Transfer of cholesteryl ester into high density lipoprotein by cholesteryl ester transfer protein: effect of HDL lipid and apoprotein content

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Abstract Recombinant high density lipoprotein (rHDL) particles were prepared by cosonication of purified lipids and human apoproteins and incubated with partly purified cholesteryl ester transfer protein (CETP) and low density lipoprotein (LDL) containing [^{3}H]cholesteryl ester. Increasing the triglyceride content relative to cholesteryl ester in rHDL significantly decreased the ability of the particles to accept cholesteryl esters transferred by CETP. Kinetic analysis of the data was performed to numerically define the maximum velocity of lipid transfer, T_{max}, and the HDL concentration required for half maximal velocity, K_H. Increases in rHDL-triglyceride content were shown to result in a significant reduction in the T_{max} without a major change in K_H. When the free cholesterol content was increased relative to phospholipid, the ability of the particles to accept cholesteryl esters was also decreased in a similar manner. Conversely, rHDL prepared from purified apoprotein A-I, A-II, or mixtures of both, had significantly elevated T_{max} and K_H values for their interaction with CETP. The results suggest that increases in triglyceride or free cholesterol content of an rHDL particle decrease the catalytic ability of CETP by noncompetitive inhibition. In addition, some component(s) of HDL apoproteins, other than A-I or A-II, were shown to uncompetitively inhibit the activity of CETP, by modifying both T_{max} and the K_H for the reaction. This study has shown that altered HDL composition may have marked effects on the transfer and equilibration of cholesteryl esters within the HDL pool. —**Sparks, D. L., and P. H. Pritchard.** Transfer of cholesteryl ester into high density lipoprotein by cholesteryl ester transfer protein: effect of HDL lipid and apoprotein content. *J. Lipid Res.* 1989. **30:** 1491–1498.

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Central to the proposed mechanism for reverse cholesterol transport is a cholesteryl ester transfer protein (CETP) that promotes the transfer/exchange of cholesteryl ester and triglycerides between different lipoprotein classes (1). This protein has been purified to homogeneity and the cDNA sequence has been determined (2,3). In his 1986 review, Tall (1) suggests that CETP may promote atherogenesis by promoting increased transfer of cholesteryl esters to apoB-containing particles in patients with low HDL-cholesterol. While studies by Fielding et al. (4) seem to contradict this suggestion, recent observations in our laboratory support Tall's theory (5). Specifically, we have shown that in several hyperlipidemic states, where the risk of vascular disease is high, increases in the triglyceride/cholesteryl ester ratios in HDL were associated with a reduction in cholesteryl ester transferred to the HDL pool and comcomitant increased transfer to LDL and VLDL (5). This indicates that that HDL composition may directly affect cholesteryl ester transport in patients with disorders of lipid metabolism and we have proposed that this may be due to impaired interaction of CETP with HDL particles. These studies, as well as observations by Morton (6), suggest that HDL may be a preferred substrate of CETP and lead us to propose that a major function of CETP may be to equilibrate cholesteryl esters within the HDL subclasses prior to transfer to VLDL/LDL (5). However, even though several investigators have attempted to elucidate the function of CETP in patients with disorders in lipid metabolism (1,4,5), the factors that regulate the interaction of this protein with the HDL particle in normal and abnormal conditions are still unclear.

While the association between reduced HDL-cholesterol levels, hyperlipidemia, and atherosclerosis is now widely accepted (7), our studies have suggested that this reduction in HDL cholesterol may be associated with a change in HDL lipid composition as well as with a decrease in HDL mass (5). Since severe reductions in HDL mass are not always associated with increased atherosclerotic risk (8), elucidation of the mechanisms resulting in

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; CETP, cholesteryl ester transfer protein; LCAT, lecithin:cholesterol acyltransferase; CE, cholesteryl ester; VHDL, very high density lipoprotein.

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the anti-atherogenic potential of HDL must involve a detailed understanding of the significance of changes in both HDL mass and composition. Studies of patients with different disorders of lipid metabolism have shown that while elevated plasma lipid levels have a marked effect on HDL composition, this may not be the only factor capable of affecting HDL metabolism (7). For example, the major role apoproteins play in maintaining HDL structure and function is now well established (9). In addition, recent reports have identified a protein that inhibits CETP and is associated with a small unique subclass of HDL that contains only a small amount of apoA-I and a trace of apoA-II (10). Other work from the same laboratory has shown that both LCAT and CETP activities are primarily associated with a subfraction of HDL that is devoid of apoA-II (11). These observations suggest that the apoA-II and CETP-inhibitor content of an HDL particle may have an important regulatory role in HDL lipid metabolism.

In this study, we have attempted to define the role of specific apoprotein and lipid components of HDL in regulating the ability of HDL to accept cholesteryl esters transferred by CETP in vitro. We have used recombinant HDL in these experiments since several reports have demonstrated that HDL, reconstituted by sonication, is identical to native HDL with respect to density and size (12, 13), their ability to interact with LCAT and CETP (Sparks, D. L., Y. Parmar, and P. H. Pritchard, unpublished results), and their ability to permit selective uptake of their cholesteryl ester by cultured cells (13).

MATERIALS AND METHODS

[^{3}H]Cholesteryl oleate (sp act 72 Ci/mmol) was obtained from NEN Research Products, Quebec, Canada. Bovine serum albumin, cholesterol, cholesteryl linoleate, and triolein were purchased from Sigma Chemical Co., St. Louis, MO. Egg phosphatidylcholine was purchased from Avanti Polar Lipids, Birmingham, AL. Total and free cholesterol determination reagent kits were purchased from Boehringer, Mannheim, F. R. G. All other chemicals were analytical grade from BDH Chemicals Canada Ltd., Vancouver, B. C.

Isolation of lipoproteins

Blood was collected from 16-h fasted, normolipidemic subjects into EDTA-containing tubes. Plasma was removed by centrifugation at 1750 *g* for 10 min. VLDL, LDL, and HDL were isolated by sequential ultracentrifugation at densities 1.006, 1.006–1.063, and 1.063–1.21 g/mL (14). HDL_2, HDL_3, and VHDL were isolated at densities 1.063–1.125, 1.125–1.21, and 1.21–1.25 g/mL, respectively. The washed lipoproteins were dialyzed four times for 4–12 h against 100-fold greater volumes of NaCl/Tris buffer (buffer A: 150 mM NaCl, 10 mM Tris/HCl, 0.3 mM EDTA, and 4.6 mM NaN_3, pH 7.4) and their purity was assessed by electrophoresis on 1% agarose. Free and total cholesterol, and triglycerides were determined enzymatically using Boehringer Mannheim kits and manufacturers' suggested procedures. Phospholipids were determined by the method of Bartlett (15) and proteins were determined by the Lowry method as modified by Peterson (16).

Assay of CETP activity

LDL was labeled with [^{3}H]cholesteryl ester by incubation of sonicated [^{3}H]cholesteryl ester/phospholipid vesicles with plasma for 24 h at 37°C (17). The radiolabeled LDL was isolated by ultracentrifugation between the densities 1.019 and 1.063 g/mL. Cholesteryl ester transfer activity was measured in the following manner: purified HDL or rHDL (0–300 μg protein/mL) was incubated with [^{3}H]LDL (43 μg total cholesterol/mL), partly pure CETP (1.9 μg/mL), bovine serum albumin (1 mg/mL), and buffer A (to a total of 700 μL) for 1.5 h at 37°C. Total [^{3}H]cholesteryl ester transferred to HDL was determined by precipitation of [^{3}H]LDL with heparin-$MnCl_2$ (90 mM $MnCl_2$, 10 mM NaCl, and 227 USP units/L of heparin) and determination of radioactivity in the supernatant. Incubations lacking CETP were used as control incubations and were subtracted from that measured for the test incubations. Incubations carried out in the absence of bovine serum albumin indicated no significant effect on the transfer; however, albumin was included to decrease the variability in the precipitation of LDL.

Partial purification of CETP

Lipoprotein-depleted plasma was prepared by addition of solid NaBr to plasma to a density of 1.21 g/mL and then ultracentrifugation and tube slicing to remove the $d < 1.21$ g/mL lipoproteins. The upper 50% of the $d > 1.21$ g/mL infranate (albumin poor), was chromatographed on phenyl-Sepharose CL-4B and CM-52 cellulose (18). Cholesteryl ester transfer activity was eluted from the phenyl-Sepharose with dH_2O and from the CM cellulose column by a linear 0–400 mmol NaCl gradient. CETP was purified approximately 2000-fold and contained no detectable LCAT activity.

Preparation of recombinant HDL

HDL was delipidated in ethanol-diethyl ether (19) and the precipitated apoproteins were washed three times with diethyl ether, dried under N_2, and resolubilized in 6 M guanidine HCl. The apoHDL was dialyzed against buffer A, and stored at −70°C. Purified apoproteins A-I and A-II were prepared by chromatofocusing as previously described (20).

The rHDL complexes were prepared by co-sonication (12) of commercially available lipids and human apoproteins (initial amounts shown in Table 1). The purified lipids were dissolved in chloroform and the appropriate amounts were dried together under N_2. The desired amount of apoHDL (or pur-

ified apoprotein) was added in buffer A containing 0.8 M NaBr, to a final volume of 3 ml. The mixture was suspended in a water bath (14°C) and sonicated 8 × 30 sec under a stream of N_2 at 75 watts with 30-sec cooling periods between sonications (Branson sonifer with microtip probe). The rHDL was immediately reisolated by sequential ultracentrifugation between densities 1.063 and 1.21 g/mL, dialyzed extensively against buffer A, and stored at 4°C.

Electron microscopy

Particle size was determined by negative staining lipoprotein electron microscopy in a manner similar to Forte and Nordhausen (21). Native or rHDL were isolated and immediately dialyzed against a buffer consisting of 0.125 M ammonium acetate, 2.6 M ammonium carbonate, and 0.26 mM tetrasodium EDTA at pH 7.4. Each sample was adjusted to a concentration of 0.5 mg protein/mL and, immediately prior to examination, the sample was mixed with an equal volume of 2% sodium phosphotungstate (pH 7.4). A small droplet (3 μL) was then applied to a Formvar- and carbon-coated grid (200–300 mesh) and after 30 sec, excess fluid was removed by blotting with a wedge of filter paper. Grids were immediately examined and micrographs were photographed at an instrument magnification of 77,000. Mean particle dimensions of 100 particles were determined from projection of each negative.

rHDL as acceptors of cholesteryl ester

rHDL were prepared with varied lipid and apoprotein compositions and compared to native HDL, HDL_2, HDL_3, and VHDL for their ability to accept cholesteryl esters transferred by partly purified CETP. Three different series of rHDL were prepared which differed in: A) core lipid (cholesteryl ester : triglyceride), B) surface lipid (phospholipid : cholesterol), and C) apoprotein (apoA-I and apoA-II). The initial composition of each preparation is indicated in **Table 1**. Initial characterization studies indicated that less than 5% of these rHDL were precipitated by heparin/$MnCl_2$. This value was similar to that observed for native HDL.

TABLE 1. Initial lipid and apoprotein composition of reconstituted HDL

Series	Batch	Composition FC	CE	TG	PL	Protein	Apoproteins Present
		% by weight					
A	I	4.6	17.4	0.0	30.0	48.0	ApoHDL
	II	4.6	12.8	4.6	30.0	48.0	ApoHDL
	III	4.6	4.6	12.8	30.0	48.0	ApoHDL
	IV	4.6	0.0	17.4	30.0	48.0	ApoHDL
B	I	6.5	12.8	4.7	28.0	48.0	ApoHDL
	II	8.7	12.8	4.7	25.8	48.0	ApoHDL
	III	11.5	12.8	4.7	23.0	48.0	ApoHDL
	IV	17.3	12.8	4.7	17.2	48.0	ApoHDL
C	I	4.6	12.8	4.6	30.0	48.0	A-I
	II	4.6	12.8	4.6	30.0	48.0	A-II
	III	4.6	12.8	4.6	30.0	48.0	A-I/A-II[a]
	IV	4.6	12.8	4.6	30.0	48.0	A-I/A-II[b]

[a]Molar ratio = 3:1.
[b]Molar ratio = 1:1.

RESULTS

Characterization of native and rHDL

Total cholesterol, free cholesterol, triglyceride, phospholipid, and protein content were determined for all reconstituted lipoproteins (**Table 2**) and for native HDL, HDL_2, HDL_3, and VHDL (**Table 3**). Final lipid compositions were similar to the initial concentrations. Thus, differences in neutral and polar lipid ratios (series A and B) were maintained in the final complexes. Protein and lipid recoveries ranged from 50 to 95% depending upon the apoproteins/lipid mixture used. The presence of individual apoproteins after reisolation was confirmed by both isoelectric focusing and SDS-PAGE; however, the final apoprotein molar ratios in complexes containing A-I and A-II were not determined. Particles prepared with purified apoproteins (series C) had lipid compositions similar to rHDL prepared from apoHDL except that rHDL prepared from pure apoA-I had a reduced cholesteryl ester content compared to those prepared from apoHDL (series A, batch II). In addition, as the apoA-II content was increased in rHDL, the relative recovery of cholesteryl ester in the rHDL also increased (series C).

The mean particle size of rHDL determined by electron microscopy is shown in Table 2. rHDL prepared from apoHDL, with a lipid composition comparable to native HDL, were spherical with mean particle sizes ranging between 8.7 and 9.4 nm (batch A-II and B-II). A similar size (9.0 ± 1.0 nm) and appearance were observed for native HDL. Particles prepared with variations in neutral lipid showed significant alterations in particle size. Those with a purely triglyceride or cholesteryl ester core were significantly ($P < 0.001$) smaller than rHDL with both neutral lipids or native HDL (series A). Increases in the free cholesterol content of rHDL, relative to phospholipid, resulted in an increased mean particle size (series B). However, rHDL prepared from mixtures of apoA-I and A-II showed no marked differences in particle size, relative to native HDL (series C).

Lipid transfer to native HDL subfractions

CETP-mediated lipid transfer from LDL to the total HDL fraction was a saturable process (**Fig. 1**). Reaction kinetics were estimated from double reciprocal plots and

TABLE 2. Lipid and apoprotein content of reconstituted HDL after sonication and isolation

Series	Batch	Composition FC	CE	TG	PL	Protein	Apoproteins Present	Particle Size[a]
		% by weight						*nm*
A	I	4.1	20.2	0.0	34.7	41.0	ApoHDL	8.30 ± 0.79
	II	3.2	18.2	6.2	29.7	42.6	ApoHDL	9.48 ± 1.11
	III	3.3	11.7	15.5	29.1	40.5	ApoHDL	10.32 ± 1.55
	IV	6.0	0.0	17.7	33.0	40.5	ApoHDL	7.44 ± 1.04
B	I	4.5	19.0	6.2	28.3	41.9	ApoHDL	8.66 ± 1.24
	II	6.0	20.9	5.7	22.2	45.3	ApoHDL	8.69 ± 1.77
	III	8.5	20.4	6.5	20.3	44.3	ApoHDL	9.18 ± 2.07
	IV	12.3	18.9	13.2	15.0	40.5	ApoHDL	10.03 ± 3.07
C	I	3.8	12.5	6.7	32.7	44.3	A-I	8.69 ± 1.94
	II	1.5	20.9	8.0	27.5	42.2	A-II	8.27 ± 1.58
	III	4.3	16.1	5.1	28.0	46.6	A-I/A-II	9.06 ± 1.55
	IV	1.6	21.6	5.4	30.8	40.5	A-I/A-II	8.44 ± 1.77

[a]Determined by negative staining electron microscopy. Mean ± 1 SD particle dimensions of 100 particles were determined from projection of each micrograph.

confirmed on direct linear plots (22). Kinetic analysis was used in an attempt to numerically characterize variations in the ability of different HDL to accept cholesteryl ester from LDL. In this context, we have determined values (analogous to V_{max} and K_m) that represent the maximum velocity of transfer, T_{max}, and the HDL concentration required for half maximal velocity, K_H. Thus, these terms are merely operational terms since CETP is not an enzyme, nor can HDL per se be considered an enzyme substrate. However, the term T_{max}, as used in this study, enables us to rank the ability of different HDL to accept cholesteryl ester. Similarly, the term K_H is used merely to rank the ability of different HDL to accept cholesteryl ester at low HDL concentrations. Determination of these reaction kinetics for the total HDL fraction indicated an apparent K_H of 17.0 μg HDL protein/mL and T_{max} of 10.0%/ml per h (Table 3). When HDL_3 was used as an acceptor of cholesteryl ester, a K_H similar to total HDL was observed but the T_{max} was shown to be increased (Fig 1; Table 3). In addition, at high substrate concentrations of HDL_3, there was an apparent substrate inhibition of the reaction (Fig. 1). This effect was not observed with HDL_2 or VHDL; however, both substrates demonstrated a slightly increased T_{max} and a marked increase in apparent K_H relative to total HDL (Table 3).

TABLE 3. Native HDL composition and kinetic parameters as a substrate for CETP

	Composition FC	CE	TG	PL	Protein	K_H[a]	T_{max}[a]
	% by weight					*μg/mL*	*%/mL/h*
HDL	3.3	27.5	4.3	28.8	36.1	17.0	10.0
HDL_2	3.0	23.9	3.6	30.7	38.8	60.7	11.4
HDL_3	3.2	21.2	2.9	26.7	46.1	19.9	14.0
VHDL	1.1	11.9	2.3	22.7	61.9	68.3	12.1

[a]Estimated from double reciprocal plots of data from each experiment.

Effect of rHDL lipid composition on lipid transfer to rHDL

rHDL with a cholesteryl ester-rich core and apoprotein composition comparable to native HDL (Table 2; batch B-I) had similar reaction kinetics with CETP to native particles (Table 3 and **Table 4**). In particles with all HDL

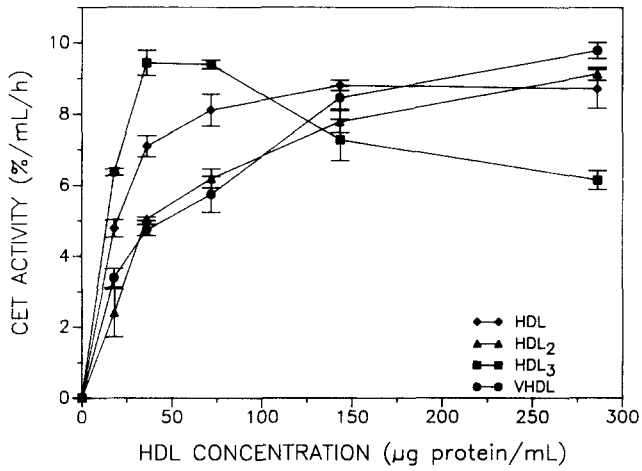

Fig. 1. Transfer of cholesteryl ester to HDL subfractions; the transfer of [^{3}H]cholesteryl ester from labeled LDL to the HDL subfractions described in Table 3. Mixtures containing partially purified CETP (1.3 μg), LDL (30 μg total cholesterol), BSA (1 mg), and the indicated amounts of HDL were incubated at 37°C for 1.5 h and the lipid transfer was determined as described in the text. Control incubations that lacked a source of cholesteryl ester transfer activity were subtracted as blanks. Values are the mean ± SD of quadruplicate determinations.

TABLE 4. Effect of apoprotein and lipid content on the kinetic parameters of rHDL[a] as a substrate for CETP

Series	Batch	Apoproteins Present	CE:TG (moles:moles)	FC:PL (moles:moles)	K_H[b]	T_{max}[b]
					μg/mL	*%/mL/h*
A	I	apoHDL	1:0	1:4.2	19.6	10.7
	II	apoHDL	4:1	1:4.6	16.1	8.9
	III	apoHDL	1:1	1:4.4	19.0	7.4
	IV	apoHDL	0:1	1:2.7	16.2	5.4
B	I	apoHDL	4.2:1	1:4.6	16.1	9.8
	II	apoHDL	5.1:1	1:1.9	19.7	8.1
	III	apoHDL	4.3:1	1:1.2	21.4	6.3
	IV	apoHDL	2.0:1	1:0.6	19.0	4.3
C	I	A-I	2.5:1	1:4.3	57.9	29.0
	II	A-II	3.6:1	1:9.0	35.7	26.4
	III	A-I/A-II	4.4:1	1:3.2	107.1	45.1
	IV	A-I/A-II	5.5:1	1:9.3	164.3	64.3

[a]rHDL prepared as described in text, composition and size described in Table 2.
[b]Estimated from double reciprocal plots of data from each experiment.

apoproteins, increases in the triglyceride content relative to cholesteryl ester resulted in significant decreases in the ability of these particles to accept cholesteryl ester (**Fig. 2A**) as indicated by a reduction in the reaction T_{max} (Table 4). This was also observed when the free cholesterol content was increased relative to phospholipid (Fig. 2B and Table 4). Regressional analysis identified a significant inverse relationship between T_{max} and rHDL triglyceride or free cholesterol content (**Fig. 3**). Regardless of changes in lipid composition, all particles prepared with apoHDL had a similar apparent K_H for the reaction (Table 4), which was similar to that for native HDL (Table 3).

Effect of apoprotein composition on lipid transfer to rHDL

rHDL prepared with a single apoprotein (apoA-I), had a higher apparent K_H and T_{max} for the reaction with CETP than did particles prepared from apoHDL (Table 4). Since the ratio of T_{max}/K_H was similar (0.5) for both apoHDL and apoA-I rHDL, it seems possible that some other component of apoHDL was acting as an uncompetitive inhibitor. Interestingly, at high substrate concentrations (greater than 100 μg protein/mL), there was an apparent substrate inhibition in the interaction between apoA-I rHDL and CETP, which was similar to that observed for HDL_3 (Fig. 3). Particles prepared with only apoA-II also demonstrated this substrate inhibition (**Fig. 4**) and a T_{max} comparable to A-I-containing rHDL but a reduced K_H (Table 4). rHDL prepared from a combination of apoproteins A-I and A-II, with an initial molar ratio of 3:1, were shown to have elevated T_{max} and K_H (Table 4), but reduced substrate inhibition (Fig. 4), relative to A-I rHDL. An increased A-II content in the recombinant particles was shown to further increase the apparent K_H and T_{max} for the interaction with CETP (Table 4).

DISCUSSION

The central role that CETP plays in the equilibration and transport of cholesteryl esters has resulted in a great deal of speculation on the role CETP in the process of atherogenesis (23). It was originally proposed that the transfer of cholesteryl ester from HDL to more rapidly turning over pools of lipoproteins was anti-atherogenic since this promoted the transfer of cholesterol from peripheral tissues to the liver (24). However, the capacity of CETP to transfer CE into

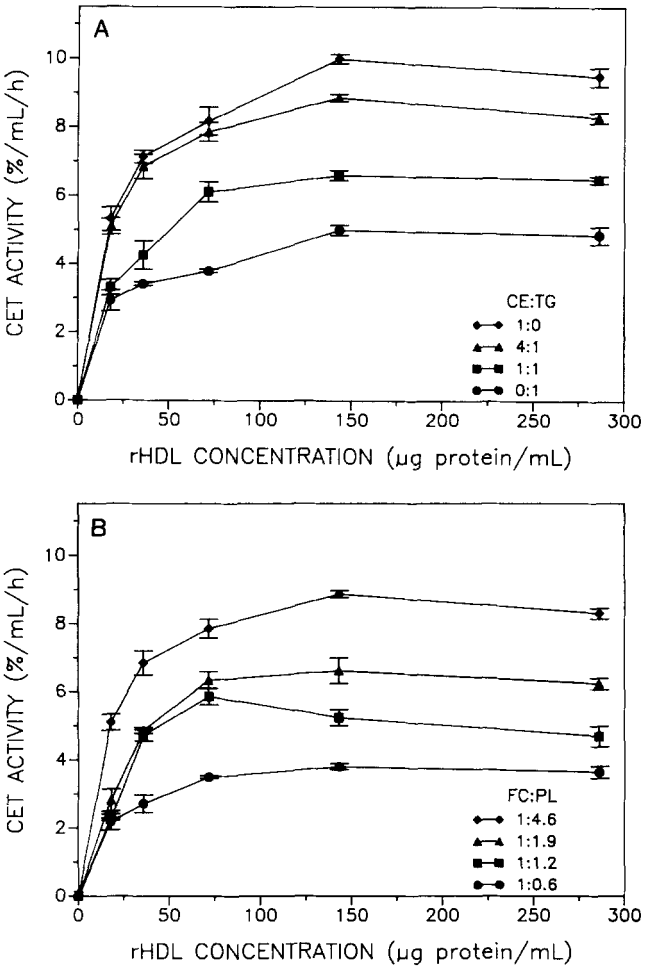

Fig. 2. Effect of lipid composition on cholesteryl ester transfer to rHDL; the transfer of [^{3}H]cholesteryl ester from LDL to recombinant HDL. rHDL were prepared, as described in the text, from a full compliment of HDL apoproteins and purified lipids, with the indicated ratios of cholesteryl ester-triglyceride (A) and unesterified cholesterol-phospholipid (B) molar contents. The lipid compositions of the rHDL used are shown in Table 2, series A and B. Incubations were as described for Fig. 1 with values being the mean ± SD of quadruplicate determinations.

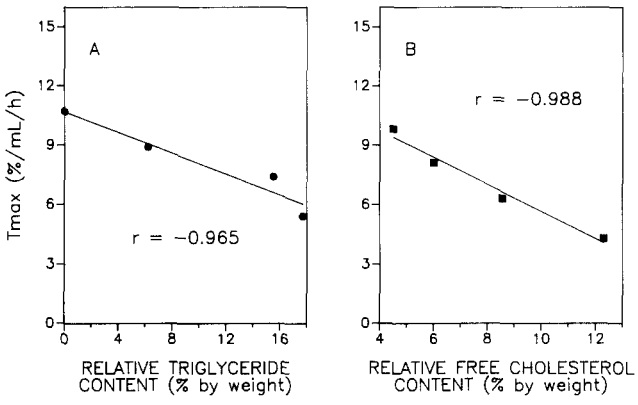

Fig. 3. Effect of rHDL lipid composition on CETP reaction kinetics. T_{max} values were estimated by double reciprocal plots of data from the incubations described in Fig. 2 and are plotted against rHDL triglyceride (A) and free cholesterol (B) contents (shown in Table 2, series A and B). Correlation coefficients (r) were determined by regressional analysis.

LDL and VLDL suggests that CETP may be directly involved in elevating plasma levels of the potentially atherogenic lipoproteins (23). Despite advances in our understanding of its protein structure, the exact role of CETP in plasma cholesterol homeostasis remains poorly understood. Specifically, the factors that regulate the ability of this protein to catalyze the exchange or net transfer of lipids within or between lipoprotein classes have not been fully elucidated.

The purpose of the present study was to identify some of the lipid and apoprotein parameters that regulate the interaction of CETP with the HDL pool. This work was prompted by our previous study in which we demonstrated that an increase in the triglyceride to cholesteryl ester ratio of HDL, isolated from patients with primary disorders of lipid metabolism, was shown to be associated with a reduced rate of transfer of CE into HDL (5). Since we observed a significant reduction of CE transfer to HDL_3 in patients with documented evidence of vascular disease, we have proposed that, in normal individuals, CE transfer within the HDL pool may prevent excessive accumulation within an atherogenic LDL pool. In the present study, we demonstrate that both the neutral and polar lipid compositions of rHDL have a marked effect on CETP activity. Specifically we have shown that an increase in rHDL triglyceride or free cholesterol (relative to cholesteryl ester or phospholipid, respectively) decreased the catalytic ability of CETP to transfer CE into HDL.

During the preparation of this report, Morton (25) also reported that the transfer of cholesteryl ester to HDL is inhibited when the free cholesterol content of HDL is increased. Interestingly, he further showed that increased free cholesterol content in lower density lipoproteins had no effect on their ability to receive CE. On the contrary, Morton (25) actually showed that increased free cholesterol in VLDL resulted in an increase in the net mass transfer of cholesteryl esters from HDL. Thus, an increase in plasma free cholesterol may promote the accumulation of cholesteryl esters in lower density lipoproteins by impairing their equilibration in HDL. This suggests that cholesteryl ester transfer activity may be governed by the free cholesterol/phospholipid ratios in donor and acceptor lipoproteins. However, in the present study we highlight the importance of core lipids as well as surface lipids in regulating the interaction of HDL with CETP. Since only the T_{max} was affected, the HDL lipid content appears to regulate the catalytic activity of CETP, apparently without modifying the interaction (K_H) between CETP and HDL. Abnormal HDL lipid composition may therefore have the potential to markedly affect cholesteryl ester equilibration within the HDL pool in vivo.

The mechanisms by which HDL lipid composition regulates the interaction with CETP is unclear. However, it seems possible that this may involve changes in the physical arrangement of the outer polar lipid monolayer. Evidence for this comes from studies of model lipoproteins that have shown that an increased concentration of cholesteryl ester relative to triglyceride results in increased order of the phospholipid acyl chains (26). As our data indicate that an increased cholesteryl ester content of HDL results in increased catalytic ability of CETP, we propose that this protein may require a highly ordered outer lipid monolayer. This may further explain our observation that HDL_3 was a better substrate for CETP, when compared to HDL_2, since HDL_3 has also been shown to have more highly ordered phospholipid acyl chains (26). Conversely, our observation that increased free cholesterol inhibits the activity of CETP cannot be explained by this hypothesis if this lipid orders the phospholipid acyl chains as it does

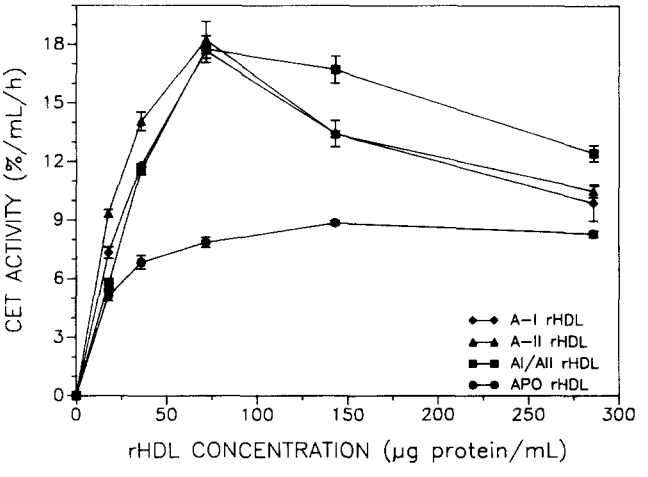

Fig. 4. Comparison of apoprotein A-I and A-II-containing rHDL as substrates for CETP; [^{3}H]cholesteryl transfer from LDL to rHDL prepared from purified apoprotein A-I, apoA-II, a mixture of both, or delipidated HDL apoproteins. Particle compositions are shown in Table 2. Incubations were as described in Fig. 1 and values are the mean ± SD of quadruplicate determinations.

in model membrane bilayers (27). However, there is no evidence that free cholesterol will order the surface lipid of a complex which is very small and is already highly ordered (26). In fact, one could suggest that the cholesterol would disorder such a complex as it does in highly ordered gel state model membranes (28). Clearly, in order to test this hypothesis, experiments on the direct effect of free cholesterol on acyl chain order in HDL must be carried out.

Alternatively, some studies have suggested that the solubility of different neutral lipids in the phospholipid layer may also be affected by the free cholesterol content (29, 30). It has been shown that increases in free cholesterol markedly reduce the solubility of cholesteryl ester, relative to triglyceride, in emulsion phospholipid monolayers (29, 30). If triglyceride is also able to reduce the solubility of cholesteryl esters phospholipid monolayer, this may explain why elevated triglyceride levels, as well as free cholesterol levels, impair the ability of rHDL to receive transferred esters. In addition, the small yet systematic increase in rHDL size observed with increasing triglyceride content (with the exception of series A, batch IV) suggests that changes in surface to volume ratios may potentially regulate this process. However, this seems less likely since marked decreases in observed T_{max} were not always associated with significant increases in particle size.

In this study, we have also used rHDL to investigate the role of specific apoproteins on the activity of CETP. We have shown that rHDL prepared from apoHDL had a significantly reduced T_{max} and K_H, relative to particles prepared from pure apoA-I, A-II, or both apoproteins. Since the ratio T_{max}/K_H was similar to that for native HDL and rHDL containing apoHDL, this suggests that some component(s) of the HDL apoproteins was acting as an uncompetitive inhibitor of CETP, which equally reduced the reaction K_H and T_{max}. Preliminary studies with other purified apoproteins have shown that inclusion of apoC-III in A-I rHDL markedly reduced the apparent K_H of the reaction to that comparable to rHDL prepared from apoHDL. Thus, we postulate that some component of the HDL apoproteins (possibly apoC-II) is associated with HDL_3 and regulates the affinity with which CETP binds to HDL. However, inclusion of apo-C-III did not completely normalize the T_{max} or abolish the apparent substrate inhibition observed at high substrate concentrations. Therefore, some other protein that is associated with HDL_2 and VHDL may be involved in a noncompetitive inhibition and act by reducing the reaction T_{max} values and preventing the apparent substrate inhibition at high substrate concentrations. This may in fact be the CETP-inhibitor protein described by others (10, 31). Localization of this inhibitor of CETP on HDL_2 and VHDL may prevent the formation of inactive complexes of CETP and two HDL particles, which could be analogous to the inactive substrate-enzyme-substrate complexes which result in substrate inhibition of enzymes. The lack of this inhibitor on HDL_3 may allow for the maturation of these particles by permitting the influx of cholesteryl esters from other lipoproteins.

In summary, we have shown that both the neutral and polar lipid and the apoprotein components of HDL are integrally involved in regulating the ability of CETP to transfer CE into HDL. Since variations in HDL lipid composition have been shown to be correlated with the incidence of ischemic heart disease (32, 33), we propose that the resultant abnormal interaction between HDL and CETP may impair the equilibration of cholesteryl esters and their subsequent removal from the HDL pool. The consequence may be increased transfer and accumulation of cholesteryl esters in lower density lipoproteins, an event which may promote atherogenesis.

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