Role of cholesteryl ester transfer protein in selective uptake of high density lipoprotein cholesteryl esters by adipocytes

Gerard Vassiliou[1] and Ruth McPherson[1]

Lipoprotein and Atherosclerosis Group, University of Ottawa Heart Institute, Ottawa, Ontario K1Y 4E9, Canada

Abstract **Previous reports attributed cholesteryl ester transfer protein (CETP)-mediated HDL cholesteryl ester (CE) selective uptake to the CETP-mediated transfer of CE from HDL to newly secreted apolipoprotein B-containing lipoproteins, which are then internalized by the LDL receptor (LDL-R). CETP has also been implicated in the remodeling of HDL, which renders it a better substrate for selective uptake by scavenger receptor class B type I (SR-BI). However, CETP-mediated selective uptake of HDL_3-derived CE was not diminished in LDL-R null adipocytes, SR-BI null adipocytes, or in the presence of the receptor-associated protein. We found that monensin treatment or energy depletion of the SW872 liposarcoma cells with 2-deoxyglucose and NaN_3 had no effect on CETP-mediated selective uptake, demonstrating that endocytosis is not required. This is supported by data indicating that CETP transfers CE into a compartment from which it can be extracted by unlabeled HDL. CETP could also mediate the selective uptake of HDL_3-derived triacylglycerol (TG) and phospholipid (PL). The CETP-specific kinetics for TG and CE uptake were similar, and both reached saturation at ~5 μg/ml HDL. In contrast, CETP-specific PL uptake did not attain saturation at 5 μg/ml HDL and was ~6-fold greater than the uptake of CE. We propose two possible mechanisms to account for the role of CETP in selective uptake.**—Vassiliou, G., and R. McPherson. **Role of cholesteryl ester transfer protein in selective uptake of high density lipoprotein cholesteryl esters by adipocytes. *J. Lipid Res.* 2004.** 45: **1683–1693.**

Supplementary key words energy depletion • monensin • scavenger receptor class B type I • SW872 liposarcoma cells

Cholesteryl ester transfer protein (CETP) is a plasma glycoprotein that promotes the net transfer of lipids between lipoproteins. In particular, cholesteryl ester (CE) from HDL and LDL is transferred to VLDL with the reciprocal transfer of triacylglycerol (TG) [for reviews, see refs. (1, 2)]. CETP also mediates the transfer of phospholipids (PLs) from TG-rich lipoproteins to HDL, principally during lipolysis of the former when excess surface is generated (3).

The overall role of CETP in atherosclerosis is unclear. CETP mediates an important step in reverse cholesterol transport. Cholesterol extracted by efflux from peripheral tissues is esterified within HDL by lecithin:cholesterol acyltransferase. This CE is subsequently transferred by CETP to apolipoprotein B (apoB)-containing lipoproteins, which are taken up and catabolized by the liver (2, 4). In this context, CETP may be antiatherogenic. However, high expression of CETP reduces plasma HDL (5), and this observation has led to the development of CETP inhibitors as a potential therapy to reduce atherosclerosis.

In addition to its established role in lipid exchange between lipoproteins, CETP has been reported to exchange lipids between cell membranes and between cell membranes and lipoproteins. For example, CETP has broad specificity for membrane surfaces and can transfer CE between membranes of the endoplasmic reticulum (6). In addition, CETP is present within the female reproductive tract, where it may enhance sperm capacitation, as it does in vitro (7), and this is attributed to the ability of CETP to alter the membrane lipid composition in a manner that increases the sperm's ability to penetrate the egg (2). The CETP-mediated efflux of cholesterol (8) or CE (9, 10) from biological membranes to lipoproteins has also been reported.

Granot, Tabas, and Tall (11) first reported that CETP mediates the selective uptake (12–15) of HDL-CE by HepG2

Abbreviations: α_2M, α_2-macroglobulin; apoB, apolipoprotein B; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; LDL-R, LDL receptor; LRP, LDL receptor-related protein; MX, 4-methyl umbelliferyl-β-D-xyloside; PL, phospholipid; RAP, receptor-associated protein; SR-BI, scavenger receptor class B type I; TG, triacylglycerol; Tmax, maximal transport rate.

[1] To whom correspondence should be addressed.
e-mail: gvass@ottawaheart.ca (G. V.);
rmcpherson@ottawaheart.ca (R. M.)

Manuscript received 12 February 2004 and in revised form 2 June 2004.

Published, JLR Papers in Press, July 1, 2004.
DOI 10.1194/jlr.M400051-JLR200

cells, by smooth muscle cells, and to a lesser extent by fibroblasts but not by endothelial cells or J774 macrophages. However, these observations were later attributed to the conventional function of CETP in mediating the transfer of HDL-CE to newly secreted apoB-containing lipoproteins followed by their internalization by the LDL receptor (LDL-R) (16, 17).

Our laboratory has shown that in human adipose tissue CETP mediates the net transfer of HDL-derived CE into cells (18). In contrast to the earlier results in HepG2 cells, we reasoned that the CETP-mediated uptake of HDL-CE is not dependent on the transfer of CE to apoB-containing lipoproteins because adipocytes do not secrete apoB-containing lipoproteins. However, the secretion of other lipoproteins to which CETP may transfer CE and the subsequent internalization of those lipoproteins could not be ruled out. In addition, Collet et al. (19) have demonstrated that the CETP-mediated enrichment of HDL with TG and the subsequent lipolysis of HDL by hepatic lipase could enhance the selective uptake of HDL-CE by scavenger receptor class B type I (SR-BI). Although adipocytes have been reported to express SR-BI mRNA in some studies (20, 21) but not others (22), they appear to express no (20, 22) or negligible amounts of (21) immunoreactive SR-BI. Nevertheless, the contribution of SR-BI in the CETP-mediated uptake of HDL-CE by adipocytes could not be excluded.

CETP might enhance the cellular uptake of CE by analogy with the proposed mechanism of lipid transfer between lipoproteins. In this regard, there are two proposed mechanisms. CETP contains binding sites for CE, TG, and PL and probably acts to shuttle these lipids between donor and acceptor lipoproteins (1, 2). In the case of selective uptake, CETP could shuttle CE between the HDL and the plasma membrane. According to a second model, CETP may enhance the exchange of lipids during the formation of a ternary collision complex consisting of donor and acceptor lipoproteins, bridged by CETP (1, 2). By analogy, CETP might bind the HDL to the plasma membrane and increase the collisions between the membrane and the lipoprotein, allowing lipids to be exchanged in the process. On the other hand, the mechanisms by which other molecules facilitate selective uptake may provide clues to the process mediated by CETP. Based on thermodynamic and kinetic data, Rodrigueza et al. (23) proposed that SR-BI forms a lipophilic channel through which CE and other lipids traverse from the bound lipoprotein to the plasma membrane. CETP has neutral lipid binding pockets that may act as a lipophilic conduit between the CETP-bound lipoprotein and the plasma membrane. Additionally, evidence for HDL recycling during selective CE transfer in *ob/ob* hepatocytes (24) implies that CE transfer may occur at an intracellular location and that after extraction of the CE the "remnant HDL" is returned to the cell exterior. It is possible that CETP mediates selective uptake by facilitating the recycling of HDL in adipocytes. Finally, molecules such as lipoprotein lipase mediate selective uptake independently of their enzymatic activity (25), and this has led to the proposal that this enzyme tethers HDL and increases its concentration at sites of selective uptake.

In this paper, we have reexamined the ability of CETP to mediate HDL-CE selective uptake and examined the possibility that CETP remodels the lipoprotein or that CE is transferred to secreted lipoproteins, which are subsequently internalized. After eliminating all of these alternative explanations, we present two possible models for CETP-mediated selective uptake, consistent with our data.

EXPERIMENTAL PROCEDURES

Materials

All common reagents were of analytical grade and purchased from Fisher (Fair Lawn NJ), Sigma (St. Louis, MO), or BDH (Poole, England). Chondroitinase ABC (EC 4.2.2.4) and heparinase I (EC 4.2.2.7) were from Sigma. Retired breeders of homozygous SR-BI null mice (strain B6;129S2-Scarb1^{tm1Kri}) and LDL-R null mice (strain B6.129S7-Ldlr^{tm1Her}) were obtained from Jackson Laboratory (Bar Harbor, ME). CETP was purchased from Cardiovascular Targets, Inc. (New York, NY).

Protein purification and labeling

ApoA-I was expressed, purified, and labeled with ^{125}I as described (26). Native α_2-macroglobulin (α_2M) was purified from human plasma by zinc-chelate chromatography (27). The α_2M was activated with methylamine and labeled with ^{125}I as described (28). Receptor-associated protein (RAP) was expressed using the IMPACT-CN system (New England Biolabs, Beverly, MA). In this system, proteins are expressed in fusion with a self-cleavable affinity tag (intein fused to a chitin binding domain) that allows a single-step purification of the fusion protein on a chitin column and elution of the target protein after dithiothreitol-induced self-cleavage of the intein. The ability to cleave the RAP from the intein system is dependent on the amino acid that is adjacent to the intein. When RAP is fused to the C terminus of intein, the amino acid of mature RAP that is adjacent to the intein is Tyr, but this does not allow efficient cleavage. Therefore, Leu was introduced at the start of the mature RAP to facilitate cleavage from intein. The RAP sequence corresponding to the mature protein was amplified by the polymerase chain reaction using a forward primer that incorporated an extra Leu and using the full-length human RAP cDNA as a template. The forward and reverse primers also included *Sap*I and *Eco*RI restriction sites. The polymerase chain reaction product and pTYB11 were double digested with *Sap*I and *Eco*RI and then ligated together. This plasmid was designated pTYB11(RAP-Leu). This cloning strategy produces full-length RAP with an extra Leu at the N terminus after cleavage from the intein-chitin binding domain. pTYB11(RAP-Leu) was amplified in DH5α. RAP was expressed in ER2566 bacteria and purified exactly as described for apoA-I (26). Typically, more than 2 mg of RAP was purified from 1 liter of bacteria, and the RAP appeared pure after SDS-PAGE and Coomassie blue staining. The cell surface binding of [^{125}I]α_2M was inhibited fully at 3 μg/ml RAP and by 50% at 0.48 μg/ml RAP.

Lipoprotein purification and labeling

Total HDL and HDL$_3$ were purified from normolipemic plasma by density gradient ultracentrifugation (29). The lipoproteins were dialyzed twice, each time against 2 liters of nitrogen-sparged PBS, pH 7.4, with 2 g of Chelex (Bio-Rad). The lipoproteins were labeled with [^{3}H]cholesteryl oleate or [^{3}H]cholesteryl

ether or with [^{125}I]apoA-I as described (26) and reisolated by density gradient ultracentrifugation (29). Typically, 1 mg of HDL_3 (protein content) used in these experiments contained 0.06 ± 0.004 mg of unesterified cholesterol, 0.22 ± 0.02 mg of CE, 0.66 ± 0.05 mg of PL, and 0.05 ± 0.004 mg of TG. CETP could not be detected by Western blotting of our HDL fractions after labeling and repurification. In one experiment, 5 mg of HDL_3 from the same batch was separately labeled with 100 μCi of [^{3}H]cholesteryl oleate ([^{3}H]CE) (Amersham Biosciences), 100 μCi of [^{3}H]triolein ([^{3}H]TG) (Perkin-Elmer, Boston, MA), or 20 μCi of L-3-phosphatidylcholine 1,2-di[1-^{14}C]oleoyl ([^{14}C]PL) (Amersham Biosciences) essentially as described (26), except that the labeled triolein or phosphatidylcholine was substituted for [^{3}H]CE. Trace amounts of unlabeled cholesteryl oleate, triolein, or phosphatidylcholine were added to each batch of labeled lipoprotein to maintain their chemical identities.

Tissue culture

All cells were cultured at 37°C in a humidified 5% CO_2 incubator. SW872 liposarcoma cells were cultured in Dulbecco's modified Eagle's medium/F-12 (3:1), 5% fetal bovine serum (Life Technologies, Inc.), 2 mM L-glutamine (Life Technologies, Inc.), and 50 μg/ml gentamicin (Schering-Plough). Primary subcutaneous human adipose tissue was obtained from healthy subjects undergoing reduction mammoplasty for cosmetic purposes, and preadipocytes were isolated, seeded at ~50% confluence onto 24-well plates, and differentiated as described (30). Mouse epididymal fat pads were isolated, and mature adipocytes were obtained after differentiation of preadipocytes by the same method used to prepare human adipocytes. Ldl-A7 CHO cells were cultured as described (31). For all cell types, there was between 60 and 100 μg of cellular protein per well when the selective uptake assays were carried out.

Selective uptake assay

This was essentially as described (26). Briefly, cells were grown on 24-well tissue culture plates and were at least 90% confluent at the time of assay. Before the selective uptake assay, the cells were washed twice at 37°C in 1 ml of ligand buffer (HBSS; 25 mM Hepes, 5 mg/ml BSA, pH 7.45) and then incubated at 37°C for 30 min in this buffer. This buffer was replaced with 300 μl of ligand buffer containing [^{3}H]CE-labeled HDL or [^{125}I]apoA-I-labeled HDL, with the addition of CETP and other compounds as indicated (all at the concentrations stated in the figure legends), and the cells were incubated at 37°C for up to 8 h. At the end of the incubation, the extracellular buffer was removed and the cells were washed five times in HBSS, 25 mM Hepes, pH 7.45, at 10°C. After the final wash, the medium was removed and the cells were solubilized with 500 μl of 200 mM NaOH for 2 h at room temperature with gentle mixing. The protein content of 40 μl from each well was measured using the bicinchoninic acid protein reagent (Pierce) according to the manufacturer's instructions with BSA for standard comparison. The ^{3}H or ^{125}I radioactivity of 440 μl of each cell lysate was measured by liquid scintillation counting using Ecolite (ICN, Costa Mesa, CA) or by γ counting, respectively. Proteolytically degraded and resecreted fragments of [^{125}I]apoA-I in the ligand-containing medium were measured according to Goldstein, Basu, and Brown (32). The cell association of [^{3}H]CE or [^{125}I]apoA-I is measured in units of the amount of these labels contained in 1 ng (protein content) of HDL. Plotted this way, an equivalent cell association of [^{3}H]CE and [^{125}I]apoA-I indicates holoparticle uptake, and any additional [^{3}H]CE cell association is attributed to selective uptake. Kinetic parameters for selective uptake were determined by nonlinear regression using the Prism computer program (GraphPad Software).

Internalization of [^{125}I]α_2M

[^{125}I]α_2M cellular association (internalization and cell surface binding) was measured in SW872 cells at 37°C as described (28). At 37°C, internalization of α_2M constitutes the majority of cell-associated α_2M in SW872 cells because cell surface binding is only ~2 fmol/mg cell protein (26).

Effect of CETP on the transport of CE to the reversible and irreversible compartments

SW872 cells were incubated at 37°C for 4 h with 100 μg/ml [^{3}H]CE-labeled HDL_3 in ligand buffer in the presence of 0.48 μg/ml CETP or without further additions. After this time, the cells were washed six times in HBSS, 25 mM Hepes, pH 7.45, at 10°C to remove the labeled HDL from the extracellular medium, and the cells were incubated for 2 h at 37°C with ligand buffer containing 400 μg/ml unlabeled HDL_3. After this incubation, the radioactivity in the extracellular medium and in the cells was counted separately. The counts in the medium and the cells were deemed to be in the reversible and irreversible compartments, respectively. The protein in the wells was not measured directly but was measured in separate, identical wells after the six washes in HBSS, 25 mM Hepes, pH 7.45.

Inhibition of membrane trafficking

SW872 cells grown on 24-well tissue culture plates were washed twice as described for the selective uptake assay. The cells were then preincubated for 30 min at 37°C in ligand buffer containing 20 mM 2-deoxyglucose and 5 mM NaN_3 to inhibit glycolysis and mitochondrial respiration, respectively (33). Alternatively, the cells were preincubated for 30 min with 10 μM monensin in ligand buffer with 1% ethanol to block clathrin-mediated endocytosis as well as the secretion and recruitment of proteins to the cell surface (34, 35). In each case, control cells were incubated in the same medium without the active ingredients. After the preincubation, the cells were incubated at 37°C for 4 h in ligand buffer with the relevant active ingredients containing either 1 μg/ml [^{125}I]α_2M (with 25 μg/ml RAP in particular samples) or 5 μg/ml [^{3}H]CE-labeled HDL_3 (with 0.48 μg/ml CETP in particular samples). After this time, the cells were washed and the cell-associated radioactivity was measured as described for the selective uptake assay.

Cell surface binding of [^{125}I]apoA-I-labeled HDL_3

SW872 cells were grown on 24-well plates and washed twice as described for the selective uptake assay. The cells were preincubated in ligand buffer for 30 min at 37°C. After the preincubation, the cells were incubated for 2 h at 4°C with up to 10 μg/ml [^{125}I]apoA-I labeled HDL_3 in ligand buffer in the presence or absence of 1 μg/ml CETP. The cells were washed five times in HBSS, 25 mM Hepes, pH 7.45, and then solubilized in 500 μl of 0.2 M NaOH. The protein content and ^{125}I radioactivity of each cell lysate were measured as described for the selective uptake assay. The cell surface binding of [^{125}I]apoA-I-labeled HDL_3 was also measured at 37°C in energy-depleted cells (incubated with 20 mM 2-deoxyglucose and 5 mM NaN_3 as described above).

Digestion and metabolic inhibition of proteoglycans

To determine whether proteoglycans were involved in CETP-dependent selective uptake, we used four separate treatments to alter the proteoglycans: *a*) Heparinase I digestion of heparan sulfate proteoglycans. Heparinase I (EC 4.2.2.7) from Sigma was incubated with the cells for 2 h at 37°C dissolved at 3 U/ml in F12 containing 5 mg/ml BSA and complete protease inhibitors (Roche Molecular Biochemicals). *b*) Chondroitinase ABC digestion of chondroitin and dermatan sulfate proteoglycans. Chondroitinase ABC (EC 4.2.2.4) from Sigma was incubated with the

cells at 1.5 U/ml exactly as described for heparinase I digestion. *c*) Growth of the SW872 cells in chlorate, which inhibits the enzyme (sulfate adenylyltransferase) that is necessary for the sulfation of all proteoglycans (36). Sodium chlorate (Fisher) was incubated with the cells at 30 mM in growth medium at the time of plating, ~2 days before the experiment. *d*) Growth of the cells in 4-methyl umbelliferyl-β-D-xyloside (MX), which substitutes for the core protein moiety of proteoglycans in the cell and significantly reduces their synthesis and appearance on the cell surface (37). MX was incubated with the cells at 2 mM in growth medium 24 h before the experiment. The efficiency of proteoglycan digestion, chlorate, or MX treatment was determined by labeling of the sulfate groups of the proteoglycans with 20 μCi/ml [^{35}S]sulfate (Amersham) for 48 h. After the proteoglycan digestion or chlorate or MX treatment, the reduction in cellular [^{35}S]sulfate was attributed to the loss of the sulfate groups (28). After the proteoglycan digestion or growth of the cells in chlorate or MX, the cells were washed once in ligand buffer and the CETP-mediated selective uptake was measured as described above.

CETP-mediated transfer of lipids from HDL to LDL

HDL_3 (2 μg of protein) that had been labeled with [^{3}H]CE, [^{3}H]TG, or [^{14}C]PL and LDL (20 μg of protein) and 5 ng of CETP were mixed into a final volume of 500 μl of ligand buffer and incubated for 1 h at 37°C with gentle rocking. The samples to measure background transfer did not include transfer protein. After the 1 h incubation, the tubes were placed on ice for 10 min. Another 20 μg (in 20 μl) of LDL was added, followed immediately by the addition of 200 μl of freshly prepared, ice-cold LDL precipitation solution [4 ml of ligand buffer, 1 ml of 2 M $MnCl_2$, 1 ml of heparin (10,000 U/ml)], and the tubes were mixed and incubated on ice for 10 min. A 200 μl aliquot of each sample was filtered using a Millititer GV 96-well filtration plate (Millipore, Bedford, MA) attached to a vacuum manifold. Each sample was washed twice with 300 μl of phosphate-buffered saline. The filters were cut from the plate and incubated overnight with 400 μl of isopropanol in 5 ml scintillation vials. After this, 4 ml of Beta-Max (ICN) was added and the radioactivity was measured by scintillation counting.

RESULTS

Characterization of CETP-mediated selective uptake in SW872 cells

Our laboratory has described CETP-mediated selective uptake in human adipose tissue (18). In this study, we have used primary human and mouse adipocytes and SW872 liposarcoma cells that we have previously characterized and shown to be a useful model for selective uptake (26). When SW872 cells were incubated with [^{3}H]CE-labeled HDL_3 or [^{125}I]apoA-I-labeled HDL_3, the [^{3}H]CE cell incorporation was ~5.8 times greater than the [^{125}I]apoA-I cell incorporation (which included cell association and degradation). Therefore, these cells mediated the selective uptake of CE (**Fig. 1**) as previously reported (26). Exogenous CETP increased the cellular incorporation of both CE and apoA-I by 140% and 53%, respectively. The absolute increase in CE and apoA-I incorporation mediated by CETP was 280 and 18 ng HDL_3 protein/mg cell protein, respectively, which represents a CETP-mediated

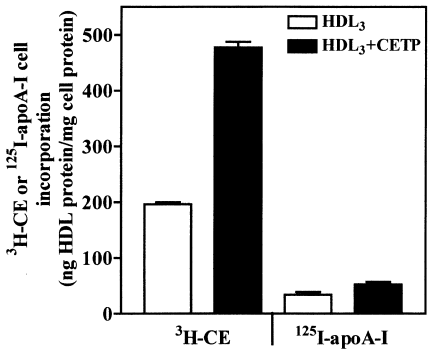

Fig. 1. Cholesteryl ester transfer protein (CETP)-mediated selective uptake of HDL_3-cholesteryl ester (CE) by SW872 cells. SW872 cells were incubated at 37°C for 8 h with 5 μg/ml [^{3}H]CE-labeled or [^{125}I]apolipoprotein A-I ([^{125}I]apoA-I)-labeled HDL_3 in the presence of 0.48 μg/ml CETP (black bars) or without CETP (white bars). After washing, the cells were solubilized and the cell-associated radioactivity and cellular protein were measured. The incorporation of [^{3}H]CE or cell-associated and degraded [^{125}I]apoA-I is plotted in units of the amount of these labels contained in 1 ng (protein content) of HDL_3 and normalized for cellular protein. Each value plotted is the mean of six measurements, and the SE is shown.

increase in selective uptake of 262 ng HDL_3 protein/mg cell protein. Essentially identical results were obtained when HDL_3 was labeled with [^{3}H]cholesteryl ether, which cannot be hydrolyzed; therefore, we assume that the CE is intact in these experiments (data not shown). Given the proportionally small increase in apoA-I uptake and degradation mediated by CETP, in the experiments to follow (unless specifically stated) we did not measure [^{125}I]apoA-I cell association and degradation. In these experiments the [^{3}H]CE uptake is referred to as "cell incorporation" rather than "selective uptake."

Kinetics of CETP-mediated selective uptake in primary human adipocytes

When CETP was added in increasing amounts to primary human adipocytes in the presence of 25 μg/ml HDL_3, the CETP-mediated cellular incorporation reached a plateau at 1.2 μg/ml CETP, with half-maximal stimulation at 0.32 μg/ml (**Fig. 2**). This is lower than the mean CETP concentration of 1.8 μg/ml in normolipemic plasma. To determine if the CETP-mediated incorporation reached a plateau because the HDL_3 was limiting, we increased the HDL_3 concentration up to 300 μg/ml and held the CETP constant at 2.4 μg/ml (**Fig. 3A**). At the lowest HDL_3 concentration, CETP increased the [^{3}H]CE cellular incorporation by a proportionally greater value, but over most of the HDL concentration range the absolute value of CETP-mediated [^{3}H]CE uptake remained fairly constant. Nonlinear regression, and analysis of the data on a Lineweaver-Burk plot (Fig. 3B), indicated that in the absence of exogenous CETP the cellular incorporation of [^{3}H]CE could be described by two distinct transport processes. The first, low-efficacy, high-capacity process has a maximal transport rate (T_{max}) of 438 ± 59 ng

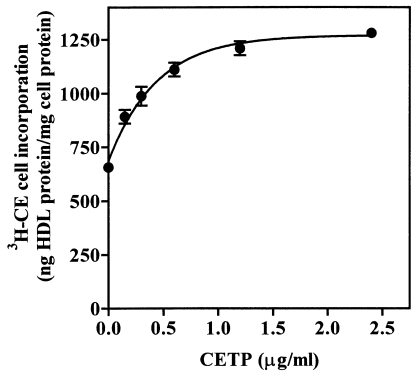

Fig. 2. Dose response of CETP-mediated cellular incorporation of HDL_3-CE. Primary human adipocytes were incubated at 37°C for 8 h with 25 μg/ml [^{3}H]CE-labeled HDL_3 in the presence of up to 2.4 μg/ml CETP. The cellular incorporation of [^{3}H]CE was measured and plotted as described in Fig. 1 without subtraction of apoA-I uptake and degradation. Each point is the mean of four measurements, and the SE is shown.

HDL protein/mg cell protein/h and a K_m of 337 ± 10 μg/ml HDL_3. The second, high-efficacy, low-capacity site has a T_{max} of 59 ± 18 ng HDL protein/mg cell protein/h and a K_m of 11 ± 6 μg/ml HDL_3. In addition to these two transport processes, a very high-efficacy transport process was evident in the presence of CETP, with a T_{max} of 68 ± 18 ng HDL protein/mg cell protein/h and a K_m of 1.3 ± 2.8 μg/ml HDL_3. The calculation of these kinetic parameters assumes that selective uptake reaches a steady state and does not attenuate over time. We have established that SW872 adipocytes accumulate CE linearly over 8 h in the presence of a high concentration (100 μg/ml) of HDL (26), and this result has been confirmed for primary adipocytes (data not shown). Given that the CETP-mediated transport process reaches a maximum when neither CETP nor HDL_3 is limiting, these two results suggests that some specific site or process conferred by the cell must curtail the CETP-mediated selective uptake.

The role of lipoprotein receptors in CETP-mediated selective uptake

LDL-R-deficient CHO cells (Ldl-A7) were capable of CETP-mediated [^{3}H]CE incorporation (**Fig. 4A**); therefore, the involvement of the LDL-R in this process can be excluded. We confirmed this result by demonstrating that primary adipocytes isolated from LDL-R-deficient mice were also capable of CETP-mediated [^{3}H]CE uptake (Fig. 4B). Therefore, contrary to previous reports using HepG2 cells (16, 17), in adipocytes CETP does not transfer CE from HDL to secreted lipoproteins, which may be internalized by the LDL-R. These results also show that CETP-mediated selective uptake may be observed in hamster ovary cells and in mouse adipocytes. It is possible that other members of the LDL-R gene family that bind to lipoproteins may compensate for the loss of LDL-R in these studies. These receptors include the LDL receptor-related protein (LRP), gp330/megalin, the VLDL receptor, and apoE receptor 2 [for reviews, see refs. (38, 39)]. All members of this family that bind lipoproteins are sensitive to inhibition by the RAP (38, 39). However, RAP had no effect on CETP-mediated selective uptake (Fig. 4C), although it was capable of preventing the cell surface binding of [^{125}I]α_2M, a ligand for LRP (data not shown). This result excludes the involvement of other members of the LDL-R gene family. Furthermore, it is apparent that selective uptake mediated by CETP and LRP (26, 40) are distinct processes.

To exclude the involvement of SR-BI, we showed that CETP-mediated selective uptake by primary adipocytes isolated from SR-BI-null mice and control mice was similar (**Fig. 5**). Therefore, enhanced SR-BI selective uptake after CETP-mediated remodeling of HDL, as proposed (19), cannot explain our observations. Furthermore, in the absence of CETP, selective uptake by SR-BI null cells was ~28% less compared with that by control cells, suggesting that SR-BI contributes to the acquisition of CE by primary adipocytes.

Our laboratory has previously reported (18) that digestion of proteoglycans reduced HDL-CE selective uptake.

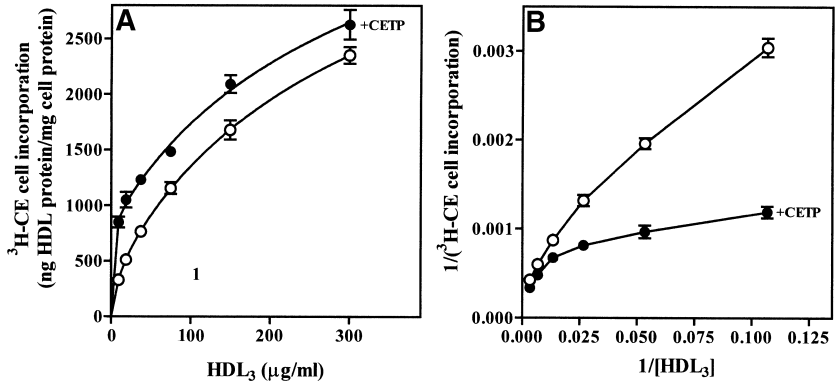

Fig. 3. CETP-mediated cellular incorporation of CE at various concentrations of HDL_3. Primary human adipocytes were incubated at 37°C for 8 h with up to 300 μg/ml [^{3}H]CE-labeled HDL_3 in the presence of 2.4 μg/ml CETP (black circles) or without CETP (white circles). The cellular incorporation of [^{3}H]CE was measured and graphed directly (A) or as a double-reciprocal plot (B). Each point is the mean of four measurements, and the SE is shown.

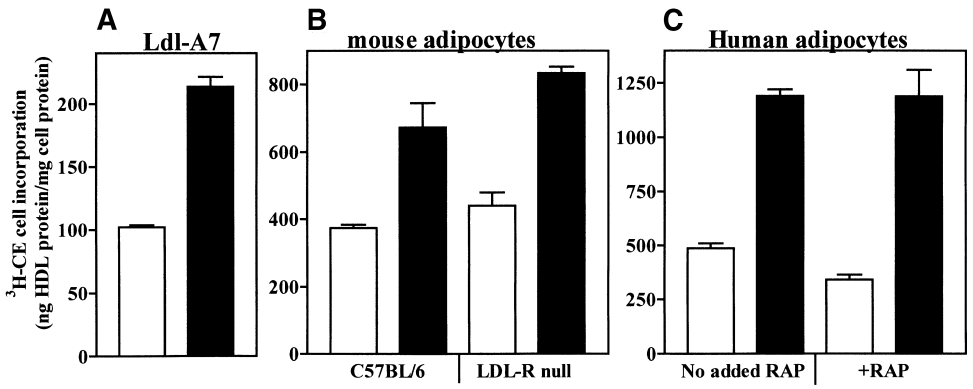

Fig. 4. Role of lipoprotein receptors in CETP-mediated cellular incorporation of HDL_3-CE. [^{3}H]CE-labeled HDL_3 (5 μg/ml) was incubated with 0.48 μg/ml CETP (black bars) or without CETP (white bars) at 37°C for 8 h with the following cells and additions: Ldl-A7 CHO cells, which are deficient in the LDL receptor (LDL-R) (A); primary mouse adipocytes from LDL-R-negative or C57BL/6 (control) mice (B); and primary human adipocytes in the presence of 25μg/ml receptor-associated protein (RAP) or without RAP (C). The cellular incorporation of [^{3}H]CE was measured and plotted as described in Fig. 1 without subtraction of apoA-I uptake and degradation. Each point is the mean of six measurements, and the SE is shown.

Although we can now attribute this effect to LRP-mediated selective uptake (40), this does not rule out the involvement of proteoglycans in CETP-mediated selective uptake. Heparin interferes with molecular interactions involving ion pairing, including interactions between ligands and the sulfated residues of proteoglycans (41, 42). We found that 10 mg/ml heparin had no effect on CETP-mediated selective uptake by human adipocytes (data not shown). Furthermore, we demonstrated that treatment of SW872 cells with heparinase I, which digests heparan sulfate proteoglycans; chondroitinase ABC, which digests chondroitin and dermatan sulfate proteoglycans; growth of the cells with chlorate, which prevents sulfation of proteoglycans; or growth of the cells in MX, which prevents proteoglycan synthesis, all had no effect on CETP-mediated selective uptake (data not shown).

Membrane traffic and CETP-mediated selective uptake

We measured the cellular uptake of HDL-derived [^{3}H]CE over time in the presence or absence of CETP (**Fig. 6**). In both cases, [^{3}H]CE accumulates linearly within the cells. Furthermore, CETP enhancement of CE uptake was observed at the earliest time point of 15 min. This is in marked contrast to LRP-dependent selective uptake, which was not measurable before 30 min (40). The rapid onset of CETP-mediated selective uptake suggests that transfer may take place at the plasma membrane.

CE is characterized by its entry into reversible and irreversible compartments (43), and this has been observed in SW872 cells (26). According to the classic view, the reversible compartment is proposed to include CE that has entered the plasma membrane and remains accessible to extraction by extracellular, unlabeled HDL. This reversible pool attains a plateau value after 2 h (26), possibly because the plasma membrane has a limited capacity to store CE or because the incorporation of CE into the plasma membrane is in equilibrium with the transfer out of this compartment. The plasma membrane CE is subsequently transferred to an irreversible compartment as it is internalized and becomes inaccessible to extraction by extracellular unlabeled HDL (44). A different interpretation (24) is that the reversible compartment constitutes CE in HDL that is in a recycling compartment. During recycling, some of the CE is transferred to other intracellular compartments and thus accumulates inside the cell in an irreversible manner. The rest of the CE is returned with the HDL to the extracellular medium, thus manifesting as a reversible phase.

Primary adipocytes were incubated with [^{3}H]CE-HDL_3 with or without CETP addition, then washed and the extracellular medium was replaced with unlabeled HDL_3 for 2 h. Under these conditions, CETP more than doubled the [^{3}H]CE that remained in the cells (i.e., in the irrevers-

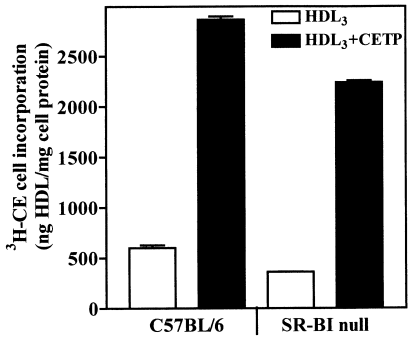

Fig. 5. Role of the scavenger receptor class B type I (SR-BI) in the CETP-mediated cellular incorporation of HDL_3-CE. Primary adipocytes from C57BL/6 (control) mice or SR-BI-negative mice were incubated at 37°C for 8 h with 5 μg/ml [^{3}H]CE-labeled HDL_3 in the presence of 0.48 μg/ml CETP (black bars) or without CETP (white bars). The cellular incorporation of [^{3}H]CE was measured and plotted as described in Fig. 1 without subtraction of apoA-I uptake and degradation. Each point is the mean of six measurements, and the SE is shown.

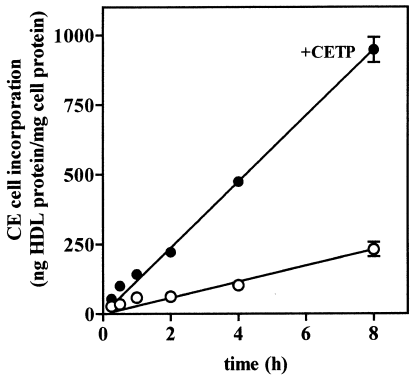

Fig. 6. Time course of CETP-mediated cellular incorporation of HDL_3-CE. SW872 cells were incubated at 37°C for the times indicated with 5 μg/ml [^{3}H]CE-labeled HDL_3 in the absence (white circles) or presence (black circles) of 0.48 μg/ml CETP. The cellular incorporation of [^{3}H]CE was measured and plotted as described in Fig. 1. Each point is the mean of four measurements, and the SE is shown.

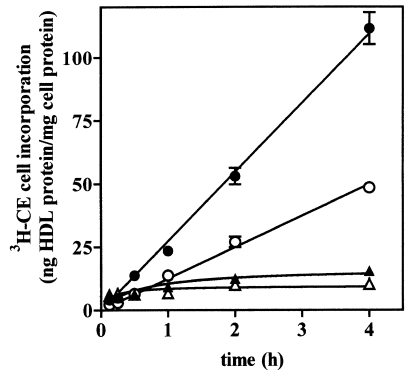

Fig. 7. Effect of CETP on the CE accumulation into reversible and irreversible compartments. Primary human adipocytes were incubated at 37°C for up to 4 h with 5 μg/ml [^{3}H]CE-labeled HDL_3 in the presence of 0.48 μg/ml CETP or without CETP. After this time, the cells were washed six times in HBSS, 25 mM Hepes, pH 7.45, and the cells were incubated for 2 h at 37°C with ligand buffer containing 400 μg/ml unlabeled HDL_3. After this incubation, the radioactivity in the extracellular medium and in the cells was counted separately. The counts in the medium were deemed to be in the reversible compartment in the absence (white triangles) or presence (black triangles) of CETP, and the cell-associated counts were deemed to be in the irreversible compartment in the absence (white circles) or presence (black circles) of CETP. The protein in the wells was not measured directly but was measured in separate, identical wells after the six washes in HBSS, 25 mM Hepes, pH 7.45. Each point shows the mean of four measurements, and the SE, which lies within the symbol in some cases, is shown.

ible compartment) (**Fig. 7**). CETP also resulted in a significant (51%) increase in the reversible compartment at the 4 h time point (according to a paired, two-tailed *t*-test: $P = 0.006$). The simplest explanation for this observation is that CETP mediates the transfer of CE into the reversible compartment but the CE does not accumulate appreciably in this compartment because it is rapidly transferred to the irreversible compartment. However, whether the reversible compartment represents the plasma membrane or a recycling compartment cannot be determined from this experiment.

To confirm that CE is first transferred to the reversible compartment and that this compartment is the plasma membrane, [^{3}H]CE cell incorporation was measured in the presence or absence of CETP using monensin to block clathrin-mediated endocytosis and recycling (34, 35). The efficacy of monensin was confirmed when it reduced the internalization of [^{125}I]α_2M to the levels seen in the presence of RAP, indicating that α_2M internalization was abolished (**Fig. 8A**). Monensin reduced the cellular incorporation of HDL_3-CE by ~30% (Fig. 8B), and this has been attributed to blocking LRP-dependent selective uptake (40). However, it is clear that monensin does not inhibit the CETP-mediated cellular incorporation of [^{3}H]CE, which suggests that CETP mediates the transfer of CE into the plasma membrane. To exclude other forms of internalization that may not be inhibited by monensin but that would undoubtedly require cellular energy, the SW872 cells were incubated in energy-depleting medium (containing 2-deoxyglucose and NaN_3). As a control for energy depletion, the [^{125}I]α_2M cell association in energy-depleting medium at 37°C was reduced to the same level seen in the presence of RAP (**Fig. 9A**), confirming that energy depletion blocked LRP internalization. As with monensin treatment, energy depletion did not inhibit the CETP-mediated cellular incorporation of [^{3}H]CE (Fig. 9B), confirming that CETP mediates the transfer of CE to the plasma membrane. These results establish that CETP does not mediate selective uptake by first transferring CE to a lipoprotein that is subsequently internalized by a receptor, irrespective of the nature of the lipoprotein or the receptor.

If CETP mediates the formation of a ternary collision complex between the plasma membrane and HDL, it should increase HDL binding to the cell surface. When [^{125}I]apoA-I-labeled HDL_3 (at concentrations up to 10 μg/ml) was incubated with SW872 cells at 4°C, cell surface binding was no different in the absence or presence of 1 μg/ml CETP (data not shown). To eliminate the possibility that the binding was temperature sensitive, the cell surface binding of [^{125}I]apoA-I-labeled HDL_3 was also measured as described above but at 37°C in energy-depleted cells (incubated with 20 mM 2-deoxyglucose and 5 mM NaN_3). Once again, CETP did not influence the cell surface binding of HDL_3 (data not shown). These observations therefore do not support a model in which CETP promotes a stable complex between HDL and the plasma membrane.

Comparisons between CETP-mediated transfer between lipoproteins and between HDL and cells

In addition to CE, CETP mediates the transfer of TG and PL between lipoproteins, possibly by shuttling these lipids between donor and acceptor lipoproteins (1, 2). If the mechanism of CE transfer between HDL and the plasma membrane is analogous to the transfer between HDL and other lipoproteins such as LDL, one might ex-

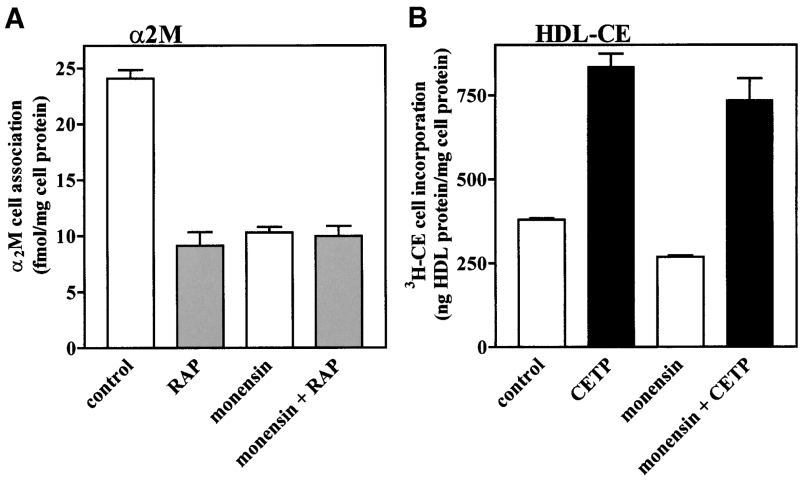

Fig. 8. Effect of monensin on CETP-mediated cellular incorporation of HDL_3-CE. SW872 cells were incubated for 30 min at 37°C in ligand buffer containing 1% ethanol (control) or 10 μM monensin in the same medium. The cells were then incubated for 4 h at 37°C in the same control or monensin-containing medium with the following additions: 1 μg/ml [^{125}I]α_2-macroglobulin ([^{125}I]α_2M) and 25 μg/ml RAP (gray bars) or without RAP (white bars) (A); or 5 μg/ml [^{3}H]CE-labeled HDL_3 and 0.48 μg/ml CETP (black bars) or without CETP (white bars) (B). After the incubation, the cells were washed and solubilized in 0.2 M NaOH, and the cell-associated radioactivity and cellular protein were measured. Each point shows the mean of six measurements, and the SE is shown.

pect CETP to mediate the selective uptake of TG and PL as well as CE. To address this question, the same batch of HDL was labeled with [^{3}H]CE, [^{3}H]TG, or [^{14}C]PL, and unlabeled lipids were included to ensure that each labeled lipoprotein was chemically identical. CETP mediated the selective uptake of [^{3}H]CE, [^{3}H]TG, and [^{14}C]PL from HDL (**Fig. 10**). The selective uptake of [^{3}H]CE was fully saturated above 3 μg/ml, confirming our earlier conclusion that some specific site or process conferred by the cell must limit the CETP-mediated selective uptake. The CETP-mediated cellular incorporation of [^{3}H]TG was similar, in magnitude and saturation kinetics, to that for [^{3}H]CE, suggesting that both of these core lipids are treated equivalently. In contrast, the T_{max} for CETP-mediated uptake of [^{14}C]PL was ~6-fold higher compared with the uptake of [^{3}H]CE (180 vs. 29 ng HDL protein/mg cell protein/h). It is known that the selectivity for CETP-mediated transfer between lipoproteins may be determined by the availability of the lipids on the lipoprotein surface (45). We confirmed that CETP was able to mediate CE, TG, and PL transfer to LDL using the same buffer that was used for the selective uptake assay (Fig. 10D). In this assay, CETP mediated [^{14}C]PL transfer from HDL to LDL 1.6-fold more efficiently compared with the transfer of [^{3}H]CE.

DISCUSSION

We have confirmed that CETP mediates the selective uptake of CE into adipocytes. We have demonstrated previously (18) that CETP-mediated uptake of HDL-derived CE into adipocytes represents a net transfer and not merely an equimolar exchange between the lipoprotein and the cells. In addition to CETP, several diverse molecules mediate selective uptake in adipocytes. In mouse and human adipocytes, the LRP (with apoE as a comediator) contributes 35–40% of the selective uptake (26). In mouse adipocytes, SR-BI contributes to ~30% of selective uptake at 5 μg/ml HDL (Fig. 5). In primary human adipocytes, a selective uptake pathway was evident with a T_{max} of 59 ng HDL protein/mg cell protein/h and a K_m of 11 μg/ml HDL_3 (Fig. 3). The K_m for this site is similar to that reported for HDL binding to SR-BI (31), and if this site is actually SR-BI, then we can calculate from Fig. 3 that SR-BI contributes ~13% of the CE selective uptake at high HDL concentrations. In addition to these molecules, lipoprotein lipase is expressed by adipocytes and is able to mediate selective uptake (26), but we have not measured its contribution at various concentrations of HDL. In primary adipocytes, CETP mediates selective uptake with a T_{max} of 68 ng HDL protein/mg cell protein/h and a K_m of

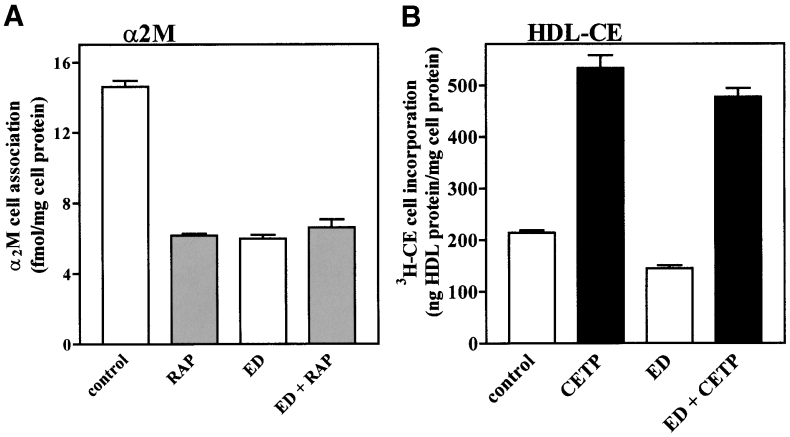

Fig. 9. Effect of cellular energy depletion on CETP-mediated cellular incorporation of HDL_3-CE. SW872 cells were incubated for 30 min at 37°C in ligand buffer or energy depletion (ED) medium (ligand buffer containing 20 mM 2-deoxyglucose and 5 mM NaN_3). The cells were then incubated for 4 h at 37°C in ligand buffer or ED medium with the following additions: 1 μg/ml [^{125}I]α_2M and 25 μg/ml RAP (gray bars) or without RAP (white bars) (A); or 5 μg/ml [^{3}H]CE-labeled HDL_3 and 0.48 μg/ml CETP (black bars) or without CETP (white bars) (B). After the incubation, the cells were washed and solubilized in 0.2 M NaOH, and the cell-associated radioactivity and cellular protein were measured. Each point shows the mean of six measurements, and the SE is shown.

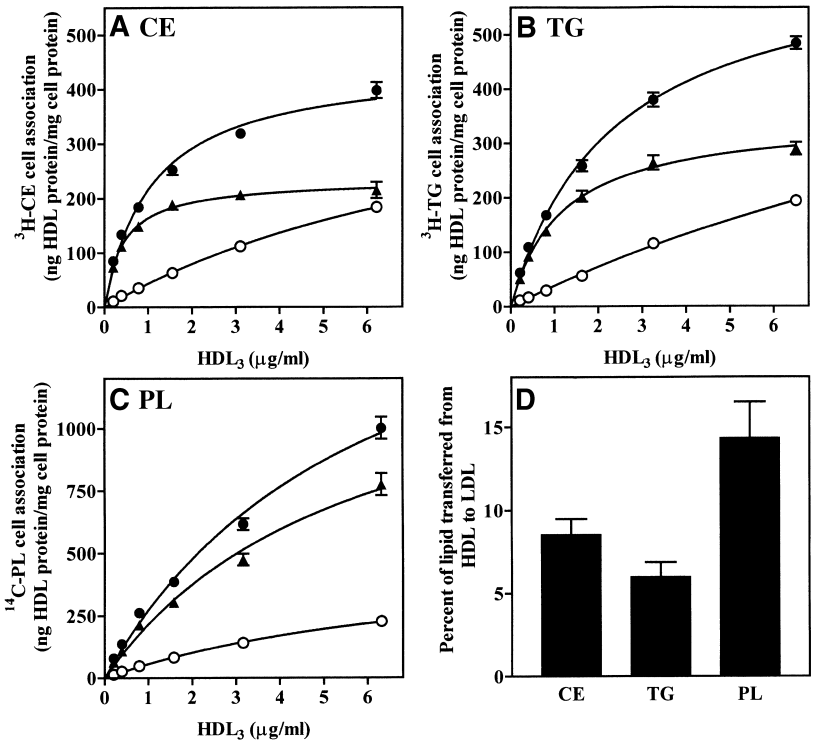

Fig. 10. CETP-mediated cellular incorporation of HDL_3-derived CE, triacylglycerol (TG), and phospholipid (PL). SW872 cells were incubated at 37°C for 8 h with 5 μg/ml HDL_3 labeled with [^{3}H]CE (A), [^{3}H]TG (B), or [^{14}C]PL (C), in each case with 0.48 μg/ml CETP (black circles) or without CETP (white circles). CETP-specific lipid incorporation (black triangles) was the lipid incorporation in the absence of CETP subtracted from the lipid incorporation in the presence of CETP. The ability of CETP to mediate the transfer of [^{3}H]CE, [^{3}H]TG, or [^{14}C]PL from HDL_3 to LDL (D) was determined as described in Experimental Procedures. The transferred lipids are plotted as a percentage of the total starting radioactivity in the radiolabeled HDL_3. Background transfer was measured in the absence of CETP, and this value has been subtracted from the measurements. In all panels, each value plotted is the mean of four measurements, and the SE is shown. This experiment was repeated entirely with essentially the same results.

1.3 μg/ml HDL_3 (Fig. 3), and we can calculate that at high concentrations of HDL, CETP contributes ~14% of selective uptake. Although this value is modest, it is misleading to base the contribution of CETP on the amount or proportion of CE that is incorporated. CE taken up by different pathways is likely to undergo different fates of hydrolysis and may be stored in distinct intracellular organelles, which in turn may have important functional consequences for the cell.

We have demonstrated that SR-BI is not required for CETP-mediated uptake; therefore, our observations are not explained by the remodeling of HDL followed by enhanced SR-BI-mediated selective uptake. Furthermore, CETP-mediated selective uptake was observed after 15 min, and although it is not apparent from the graph, it was also observed after just 7.5 min (Fig. 6). If remodeling of HDL_3 was necessary, we might expect to see some delay before CETP-mediated selective uptake was evident. Most importantly, we found that CETP transfers CE into the plasma membrane in a process that does not require cellular energy, contrary to the model (16, 17) that CE is first transferred by CETP to a lipoprotein that subsequently undergoes internalization.

We propose two models to explain CETP-mediated selective uptake of CE, TG, and PL. First, given the diversity of lipoproteins and lipid vesicles between which CETP can mediate lipid transfer (1, 2), it is tempting to speculate that CETP may mediate selective uptake by shuttling CE from HDL to the plasma membrane. Our observation that the CETP-mediated transfer of CE saturates when neither CETP nor HDL_3 is limiting suggests that this transfer must be limited in some way by the cell. One possibility is that this process requires another protein on the cell surface. Alternatively, CETP may transfer lipids to particular membrane structures such as microvilli or protrusions (46) that have a high curvature and therefore may, at a molecular level, be comparable to a lipoprotein. The transfer activity of other lipid transfer proteins (PL transfer protein and phosphatidylinositol transfer protein) has been positively correlated with high bilayer curvature (47, 48), but data for CETP are lacking.

According to a second model, CETP may transfer lipid to the plasma membrane by mediating the transient fusion of the HDL_3 amphipathic coat with the membrane outer leaflet. This would create a membrane bridge through which CE and TG can traverse from the lipopro-

tein core into the plasma membrane. The HDL could then dissociate from the membrane. CETP contains a C-terminal peptide that has a tilted orientation relative to the lipid-water interface, and this peptide has fusogenic properties similar to those of viral fusion peptides in a lipid-mixing assay (49). In the case of CETP, the C-terminal tail is suggested to be important for disrupting the lipoprotein surface to render CE accessible (50). Nevertheless, CETP has been proposed to mediate lipoprotein fusion, and this has been demonstrated under certain circumstances (51). The C terminus of CETP is required for selective uptake because TP2, an antibody that binds this region, was inhibitory (18). Our observation that CETP-mediated PL transfer was more efficient than the transfer of core lipids supports this model, in which CETP mediates the fusion of the lipoprotein amphipathic coat with the plasma membrane. However, this model also predicts that CETP would increase the cell surface binding of [^{125}I]apoA-I-labeled HDL_3 (which we did not observe) unless the dissociation of the HDL is very efficient. Further work will be required to dissect the molecular mechanisms of CETP-mediated selective uptake, although these two models are not mutually exclusive.

CETP is a crucial component of reverse cholesterol transport (2, 4). Cholesterol extracted by efflux from peripheral tissues is esterified within HDL by lecithin:cholesterol acyltransferase. This CE is subsequently transferred to apoB-containing lipoproteins that are taken up and catabolized by the liver. However, this study and our previous study (18) indicate that in addition to this reverse cholesterol transport pathway, CETP transfers some HDL-CE, derived from peripheral tissues, to adipocytes for storage. In particular, cholesterol from cells such as macrophages may ultimately be transferred by CETP to adipocytes in which the cholesterol is safely sequestered. This "lateral" cholesterol transport may be a significant antiatherosclerotic pathway given that adipocytes synthesize and secrete CETP (52). In this respect, it will be important to determine whether drugs that are used to inhibit CETP transfer activity alter selective uptake.

The authors thank Nihan Gul Kavaslar, Paulina Lau, and Thet Naing for excellent technical assistance. The authors also thank Ross Milne for helpful discussions and Jim Burgess and Yves Marcel for critically reviewing the manuscript. R.M. was supported by Canadian Institutes for Health Research (CIHR) Grant MOP-44360 and a CIHR/Wyeth-Ayerst Chair in Cardiovascular Disease.

REFERENCES

1. Barter, P., and K. A. Rye. 1994. Cholesteryl ester transfer protein: its role in plasma lipid transport. *Clin. Exp. Pharmacol. Physiol.* **21:** 663–672.
2. Tall, A. 1995. Plasma lipid transfer proteins. *Annu. Rev. Biochem.* **64:** 235–257.
3. Albers, J. J., J. H. Tollefson, C. H. Chen, and A. Steinmetz. 1984. Isolation and characterization of human plasma lipid transfer proteins. *Arteriosclerosis.* **4:** 49–58.
4. Bruce, C., R. A. Chouinard, Jr., and A. R. Tall. 1998. Plasma lipid transfer proteins, high-density lipoproteins, and reverse cholesterol transport. *Annu. Rev. Nutr.* **18:** 297–330.
5. Marotti, K. R., C. K. Castle, T. P. Boyle, A. H. Lin, R. W. Murray, and G. W. Melchior. 1993. Severe atherosclerosis in transgenic mice expressing simian cholesteryl ester transfer protein. *Nature.* **364:** 73–75.
6. Hashimoto, S., R. E. Morton, and D. B. Zilversmit. 1984. Facilitated transfer of cholesteryl ester between rough and smooth microsomal membranes by plasma lipid transfer protein. *Biochem. Biophys. Res. Commun.* **120:** 586–592.
7. Ravnik, S. E., J. J. Albers, and C. H. Muller. 1993. A novel view of albumin-supported sperm capacitation: role of lipid transfer protein-I. *Fertil. Steril.* **59:** 629–638.
8. Ruiz-Noriega, M., I. Silva-Cardenas, B. Delgado-Coello, A. Zentella-Dehesa, and J. Mas-Oliva. 1994. Membrane bound CETP mediates the transfer of free cholesterol between lipoproteins and membranes. *Biochem. Biophys. Res. Commun.* **202:** 1322–1328.
9. Stein, Y., O. Stein, T. Olivecrona, and G. Halperin. 1985. Putative role of cholesteryl ester transfer protein in removal of cholesteryl ester from vascular interstitium, studied in a model system in cell culture. *Biochim. Biophys. Acta.* **834:** 336–345.
10. Stein, O., G. Halperin, and Y. Stein. 1986. Cholesteryl ester efflux from extracellular and cellular elements of the arterial wall. Model systems in culture with cholesteryl linoleyl ether. *Arteriosclerosis.* **6:** 70–78.
11. Granot, E., I. Tabas, and A. R. Tall. 1987. Human plasma cholesteryl ester transfer protein enhances the transfer of cholesteryl ester from high density lipoproteins into cultured HepG2 cells. *J. Biol. Chem.* **262:** 3482–3487.
12. Stein, Y., Y. Dabach, G. Hollander, G. Halperin, and O. Stein. 1983. Metabolism of HDL-cholesteryl ester in the rat, studied with a non-hydrolyzable analog, cholesteryl linoleyl ether. *Biochim. Biophys. Acta.* **752:** 98–105.
13. Glass, C., R. C. Pittman, D. B. Weinstein, and D. Steinberg. 1983. Dissociation of tissue uptake of cholesterol ester from that of apoprotein A-I of rat plasma high density lipoprotein: selective delivery of cholesterol ester to liver, adrenal, and gonad. *Proc. Natl. Acad. Sci. USA.* **80:** 5435–5439.
14. Rigotti, A., B. Trigatti, J. Babitt, M. Penman, S. Xu, and M. Krieger. 1997. Scavenger receptor BI—a cell surface receptor for high density lipoprotein. *Curr. Opin. Lipidol.* **8:** 181–188.
15. Krieger, M. 1998. The "best" of cholesterols, the "worst" of cholesterols: a tale of two receptors. *Proc. Natl. Acad. Sci. USA.* **95:** 4077–4080.
16. Rinninger, F., and R. C. Pittman. 1989. Mechanism of the cholesteryl ester transfer protein-mediated uptake of high density lipoprotein cholesteryl esters by Hep G2 cells. *J. Biol. Chem.* **264:** 6111–6118.
17. Francis, G. A., K. W. Ko, H. Hara, and S. Yokoyama. 1991. Regulation of the uptake of high-density lipoprotein-originated cholesteryl ester by HepG2 cells: role of low-density lipoprotein and plasma lipid transfer protein. *Biochim. Biophys. Acta.* **1084:** 159–166.
18. Benoist, F., P. Lau, M. McDonnell, H. Doelle, R. Milne, and R. McPherson. 1997. Cholesteryl ester transfer protein mediates selective uptake of high density lipoprotein cholesteryl esters by human adipose tissue. *J. Biol. Chem.* **272:** 23572–23577.
19. Collet, X., A. R. Tall, H. Serajuddin, K. Guendouzi, L. Royer, H. Oliveira, R. Barbaras, X. C. Jiang, and O. L. Francone. 1999. 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.* **40:** 1185–1193.
20. Acton, S. L., P. E. Scherer, H. F. Lodish, and M. Krieger. 1994. Expression cloning of SR-BI, a CD36-related class B scavenger receptor. *J. Biol. Chem.* **269:** 21003–21009.
21. Landschulz, K. T., R. K. Pathak, A. Rigotti, M. Krieger, and H. H. Hobbs. 1996. Regulation of scavenger receptor, class B, type I, a high density lipoprotein receptor, in liver and steroidogenic tissues of the rat. *J. Clin. Invest.* **98:** 984–995.
22. Wang, N., W. Weng, J. L. Breslow, and A. R. Tall. 1996. Scavenger receptor BI (SR-BI) is up-regulated in adrenal gland in apolipoprotein A-I and hepatic lipase knock-out mice as a response to depletion of cholesterol stores. In vivo evidence that SR-BI is a functional high density lipoprotein receptor under feedback control. *J. Biol. Chem.* **271:** 21001–21004.
23. Rodrigueza, W. V., S. T. Thuahnai, R. E. Temel, S. Lund-Katz, M. C. Phillips, and D. L. Williams. 1999. Mechanism of scavenger recep-

tor class B type I-mediated selective uptake of cholesteryl esters from high density lipoprotein to adrenal cells. *J. Biol. Chem.* **274:** 20344–20350.
24. Silver, D. L., N. Wang, and A. R. Tall. 2000. Defective HDL particle uptake in ob/ob hepatocytes causes decreased recycling, degradation, and selective lipid uptake. *J. Clin. Invest.* **105:** 151–159.
25. Rinninger, F., T. Kaiser, W. A. Mann, N. Meyer, H. Greten, and U. Beisiegel. 1998. Lipoprotein lipase mediates an increase in the selective uptake of high density lipoprotein-associated cholesteryl esters by hepatic cells in culture. *J. Lipid Res.* **39:** 1335–1348.
26. Vassiliou, G., F. Benoist, P. Lau, G. N. Kavaslar, and R. McPherson. 2001. The low density lipoprotein receptor-related protein contributes to selective uptake of high density lipoprotein cholesteryl esters by SW872 liposarcoma cells and primary human adipocytes. *J. Biol. Chem.* **276:** 48823–48830.
27. Porath, J., J. Carlsson, I. Olsson, and G. Belfrage. 1975. Metal chelate affinity chromatography, a new approach to protein fractionation. *Nature.* **258:** 598–599.
28. Vassiliou, G., and K. K. Stanley. 1994. Exogenous receptor-associated protein binds to two distinct sites on human fibroblasts but does not bind to the glycosaminoglycan residues of heparan sulfate proteoglycans. *J. Biol. Chem.* **269:** 15172–15178.
29. Sattler, W., D. Mohr, and R. Stocker. 1994. Rapid isolation of lipoproteins and assessment of their peroxidation by high-performance liquid chromatography postcolumn chemiluminescence. *Methods Enzymol.* **233:** 469–489.
30. Gauthier, B., M. Robb, and R. McPherson. 1999. Cholesteryl ester transfer protein gene expression during differentiation of human preadipocytes to adipocytes in primary culture. *Atherosclerosis.* **142:** 301–307.
31. Acton, S., A. Rigotti, K. T. Landschulz, S. Xu, H. H. Hobbs, and M. Krieger. 1996. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science.* **271:** 518–520.
32. Goldstein, J. L., S. K. Basu, and M. S. Brown. 1983. Receptor-mediated endocytosis of low-density lipoprotein in cultured cells. *Methods Enzymol.* **98:** 241–260.
33. Hao, M., S. X. Lin, O. J. Karylowski, D. Wustner, T. E. McGraw, and F. R. Maxfield. 2002. Vesicular and non-vesicular sterol transport in living cells. The endocytic recycling compartment is a major sterol storage organelle. *J. Biol. Chem.* **277:** 609–617.
34. Basu, S. K., J. L. Goldstein, R. G. Anderson, and M. S. Brown. 1981. Monensin interrupts the recycling of low density lipoprotein receptors in human fibroblasts. *Cell.* **24:** 493–502.
35. Steer, C. J., and R. D. Klausner. 1983. Clathrin-coated pits and coated vesicles: functional and structural studies. *Hepatology.* **3:** 437–454.
36. Baeuerle, P. A., and W. B. Huttner. 1986. Chlorate—a potent inhibitor of protein sulfation in intact cells. *Biochem. Biophys. Res. Commun.* **141:** 870–877.
37. Lucas, M., and T. Mazzone. 1996. Cell surface proteoglycans modulate net synthesis and secretion of macrophage apolipoprotein E. *J. Biol. Chem.* **271:** 13454–13460.
38. Gliemann, J. 1998. Receptors of the low density lipoprotein (LDL) receptor family in man. Multiple functions of the large family members via interaction with complex ligands. *Biol. Chem.* **379:** 951–964.
39. Willnow, T. E., A. Nykjaer, and J. Herz. 1999. Lipoprotein receptors: new roles for ancient proteins. *Nat. Cell Biol.* **1:** E157–E162.
40. Vassiliou, G., and R. McPherson. 2004. A novel "efflux-recapture" process underlies the mechanism of high density lipoprotein-cholesteryl ester selective uptake by the low-density lipoprotein receptor-related protein. *Arterioscler. Thromb. Vasc. Biol.* In press.
41. Goldstein, J. L., S. K. Basu, G. Y. Brunschede, and M. S. Brown. 1976. Release of low density lipoprotein from its cell surface receptor by sulfated glycosaminoglycans. *Cell.* **7:** 85–95.
42. Vassiliou, G. 1997. Pharmacological concentrations of suramin inhibit the binding of alpha2-macroglobulin to its cell-surface receptor. *Eur. J. Biochem.* **250:** 320–325.
43. Rinninger, F., and R. C. Pittman. 1987. Regulation of the selective uptake of high density lipoprotein-associated cholesteryl esters. *J. Lipid Res.* **28:** 1313–1325.
44. Knecht, T. P., and R. C. Pittman. 1989. A plasma membrane pool of cholesteryl esters that may mediate the selective uptake of cholesteryl esters from high-density lipoproteins. *Biochim. Biophys. Acta.* **1002:** 365–375.
45. Morton, R. E., and D. J. Greene. 2003. The surface cholesteryl ester content of donor and acceptor particles regulates CETP: a liposome-based approach to assess the substrate properties of lipoproteins. *J. Lipid Res.* **44:** 1364–1372.
46. Drobnik, W., H. Borsukova, A. Bottcher, A. Pfeiffer, G. Liebisch, G. J. Schutz, H. Schindler, and G. Schmitz. 2002. Apo AI/ABCA1-dependent and HDL3-mediated lipid efflux from compositionally distinct cholesterol-based microdomains. *Traffic.* **3:** 268–278.
47. Sweeny, S. A., and A. Jonas. 1985. Substrate specificity of human plasma phospholipid transfer protein. *Biochim. Biophys. Acta.* **835:** 279–290.
48. Komatsu, H., B. Bouma, K. W. Wirtz, T. F. Taraschi, and N. Janes. 2000. Activity of phosphatidylinositol transfer protein is sensitive to ethanol and membrane curvature. *Biochem. J.* **348:** 667–673.
49. Brasseur, R., T. Pillot, L. Lins, J. Vandekerckhove, and M. Rosseneu. 1997. Peptides in membranes: tipping the balance of membrane stability. *Trends Biochem. Sci.* **22:** 167–171.
50. Wang, S., X. Wang, L. Deng, E. Rassart, R. W. Milne, and A. R. Tall. 1993. Point mutagenesis of carboxyl-terminal amino acids of cholesteryl ester transfer protein. Opposite faces of an amphipathic helix important for cholesteryl ester transfer or for binding neutralizing antibody. *J. Biol. Chem.* **268:** 1955–1959.
51. Rye, K. A., N. J. Hime, and P. J. Barter. 1997. Evidence that cholesteryl ester transfer protein-mediated reductions in reconstituted high density lipoprotein size involve particle fusion. *J. Biol. Chem.* **272:** 3953–3960.
52. Jiang, X. C., P. Moulin, E. Quinet, I. J. Goldberg, L. K. Yacoub, L. B. Agellon, D. Compton, R. Schnitzer-Polokoff, and A. R. Tall. 1991. Mammalian adipose tissue and muscle are major sources of lipid transfer protein mRNA. *J. Biol. Chem.* **266:** 4631–4639.