable than LpA-I/A-II concentration (3, 20, 27), intra-plasmatic distribution of CETP is largely determined by LpA-I concentration. Males, particularly when they are hyperlipidemic, have lower LpA-I concentrations and consequently a greater proportion of their CETP is found in LpA-I/A-II. Although we did not find an overall significant effect of hyperlipidemia on CETP distribution, the differences in LpA-I concentration between hyperlipidemic and normolipidemic subjects were less than between males and females, so that an effect of hyperlipidemia might have been more difficult to detect. Our study did not include severely hypertriglyceridemic subjects who might be expected to show larger reductions in LpA-I.

Multiple regression analysis revealed that it was exclusively the apoA-I content of LpA-I that was independently correlated with the percentage of plasma CETP in LpA-I. As CETP is found in a broad size range and as there is a wide overlap in size and lipid composition of apoA-I-defined particles (Fig. 5), it is unlikely that the higher affinity of LpA-I is due to an overall effect of particle size or lipid composition. The high affinity could be due to a direct interaction of apoA-I with CETP that is inhibited by apoA-II or to some other subtle compositional or physical difference between particles dictated by the presence of apoA-I (31).

Despite the higher affinity of CETP for LpA-I, both types of apoA-I-defined particles appear to act in a similar fashion when compared as substrates for CETP (Fig. 6). The in vitro assays were set up under conditions where HDL, LDL, and CETP were close to physiological concentrations. These studies do not exclude the possibility of slight differences in the properties of the particles. However, our findings obtained with native particles are consistent with the results of Sparks and Pritchard (32), who found no difference between recombinant LpA-I and LpA-I/A-II when compared as CETP substrates over a range of concentrations. The apparent high affinity of CETP for LpA-I, despite the similar abilities of the two kinds of the LpAs to serve as substrates for CETP, suggests that the simple association/dissociation of CETP with LpAs is not a rate-limiting step in the transfer of CE. It has been reported by Morton (33) that there is rapid binding and equilibration between free and HDL-bound CETP. A step in the transfer mechanism that is different from the binding of CETP to HDL, such as the transfer of a CE molecule from the donor lipoprotein to CETP or from a CETP-CE complex to an acceptor molecule, must be a slower process that is equal in rate for the two LpAs we examined.

Recently it has been shown that transgenic mice expressing both human CETP and human apoA-I genes show much larger reductions in HDL cholesterol than animals expressing the human CETP gene alone, indicating that particles containing human apoA-I are better substrates than mouse HDL in vivo (23). The expression of human apoA-I in mice results in human-like speciation of HDL into distinct larger and smaller subclasses, unlike the largely monodisperse mouse HDL (34). The present finding that both types of apoA-I-defined particles act comparably as CETP substrates suggests that the enhanced transfer efficiency found in apoA-I/CETP transgenic mice reflects a species-specific property of human apoA-I, rather than an imbalance in the production of apoA-I (and consequently LpA-I) relative to apoA-II. However, this hypothesis will require further testing in vivo, for example, by carrying out experiments with mice transgenic for human apoA-I and/or apoA-II.

In isolated LpA-I and LpA-I/A-II, CETP was found broadly distributed in particles with a wide spectrum of sizes. These data provide little support to the notion that CETP activity is confined to a highly specific HDL subclass as determined by HDL composition or size. However, owing to the preferential association of CETP with LpA-I, CETP activity will be predominantly found in LpA-I in females whereas it will be shared between the different apoA-I-defined particles in normolipidemic males or predominantly found in LpA-I/A-II in hyperlipidemic males. The consequences of this finding in terms of its potential effect on atherogenesis or the efficiency of reverse cholesterol transport remain to be determined. However, there are two potential ways that the preferential association of CETP with LpA-I could influence the reverse cholesterol transport pathway. First, CETP activity coupled to LCAT activity within LpA-I could result in more efficient cellular free cholesterol efflux into LpA-I in females (16, 35). Although controversial (3, 10–12) LpA-I/A-II could be less effective than LpA-I in promoting cellular cholesterol efflux, so that the overall effect of CETP on cellular cholesterol efflux might be less in males than females. A second effect of the differential distribution of CETP could be related to the different fates of apoA-I-defined particles subsequent to lipid exchange. The triglyceride-enriched LpAs are thought to be acted on by hepatic lipase, a step that could be important in HDL cholesterol and cholesteryl ester uptake by hepatocytes (1). However, apoA-II appears to be a potent inhibitor of hepatic lipase (36), so that LpA-I/A-II might be resistant to remodeling by hepatic lipase. By either of these two

mechanisms, the greater proportion of the plasma pool of CETP within LpA-I/A-II in male hyperlipidemic subjects could be a factor contributing to a general impaired reverse cholesterol transport caused by low levels of LpA-I.

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