Remodeling of HDL by CETP in vivo and by CETP and hepatic lipase in vitro results in enhanced uptake of HDL CE by cells expressing scavenger receptor B-I

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Abstract The transport of HDL cholesteryl esters (CE) from plasma to the liver involves a direct uptake pathway, mediated by hepatic scavenger receptor B-I (SR-BI), and an indirect pathway, involving the exchange of HDL CE for triglycerides (TG) of TG-rich lipoproteins by cholesteryl ester transfer protein (CETP). We carried out HDL CE turnover studies in mice expressing human CETP and/or human lecithin:cholesterol acyltransferase (LCAT) transgenes on a background of human apoA-I expression. The fractional clearance of HDL CE by the liver was delayed by LCAT transgene, while the CETP transgene increased it. However, there was no incremental transfer of HDL CE radioactivity to the TG-rich lipoprotein fraction in mice expressing CETP, suggesting increased direct removal of HDL CE in the liver. To evaluate the possibility that this might be mediated by SR-BI, HDL isolated from plasma of the different groups of transgenic mice was incubated with SR-BI transfected or control CHO cells. HDL isolated from mice expressing CETP showed a 2- to 4-fold increase in SR-BI-mediated HDL CE uptake, compared to HDL from mice lacking CETP. The addition of pure CETP to HDL in cell culture did not lead to increased selective uptake of HDL CE by cells. However, when human HDL was enriched with TG by incubation with TG-rich lipoproteins in the presence of CETP, then treated with hepatic lipase, there was a significant enhancement of HDL CE uptake. Thus, the remodeling of human HDL by CETP, involving CE–TG interchange, followed by the action of hepatic lipase (HL), leads to the enhanced uptake of HDL CE by cellular SR-BI. These observations suggest that in animals such as humans in which both the selective uptake and CETP pathways are active, the two pathways could operate in a synergistic fashion to enhance reverse cholesterol transport.—Collet, X., A. R. Tall, H. Serajuddin, K. Guendouzi, L. Royer, H. Oliveira, R. Barbaras, X-c. Jiang, and O. L. Francone. **Remodeling of HDL by CETP in vivo and by CETP and hepatic lipase in vitro results in enhanced uptake of HDL CE by cells expressing scavenger receptor B-I.** ***J. Lipid Res.*** **1999.** 40: **1185–1193.**

Supplementary key words HDL-cholesterol • transgenic mice • reverse cholesterol transport • cholesterol metabolism • carrier proteins

In humans, plasma high density lipoprotein (HDL) levels show an inverse relationship to the incidence of coronary heart disease (1). Although the mechanism is uncertain, several lines of evidence suggest that the HDL-mediated transport of cholesterol from the extrahepatic tissues to the liver, called reverse cholesterol transport, may account in part for the protective role of HDL in atherosclerosis (2). After initial steps of cellular free cholesterol efflux into HDL and conversion of free cholesterol into CE by the enzyme lecithin:cholesterol acyltransferase (LCAT), HDL CE may be returned to the liver by several different routes. In humans, a major pathway for returning HDL CE to the liver involves the cholesteryl ester transfer protein (CETP), which is thought to mediate the transfer of HDL CE to triglyceride-rich lipoproteins (TRL) by stimulating CE–TG interchange, followed by the uptake of TRL remnants in the liver. The overall importance of CETP in HDL CE clearance is illustrated by a human genetic deficiency of CETP which results in markedly increased plasma HDL levels (3).

Mice normally lack CETP activity in plasma and when a human CETP transgene is introduced there is a lowering of plasma HDL levels, associated with increased catabolism of HDL CE due to enhanced clearance by the liver (4). Conversely, overexpression of the human LCAT transgene leads to an increase in HDL CE (5) and a decrease

Abbreviations: apoA-I, apolipoprotein A-I; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; CHO, Chinese hamster ovary; FCR, fractional catabolic rate; HL, hepatic lipase; HDL, high density lipoprotein; HuAITg, transgenic mice expressing the human apoA-I transgene; HuAILCATTg, transgenic mice expressing the human apoA-I and LCAT transgenes; HuAICETPTg, transgenic mice expressing the human apoA-I and CETP transgenes; HuAILCATCETPTg, transgenic mice expressing the human apoA-I, LCAT, and CETP transgenes; KO, knockout; LCAT, lecithin:cholesterol acyltransferase; TRL, triglyceride-rich lipoproteins; TG, triglycerides; SR-BI, scavenger receptor BI; TR, transport rate; VLDL, very low density lipoprotein.

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in the fractional clearance of HDL CE by the liver (6). Effects of human LCAT and human CETP transgenes on HDL CE are much greater on a background of human apoA-I transgene expression (4, 7).

In mice, the return of HDL CE to the liver is mediated in part by a selective uptake pathway, in which there is uptake of HDL CE by liver cells without the accompanying uptake and degradation of HDL protein (8). Recently, scavenger receptor BI (SR-BI) has been shown to play a crucial role in mediating the selective uptake of HDL CE by tissues. Cultured Chinese hamster ovary (CHO) cells transfected with SR-BI show enhanced selective uptake (9) and SR-BI is highly expressed in liver and steroidogenic tissues where selective uptake occurs (10, 11). Moreover, mice with decreased or attenuated expression of SR-BI have a marked increase in HDL levels (12) and a proportional reduction in HDL CE selective uptake by the liver (13). These findings suggest that SR-BI is the major pathway mediating selective uptake of HDL CE in the liver. Moreover, mutations in genes involved in HDL biogenesis lead to increased SR-BI expression in the adrenal glands associated with depletion of adrenal cholesterol stores (14, 15). Hepatic lipase (HL) knockout (KO) mice have decreased CE stores and increased adrenal SR-BI, raising the possibility that remodeling of HDL by HL might enhance tissue HDL CE delivery by the SR-BI pathway. In animals expressing CETP, the remodeling of HDL is thought to involve a CE–TG interchange (mediated by CETP), followed by hydrolysis of TG by HL (16, 17). This raises the possibility that entry of HDL CE into the SR-BI pathway might be facilitated by the remodeling of HDL involving combined activities of CETP and HL.

In the initial phase of the present study we investigated the effects of LCAT and CETP transgene expression, singly or in combination, on plasma lipoprotein levels and HDL CE clearance from plasma by the liver. Surprisingly, we discovered that CETP enhanced hepatic HDL CE clearance without a measurable increase in HDL CE radioactivity in TRL. This suggested the hypothesis that remodeling of HDL by CETP activity in vivo might lead to increased uptake of HDL CE by hepatic SR-BI, giving rise to a direct enhancement of hepatic HDL CE uptake by CETP. To test this idea, we studied the ability of ex vivo HDL from transgenic mice to interact with SR-BI in cell culture. Finding an enhanced uptake of CETP Tg HDL CE by SR-BI, we then sought to dissect the mechanism by which in vivo remodeling of HDL by CETP action leads to increased uptake of HDL CE by SR-BI. The results suggest the novel hypothesis that CETP activity in vivo leads to increased uptake of HDL CE by the selective uptake pathway.

METHODS

Transgenic mice

The transgenic mice used in the present study were described previously (7, 18). Mice expressing the human apoA-I and LCAT transgenes (HuAILCATTg) were obtained by mating transgenic mice expressing the human apoA-I transgene (HuAITg) with LCAT heterozygous mice (HuLCATTg). Transgenic mice expressing both the human apoA-I and CETP transgenes (HuAICETPTg) and triple transgenics (HuAILCATCETPTg) were generated by mating HuAILCATTg mice with mice carrying the human CETP transgene.

Plasma lipids and lipoprotein analysis

Mouse serum was isolated from blood collected retro-orbitally with heparinized hematocrit tubes after mice were fasted overnight. Total cholesterol, free cholesterol, TG, and phospholipids levels were determined using enzymatic colorimetric assays (Wako, Osaka, Japan).

To isolate lipoproteins from plasma, 400 μl of pooled plasma containing 1.5 mm 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) from HuAITg, HuAICETPTg, HuAILCATTg, and HuAILCATCETPTg mice on a chow diet was fractionated using two tandem Superose 6 columns (Pharmacia, LKB Biotechnology, Piscataway, NJ) as previously described (5). Cholesterol was determined enzymatically as described above while human apoA-I was determined by ELISA.

Human HDL_2 were isolated in the d 1.085–d 1.125 g/ml density interval and enriched in TG as previously described (19, 20). After dialysis against Tris/NaCl (10 mm/135 mm), HDL_2 (0.2 mm total cholesterol) were enriched in triacylglycerol in the presence of VLDL (0.145 mm free cholesterol), of the d > 1.21 g/ml plasma fraction (25% of total volume), as a source of CETP, coincubated during 6 h at 37°C in the presence of 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB) as an LCAT activity inhibitor. After removing VLDL at 1.07 g/ml, modified HDL_2 were reisolated by ultracentrifugation at d 1.21 g/ml, and were further dialyzed against Tris/NaCl (10 mm/135 mm), pH 7.4. TG-rich HDL_2 (300 mg/ml of protein) were then incubated with or without HL (80 mU/ml) during 2 h at 37°C in the presence of fatty acid-free albumin. Control TG-rich HDL_2 and remnant-HDL_2 were reisolated by ultracentrifugation at d 1.19 g/ml. ^{125}I-labeling of TG-rich HDL_2 and remnant HDL_2 was performed by the N-bromosuccinimide method according to Sinn et al. (21). Specific radioactivity ranged from 1000 to 3000 cpm/ng of protein.

Plasma levels of apoA-I and CETP levels were determined by enzyme-linked immunosorbent assays as described previously (5, 22).

Determination of LCAT activity

LCAT activity was measured on fasting plasma as the rate of synthesis of [^{3}H]cholesteryl esters from unilamellar vesicles prepared with a French pressure cell and activated with human apoA-I (Sigma) as described previously (7).

In vivo HDL CE turnover studies

HDL was isolated from each group of transgenic mice and labeled with of [^{3}H]cholesteryl-oleoyl ether as previously described (4). Mice were injected in the femoral vein with 2×10^5 dpm of [^{3}H]cholesteryl-oleoyl ether-labeled HDL. The injected HDL mass was less than 5% of the mouse HDL CE pool. Mice were fasted throughout the 24-h study period but had free access to water. Blood (50 μl) was withdrawn from the tail vein under methoxyflurane anesthesia at 2 min, 1 h, 3 h, 6 h, 9 h, 12 h, and 24 h for determination of radioactivity. The fractional catabolic rate (FCR) was calculated from plasma disappearance curves analyzed by a two-pool model described by Matthews, and kinetic parameters were estimated using the nonlinear least-squares curve fitting program kinetic (G. A. McPerson, Elsevier-Biosoft, Cambridge, UK). Plasma transport rates (TR) were calculated using the equation TR = FCR × M, where M is the HDL-CE plasma pool size (23).

Twenty-four hours after the injection of the tracer, mice were

anesthetized with methoxyflurane. The liver was perfused through the heart with Dulbecco's phosphate-buffered saline (Gibco, BRL), excised, and weighed immediately. The radioactivity present in the liver was determined by liquid scintillation spectrometry after lipid extraction. Liver FCR and the absolute amount of CE mass taken up by the liver (liver flux) were calculated as previously described (24).

Generation of SR-BI expressing cells

Transfection of the murine SR-BI cDNA into CHO cells and selection of overexpressing cells were carried out as previously described (25). Stably transfected cells were maintained at 37°C in Ham's F-12 medium containing geneticin (0.3 mg/ml), penicillin (100 units/ml), streptomycin (100 μg/ml), and 2 mm glutamine supplemented with 5% fetal bovine serum. Cells were seeded in 6-well plates at $2–3 \times 10^5$ cells/well. Forty-eight h later cells were washed with PBS and preincubated at 37°C, 5% CO_2 for 2 h in Ham's F-12 medium supplemented with bovine serum albumin (BSA) (5 mg/ml). [^{3}H]cholesteryl oleoyl ether-labeled HDL were diluted (1–3 μg esterified cholesterol/ml) in Ham's F-12 medium supplemented with BSA (5 mg/ml) and incubated for 2 h. Culture medium was removed and cells were washed 4 times with PBS-containing BSA (2 mg/ml) and 4 times in PBS. Cells were harvested and lipids were extracted with hexane–isopropanol 3:2 (v/v) during 30 min under mild shaking. When the cells were incubated with ^{125}I-labeled HDL_2, the cells were washed in the same conditions. To determine cell-associated radioactivity, 500 μl of 0.1 N NaOH was added to the washed monolayer and aliquots were taken to detemine protein concentration and cell-associated radioactivity.

Miscellaneous

Partial purification of HL from human plasma was achieved by heparin affinity chromatography as described previously (26). HL activity was assayed according to Nilsson-Ehle and Eckman (27) using [^{3}H]triolein as a substrate in the presence of 1 m NaCl. The enzymatic activity is expressed as mIU (nmol free fatty acid released per min).

RESULTS

Lipid and lipoprotein analysis

Table 1 summarizes plasma lipid and apoA-I levels in the four groups of mice (HuAITg, HuAICETPTg, HuAILCATTg, and HuAILCATCETPTg), and **Fig. 1** shows the distribution of cholesterol and apoA-I in the plasma lipoproteins, as determined by FPLC. As previously shown (4), the expression of the human CETP transgene leads to a decrease in plasma HDL cholesterol and apoA-I levels, while the expression of human LCAT is associated with increased plasma HDL cholesterol and apoA-I (Fig. 1 and Table 1). Compared to HuAILCATTg mice, HuAILCATCETPTg mice showed a marked decrease in HDL choles-

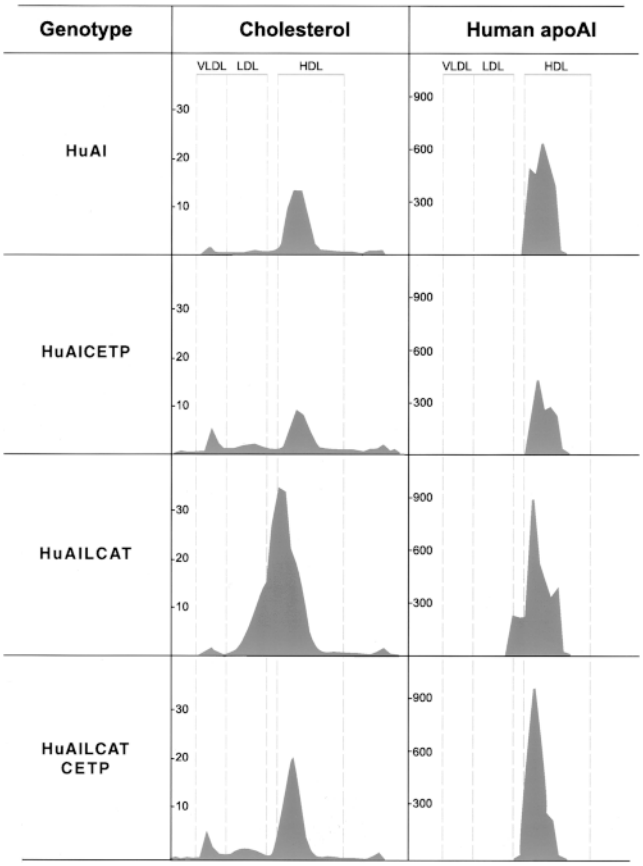

Fig. 1. Plasma lipoprotein cholesterol and human apoA-I distribution in HuAITg, HuAICETPTg, HuAILCATTg, and HuAILCATCETPTg mice fed a chow diet. Pooled mouse plasma (400 μl) containing 1.5 mm DTNB was fractionated using Superose 6 columns as described under Methods. Total cholesterol, human apoA-I were determined and plotted as a function of column fraction. The positions at which known lipoproteins eluted from the column are indicated. HuAI, transgenic mice expressing the human apoA-I transgene; HuAICETP, transgenic mice expressing the human apoA-I and CETP transgenes; HuAILCAT, transgenic mice expressing the human apoA-I and LCAT transgenes; HuAILCATCETP, transgenic mice expressing the human apoA-I, LCAT, and CETP transgenes; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins.

TABLE 1. Lipid profile of plasma and LCAT activities, human apoA-I and CETP levels in HuAITg, HuAICETPTg, HuAILCATTg, and HuAILCATCETPTg mice

Transgenic Mice	Cholesterol	Cholesteryl Ester	Phospholipids	Triglycerides	ApoA-I	LCAT Activity	CETP
	mg/dl				*mg/ml*	*nmol/ml/h*	*μg/ml*
HuAI	126.6 ± 28.3	90.2 ± 22.1	270.6 ± 43.9	103.2 ± 67.0	2.07 ± 0.65	23.7 ± 5.5	—
HuAICETP	71.8 ± 15.0[b]	52.0 ± 21.6[a]	170.6 ± 18.2[b]	81.0 ± 39.4	1.26 ± 0.28[a]	30.5 ± 7.6	2.9 ± 0.2
HuAILCAT	482.8 ± 161.0[b]	347.3 ± 136.3[b]	549.3 ± 147.4[b]	165.0 ± 56.6	3.98 ± 0.77[b]	82.8 ± 13.2[b]	—
HuAILCATCETP	208.7 ± 62.2[a]	154.7 ± 51.6[a]	327.7 ± 15.5	133.3 ± 88.9	3.50 ± 0.63[b]	80.4 ± 3.7[b]	4.8 ± 1.6

Values shown are mean ± SD (n: 4–8 mice per group). All lipid values are expressed in milligrams per deciliter of plasma. Mouse apoA-I was determined from pooled plasma from 4–8 mice and values (expressed in mg/ml plasma) are 0.03, 0.03, 0.14, and 0.07 for HuAI, HuAICETP, HuAILCAT, and HuAILCATCETP transgenic mice, respectively.

[a] $P < 0.05$, [b] $P < 0.005$ compared to HuAI.

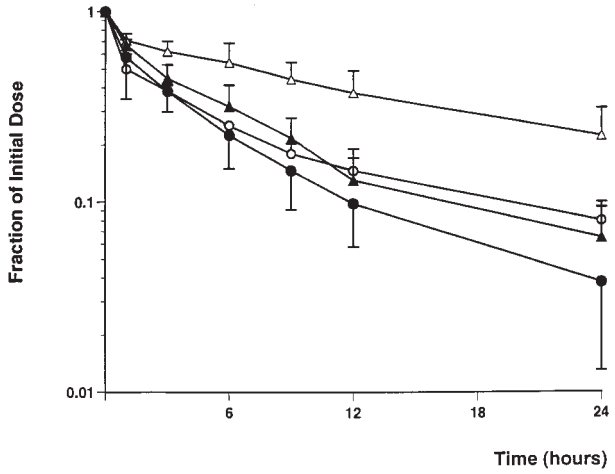

Fig. 2. Radiolabeled HDL decay curve in HuAITg, HuAICETPTg, HuAILCATTg, and HuAILCATCETPTg mouse plasma. Mice of different genotypes were injected intravenously with autologous [^{3}H]ether-labeled HDL. Blood (50 μl) was withdrawn from the tail vein for determination of radioactivity. HuAITg, open circles; HuAICETPTg, closed circles; HuAILCATTg, open triangles; HuAILCATCETPTg, closed triangles.

terol levels almost to the levels of HuAITg. The absolute and percentage reductions of HDL cholesterol due to the CETP transgene were greater in HuAILCATTg mice than in HuAITg mice indicating a coordinate role of CETP and LCAT in the modulation of HDL cholesterol levels.

HDL CE turnover studies

To evaluate the in vivo effects of LCAT and CETP on the metabolism of HDL CE, autologous HDL labeled with [^{3}H]cholesteryl oleoyl ether was injected into mice of different genotypes. The disappearance of radioactivity from plasma was followed for 24 h. The plasma clearance rate for labeled HDL (**Fig. 2** and **Table 2**) was significantly different among the groups studied. In close agreement with previous studies (4, 24), the HDL CE FCR in HuAITg mice was 0.166 ± 0.032 pools/h. As illustrated in Fig. 2, HDL from HuAILCATTg mice had a significantly slower rate of disappearance from plasma as compared with HuAITg mice (0.072 ± 0.022 pools/h, $P < 0.05$), suggesting that the increase in the plasma HDL CE pool size (Table 2) observed in HuAILCATTg is attributable not only to an increase in the production rate of CE but also to a delayed clearance of HDL CE. To test the hypothesis that when HDL levels are high, the CETP-induced remodeling of HDL is the rate-limiting step in modulating the clearance of HDL and the reverse cholesterol transport process, CETP was expressed alone (HuAICETPTg) or in combination with LCAT (HuAILCATCETPTg). As shown in Table 2, the presence of CETP induced a 40 and 70% decrease in HDL CE pool size compared to HuAITg and HuAILCATTg mice, respectively, with parallel increases in HDL CE FCR. While LCAT overexpression markedly increased the transport rate of HDL CE (equivalent to synthesis), this parameter was not affected by the expression of CETP. These results show that CETP is a major determinant of the rate at which HDL CE is cleared from the plasma compartment and show that the impairment of clearance associated with LCAT expression is completely reversed by CETP.

TABLE 2. In vivo HDL CE metabolism in HuAITg, HuAILCATTg, HuAILCATCETPTg, and HuAICETPTg mice fed a chow diet

Mice	HDL CE Pool Size	HDL CE FCR	HDL CE TR
	μg	*pool/h*	*μg/h*
HuAI	566.1 ± 87.9	0.166 ± 0.032	79.5 ± 7.4
HuAICETP	338.6 ± 58.1[a]	0.239 ± 0.094	87.7 ± 18.7
HuAILCAT	3042 ± 631.2[a]	0.072 ± 0.022[a]	208.7 ± 43.2[a]
HuAILCATCETP	966.2 ± 162.2[a]	0.184 ± 0.06	193.8 ± 8.8[a]

Mouse HDL was labeled with [^{3}H]cholesteryl oleoyl ether as described under Methods and injected in the femoral vein of mice expressing the various combinations of transgenes. Blood was withdrawn at various time points and radioassayed for ^{3}H. HDL CE TR is calculated using the equation TR = FCR × M, where M is the HDL CE plasma pool size. Statistically significant differences were calculated by Student *t*-test.

[a] $P < 0.01$ compared to HuAI.

To determine to what extent enhanced clearance of the HDL CE from plasma involves the CETP-mediated transfer of HDL CE to TRL, lipoproteins were isolated by ultracentrifugation of pooled plasma at d < 1.063 and the amount of [^{3}H]cholesteryl oleoyl ether present in the VLDL + LDL fraction was determined at 2 min, 1 h, 3 h, 6 h, 9 h, and 12 h after tracer injection in the different groups of mice. Two minutes after the injection of labeled HDL, 4% and 7% of the total radioactivity present in plasma was detected in the d < 1.063 g/ml lipoproteins in HuAILCATCETPTg and HuAICETPTg mice, respectively. This amount remained constant over the time interval studied (**Fig. 3**) and did not increase over the values obtained in mice without CETP (5.5% and 11.6% for HuAITg and HuAILCATTg mice, respectively), indicating that the changes observed in the plasma HDL CE pool size and HDL CE FCR are not reflected by an equivalent transfer of HDL CE into the d < 1.063 g/ml fraction. This suggests that the enhanced clearance of HDL CE in mice expressing the human CETP transgene may not be entirely mediated by enhanced transfer to d < 1.063 g/ml lipoproteins.

Hepatic uptake of HDL CE

The uptake of HDL CE by the liver of the different groups of mice was assessed by determining the uptake of the nondegradable cholesteryl oleoyl ether label from HDL. As no appreciable amount of cholesteryl ether was observed in the apoB-containing lipoproteins in all transgenic mice studied, the radioactivity present in the liver reflects the uptake of [^{3}H]cholesteryl ether from HDL itself, not from other lipoprotein fractions labeled secondarily.

Compared to HuAITg mice, HuAICETPTg mice had a markedly increased fractional clearance and net mass flux of HDL CE to the liver (3.5- and 2.3-fold, $P < 0.05$, respectively) (**Table 3**). The expression of the human LCAT transgene resulted in a decrease in HDL CE fractional

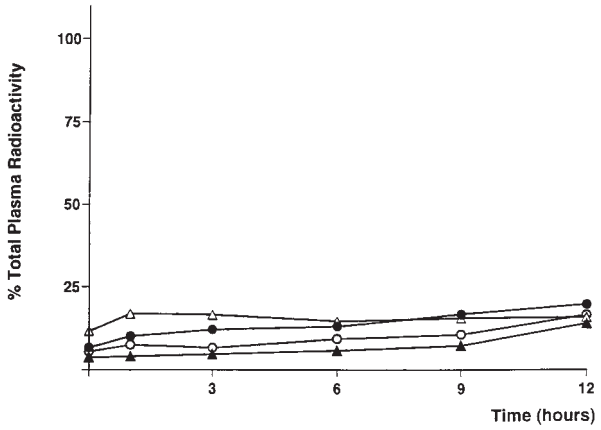

Fig. 3. Transfer of [^{3}H]cholesteryl ether from HDL to apoB-containing lipoproteins. Pooled plasma from HuAITg, HuAICETPTg, HuAILCATTg, and HuAILCATCETPTg mice was collected after the injection of labeled HDL and apoB-containing lipoproteins were isolated by ultracentrifugation. The amount of [^{3}H]cholesteryl oleoyl ether present in apoB-containing lipoproteins was determined at 2 min, 1 h, 3 h, 6 h, 9 h, and 12 h after tracer injection and expressed as percentage of total plasma radioactivity. HuAITg, open circles; HuAICETPTg, closed circles; HuAILCATTg, open triangles; HuAILCATCETPTg, closed triangles.

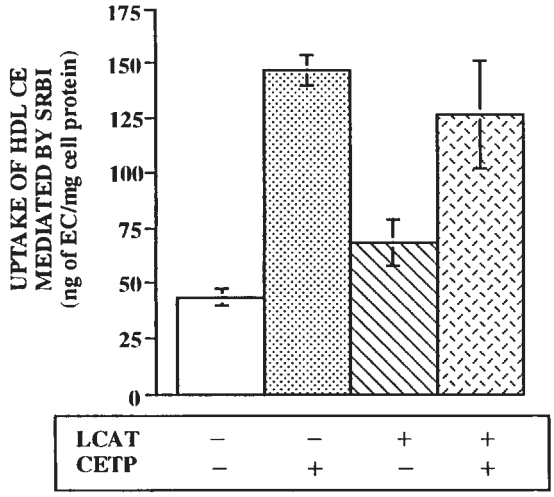

Fig. 4. SR-BI-mediated CE uptake from HDL isolated from HuAITg, HuAICETPTg, HuAILCATTg, and HuAILCATCETPTg mouse plasma. CHO cells expressing the murine SR-BI cDNA and vector transfected (control) CHO cells were incubated with HDL isolated from mice of different genotypes for 2 h as described under Methods. Values shown are mean ± SD on three separate experiments. SR-BI-mediated selective CE uptake represents the difference between the uptake found in SR-BI and control cells. CE uptake in control cells is 133.7 ± 4.2, 151.0 ± 3.4, 124.1 ± 4.9, and 157.4 ± 18.3 ng of CE/mg of cell protein for HuAITg, HuAICETPTg, HuAILCATTg, and HuAILCATCETPTg mouse HDL, respectively.

clearance, but increased net mass flux to the liver reflecting the expanded plasma pool of HDL CE (Tables 2 and 3). In HuAILCATCETPTg mice, liver FCR was increased (3.7-fold) but net mass flux was similar to HuAILCATTg mice. Together with the data of Fig. 3, these results suggest that CETP enhances the direct removal of HDL CE by the liver. The failure to further increase flux in the triple transgenic mice could reflect saturation of the hepatic removal mechanism.

Interaction of transgenic mouse HDL with CHO cells expressing the murine SR-BI cDNA

The stimulation of hepatic clearance of HDL CE by CETP, without a significant increment in the transfer to other lipoprotein fractions, suggested the possibility of enhanced clearance by the SR-BI pathway. To ascertain a potential role for SR-BI, HDL was isolated from plasma from the different groups of transgenic mice and incubated with CHO cells overexpressing murine SR-BI cDNA or with control CHO cells that have very low SR-BI expression (25). The difference in HDL CE uptake between the cell types, representing selective uptake by SR-BI, is shown in **Fig. 4**. Compared to HuAITg HDL, incubation of SR-BI expressing cells with HDL isolated from mice expressing CETP resulted in a marked 3- to 4-fold stimulation in selective uptake of CE. Similarly, HDL from HuAILCATCETPTg mice showed enhanced SR-BI-mediated HDL CE uptake, compared to HDL from HuAITg or HuAILCATTg mice. The uptake of HDL CE by CHO cells expressing SR-BI was not affected by the addition of either purified CETP (4.5 μg/well) or heparin (10 units/well) to the medium suggesting that the enhanced uptake of HDL CE from mice expressing the CETP transgene requires in vivo remodeling of HDL and that the effects are independent of LDL receptor activity.

TABLE 3. Liver HDL CE uptake in HuAITg, HuAICETPTg, HuAILCATTg, and HuAILCATCETPTg mice

Mice	Liver FCR	Mass Flux to Liver
HuAI	20.0 ± 4.0	393.9 ± 41.7
HuAICETP	69.0 ± 16.0[a]	920.6 ± 171.5[a]
HuAILCAT	6.0 ± 3.0[a]	745.7 ± 131.7[a]
HuAILCATCETP	22.0 ± 8.0	828.3 ± 76.3[a]

Labeled HDL were injected into the femoral vein of mice as described under Methods. Plasma decays were followed for 24 h after which the livers were perfused with PBS, removed, and assayed for radioactivity after lipid extraction. Statistically significant differences were calculated by Student *t*-test. Liver FCR is calculated as the fraction of plasma pool cleared per hour per gram of liver × 10^3.

[a] $P < 0.01$.

To further evaluate potential changes in HDL composition that could lead to enhanced uptake of HDL CE by SR-BI, a more detailed compositional analysis was performed. As shown in **Table 4**, there was a significant increase in the TG content of HDL isolated from mice expressing human CETP. Compared to the HDL isolated from HuAITg mice, HuAICETPTg mice HDL contain significantly more TG (TG/TG + CE) 9.3% ± 0.7 vs. 3.2 ± 0.1, for HuAICETP and HuAITg, respectively ($P < 0.05$, n = 5). The amounts of triacylglycerols expressed as μmol/mg apoA-I in HuAICETPTg HDL compared to HuAITg HDL were 0.07 ± 0.01 vs. 0.03 ± 0.001, respectively ($P < 0.05$, n = 5), whereas esterified cholesterol content relative to apoA-I was not significantly different (0.69 ±

TABLE 4. Lipid composition of HDL isolated from HuAITg, HuAICETPTg, HuAILCATTg, and HuAILCATCETPTg mice fed a chow diet

Mice	n	TC	FC	CE	TG	% TG/(TG + CE)
			μmol/mg human apoA-I			*%*
HuAI	6	1.03 ± 0.03	0.24 ± 0.02	0.79 ± 0.10	0.03 ± 0.01	3.17 ± 0.19
HuAICETP	7	0.76 ± 0.11	0.07 ± 0.01[a]	0.69 ± 0.07	0.07 ± 0.01[a]	9.26 ± 0.7[c]
HuAILCAT	5	2.23 ± 0.27[a]	0.43 ± 0.03[b]	1.80 ± 0.20[a]	0.03 ± 0.01	1.51 ± 0.66
HuAILCATCETP	2	1.69	0.57	1.12	0.09	7.43

HDL was isolated as described under Methods. Lipids were analyzed by gas–liquid chromatography. Human apoA-I was determined by ELISA as described under Methods. The abbreviations used are: TG, triglycerides; FC, free cholesterol; CE, cholesteryl ester; TC, total cholesterol. TC, FC, CE, and TG are expressed as μmol/mg human apoA-I.

[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$.

0.07 vs. 0.79 ± 0.10). The TG enrichment of HDL occurred without transfer of the bulk of HDL CE radioactivity with TRL (Fig. 3). This could occur if small amounts of CE and TG interchange (below detection limits) initiate a remodeling of HDL that leads to increased hepatic uptake of the bulk of HDL CE. Alternatively, CETP could enhance TG transfer into HDL without equivalent loss of HDL CE, as suggested by the compositional data. The significant enrichment of HDL from CETP transgenic mice in TG suggests that these particles should be an excellent substrate for HL.

Effects of the remodeling of human HDL by CETP and HL on HDL CE uptake by CHO expressing the murine SR-BI cDNA

The lack of a direct effect of CETP on HDL CE uptake by cells overexpressing SR-BI suggested that in vivo remodeling of HDL by CETP might be involved. As the remodeling of HDL is thought to involve coordinate actions of CETP and HL, an in vitro remodeling study was performed using human HDL. We used a well-characterized system using human HDL (19, 20). HDL_2 were isolated by ultracentrifugation and enriched in TG (TG-rich HDL_2) by incubation with TRL in the presence of CETP and then treated by HL. HL transforms the TG-rich HDL into preβ1-HDL and a lipolysis modified HDL, called remnant HDL (19, 20). In these two independent experiments, TG-rich HDL_2 contained 20–27% TG in their lipid core as compared to 8–10% in non-enriched HDL_2. After treatment by HL, 60–70% of TG were hydrolyzed, whereas the amount of CE (μmol/mg apoA-I) was unchanged.

The uptake of HDL CE was assessed by determining the uptake of the nondegradable cholesteryl oleoyl ether label from TG rich HDL_2 treated or not with HL. The difference in HDL CE uptake between the cell types, representing selective uptake by SR-BI, is shown in **Fig. 5**. Compared to HDL (TG-rich HDL) not treated with HL, there was a 2-fold increase in the SR-BI-mediated uptake of CE from TG-rich HDL_2 incubated with HL (remnant HDL_2). The cell association of HDL protein at 37°C (2 h) was also assessed using TG-rich HDL_2 treated or not with HL. The difference in HDL protein association between the cell types, corresponds to 37.5 for TG-rich HDL_2 and 31.7 ng of protein/mg of cell protein for remnant HDL_2. Thus, the marked increase of HDL CE uptake by cells expressing SR-BI was not due to an increase of the HDL apoprotein cell association.

DISCUSSION

In the initial phase of this study we examined the effects of LCAT or CETP transgenes, singly or together, on HDL CE turnover and hepatic uptake in mice. Consistent with earlier reports (4, 6), these studies showed that CETP increases and LCAT decreases the fractional clearance of HDL CE by the liver. Moreover, the results show a coordinate effect of LCAT and CETP, leading to more marked reduction in HDL CE levels and pool size and increases in HDL CE FCR in mice expressing CETP and LCAT than in mice expressing CETP alone. We then evaluated the hypothesis that these effects of CETP on HDL CE clearance might involve an enhancement of selective uptake by the

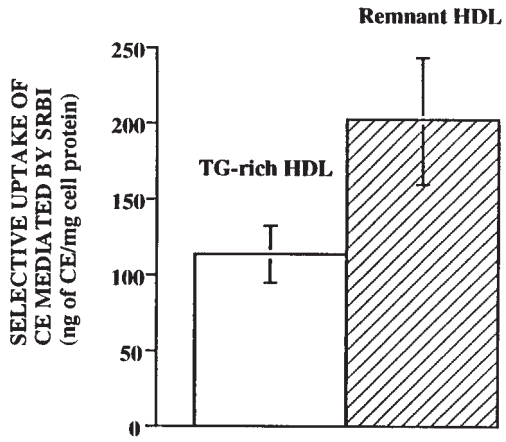

Fig. 5. SR-BI-mediated HDL CE uptake from HDL_2 remodeled by HL. TG-rich and remnant HDL_2 were prepared as described under Methods. CHO cells expressing the murine SR-BI cDNA and vector transfected CHO cells (control) were incubated with 30 μg of HDL CE of either TG-rich or remnant HDL_2 for 2 h at 37°C. Values shown are mean ± SD of three separate experiments. SR-BI-mediated selective HDL CE uptake represents the difference between the uptake found in SR-BI and control cells. HDL CE uptake in control cells represents 206.0 ± 27.7 for TG-rich HDL_2 and 259.6 ± 28.8 for remnant HDL_2 ng of CE/mg of cell protein.

SR-BI. In view of the recent evidence that hepatic SR-BI plays a major role in mediating the clearance of HDL CE by the selective uptake pathway in mice (13, 28), we investigated the possibility that CETP might modify HDL in vivo in a way that results in enhanced uptake of HDL CE by SR-BI. HDL from mice expressing the CETP transgene incubated with cultured cells expressing SR-BI showed a 2- to 4-fold increase in SR-BI-mediated HDL CE uptake. This effect could not be reproduced by direct addition of CETP to culture medium. Further mechanistic studies showed that the CETP-mediated enrichment of human HDL with TG and the subsequent lipolysis of HDL by HL could similarly enhance the uptake of HDL CE by SR-BI in cell culture. Thus, it appears that the in vivo remodeling of HDL by CETP acting in conjunction with HL enhances the uptake of HDL CE by hepatic SR-BI. This suggests that at least part of the mechanism by which CETP enhances reverse cholesterol transport involves enhanced remodeling of HDL followed by uptake of hepatic SR-BI.

In order to elucidate the mechanisms responsible for the increased cellular uptake of CE from CETP transgenic mouse HDL, we carried out in vitro remodeling studies of human HDL using purified components. Whereas CETP alone had no effect, incubation of HDL with TRL and CETP, followed by direct treatment by HL, led to increased SR-BI-dependent cellular uptake of HDL CE. Thus, it appears likely that compositional and physical changes of HDL that are mediated by CETP and HL result in increased HDL CE uptake by the SR-BI pathway. There are some obvious shortcomings of using human HDL as a model for mouse HDL. Thus, further studies will be needed to define precisely which structural or compositional changes of HDL are most important for enhanced selective uptake.

The results with in vitro remodeled HDL provide a reasonable explanation for the increased uptake of CE from CETP Tg mice HDL by cells expressing SR-BI. Deckelbaum et al. (29) first showed that lipoproteins (LDL and HDL) could be remodeled by lipid exchange of CE for TG followed by hydrolysis of TG by lipoprotein or HL; this concept has been repeatedly validated, and has been shown to occur in CETP Tg mice (30). Thus, HDL from CETP Tg mice is known to be enriched with TG (and reproduced in this study), reflecting the ability of CETP to mediate the hetero-exchange of HDL CE with TG of TRL. Moreover, the HDL from CETP Tg mice contains an increased proportion of preβ-HDL, reflecting in part the modification of TG-enriched HDL by HL activity (22, 31). Studies in HL transgenic and KO mice and HL transgenic rabbits clearly show a major role of HL in the determination of HDL CE levels (32–34). Furthermore, a recent study shows that it is the catalytic activity of HL that is important in facilitating removal of HDL CE in the mouse (35), whereas the bridging function of HL may be involved in mediating the removal of remnants from the circulation (36).

The effect of CETP activity to increase the selective uptake of HDL CE by the SR-BI pathway could play an important role in the clearance of HDL CE in vivo. The results of the cellular SR-BI uptake study (Fig. 4) were well correlated with hepatic FCR of HDL CE for the different genotypes (Table 3). LCAT overexpression appears to saturate the hepatic clearance pathway of HDL CE by increasing the substrate pool, rather than by decreasing the efficiency of uptake of HDL CE by SR-BI (Fig. 4). The increased clearance of HDL CE mediated by CETP was accompanied by no detectable increment in transfer of HDL CE radioactivity into the $d < 1.063$ g/ml fraction (containing TRL). Although the fact that HDL from CETP Tg mice is TG-enriched suggests that some CE–TG exchange may occur, the present studies raise the intriguing possibility that this process may trigger uptake of a part of HDL CE by the SR-BI pathway in the liver. Thus, a fraction of HDL CE may be cleared from plasma without transfer to VLDL. An alternative explanation for our findings is that the turnover of VLDL CE is so rapid that the transfer of HDL CE into that fraction cannot be measured. Although there is a paucity of data on the turnover rate of VLDL CE in mice, the disappearance of retinyl esters from the TRL fraction suggests a half-life measured in hours (1–2 h) (37). This is only slightly faster than the turnover of HDL CE and thus this explanation appears to be unlikely. Furthermore, a previous study using freshly isolated human plasma incubated with fibroblasts has shown that most of cholesteryl esters generated by LCAT are initially redistributed among different HDL subfractions (38). However, further in vivo testing at the quantitative importance of the remodeling of HDL in the selective uptake pathway is desirable, for example using SR-BI knockout mice.

Our data suggest that lipid exchange processes mediated by CETP remodel HDL and trigger a pathway of HDL CE removal involving hepatic SR-BI. This concept is consistent with earlier work suggesting that HL increases the selective uptake of HDL CE by liver cells (19) and the uptake of HDL CE by the adrenals (39). Recent evidence shows that SR-BI plays the major role in mediating the selective uptake of HDL CE in the liver and adrenal (11, 13). Therefore, HL and SR-BI are likely to act in the same metabolic pathway, rather than in parallel pathways. In CETP transgenic mice, the combined activities of CETP and HL may optimize HDL for clearance by the SR-BI pathway in the liver. Although mice lack CETP activity, it is possible that the homologous phospholipid transfer protein (PLTP) subserves a similar role: PLTP is highly expressed in mice and PLTP mediates inter-lipoprotein transfer of diglycerides, which may act as substrates for HL activity (40). In the rabbit, a species with a significant amount of plasma CETP, it appears that the selective uptake pathway plays a significant role in HDL CE catabolism, although this is less quantitatively important than catabolism via the VLDL/LDL fractions (41). These observations could be related to the very low activity of HL in this animal (41) and suggest again an important role of HL in the remodeling of HDL species.

Although previously considered as distinct pathways, the present results suggest that at least part of the CETP-mediated HDL clearance pathway in rabbits and humans

could occur via hepatic SR-BI. The hepatic expression of the human SR-BI equivalent, CLA-1, appears substantial (42), suggesting a role for the selective uptake pathway in humans. However, it is puzzling that human genetic CETP deficiency results in marked increases in HDL CE, as the selective uptake pathway is a high capacity process that should compensate for the rise in HDL CE (43). This could be explained if the activity of CETP (interchange of HDL CE with TG of TRL, and subsequent lipolysis of HDL TG by HL) also serves to optimize the uptake of HDL CE by the selective uptake pathway in humans.

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