Elevated Triglyceride Content Diminishes the Capacity of High Density Lipoprotein to Deliver Cholesteryl Esters via the Scavenger Receptor Class B Type I (SR-BI)*

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The selective uptake of high density lipoprotein (HDL) cholesteryl ester (CE) by the scavenger receptor class B type I (SR-BI) is well documented. However, the effect of altered HDL composition, such as occurs in hyperlipidemia, on this important process is not known. This study investigated the impact of variable CE and triglyceride (TG) content on selective uptake. CE selective uptake by Y1 and HepG2 cells was strongly affected by modification of either the CE or TG content of HDL. Importantly, TG, like CE, was selectively taken up by a dose-dependent, saturable process in these cells. As shown by ACTH up-regulation and receptor overexpression experiments, SR-BI mediated the selective uptake of both CE and TG. With in vitro modified HDLs of varying CE and TG composition, the selective uptake of CE and TG was dependent on the abundance of each lipid within the HDL particle. Furthermore, total selective uptake (CE + TG) remained constant, indicating that these lipids competed for cellular uptake. These data support a novel mechanism whereby SR-BI binds HDL and mediates the incorporation of a nonspecific portion of the HDL lipid core. In this way, TG directly affects the ability of HDL to donate CE to cells. Processes that raise the TG/CE ratio of HDL will impair the delivery of CE to cells via this receptor and may compromise the efficiency of sterol balancing pathways such as reverse cholesterol transport.

High density lipoprotein (HDL) exists in plasma as several distinct species defined by their size and density properties. Although synthesized initially as CE- and TG-deficient discs, HDL rapidly becomes spherical as it acquires these core lipids (1, 2). The transition to spherical particles and the repetitive cycling of individual HDL species among the various size/density subfractions are mediated in plasma by the actions of LCAT, CETP, phospholipid transfer protein, and hepatic lipase (2–6). Together these factors control the levels of HDL CE and TG, which directly influence the subtraction content of the HDL pool.

HDL plays an important role in sterol metabolism. In non-steroidogenic tissues, cholesterol homeostasis is maintained through the capacity of HDL to promote the net removal of free cholesterol from cells. HDL-associated free cholesterol is converted to CE through the action of LCAT (3). CE is in turn delivered to the liver for excretion. The essential role of HDL in this process, known as reverse cholesterol transport, is thought to explain, at least in part, the epidemiologic evidence that HDL is anti-atherogenic (7, 8). In steroidogenic tissues, the delivery of HDL cholesterol to cells is essential to the maintenance of normal hormone biosynthesis (9).

The uptake of HDL-associated cholesterol in both hepatic and steroidogenic tissues is mediated by the SR-BI receptor (10). SR-BI, most highly expressed in the liver, adrenal gland, and ovary (10, 11), is the only known receptor for HDL (10, 12, 13). The importance of SR-BI in cholesterol homeostasis is readily observed in genetically altered animals. Mice deficient in SR-BI display marked hypercholesterolemia characterized by enlarged, cholesterol-rich HDL particles, impaired HDL cholesterol clearance, and dramatically reduced sterol content of adrenal tissue (14, 15). Conversely, hepatic overexpression of SR-BI results in decreased HDL cholesterol content and increased delivery of HDL-associated lipid to hepatocytes and the bile.

Unlike the well characterized uptake of lipoproteins by receptor-mediated endocytosis (16), SR-BI facilitates a nonendocytic process known as selective uptake. In selective uptake, HDL binds to SR-BI on the cell surface, a portion of its CE is transferred to the cell without concomitant whole particle uptake, and then the CE-depleted HDL is released into the extracellular fluid (10, 17, 18). Little is known about the properties of HDL that provide optimum interaction and cholesterol delivery through SR-BI. Because binding to SR-BI does not require a specific apolipoprotein (19, 20), the variations in apoprotein content of HDL subfractions are not likely to influence markedly their substrate potential. Particle size has been suggested to be an important determinant of selective uptake, but two studies with synthetic HDL particles containing CE as their only core lipid have given contradictory results (18, 19).

However, unlike these artificial lipoproteins, physiologic and pathophysiologic changes in human HDL particle size are accompanied by alterations in both the absolute level of CE within the particle and the relative amounts of CE compared with the other major core lipid, TG. Although TG has been reported to be a major regulator of cholesterol esterification and reverse cholesterol transport (21), the influence of HDL TG content on the capacity of HDL to donate CE via SR-BI is

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1 The abbreviations used are: HDL, high density lipoprotein; CETP, cholesteryl ester transfer protein; LCAT, lecithin:cholesterol acyltransferase; VLDL, very low density lipoprotein; CE, cholesteryl ester; COE, cholesteryl oleyl ether; TG, triacylglyceride; TGE, trialkylglycerol ether; BSA, bovine serum albumin; DME medium, Dulbecco’s modified Eagle’s medium; PBS, phosphate-buffered saline; ACTH, adrenocorticotropic hormone; SR-BI, scavenger receptor class B type I.

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unknown. In this study we have examined the influence of HDL core lipid (CE and TG) composition on CE selective uptake in cultured hepatocytes and adrenal cells. The findings shed light on the selective uptake mechanism and suggest that the beneficial functions of HDL may be compromised under conditions of excess HDL TG.

**EXPERIMENTAL PROCEDURES**

**Materials**

[9,10-3H]Triolein (15 Ci/mmol) and carrier-free 125I in NaOH were purchased from PerkinElmer Life Sciences. [1,2-3H]Cholesteryl oleyl ether ([H-COE, 49 Ci/mmol] was purchased from Amersham Pharma- cia Biotech. [H]-Labeled trialkylglycerol ether ([H-TGE], a triether analogue of TG, was synthesized from 3H-glycerol (1(3)-[3H]glycerol, 3 Ci/mmol), ACTH, egg phosphatidylcholine, sodium cholate, trichloroacetic acid (Costa Mesa, CA), and cholesterol was from Nu-Chek Prep. BSA (fraction V), from ICN) for that 37 ° C. Lipolytic activity was determined as previously reported (49). In this case, specific activities ranged from 18.9 to 68.7 cpm/ng of CE for [H-COE] and 17.1 to 57.5 cpm/ng of TG for [H-TG].

In some instances, [H-COE] or [H-TG]-labeled HDL was also labeled with the nondegradable conjugate [125I]-tyramine-cellobiose (28). The tyramine cellobiose was first iodinated using the iodogen reagent (Pierce). The labeled adduct was then conjugated to HDL protein with cyanuric chloride. The specific activities of the HDL ranged from 4.7 to 24.3 cpm/106 ng protein. Alternatively, the proteins of [H-COE] or [H-TG]-labeled HDL were labeled directly with 125I using the iodine monochloride method of Bilheimer et al. (29). In this case, specific activities ranged from 147 to 270 cpm/125I/ng of protein.

**Preparation of Liposomes and Synthesis of Radiolabeled HDL**

Phospholipid/cholesterol liposomes containing either 50 µCi of [H-COE] or [H-TG] were prepared by a modification (26) of the cholate dialysis method of Brunner et al. (27). To radiolabel native or modified HDL, lipoproteins were incubated with either liposome preparation (4 cpm/ng of protein) in the presence of excess CETP and 0.5% BSA at 37 ° C for 18 h. HDL was re-isolated within its original density limits and dialyzed as described above. Final specific activities ranged from 18.9 to 68.7 cpm/ng of CE for [H-COE] and 17.1 to 57.5 cpm/ng of TG for [H-TG].

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**Selective Uptake of CE and TG by Cultured Cells**

Y1 and HepG2 cells were obtained from American Type Culture Collection (Manassas, VA) and maintained in DME/F12 medium containing 10% fetal bovine serum and penicillin/streptomycin (100 units/ml, 100 µg/ml, respectively) at 37 ° C in a 5% CO2 environment. For experiments, cells were seeded in 12-well plates and grown to 80% confluence. Y1 adrenal cells were pretreated for 18 h with 0.1 µM ACTH in DME/F12 media containing 5% human lipoprotein-deficient plasma to up-regulate steroidogenesis (30). Two different approaches were taken to assess the selective uptake of cholesteryl esters and triglycerides from HDL. In general, these approaches follow the methods described by Pittman et al. (18) and Rodriguez et al. (31).

**Procedure 1—**Cells were incubated with double-labeled HDL ([H-COE]125I, [H-TG]-labeled HDL, 1 mg protein) prepared as described below, or assayd for protein, total cholest erol, free cholesterol, TG, and phospholipid content as described above. CE was calculated as the difference between total and free cholesterol times 1.69 to correct for its fatty acid content.

In addition to CETP modification of HDL composition, the levels of TG and CE were altered independently. To enhance CE content, fresh human plasma was incubated for 18 h at 37 ° C, allowing LCAT to act on the lipoproteins and thus increasing their CE concentration. Control plasma was incubated similarly but received paraaxon to block the action of LCAT. HDL was then isolated by ultracentrifugation and its lipid constituents assayed as described below. To reduce the TG content of HDL, doubly labeled HDL ([H-COE]/[125I]-HDL, 1 mg protein), prepared as described below, was treated with 30 units of lipoprotein lipase (Pseudomonas, from ICN) for 2 h at 37 ° C. Lipolytic activity was stopped by the addition of paraaxon (1 mM); control HDL received paraaxon but no lipase. The extent of the lipolytic effect was determined from the degradation of identically treated [H-TG]-labeled HDL. Treatment with this bacterial lipase did not affect the phospholipid content of the HDL.

The particle size of native and modified HDL was determined using nondenaturing polyacrylamide gel electrophoresis on 4-30% gradient gels (Isolab, Inc., Akron, OH) as previously described (25). Gels were stained with colloidal Coomassie Blue G-250 (Gradiopore LTD, Sydney, NS, Australia). Images were captured using a ScanMaker III scanner (Microtek Lab Inc., Redondo Beach, CA), and bands were quantitated by analysis with NIH Image software (version 1.6). Thryoglobulin was added as an internal standard to each sample. The lipoprotein particle size was determined as previously reported (25) with high molecular weight standards (Amersham Pharmacia Biotech). HDLs were assigned to subfractions based on the criteria reported by Nichols et al. (25).
and 125I-labeled HDLs of identical chemical composition. In this instance, following incubation and rigorous washing of cells as described above, aliquots of cell lysate were counted directly to determine lipid (3H) or protein (125I) uptake. Post-incubation medium was also collected from 125I-HDL-treated cells, and an aliquot was precipitated with trichloroacetic acid (33) to determine the amount of 125I-protein degradation products in the medium. Total uptake of HDL protein was calculated as the sum of the 125I in the cells plus the noniodide-, nontrichloroacetic acid-precipitable 125I in the medium. Selective uptake was calculated as the difference between total lipid uptake, as determined from cellular 3H radioactivity content, and the amount of lipid incorporation because of whole particle uptake, as determined from cell-associated 125I plus 125I degradation products in the media. To minimize the contribution of low levels of radiolabeled degradation products present initially in lipoprotein preparations on calculated uptake values, cellular lipid and protein uptake were determined at both 0.5 and 5 h. Reported uptake values are equal to the difference between these two values, which reflects cellular uptake over a 4.5-hour time span. Cell protein levels were quantitated (34) and all uptake values normalized for cellular protein mass.

SR-BI Expression in COS7 Cells

Recombinant adenovirus expressing mouse SR-BI, or a recombinant virus with analogous adenoviral sequences but containing no transgene (Adnull), was prepared as previously described (35). These reagents and a protocol for their use were generously provided by Dr. Denyes van der Westhuysen (University of Kentucky Medical Center). COS7 cells were grown in 12-well plates containing DMEM medium with 10% fetal bovine serum and penicillin/streptomycin (100 units/ml, 100 μg/ml, respectively) until ~80% confluent (~0.8 × 10^6 cells/well). Adenovirus, stored at −80 °C, was thawed on ice, diluted in DMEM medium to 8 × 10^6 plaque-forming units/ml, and filtered (0.45 μm), and 1 ml was added per well. After 24 h, the treatment medium was removed and cells were incubated for 0.5 or 5 h with radiolabeled HDL as described above. TG and CE uptake was determined as detailed above, and SR-BI protein levels were determined by Western blot using rabbit anti-mouse SR-BI antiserum (35).

Analytical Methods

Protein was quantitated by the method of Lowry et al. (36) as modified by Peterson (34) with BSA as standard. Lipoprotein cholesterol levels were determined by Western blot using rabbit anti-mouse SR-BI antiserum (35).

**FIG. 1.** Separation of HDL subclasses by nondenaturing polyacrylamide gradient gel electrophoresis. HDL subpopulations were separated on 4–30% gradient gels as described under "Experimental Procedures." Numbers represent the relative percentage of each subpopulation contained within the HDL fraction. Subpopulations are noted by vertical dashed lines. **A**, native HDL. **B**, HDL modified with VLDL and CETP for 24 h.

**FIG. 2.** Dose-dependent uptake of cholesterol ester from native HDL or CE-depleted HDL in Y1 and HepG2 cells. HDL were depleted of CE by incubation with CETP and VLDL as described under "Experimental Procedures." Native and modified HDLs were doubly labeled with 3H-COE and 125I-tyramine-cellobiose. A and C, total CE (3H-COE) uptake from native HDL (CE/Prot = 0.32) (closed squares) and from two different CETP-treated HDLs (CE/Prot = 0.29 (closed circles) or CE/Prot = 0.19 (closed triangles)). CE uptake attributable to whole HDL uptake is shown by the respective open symbols. B and D, selective CE uptake calculated as the difference between total CE uptake and whole HDL uptake. Results are the mean ± S.D. and are representative of four experiments each for Y1 and HepG2 cells.
was assayed by a colorimetric, enzymatic method using a Cholesterol 100 reagent kit (Sigma). Free cholesterol was determined by a free cholesterol kit from Wako Diagnostics (Richmond, VA). Triglyceride was assayed using the GPO-Trinder reagent (Sigma) with glycerol as standard. Lipid phosphorus was determined by the method of Bartlett (37); phospholipid mass was calculated assuming an average molecular weight of 800.

RESULTS

HDL Modification—Plasma lipoproteins undergo continual remodeling of their composition during their circulatory lifetime. It is widely recognized that CETP remodels HDL in vivo through a heteroexchange reaction in which TG from triglyceride-rich lipoproteins is exchanged for HDL CE. The effect of this modification on the ability of HDL to donate CE to the SR-BI receptor was studied herein. When incubated with CETP and VLDL as a source of TG, HDL was enriched in TG and depleted of CE in a time-dependent manner (Table I). HDL, initially containing predominately CE in its core, became a TG-rich lipoprotein with the mole ratio of TG to CE exceeding 1 at longer incubation times. This remodeling of HDL composition was virtually limited to modification of CE and TG content. Free cholesterol (FC) and phospholipid (PL) increased only modestly, whereas the FC/PL ratio of modified HDL remained nearly constant (Table I). The equimolar heteroexchange of CE for the larger TG molecule (38) also modified HDL size. As shown by nondenaturing gradient gel electrophoresis, native HDL was composed of approximately equal populations of HDL3, HDL2a, and HDL2b (34.1, 37.4, and 28.5%, respectively) (Fig. 1). With TG enrichment this distribution shifted. A 2-fold decrease in the content of smaller HDL3 particles and a marked rise in the largest HDL subfraction, HDL2b, were observed. The mean HDL particle size increased from ~8.9 to ~9.7 nm after extensive CETP modification. Thus, CETP activity resulted in larger HDL particles that were CE-depleted and TG-enriched.

CE Selective Uptake from Modified HDL—The dose-dependent uptake of CE was measured with COE, a nondegradable analogue of CE, from native and modified HDL (15–60 μg of HDL protein) into Y1 adrenal cells (Fig. 2A) and HepG2 hepatocytes (Fig. 2C). Compared with that for native HDL (CE/TG = 9.1), total CE uptake from CE-depleted particles was markedly reduced and directly related to the residual CE content of the modified HDL (CE/TG = 4.2 and 3.3) in both cell types. Over this range of CE/TG compositions, although the dose dependence of CE uptake was similar regardless of the HDL substrate, CE uptake declined by 73–76% in both Y1 and HepG2 cells. The portion of total CE uptake attributed to whole HDL uptake (Fig. 2, A and C, open symbols) was small in all cases and accounted for little of the total CE incorporated by cells. Thus, the extent of CE selective uptake (Fig. 2, B and D), the difference between total uptake and whole-HDL uptake by Y1 and HepG2 cells, known to be mediated by the SR-BI receptor, was related directly to the CE content of the HDL substrate.

To further investigate the relationship between the CE content of HDL and its uptake, HDL was modified by an alternate strategy whereby HDL CE was increased through the action of the LCAT enzyme. Compared with HDL isolated in the absence of LCAT modification, LCAT-modified HDL was CE-enriched.
(CE/protein = 0.66 versus 0.47) but had similar TG content (TG/protein = 0.10 versus 0.09). CE-enriched HDL was more effective donors of CE via the selective uptake mechanism than control HDL (Fig. 3). In both Y1 adrenal cells (panel A) and HepG2 hepatocytes (panel B), LCAT-modified HDL was a 2-fold better donor of CE than control.

The foregoing data indicated that the capacity of HDL to donate CE to the selective uptake pathway was dependent on the CE content of HDL. However, because both CETP and LCAT modification altered the level of CE in HDL, it remained undetermined whether the relationship between CE selective uptake and HDL composition reflected a dependence on the absolute concentration of CE in HDL or on its relative abundance compared with TG. To investigate these possibilities, HDL was treated with a TG-specific bacterial lipase to selectively reduce its TG content. Despite a constant CE content, TG-deficient HDL was significantly more effective at donating CE to both Y1 and HepG2 cells than control HDL (Fig. 3, panels C and D, respectively). Hydrolysis of ~50% of the TG in HDL led to a 34 (Y1) and 73% (HepG2) increase in CE selective uptake.

**TG as a Selective Uptake Substrate**—The above detailed studies demonstrate the importance of the relative CE and TG content of HDL as a determinant of the capacity of various HDLs to donate CE to the selective uptake process. The importance of TG content, as specifically demonstrated with lipase-modified HDL, suggests one of two possible roles for HDL TG. First, HDL TG may serve as an inert diluent for CE, thus decreasing CE selective uptake simply by controlling the apparent concentration of CE. Alternatively, HDL TG may also be a substrate for selective uptake such that CE and TG compete for uptake via a common pathway.

To delineate these possibilities, the substrate potential of TG was investigated in cultured Y1 cells. Like CE, TG in native HDL was taken up in a dose-dependent fashion (Fig. 4A). The portion of TG uptake attributable to the uptake of the whole HDL particle was small, representing <10–15% of total TG uptake, indicating that most TG uptake occurred by a selective process. Thin layer chromatographic analysis of cell lysates showed that cell-associated TG was degraded extensively (>64%) to fatty acids and intermediate acylglycerols by both Y1 and HepG2 cells. The possibility that extracellular degradation products were the source of apparent TG uptake was examined using a nondegradable ether analogue of TG (TGE). Although performed with different HDL preparations, when compared with the uptake of CE from the same HDL, the kinetics of TG uptake were similar to that of the ester (Fig. 4A).

This finding suggests that TG was incorporated selectively into cells prior to degradation. In addition, within this time frame it does not appear that significant degradation products were lost from cells because the apparent uptake of TG was not increased when the extracellular fatty acid acceptor, BSA, was omitted from the culture medium (78.8% of control in Y1 cells and 95.9% of control in HepG2 cells). CE selective uptake was similarly affected by the deletion of BSA. Thus, SR-BI-expressing cells selectively incorporated both CE and TG from HDL.

The dependence of TG selective uptake on HDL concentration was similar to that for CE. The presence of a common pathway for the selective uptake of these two lipids was further supported by the finding that the addition of a 10-fold excess of unlabelled HDL to the media suppressed the selective uptake of CE and TG to similar extents (52.7 and 52.8%, respectively, for Y1 and 54.8 and 58.6%, respectively, for HepG2). That this pathway was mediated by the SR-BI receptor was supported by several observations. First, up-regulation of SR-BI on Y1 adrenal cells by pretreatment with the steroidogenic hormone

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**TABLE II**

Response of CE and TG uptake by Y1 cells to ACTH

| Expt. | ACTH | CE± | TG± | TGE± | % of Control |
|-------|------|-----|-----|------|-------------|
|       |      |     |     |      | CE          |
| 1     | −    | 330.6 ± 59.5 | 126.2 ± 12.4 | 337 | 309 |
|       | +    | 1116.4 ± 55.8 | 389.8 ± 15.6 |      |             |
| 2     | −    | 685.5 ± 168 | 215.9 ± 20.1 | 270 | 242 |
|       | +    | 1849.9 ± 51.0 | 523.4 ± 104 |      |             |

*Quantitated as ng uptake/mg cell protein.*
ACTH equally stimulated the selective uptake of both CE and TG, as well as TGE, from HDL (Table II). Additionally, the overexpression of murine SR-BI in COS7 cells, as confirmed by Western analysis (Fig. 5, inset), enhanced the selective uptake of both CE and TG to similar extents compared with control cells (Fig. 5). Collectively these data show that TG is a selective uptake substrate like CE and that the uptake of these lipids proceeds through SR-BI.

**Competition of CE and TG for Selective Uptake**—Given the observed effects of variable HDL TG/CE composition on CE selective uptake and our finding that TG is also a substrate for this process, we investigated the relationship between TG and CE selective uptake as a function of varied HDL core lipid composition. CETP-modified HDL particles were altered in their capacity to support CE and TG selective uptake. Continuous TG enrichment and CE depletion by CETP resulted in particles that were progressively more efficient donors of TG and less effective donors of CE. Over a 4-fold increase in HDL TG/CE content, TG selective uptake increased nearly 2-fold (Fig. 6A). Concurrently, as the CE content of HDL decreased, CE selective uptake decreased by almost 50%. Overall, the selective uptake of TG or CE was proportional to the mole fraction of that lipid in the HDL core (i.e. TG/CE + TG or CE/CE + TG, respectively). This resulted in a near linear relationship between the TG/CE content of the donor HDL particle and the ratio of these lipids selectively taken up by cells (Fig. 6B). The slope of this line (panel B) was near a value of 1, suggesting that CE and TG were equivalent substrates for selective uptake. Notably, the total amount of lipid selectively imported from HDL (CE + TG) remained rather constant.

Together, these data illustrate that TG and CE compete for selective uptake, with the relative rate of uptake for each lipid being defined by the relative abundance of TG and CE in the HDL core.

**DISCUSSION**

Few studies have investigated the properties of HDL that influence its capacity to donate CE to cells through the selective uptake pathway. Early work with synthetic HDL suggests that the rate of selective uptake is a function of particle size and density, with small, dense HDL particles being more effective donors of CE (19). Additionally, selective uptake has been reported to be more extensive with particles containing apoA-I (19, 39). Consistent with this finding, selective uptake of CE from endogenous lipoproteins is nearly absent in knockout mice lacking apoA-I but not in apoA-II or apoE knockout animals (40). An interpretation of this finding is complex, however, because apoE-deficient mice fail to synthesize HDL. In contrast, binding studies show that multiple apoproteins can mediate particle interaction with SR-BI (41). These different results may be explained in part by the observation that particle electronegativity is a key determinant of SR-BI binding (42, 43). Nevertheless, it is important to note that although particle binding is essential, fruitful selective uptake also requires subsequent, distinct interactions between SR-BI and the bound ligand to facilitate lipid incorporation (44). Features of HDL that influence the availability of CE for selective uptake during this second interaction are addressed in this study.

Physiologically, CETP is an important determinant of HDL core lipid composition. Its remodeling of HDL composition alters the balance of HDL subclasses and directly influences the

**Fig. 5. Effect of murine SR-BI overexpression on selective uptake.** COS7 cells were transiently transfected with empty adenovirus (Adnull) or adenovirus containing murine SR-BI (AdmSRBI), and lipid uptake from 3H-TG- or 3H-COE-labeled HDL (60 μg) was measured as described under “Experimental Procedures.” Data (mean ± S.D.) show the increase in lipid uptake over control COS7 cells not transfected. Inset, Western analysis of SR-BI levels in control and transfected COS7 cells detected with anti-mouse SR-BI. Ctl, control; Ad, Adnull; AdSR, AdmSRBI.

**Fig. 6. Competition of CE and TG for selective uptake.** Lipid uptake from CETP-modified HDL was measured as described under “Experimental Procedures” from 3H-TG- and 3H-COE-labeled HDL. A, CE (3H-COE, closed squares) and TG (closed circles) uptake as a function of the mole lipid ratio of these lipids in the donor HDL. B, ratio of TG/CE uptake, calculated from the data given in panel A, as a function of particle composition. Inset, total neutral lipid uptake (TG + CE). Results are the mean ± S.D. and are representative of five similar experiments.
functionality of the HDL fraction (4, 45, 46). In this study we have used CETP as a tool to investigate the importance of core lipid composition on the selective uptake of CE from HDL. Our data demonstrate that the neutral lipid composition of the HDL core has a direct effect on the ability of HDL to donate CE to the selective uptake pathway. As HDL is made progressively CE-deficient and TG-enriched, CE selective uptake diminishes. Although these modified HDL are increased in size, this appears to contribute little to the decline in CE selective uptake because enlarged, CE-enriched HDL, produced by LCAT modification, are also more effective donors of CE. CE selective uptake was also found to increase when HDL TG is hydrolyzed by lipase even though CE content is not affected. Taken together, these data demonstrate that HDL TG levels influence CE selective uptake.

Our observation that HDL TG and its nonhydrolyzable analogue, TGE, are selectively taken up by Y1 and HepG2 cells supports previous studies with Y1-BS1 cells in which TG was selectively taken up from reconstituted HDL (22). Like CE, TG selective uptake is mediated by SR-BI, as demonstrated by the costimulation of CE and TG selective uptake in ACTH-treated cells and by overexpression of the murine SR-BI receptor in COS7 cells. Although selectively incorporated TG is rapidly degraded by cells, the similarity between TG and TGE uptake kinetics suggests that TG degradation products are quantitatively retained, perhaps through their efficient incorporation into cellular lipids as reported by Hilaire et al. (47).

Rodrigueza et al. (31) reported that CE selective uptake is directly dependent on the concentration of CE within the HDL core, suggesting a mechanism whereby CE moves down a concentration gradient from HDL particles docked on SR-BI into the cell plasma membrane. Although this mechanism can explain some of our findings with CETP-modified HDLs, it does not appear to explain the higher transfer rates mediated by HDL that have been modified by bacterial lipase (reported here), hepatic lipase (48–50), or a combination of CETP and hepatic lipase (51), since CE in these particles is either unchanged or reduced. Our observation that TG is also a selective uptake substrate suggests that CE selective uptake may be modulated by the uptake of TG. This possibility was investigated directly with a series of HDL particles of varying TG/CE composition. Our data demonstrated that the rates of CE and TG selective uptake were directly related to the content of these lipids in the donor HDL. Even though the selective uptake of TG or CE differed by several-fold over the range of modified HDL studied, the sum of these two lipids transferred to cells remained essentially constant. This strongly suggests that TG and CE compete for transfer by SR-BI. In general, the extent of TG and CE selective uptake was the same when the mole ratio of these two lipids in HDL was near 1, indicating little preference for CE or TG in selective uptake. Also, because total lipid uptake (TG + CE) remained constant, these data further suggest that modest changes in HDL size, which resulted from TG enrichment, did not influence selective uptake rates.

In conclusion, the data reported here demonstrate that TG, like CE, is a substrate for selective uptake by the SR-BI receptor. The selection of CE or TG for transfer is related directly to the mole ratio of these lipids. This relationship was observed with HDL of widely varying TG/CE content, including particles more CE-rich and particles more TG-rich than native HDL. Based on the results obtained here, we propose a modification of the nonaqueous channel model described for SR-BI (31). Our data suggest a mechanism in which HDL binds to SR-BI, creating a channel through which a nonspecific portion of core lipid, defined by its TG/CE ratio, diffuses into the cell plasma membrane and is incorporated into the cell (Fig. 7). Although this channel does not appear to discriminate between molecules of similar size, such as CE and TG, it apparently cannot accommodate very large hydrophobic molecules (18). HDL processed by such a mechanism is depleted of both CE and TG, with the resulting HDL product retaining the same TG/CE ratio of the parent molecule (Fig. 7). Such a mechanism provides a plausible explanation for the higher CE selective uptake from HDL depleted of TG by hepatic lipase (48–51). In this way, the ability of HDL to donate cholesterol for steroidogenesis or to promote reverse cholesterol transport may be augmented when HDL contain a high CE/TG ratio, such as that promoted by the combined actions of CETP and hepatic lipase (51) and enhanced by lipid transfer inhibitor protein (24, 52). Conversely, these beneficial functions of HDL may be impaired when these particles contain elevated TG, such as occurs in hypertriglyceridemia and noninsulin-dependent diabetes mellitus. In this regard our data suggest a novel mechanism by which elevated TG may promote atherosclerosis.

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