Lipoprotein lipase mediates an increase in selective uptake of HDL-associated cholesteryl esters by cells in culture independent of scavenger receptor BI

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Abstract **Scavenger receptor class B type I (SR-BI) mediates the selective uptake of HDL cholesteryl esters (CEs) by the liver. LPL promotes this selective lipid uptake independent of lipolysis. In this study, the role of SR-BI in the mechanism of this LPL-mediated increase in selective CE uptake was explored. Baby hamster kidney (BHK) cells were transfected with the SR-BI cDNA, and significant SR-BI expression could be detected in immunoblots, whereas no SR-BI was visualized in control cells. Y1-BS1 murine adrenocortical cells were cultured without or with adrenocorticotropic hormone, and cells with no detectable or with SR-BI were obtained. These cells incubated without or with LPL in medium containing ^{125}I/[^{3}H]cholesteryl oleyl ether-labeled HDL_3; tetrahydrolipstatin inhibited the catalytic activity of LPL. In BHK and in Y1-BS1 cells without or with SR-BI expression, apparent HDL_3 selective CE uptake ([^{3}H]CEt − ^{125}I) was detectable. Cellular SR-BI expression promoted HDL_3 selective CE uptake by ~250–1,900%. In BHK or Y1-BS1 cells, LPL mediated an increase in apparent selective CE uptake. Quantitatively, this stimulating LPL effect was very similar in control cells and in cells with SR-BI expression. The uptake of radiolabeled HDL_3 was also investigated in human embryonal kidney 293 (HEK 293) cells that are an established SR-BI-deficient cell model. LPL stimulated [^{3}H]cholesteryl oleyl ether uptake from labeled HDL_3 by HEK 293 cells substantially, showing that LPL can induce selective CE uptake from HDL_3 independent of SR-BI. To explore the role of cell surface proteoglycans on lipoprotein uptake, we induced proteoglycan deficiency by heparinase treatment. Proteoglycan deficiency decreased the LPL-mediated promotion of HDL_3 selective CE uptake. In summary, evidence is presented that the stimulating effect of LPL on HDL_3 selective CE uptake is independent of SR-BI and lipolysis. However, cell surface proteoglycans are required for the LPL action on selective CE uptake. It is suggested that pathways distinct from SR-BI mediate selective CE uptake from HDL.**—Rinninger, F., M. Brundert, I. Brosch, N. Donarski, R. M. Budzinski, and H. Greten. **Lipoprotein lipase mediates an increase in selective uptake of HDL-associated cholesteryl esters by cells in culture independent of scavenger receptor BI.** ***J. Lipid Res.*** **2001.** 42: **1740–1751.**

Supplementary key words adrenal • atherosclerosis • BHK • HEK 293 • liver • metabolism • Y1-BS1

HDL-associated cholesteryl esters (CEs) are taken up by hepatocytes and steroidogenic cells selectively, that is, independently of HDL holo-particle internalization (1). Scavenger receptor class B type I (SR-BI), an HDL receptor protein, plays a central role in this pathway (2). The physiologic function of SR-BI in HDL metabolism is illustrated in genetically modified mice that have no or a reduced SR-BI expression in the liver (3, 4). SR-BI deficiency in these rodents increases plasma HDL cholesterol and decreases HDL selective CE uptake by the liver (3, 4). These investigations provide evidence for a physiologic function of SR-BI in HDL metabolism in vivo.

Besides cellular receptors, lipoprotein metabolism in plasma is modified by lipolytic enzymes. LPL is bound to the luminal side of capillaries and arteries and is abundant in muscle and adipose tissue (5). This enzyme hydrolyzes chylomicron- and VLDL-associated triglycerides to provide fatty acids to tissues as an energy source (5). Hepatic lipase (HL) is related to LPL with respect to function and structure. This enzyme originates from the liver and is attached to the vascular endothelium of adrenals, ovaries, and sinusoids of the liver (6–8). HL catalyzes the hydrolysis of HDL phospholipids and triglycerides.

Distinct from lipolysis, LPL and HL have been implicated in cellular lipoprotein holo-particle metabolism. In

Abbreviations: ACTH, adrenocorticotropic hormone; apoA-I, apolipoprotein A-I; BHK, baby hamster kidney; BN, Brown Norway rat yolk sac epithelial; CE, cholesteryl ester; CMV, cytomegalovirus; [^{3}H]CEt, [^{3}H]cholesteryl oleyl ether; HEK, human embryonal kidney; HL, hepatic lipase; ^{125}I-NMTC, radio-iodinated *N*-methyl-tyramine cellobiose ligand; LPDS, lipoprotein-deficient serum; SR-BI, scavenger receptor class B type I; THL, tetrahydrolipstatin.

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cultured cells, LPL promotes the uptake of apolipoprotein B-containing lipoproteins independent of lipolysis (9–12). LPL binds to cell surface proteoglycans and associates with lipoproteins as well (5, 10, 13). Presumably this "bridging" concentrates the lipoprotein particles on the cell surface and thereby facilitates their uptake. The latter may be mediated by cell surface heparan sulfate proteoglycans (10, 14) or by lipoprotein receptors (11, 12). Established ligands for this lipase-mediated particle uptake are chylomicrons, chylomicron remnants, β-VLDL, and LDL (10–12, 14).

Distinct from holo-particle internalization, LPL and HL stimulate the selective lipid uptake from lipoproteins by cells and tissues. Initial evidence established that these enzymes facilitate the selective CE uptake from HDL by hepatic and extrahepatic cells in vitro (13, 15–17). This lipase-mediated increase in selective CE uptake is independent of lipolysis but dependent on cell surface proteoglycans (15–17). Besides HDL, recent investigations suggested that LPL mediates an increase in selective CE uptake from LDL (18). Taken together, these observations show that LPL and HL can modulate the selective lipid uptake pathway.

Considering the LPL- and HL-mediated promotion of the selective CE uptake pathway, the question arises whether SR-BI is involved in the mechanism of these lipase actions. Recently, the role of SR-BI in the lipase-promoted increase in selective CE uptake has been explored (18, 19). In human embryonal kidney 293 (HEK 293) cells, SR-BI and HL synergistically facilitated selective CE uptake from HDL, and this effect was independent of lipolysis (19). In Chinese hamster ovary cells, LPL stimulated the selective CE uptake from LDL (18). In contrast with HDL (19), this LPL effect on LDL selective CE uptake was independent of SR-BI (18). In summary, with respect to the mechanism, it seems at present that SR-BI is involved in the HL-mediated increase in selective CE uptake from HDL, whereas this is not true for LPL and LDL (18, 19).

In the current study, the question was addressed whether SR-BI plays a role in the mechanism of the LPL-mediated increase in HDL selective CE uptake. Baby hamster kidney (BHK) cells, Y1-BS1 murine adrenocortical cells, and HEK 293 cells with no detectable or with substantial SR-BI expression made up the experimental model (19, 20), and HDL_3 (d = 1.125–1.21 g/ml) was radiolabeled in the protein and lipid moieties (21). Results showed that LPL stimulates HDL_3 selective CE uptake by cells with no detectable or with substantial SR-BI expression. Quantitatively, this lipase-mediated increase in HDL_3 selective CE uptake is very similar in cells without or with SR-BI expression. Therefore, it is suggested that a pathway(s) distinct from SR-BI contributes to the lipase-mediated increase in HDL selective CE uptake.

MATERIALS AND METHODS

Preparation of unlabeled HDL_3 and lipoprotein-deficient serum

Human HDL_3 (d = 1.125–1.21 g/ml) was isolated by ultracentrifugation from pooled plasma of healthy donors (22, 23). Heparin-Sepharose (Amersham Pharmacia) affinity chromatography was used to remove any HDL_3 particles containing apolipoprotein E (23, 24). Lipoprotein-deficient serum (LPDS) was prepared by ultracentrifugation as the d > 1.25 g/ml fraction of human plasma (16).

Preparation of doubly radiolabeled HDL_3

Human apolipoprotein A-I (apoA-I) was traced with radioiodinated *N*-methyl-tyramine cellobiose ligand (^{125}I-NMTC-apoA-I) (21). Na^{125}I and [1-α, 2α (n)-^{3}H]cholesteryl oleyl ether ([^{3}H]CEt) were supplied by Amersham Pharmacia. Doubly radiolabeled HDL_3 was prepared exactly as described previously (16, 23). ^{125}I-NMTC-apoA-I and [^{3}H]CEt were incorporated into apolipoprotein E-deficient HDL_3 by exchange (16, 23).

Preparation of LPL

LPL was a kind gift from Dr. Ira J. Goldberg, Columbia University, New York. This enzyme was purified from unpasteurized bovine milk and stored at −70°C (25). Tetrahydrolipstatin (THL; Orlistat®) was used to covalently inhibit the active site and thus the lipolytic activity of LPL (26). Media for the uptake assays with cells containing doubly radiolabeled HDL_3 and LPL were prepared, and THL (final concentration 50 μg/ml, dissolved in ethanol; final ethanol concentration 0.1% v/v) was added to these media if LPL was absent or present. Under these conditions, the lipolytic activity of LPL was blocked as demonstrated previously (16, 27).

Cloning of SR-BI and transfection of BHK cells

Total RNA from human HepG2 hepatoma cells was used for RT-PCR. One microgram of total RNA was subjected to RT-PCR according to the SuperScript One-Step Kit protocol (Life Technologies). The sequence of the primers used in the amplification of the cDNA were 5′-CCCAGGCGCGCAGACATGG (forward primer) and 5′-CCTACAGTTTTGCTTCCTGCAG (reverse primer), and these were selected according to the sequence of human SR-BI (28). The main amplification product was a fragment of 1546 bp consisting of the complete coding region plus 15 bp and 1 bp of the 5′ and 3′ untranslated region, respectively. The sequence was verified by cycle sequencing with the ABI PRISM BigDye Terminator Cycle Sequencing Kit (PE Biosystems). The cDNA fragment was cloned into the mammalian expression vector pBK-CMV (Stratagene).

BHK-21 cells were obtained from American Type Culture Collection and are designated BHK cells in this study. These cells were transfected using the Lipofectamine® reagent (Life Technologies) (29). Briefly, lipid and DNA (1 μg) were diluted into OPTI-MEM (100 μl; Life Technologies), thereafter gently mixed and incubated for 30 min at room temperature. The lipid-DNA mixture then was diluted with 0.8 ml medium and added to subconfluent cells in six well (35 mm) tissue culture dishes (Costar). After 6 h in culture, this medium was replaced by DMEM (Bio Whittaker), which was supplemented with FBS (10% v/v; Life Technologies) and incubated further for 72 h. Stable cell clones were selected in DMEM supplemented with FBS (10% v/v) and G418 sulfate (0.8 mg/ml; Geneticin®, Life Technologies). In control cells that were transfected with the plasmid pBK-CMV, no expression of SR-BI was detectable at the RNA or the protein level as determined in Northern blots and specific immunoblots as described below.

To express human LPL, we transfected BHK cells with pRK5 (vector) or pRK5-hLPL; this LPL expression plasmid was constructed by insertion of the LPL cDNA (full-length) downstream from the CMV promoter in vector pRK5 (30). These plasmids were a kind gift from Dr. Hans Will, University of Hamburg, Germany. For transfection, plasmid DNA (1 μg) and Fugene®

(6 μl; Roche) were used according to the manufacturer's instructions.

Culture of BHK cells

BHK cells were cultured (37°C) in DMEM (Life Technologies) containing FBS (5% v/v), penicillin (100 IU/ml; Life Technologies), streptomycin (100 μg/ml; Life Technologies), glutamine (2 mM; Life Technologies), and G418 sulfate (0.8 mg/ml).

For lipoprotein uptake experiments, BHK cells (500,000 per well) were plated in wells (35 mm, multiwell tissue culture plates; Nunc). Twenty-four or 48 h after plating when the cells were near confluency, the cells were washed (PBS, two times), and the culture medium was replaced by DMEM supplemented with LPDS (5% v/v), antibiotics (see above), and G418 sulfate (0.8 mg/ml). After culture (37°C, 20 h) in this medium followed the preincubations and the uptake assays as outlined below.

Culture of Y1-BS1 murine adrenocortical cells

Y1-BS1 cells were a kind gift from Dr. B. P. Schimmer, University of Toronto, Toronto, ON, Canada (20). These cells were cultured (37°C) in Ham's F-10 nutrient mixture (Life Technologies) supplemented with horse serum (12.5% v/v; Life Technologies), FBS (2.5% v/v), glutamine (2 mM), and antibiotics (see above). For HDL_3 uptake experiments, Y1-BS1 cells were plated in tissue culture wells (35 mm, six well plates). Twenty hours before the uptake assays, when the cells were close to confluency, Y1-BS1 cells were cultured (37°C) in α-MEM (Life Technologies) containing BSA (5 mg/ml, Sigma) and antibiotics (see above); adrenocorticotropic hormone (ACTH, fragment 1–24, 100 nmol/l; Sigma) was absent or present as indicated (31, 32).

Culture of HEK 293 cells

HEK 293 cells originated from American Type Culture Collection. These cells were cultured (37°C) in DMEM supplemented with FBS (10% v/v), glutamine (2 mM), and antibiotics (see above). For uptake experiments, HEK 293 cells were plated in 35 mm culture wells (six well plates). Twenty hours before the HDL_3 uptake assays, the cells were washed (PBS, two times), and the culture medium was replaced by DMEM containing LPDS (5% v/v) and antibiotics (see above).

Culture of Brown Norway cells

Cubilin expressing Brown Norway (BN) yolk sac epithelial cells that were transformed with mouse sarcoma virus were grown in MEM (Life Technologies) that was supplemented with FBS (10% v/v) (33). Postnuclear extracts were prepared from these cells and used in immunoblots as described below (32).

Preincubation of the cells

Before initiating the lipoprotein uptake assays, BHK cells and Y1-BS1 cells preincubated in serum-free and lipoprotein-free medium. These preincubations were performed to allow internalization or dissociation of membrane-associated serum or protein components originating from culture in the presence of LPDS, from BSA or from cell secretion. After aspiration of the culture medium, the cells were washed with PBS (two times). Thereafter followed the preincubation (37°C, 30 min) in DMEM (BHK cells) or α-MEM (Y1-BS1 cells) containing BSA (5 mg/ml) and antibiotics (see above).

Uptake of doubly radiolabeled HDL_3 by cells in culture

To investigate HDL_3 uptake, we incubated BHK cells or HEK 293 cells (37°C) in DMEM, and Y1-BS1 cells (37°C) were incubated in α-MEM containing BSA (5 mg/ml) and doubly radiolabeled HDL_3; LPL was absent or present as indicated in the respective legends (23). In these assays, the catalytic activity of LPL was blocked with THL (see above). After incubation (37°C) for the indicated time periods, the medium was aspirated, and the cells were washed (PBS, four times). Then, DMEM (BHK cells) or α-MEM (Y1-BS1 cells) containing BSA (5 mg/ml) and unlabeled HDL_3 (100 μg HDL_3 protein/ml) was added for a "chase" incubation (37°C, 2.0 h) to remove reversibly cell-associated tracers (34). After this chase period, the medium was aspirated, and the cells were washed again (PBS, one time). The cells then were released from the wells with trypsin/EDTA solution (trypsin 0.5 g/l, EDTA 0.2 g/l, 1.0 ml/well; Life Technologies). Trypsin activity was quenched by addition of PBS containing excess BSA (50 mg/ml). The cell suspensions were transferred to tubes with a PBS (4°C) rinse of the wells. The cells then were pelleted by centrifugation (2,000 *g*, 4°C, 15 min) followed by aspiration of the supernatant. The cell pellet thereafter was resuspended in PBS (4°C, 5.0 ml) followed by centrifugation (2000 *g*, 4°C, 15 min). The final cell pellet was dissolved in NaOH solution (0.1 N, 1.0 ml) and sonicated, and aliquots were used for protein determination (35), direct ^{125}I radioassay, and ^{3}H radioassay after lipid extraction (23). In the case of HEK 293 cells, no chase incubation was performed, and cells were harvested by cell scraping.

Immunoblot analysis

An anti-SR-BI antiserum was prepared by immunization of rabbits with a recombinant murine SR-BI fragment (amino acids 315–412) that was produced in a bacterial expression system (36). Alternatively, an anti-SR-BI antiserum was raised in rabbits using a synthetic peptide corresponding to residues 495–509 of human SR-BI (37). A polyclonal antibody directed against the LDL receptor that was generated in rabbits was a kind gift from Dr. Joachim Herz, University of Dallas, Dallas, Texas. A polyclonal antibody directed against rat cubilin has been prepared in rabbits (33).

For immunoblot analysis, postnuclear supernatants were prepared from BHK, Y1-BS1, HEK 293, or BN cells (32). Samples containing the indicated amount of protein were reduced with 2-mercaptoethanol in gel loading buffer, fractionated by SDS-PAGE, and transferred to nitrocellulose membranes. Finally, the membranes were incubated in buffer containing the respective antiserum. The primary antibody was detected using a peroxidase-conjugated goat anti-rabbit IgG (dilution 1:5,000; Amersham Pharmacia). Antibody binding was visualized by enhanced chemiluminescence detection (Amersham Pharmacia) and autoradiography. Ponceau S staining of membranes was used to detect molecular mass standards (Amersham Pharmacia). SR-BI protein immunoreactivity was identified at its authentic molecular mass (85 kDa) (28).

Miscellaneous

Heparinase I (EC 4.2.2.7, from *Flavobacterium heparinum*) and heparinase III (EC 4.2.2.8, from *F. heparinum*) were obtained from Sigma (St. Louis, MO) (13). LPL activity was measured as outlined previously using [^{14}C]triolein as tracer (16).

Statistics and calculations

Data are expressed as means ± SEM. Significance of differences was examined using Student's *t*-test.

For cellular uptake of doubly radiolabeled HDL_3, uptake of each tracer is shown in terms of apparent lipoprotein particle uptake, expressed as lipoprotein protein (1, 16, 23). This is done to compare the uptake of both tracers on a common basis. Outlined in this way, uptake of HDL_3 holo-particles is represented by equal uptake of both tracers. In the figures, ^{125}I represents apparent lipoprotein particle uptake according to the protein tracer (^{125}I-NMTC-apoA-I), [^{3}H]CEt that due to the CE tracer,

and [^{3}H]CEt − ^{125}I shows the difference in apparent HDL_3 particle uptake, that is, apparent selective CE uptake (23).

RESULTS

Stable cell clones were derived from BHK cells by transfection either with an expression plasmid containing the human SR-BI cDNA under the control of the CMV promotor (pBK-CMV-hSR-BI) or with the control vector (pBK-CMV). Immunoblots using a murine anti-SR-BI antiserum (32, 36) revealed a strong band at the appropriate molecular weight in cells transfected with pBK-CMV-hSR-BI (**Fig. 1**, right). An identical signal was obtained in such blots by using an antiserum that is directed against human SR-BI (data not shown) (37). As expected, no SR-BI expression could be visualized in BHK cells transfected with the control vector (Fig. 1, right). In parallel, the identical postnuclear supernatants from both types of BHK cells were probed with an antiserum directed against the LDL receptor (Fig. 1, left). A band corresponding to this receptor could be detected in control BHK cells and in BHK cells with SR-BI expression, and the respective signal was identical in both cell types.

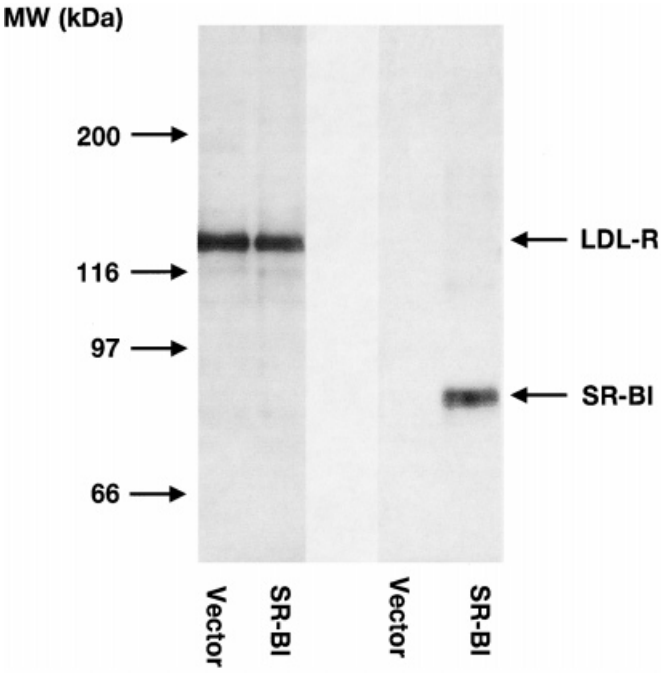

Fig. 1. Immunoblot analysis for scavenger receptor class B type I (SR-BI) and LDL receptors in baby hamster kidney (BHK) cells. BHK cells were transfected with vector pBK-CMV or with pBK-CMV-hSR-BI (human SR-BI cDNA). Postnuclear supernatants were prepared from these cells, and thereafter 80 μg protein (per lane) was fractionated by SDS-PAGE (7.5%). Finally, the proteins were transferred to nitrocellulose membranes. These membranes were immunoblotted with an anti-SR-BI antiserum (murine, right lanes, dilution 1:1,000) or an anti-LDL receptor antiserum (rabbit, left lanes, dilution 1:1,000). IgG binding was visualized as outlined in Materials and Methods. Four similar blots for SR-BI yielded qualitatively identical results. MW, molecular weight.

To investigate the role of SR-BI in HDL metabolism of BHK cells, we explored the uptake of doubly radiolabeled HDL_3. HDL_3 was labeled in the protein and in the lipid moieties with ^{125}I-NMTC-apoA-I and [^{3}H]CEt, respectively (21, 23). Control BHK cells or BHK cells with SR-BI expression were incubated in parallel in medium containing doubly radiolabeled HDL_3 (**Fig. 2**). After a chase incubation, which removes reversibly cell-associated HDL_3 tracers (34), cellular tracer content and apparent HDL_3 particle uptake according to ^{125}I-NMTC-apoA-I or [^{3}H]CEt were analyzed (1, 23, 40). In control BHK cells (no SR-BI expression), apparent HDL_3 particle uptake according to ^{125}I-NMTC-apoA-I was 27.4 ± 4.2 ng HDL_3 protein/mg cell protein (mean ± SEM, n = 5 independent experiments) (Fig. 2). In these control cells, apparent HDL_3 particle uptake due to [^{3}H]CEt was 165.5 ± 19.2 ng HDL_3 protein/mg cell protein, and this uptake was in significant excess on that according to the protein tracer. The difference in apparent HDL_3 particle uptake between [^{3}H]CEt and ^{125}I-NMTC-apoA-I yields apparent selective CE uptake, and this was 138.0 ± 16.3 ng HDL_3 protein/mg cell protein in these control cells (Fig. 2). In BHK cells with SR-BI expression, apparent HDL_3 particle uptake according to ^{125}I-NMTC-apoA-I was 149.9 ± 12.2 ng HDL_3 protein/mg cell protein (mean ± SEM, n = 5 independent experiments) (Fig. 2). In these cells, apparent HDL_3 particle uptake due to [^{3}H]CEt was 2,789.9 ± 293.1 ng HDL_3 protein/mg cell protein, and apparent selective CE up-

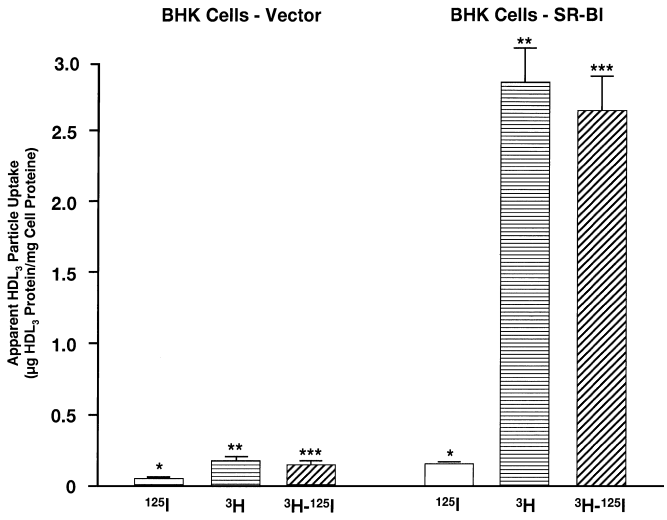

Fig. 2. Uptake of doubly radiolabeled HDL_3 by baby hamster kidney (BHK) cells. Control BHK cells (vector, left) or BHK cells with scavenger receptor class B type I (SR-BI) expression (right) were incubated (37°C, 4.0 h) in parallel in DMEM containing doubly radiolabeled HDL_3 (40 μg HDL_3 protein/ml). After a chase incubation, cellular tracer content and apparent HDL_3 particle uptake were analyzed. ^{125}I represents apparent HDL_3 particle uptake according to ^{125}I-NMTC-apoA-I; ^{3}H demonstrates apparent HDL_3 particle uptake due to [^{3}H]cholesteryl oleyl ether ([^{3}H]CEt), and ^{3}H − ^{125}I shows the difference, i.e., apparent HDL_3 selective CE uptake. Values are means ± SEM of five independent experiments; within each experiment, three or four independent incubations were performed. Unpaired two-tailed Student's *t*-test, * ^{125}I, $P < 0.0001$; ** ^{3}H, $P < 0.0001$; *** ^{3}H − ^{125}I, $P < 0.0001$.

take ([^{3}H]CEt − ^{125}I-NMTC-apoA-I) was 2,631.7 ± 279.1 ng HDL_3 protein/mg cell protein (Fig. 2). Thus, SR-BI expression in BHK cells promoted an increase of 1,907% in apparent selective CE uptake from HDL_3 (control cells correspond to 100%), and for [^{3}H]CEt this relative increase was 1,686%. Therefore, these SR-BI-transfected BHK cells are an appropriate model for studies on the role of this receptor protein in HDL metabolism.

To elucidate whether SR-BI plays a role in the LPL-mediated increase in HDL_3 selective CE uptake (16), we incubated control BHK cells or BHK cells with SR-BI expression in parallel in medium that contained doubly radiolabeled HDL_3, and LPL was absent or present; THL was added to this medium also to block the catalytic enzyme activity (**Fig. 3**) (16, 26). In the absence of LPL, in both cell types apparent HDL_3 particle according to [^{3}H]CEt was in substantial excess of that due to ^{125}I-NMTC-apoA-I. The difference in uptake ([^{3}H]CEt − ^{125}I-NMTC-apoA-I) yields apparent selective CE uptake from HDL_3, and this rate is greater for BHK cells with SR-BI expression (Fig. 3, right, bottom panel) compared with control cells (Fig. 3, left, bottom panel). LPL addition to the assay medium of both types of BHK cells containing labeled HDL_3 had virtually no effect on uptake of ^{125}I-NMTC-apoA-I (Fig. 3, bottom panels). However, LPL increased the uptake of HDL_3-associated [^{3}H]CEt in a dose-dependent manner, and this effect was close to saturation at 500 ng LPL protein/ml (Fig. 3). As a result, apparent HDL_3 selective uptake ([^{3}H]CEt − ^{125}I-NMTC-apoA-I) was stimulated by LPL in a concentration-dependent manner in both types of BHK cells (Fig. 3).

The difference in apparent HDL_3 selective CE between the absence and the presence of LPL was calculated (Fig. 3, top panels). In control BHK cells, LPL (500 ng protein/ml) induced an absolute increase in apparent HDL_3 selective CE uptake of 602.6 ± 5.5 ng HDL_3 protein/mg cell protein (mean ± SEM, n = 3 incubations) (Fig. 3, left, top panel). In BHK cells with SR-BI expression, the identical LPL concentration promoted an absolute increase in apparent HDL_3 selective CE uptake of 413.3 ± 78.7 ng HDL_3 protein/mg cell protein (mean ± SEM, n = 3 incubations) (Fig. 3, right, top panel). Thus, quantitatively LPL stimulated the selective uptake of HDL_3-associated CE by control BHK cells or by BHK cells with SR-BI expression to a very similar extent.

To investigate the effect of LPL on the kinetics of selective CE uptake form HDL_3, we incubated control BHK cells or BHK cells with SR-BI expression (37°C) for 10, 60, or 240 min in medium containing doubly radiolabeled HDL_3 (40 μg HDL_3 protein/ml), and LPL (750 ng protein/ml) was absent or present (data not shown). Finally, apparent HDL_3 particle uptake (^{125}I-NMTC-apoA-I and [^{3}H]CEt) was analyzed as outlined in Materials and Methods. In the absence of LPL, apparent selective CE uptake ([^{3}H]CEt − ^{125}I-NMTC-apoA-I) by both types of BHK cells was evident after 10 min of incubation, and this uptake increased in a time-dependent manner at linear rates up to 240 min. Throughout the entire time course, this rate of

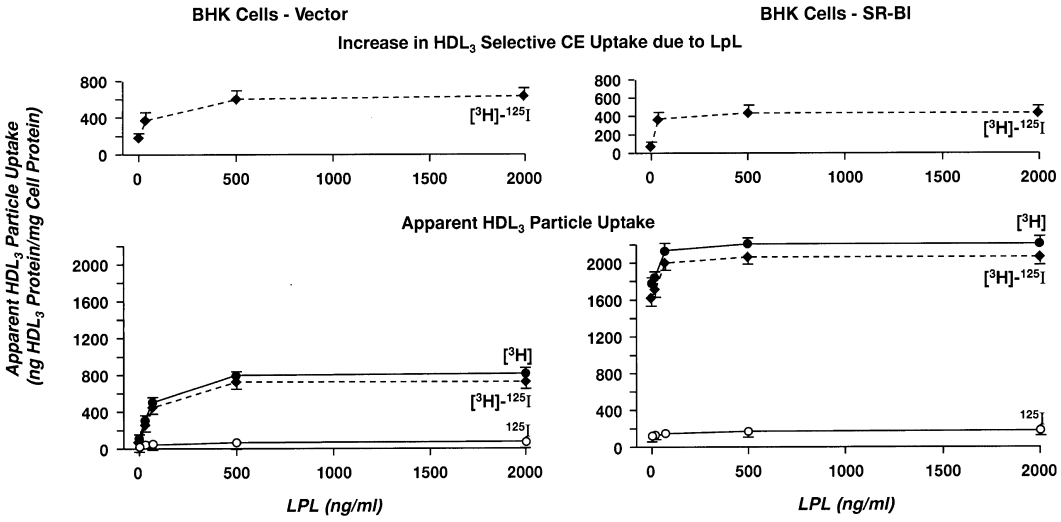

Fig. 3. LPL dose-response curve on uptake of doubly radiolabeled HDL_3 by baby hamster kidney (BHK) cells. Control BHK cells (vector, left panels) or BHK cells with scavenger receptor class B type I (SR-BI) expression (right panels) were incubated (37°C, 4.0 h) in parallel in DMEM containing doubly radiolabeled HDL_3 (40 μg HDL_3 protein/ml), and LPL was absent or present as indicated in the abscissa. After a chase incubation, cellular tracer content and apparent HDL_3 particle uptake were analyzed. In the bottom panels, ^{125}I represents apparent HDL_3 particle uptake according to ^{125}I-NMTC-apoA-I; ^{3}H demonstrates apparent particle uptake due to [^{3}H]CEt and ^{3}H − ^{125}I shows the difference, i.e., apparent HDL_3 selective cholesteryl ester (CE) uptake. The difference in apparent HDL_3 selective CE uptake between the absence and the presence of LPL was calculated. This LPL-induced absolute increase in apparent HDL_3 selective CE uptake is shown in the top panels. Values are means ± SEM of four incubations (without LPL) or three incubations (with LPL). Two independent similar experiments yielded qualitatively identical results.

apparent HDL_3 selective CE uptake was higher in BHK cells with SR-BI expression compared with control cells (data not shown). In parallel, both types of BHK cells were incubated in the additional presence of LPL (750 ng protein/ml) in the medium. LPL significantly increased apparent selective CE uptake ($[^3H]CEt - {}^{125}I$-NMTC-apoA-I) from HDL_3 by both types of BHK cells, and this effect was evident throughout the entire time course. The absolute increase in apparent selective CE uptake mediated by LPL was calculated as well (see above). Quantitatively, this LPL-induced increase in apparent HDL_3 selective CE uptake was very similar in control BHK cells and in BHK cells with SR-BI expression (data not shown).

A dose-response curve for the uptake of radiolabeled HDL_3 by control BHK cells or by BHK cells with SR-BI expression is shown in **Fig. 4** (bottom panels). Both cell types were incubated in parallel in medium that contained increasing concentrations of doubly radiolabeled HDL_3, and LPL was absent or present. Finally, cellular tracer content and selective CE uptake ($[^3H]CEt - {}^{125}I$-NTMC-apoA-I) were analyzed. Increasing concentrations of radiolabeled HDL_3 yielded a dose-dependent increase in apparent selective CE uptake from HDL_3, and this was true for both types of BHK cells (Fig. 4). LPL stimulated apparent selective CE uptake by control BHK cells and by BHK cells with SR-BI expression (Fig. 4). The absolute increase in apparent HDL_3 selective CE uptake mediated by LPL is shown in Fig. 4 (top panels) also. Quantitatively, this LPL-mediated increase in apparent selective CE uptake was similar in control BHK cells (Fig. 4, left) and in BHK cells with SR-BI expression (Fig. 4, right), and this LPL effect was observed at each HDL_3 concentration examined.

LPL was from exogenous, bovine origin in the experiments, which are presented above. To explore the action of endogenous, cell-secreted LPL on selective CE uptake from HDL_3, we transfected control BHK cells or BHK cells with SR-BI expression with pRK5 (control vector), or pRK5-hLPL (full-length human LPL cDNA) (**Fig. 5**). Forty-eight hours after transfection, media of the cells were harvested for determination of LPL catalytic activity (see Materials and Methods). In the experiment shown in Fig. 5, in control BHK cells transfected with the vector pRK5 (control), LPL activity was 0.23 nmol FFA $\times$ min^{-1} $\times$ mg^{-1} cell protein (background); in control BHK cells transfected with pRK5-hLPL, this value was 8.3. In BHK cells with SR-BI expression that were transfected with the vector pRK5 (control), LPL activity was 0.18 nmol FFA $\times$ min^{-1} $\times$ mg^{-1} cell protein; in these cells transfected with pRK5-hLPL, this value was 10.6 (means of n = 2 determinations). Thus, substantial lipase activity was detectable in the media of the cells after the respective transfection.

In parallel, 48 h after transfection with pRK5 or pRK5-hLPL, doubly radiolabeled HDL_3 and THL were added to the medium of both types of BHK cells (Fig. 5). After incubation (37°C, 4.0 h), apparent HDL_3 particle uptake was analyzed. Apparent HDL_3 selective uptake ($[^3H]CEt - {}^{125}I$-NMTC-apoA-I) increased in a dose-dependent manner in both types of BHK cells (transfected with pRK5, control), and this increase was greater in BHK cells with SR-BI expression. Expression of the LPL transgene stimulated selective CE uptake from HDL_3 in both groups of BHK cells (Fig. 5, bottom panels). The absolute increase in selective CE uptake due to LPL was calculated (Fig. 5, top panels). Quantitatively, LPL of endogenous origin stimulated HDL_3 selective CE uptake to a very similar extent in control BHK cells and in BHK cells with SR-BI

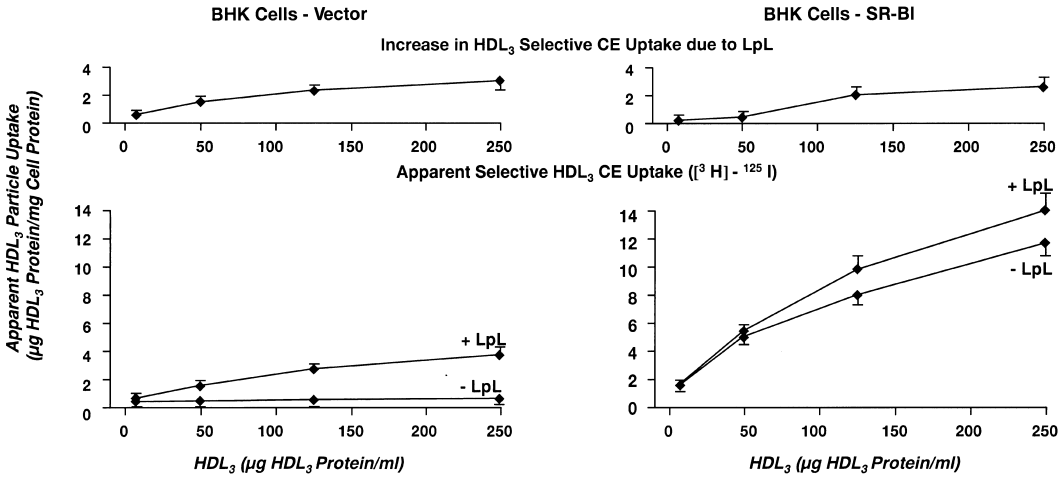

Fig. 4. Dose-response curve for uptake of doubly radiolabeled HDL_3 by baby hamster kidney (BHK) cells and the effect of LPL. Control BHK cells (vector, left panels) or BHK cells with scavenger receptor class B type I (SR-BI) expression (right panels) were incubated (37°C, 4.0 h) in parallel in DMEM containing doubly radiolabeled HDL_3, and the respective HDL_3 concentrations are indicated in the abscissa; LPL (750 ng protein/ml) was absent or present as shown. After a chase incubation, cellular tracer content and apparent HDL_3 selective cholesteryl ester (CE) uptake ($[^3H]CEt - {}^{125}I$-NMTC-apoA-I, bottom panels) were analyzed. The difference in apparent HDL_3 selective CE uptake between the absence and the presence of LPL was calculated, and this LPL-induced absolute increase in selective CE uptake is shown in the top panels. Values are means ± SEM of three incubations. One independent experiment yielded qualitatively identical results.

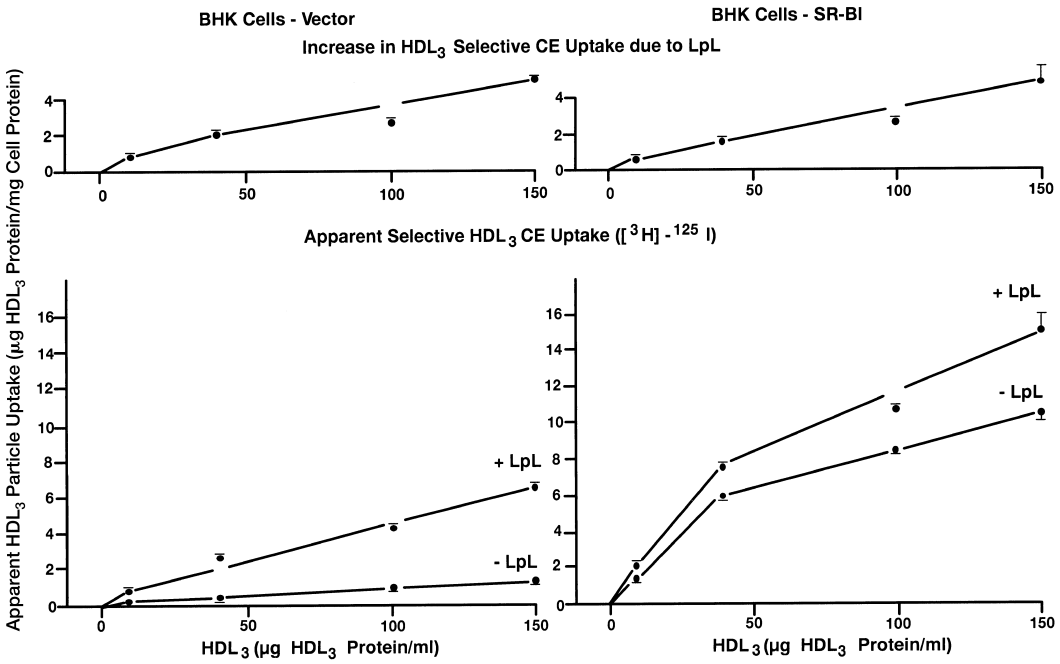

Fig. 5. Effect of endogenous, cell-secreted LPL on uptake of doubly radiolabeled HDL_3 by baby hamster kidney (BHK) cells. Control BHK cells (vector, left panels) or BHK cells with SR-BI expression (right panels) were transfected with vector pRK5 (1 μg DNA, control) or pRK5-hLPL (1 μg DNA, full-length human LPL cDNA). After culture (37°C, 48 h), either doubly radiolabeled HDL_3 and tetrahydrolipstatin (50 μg/ml) were added to the media or in parallel wells or media were harvested for LPL activity determination. After the incubation (37°C, 4.0 h) with HDL_3, cellular tracer content and apparent HDL_3 selective cholesteryl ester (CE) uptake ($[^3H]$CEt − ^{125}I-NMTC-apoA-I) were analyzed (bottom panels). The difference in apparent HDL_3 selective CE uptake between the absence and the presence of LPL was calculated, and this LPL-induced absolute increase in selective CE uptake is shown in the top panels. Values are means ± SEM of three incubations. Two independent similar experiments yielded qualitatively identical results. The lipolytic activity in the media was measured as outlined in Materials and Methods, and the respective data are presented in Results.

expression. Thus, LPL from endogenous and exogenous origin elicited qualitatively identical results.

Y1-BS1 murine adrenocortical cells express SR-BI, selectively take up HDL CE, and synthesize steroid hormones (31, 32, 38). Besides, these parameters can be up-regulated by ACTH. To explore SR-BI expression in this physiologic model for the HDL selective CE uptake pathway (39), we cultured Y1-BS1 cells in the absence or presence of ACTH, and thereafter postnuclear supernatants were prepared (**Fig. 6**). In proteins from ACTH-treated cells, immunoblot analysis showed a strong band corresponding to SR-BI (Fig. 6). No SR-BI signal was visible in several immunoblots of cells that were cultured in the absence of ACTH (Fig. 6). However, in a few cases, in postnuclear supernatants from Y1-BS1 cells that were cultured in the absence of this tropic compound, a very faint signal for SR-BI was detectable (data not shown). Thus, Y1-BS1 cells with virtually no SR-BI or with substantial SR-BI expression were available.

The effect of LPL on HDL_3 metabolism of Y1-BS1 adrenocortical cells with no detectable SR-BI or with SR-BI expression was explored (**Fig. 7**). Y1-BS1 cells were cultured in the absence or presence of ACTH. Thereafter followed an incubation in medium that contained doubly radiolabeled HDL_3 in the continued absence or presence of ACTH. Under both conditions, apparent HDL_3 particle uptake according to $[^3H]$CEt was in substantial excess of that due to ^{125}I-NTMC-apoA-I, and the difference in uptake reveals apparent selective CE uptake (Fig. 7, bottom panels). As expected, apparent HDL_3 selective CE uptake ($[^3H]$CEt − ^{125}I-NMTC-apoA-I) was higher in ACTH-stim-

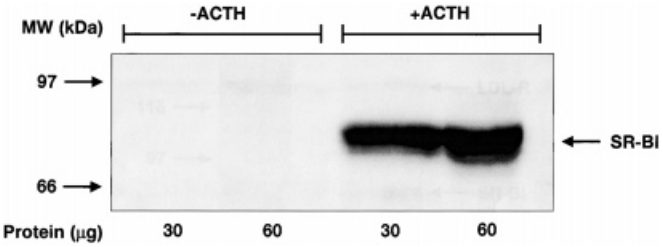

Fig. 6. Immunoblot analysis for SR-BI in Y1-BS1 murine adrenocortical cells. Y1-BS1 cells were cultured (37°C, 20 h) in the absence or presence of adrenocorticotropic hormone (ACTH; 100 nmol/l). Thereafter, postnuclear supernatants were prepared from both groups of cells, and 30 or 60 μg protein (per lane) was fractionated by SDS-PAGE (10%). Finally, the proteins were transferred to nitrocellulose membranes. The membranes were immunoblotted with an anti-SR-BI antiserum (mouse, dilution 1:500). IgG binding was visualized as outlined in Materials and Methods. Two similar immunoblots yielded qualitatively identical results. MW, molecular weight.

ulated Y1-BS1 cells (271%, absence of ACTH = 100%). LPL addition to the assay medium of Y1-BS1 cells had virtually no effect on ^{125}I-NTMC-apoA-I uptake from labeled HDL_3 by either group of cells (Fig. 7). However, LPL increased the uptake of HDL_3-associated [^{3}H]CEt in cells that were cultured in the absence (Fig. 7, left) or in the presence of ACTH (Fig. 7, right); as a result, LPL mediated an increase in apparent selective CE uptake from HDL_3 ([^{3}H]CEt − ^{125}I-NMTC-apoA-I). The absolute LPL-mediated increase in apparent HDL_3 selective CE uptake was calculated (Fig. 7, top panels). Quantitatively, this LPL-induced increase in selective CE uptake was very similar in cells that were cultured in the absence or presence of ACTH. Thus, quantitatively LPL induced a very similar increase in HDL_3 selective CE uptake in Y1-BS1 cells independent of the respective SR-BI status.

As a complementary approach to explore whether SR-BI is involved in the lipase-mediated increase in HDL_3 selective CE uptake, HEK 293 cells were used that represent an established SR-BI-deficient cell model (18, 19). To explore SR-BI expression in these cells, we performed immunoblots using a human SR-BI-specific antiserum. No band corresponding to SR-BI could be detected in these HEK 293 cells in three independent experiments (data not shown).

To investigate HDL_3 metabolism of HEK 293 cells, we examined the dose-response curves for the uptake of radiolabeled HDL_3. In a typical experiment, HEK 293 cells were incubated (37°C, 4.0 h) in medium that contained doubly radiolabeled HDL_3 (20, 40, 100, 200 μg HDL_3 protein/ml), and thereafter cellular tracer content was determined. Increasing the concentration of radiolabeled HDL_3 yielded a dose-dependent increase in apparent HDL_3 particle according to ^{125}I-NMTC-apoA-I and due to [^{3}H]CEt (data not shown). The difference in apparent HDL_3 particle uptake between [^{3}H]CEt and ^{125}I-NMTC-apoA-I was calculated. At an HDL_3 concentration of 10 or 40 μg HDL_3 protein/ml, apparent HDL_3 selective CE uptake by HEK 293 cells was detectable, however, at a very low rate (21.3 ± 1.1 or 80.3 ± 19.0 ng HDL_3 protein/mg cell protein, means ± SEM, n = 3 incubations). No apparent selective CE uptake from HDL_3 could be measured at an HDL_3 concentration of 100 or 200 μg HDL_3 protein/ml. Qualitatively, similar results were obtained in three independent experiments with HEK 293 cells, and in each case apparent HDL_3 selective CE uptake was very low or not detectable.

An LPL dose-response curve on the uptake of radiolabeled HDL_3 by HEK 293 cells is shown in **Fig. 8**. HEK 293 cells were incubated in medium that contained doubly radiolabeled HDL_3, and LPL was absent or present. In the absence of LPL, apparent HDL_3 particle uptake according to ^{125}I-NMTC-apoA-I was 273.0 ± 26.0 ng HDL_3 protein/mg cell protein (mean ± SEM, n = 6 incubations); apparent HDL_3 selective CE uptake ([^{3}H]CEt − ^{125}I-NMTC-

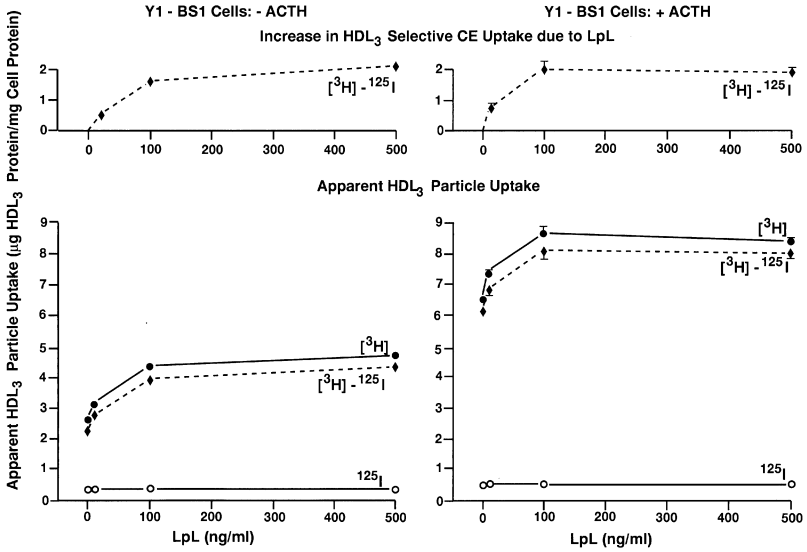

Fig. 7. Uptake of doubly radiolabeled HDL_3 by Y1-BS1 murine adrenocortical cells and the effects of adrenocorticotropic hormone (ACTH) and LPL. Y1-BS1 cells were cultured (37°C, 20 h) in the absence (left panels) or presence (right panels) of ACTH (100 nmol/l). After a preincubation (37°C, 0.5 h) in the absence or presence of ACTH (100 nmol/l), the cells were incubated (37°C, 4.0 h) in medium that contained doubly radiolabeled HDL_3 (40 μg HDL_3 protein/ml) in the continued absence or presence of ACTH (100 nmol/l); LPL was added to this medium or not as indicated in the abscissa. Finally, cellular tracer content and apparent HDL_3 particle uptake were analyzed. In the bottom panels, ^{125}I represents apparent HDL_3 particle uptake according to ^{125}I-NMTC-apoA-I; ^{3}H demonstrates apparent particle uptake due to [^{3}H]CEt, and ^{3}H − ^{125}I shows the difference, i.e., apparent selective cholesteryl ester (CE) uptake. The difference in apparent HDL_3 selective CE uptake between the absence and the presence of LPL was calculated, and this absolute LPL-mediated increase in selective CE uptake is presented in the top panels. Values are means ± SEM of six incubations (without LPL) or three incubations (with LPL). Where no error bars are shown, SEMs are smaller than the respective symbols. Three independent experiments yielded qualitatively identical results.

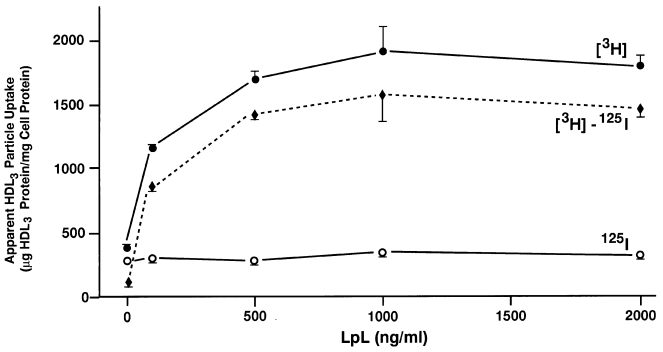

Fig. 8. LPL dose-response curve on uptake of doubly radiolabeled HDL_3 by HEK 293 cells. HEK 293 cells incubated (37°C, 4.0 h) in DMEM containing doubly radiolabeled HDL_3 (40 μg HDL_3 protein/ml), and LPL was absent or present as indicated in the abscissa. Thereafter, cellular tracer content and apparent HDL_3 particle uptake were analyzed. ^{125}I represents apparent HDL_3 particle uptake according to ^{125}I-NMTC-apoA-I; ^{3}H demonstrates apparent particle uptake due to [^{3}H]CEt, and $^{3}H - ^{125}I$ shows the difference, i.e., apparent selective cholesteryl ester uptake. Values are means ± SEM of six (without LPL) or three (with LPL) incubations. Two similar independent experiments yielded qualitatively identical results.

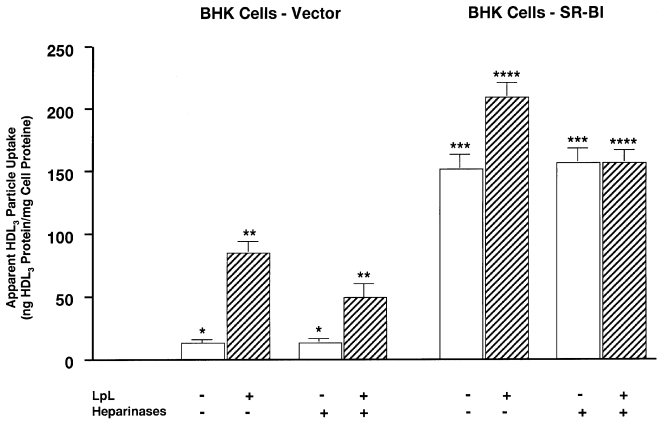

Fig. 9. Effect of preincubation with heparinases on the LPL-mediated increase in selective cholesteryl ester (CE) uptake from doubly radiolabeled HDL_3 by baby hamster kidney (BHK) cells. After preincubation (37°C, 0.5 h, see Materials and Methods), control BHK cells (vector, left) or BHK cells with SR-BI expression (right) were incubated (37°C, 2.0 h) in parallel in DMEM that contained heparinase I (6.0 U/ml) and heparinase III (1.8 U/ml) or not as indicated in the abscissa. Thereafter, this medium was aspirated, and both groups of cells were washed (PBS, two times) followed by an incubation (37°C, 0.5 h) in DMEM containing doubly radiolabeled HDL_3 (40 μg HDL_3 protein/ml); LPL (1,000 ng/ml) was absent or present during this incubation as indicated in the abscissa. Finally, cellular tracer content and apparent HDL_3 selective CE uptake ([^{3}H]CEt − ^{125}I-NMTC-apoA-I) were analyzed. Values are means ± SEM of three incubations. Unpaired one-tailed Student's *t*-test, * $P < 0.377$; ** $P < 0.0183$; *** $P < 0.496$; **** $P < 0.0067$. Two independent experiments yielded qualitatively identical results.

apoA-I) was 135.0 ± 12.0 ng HDL_3 protein/mg cell protein; thus under basal conditions, selective CE uptake was very low (Fig. 8). LPL in the medium of HEK 293 cells had no effect on uptake of ^{125}I-NMTC-apoA-I. However, LPL mediated a dose-dependent increase in cellular [^{3}H]CEt uptake from HDL_3. As a result, this lipolytic enzyme induced a dose-dependent apparent HDL_3 selective CE uptake ([^{3}H]CEt − ^{125}I-NMTC-apoA-I) in HEK 293 cells. In the presence of LPL (1,000 ng protein/ml), apparent HDL_3 selective CE uptake was 1,573 ± 55 ng HDL_3 protein/mg cell protein (mean ± SEM, n = 3 incubations), and this corresponds to a relative increase of 1,165% compared with the absence of LPL (corresponding to 100%) (Fig. 8). These experiments with HEK 293 cells suggest that the LPL-mediated increase in HDL_3 selective CE uptake is independent of SR-BI.

Cell surface proteoglycan deficiency diminishes the lipase-mediated increase in selective CE uptake from HDL and LDL (10, 16, 18). Next, we addressed the question whether the effect of LPL on HDL_3 selective CE uptake is dependent on proteoglycans in cells without or with SR-BI expression. Both types of BHK cells were incubated in parallel in the absence or presence of heparinase I and heparinase III in the medium to deplete the cells from surface proteoglycans (**Fig. 9**) (10, 13). Previously, it was established that the cells are, in fact, depleted from proteoglycans by heparinase digestion under these conditions (16). Thereafter followed an incubation of both types of BHK cells in medium that contained doubly radiolabeled HDL_3, and LPL was absent or present. Finally, apparent HDL_3 selective CE uptake ([^{3}H]CEt − ^{125}I-NMTC-apoA-I) was analyzed. In control BHK cells or in BHK cells with SR-BI expression, heparinase digestion per se had no effect on apparent HDL_3 selective CE uptake (Fig. 9). As expected, LPL stimulated apparent selective CE uptake from HDL_3 in control BHK cells (vector) or in BHK cells with SR-BI expression. However, preincubation of the cells with heparinases decreased the stimulatory action of LPL on apparent HDL_3 selective CE uptake significantly, and this was observed in control BHK cells or in BHK cells with SR-BI expression (Fig. 9). Thus, independent of the cellular SR-BI status, the LPL-mediated stimulation of HDL_3 selective CE uptake is dependent on proteoglycans.

The cellular uptake of HDL_3-associated ^{125}I-NMTC-apoA-I represents HDL_3 holo-particle internalization (1, 40). Accordingly, the experiments presented in Fig. 2 and Fig. 3 demonstrate that BHK cells take up HDL_3 holo-particles. Cubilin, which is the intestinal receptor for the endocytosis of the intrinsic factor-vitamin B12 complex (33), mediates HDL holo-particle uptake in kidney cells (33, 41). To explore whether cubilin is involved in lipoprotein particle uptake by kidney-derived BHK cells, we performed cubilin-specific immunoblots. Postnuclear supernatants were prepared from control BHK cells or from BHK cells with SR-BI expression; as reference, such supernatants were isolated from BN rat yolk sac epithelial cells (33). In specific immunoblots from both types of BHK cells, no signal corresponding to cubilin could be detected (data not shown). However, in immunoblots from BN cells, the respective band was visible at the appropriate molecular weight (data not shown). These experiments yield no evidence that cubilin is involved in HDL_3 holo-particle uptake by BHK cells.

DISCUSSION

To elucidate the role of SR-BI in the mechanism of the LPL-mediated increase in HDL selective CE uptake, we have generated by transfection BHK cells, which stably express human SR-BI, and significant expression of this protein was detectable in immunoblots. In contrast, SR-BI could not be visualized in control BHK cells. As a physiological model for the HDL selective CE uptake pathway, Y1-BS1 murine adrenocortical cells were used, and these cells demonstrated no SR-BI expression or substantial SR-BI expression in immunoblots depending on the experimental conditions (31, 32, 38, 39). An established model for cells that lack SR-BI are HEK 293 cells, and the absence of this receptor protein has been confirmed in immunoblots (18, 19).

Control BHK cells and Y1-BS1 cells that were cultured in the absence of ACTH demonstrated apparent HDL_3 selective CE uptake from doubly radiolabeled HDL_3; however, quantitatively, this lipid uptake was low. SR-BI-deficient HEK 293 cells showed no or only a minor rate of selective CE uptake from HDL_3 depending on the experimental conditions. These observations suggest that also in the absence of cellular SR-BI, HDL-associated lipid can be selectively taken up, however, at low rates. As expected, SR-BI expression in BHK cells or in Y1-BS1 cells facilitated HDL_3 selective CE uptake up to ~1,900%.

LPL promoted HDL_3 selective CE uptake by cells without or with SR-BI expression. Quantitatively, this lipase effect was very similar in the absence or presence of SR-BI. Exogenous LPL of bovine origin that was added to the assay medium provoked qualitatively identical effects compared with LPL, which was synthesized and secreted by the cells. Besides, LPL induced HDL_3 selective CE uptake in SR-BI-deficient HEK 293 cells (18, 19). This qualitatively identical and quantitatively very similar LPL-mediated stimulation of HDL_3 selective CE uptake in the absence or presence of SR-BI provides strong evidence that the lipase action on the selective HDL_3 lipid uptake pathway occurs via a mechanism that is distinct from SR-BI. Consistent with this proposal is the selective CE uptake from HDL_3 by control BHK cells and basal Y1-BS1 cells that are SR-BI deficient.

To investigate the effect of LPL on HDL_3 metabolism independent of lipolysis, an inhibitor of the catalytic activity of this enzyme, that is, THL, was present in the assay medium along with HDL_3 and this lipase (26, 27). This compound blocks the LPL-induced lipolysis (16). Because of these conditions, it is concluded that LPL stimulates the HDL selective uptake pathway independent of lipolysis. This proposal is in line with recent investigations (18, 19). However, in vivo both lipolytic and nonlipolytic actions of LPL may modify HDL metabolism (19, 42).

The LPL-mediated stimulation of the HDL selective CE uptake pathway was dependent on cell surface proteoglycans, and this was true in control BHK cells and in BHK cells with SR-BI expression. However, proteoglycan deficiency had no effect on selective CE uptake in the absence of LPL. This result with BHK cells is consistent with previous observations, that is, the actions of lipases on selective CE uptake are dependent on cell surface proteoglycans, and this is true independent of the cellular SR-BI status (13, 16, 18). Lipase binding to cell surface proteoglycans presumably anchors lipoproteins close to the plasma membrane (5, 9). Most likely, proteoglycan deficiency impairs lipase binding to the cell surface and thus reduces the association of HDL close to the membrane. Such a mechanism probably explains the reduced LPL-mediated stimulation of HDL_3 selective CE uptake in proteoglycan-deficient cells.

The LPL-mediated and SR-BI-independent stimulation of HDL selective CE uptake that was observed here is in basic agreement with an investigation using LDL as lipoprotein particle (18). LPL stimulated selective CE uptake from LDL independent of SR-BI and lipolysis, and this was true whether this lipase was of exogenous or endogenous, cell-secreted origin (18). Even though HL is not identical with LPL, the role of SR-BI in the mechanism of the HL-mediated increase in selective CE uptake was explored recently (19). Cell-secreted HL and SR-BI had a synergistic function in the mechanism of the lipase-mediated promotion of selective HDL CE uptake (19). This result is contrast with the conclusion of this study and with LPL-mediated stimulation of selective CE uptake from LDL (18). However, significant methodological differences have to be considered. For example, the radiolabeling protocol for HDL was significantly different between the investigation of Lambert and co-workers (19) and the one that was applied here. Besides, in the study with HL, the HEK 293 cells only transiently expressed SR-BI (19). In contrast, here transfected or hormone-stimulated cells with stable SR-BI expression were the experimental model. Such methodological differences may explain the differences in results (19).

The stimulation of the HDL selective CE uptake pathway by LPL in cells without or with SR-BI expression that is observed here and the LPL-induced selective CE uptake from LDL independent of SR-BI (18) suggest that (a) pathway(s) distinct from SR-BI mediate(s) this lipase-induced increase in selective lipid uptake. The question arises which specific cellular mechanism(s) is (are) responsible for the LPL-mediated stimulation of selective CE uptake. LPL binds to cell surface proteoglycans and lipoproteins, and these interactions closely associate these particles with cell membranes (5, 9, 10). For holo-lipoprotein uptake, presumably this "bridging" facilitates particle internalization (5, 11, 12, 14). Analogously, the results of this HDL study and those presented for LDL (18) are in line with the hypothesis that LPL "anchors" lipoproteins in close association with the plasma membrane, and this facilitates the selective lipid transfer into cells. This model is in agreement with recent results on the molecular mechanism of the selective uptake pathway (39, 43). In transfected cells that express SR-BI or CD36, a class B scavenger receptor that is closely related to SR-BI, both SR-BI or CD36 bind HDL with high affinity (43). This HDL binding to SR-BI or CD36 mediates lipid transfer from this lipoprotein to cells, and this presumably constitutes one component in the molecular mechanism of the selective

CE uptake pathway. Analogously, in Y1-BS1 adrenocortical cells a tight correlation between HDL binding and HDL selective CE uptake has been established (39). These observations are in line with the above presented hypothesis, that is, the LPL-induced close association between HDL and the plasma membrane presumably facilitates the selective CE transfer from lipoproteins into cells.

By which molecular mechanism(s) are the hydrophobic CE molecules finally transferred from the neutral lipid core of the HDL particle into the cell after a lipase-mediated HDL tethering to the plasma membrane? The mechanism(s) of this lipid transfer is not defined at present. Pittman and co-workers (44) explored the CE transfer from HDL particles to plasma membranes. Experiments using model membranes, purified plasma membranes, native radiolabeled HDL, and recombinant HDL suggested that CE molecules are transferred to membranes in a collision-mediated process. The latter process involves lipid-lipid interactions independent of membrane proteins (44). According to an alternative model, SR-BI forms a nonaqueous "channel" that mediates the CE movement from a lipoprotein into the plasma membrane (39). However, in view of the results of this study, it is very unlikely that this channel contributes to the lipase-mediated CE transfer. Taken together, future experiments have to define the molecular mechanism(s) that mediate(s) the lipase-induced selective CE transfer from lipoproteins to cells. Of course, besides lipid–lipid interactions, a plasma membrane protein(s) distinct from SR-BI may play a role in this lipid transfer.

The interaction between LPL, SR-BI, and HDL was investigated in this investigation in vitro. However, which physiological implications have these results for reverse cholesterol transport to the liver in vivo? SR-BI has a physiologic role for reverse cholesterol transport to the liver (2, 4). In LPL transgenic mice, expression of human LPL in skeletal muscle reduces plasma HDL cholesterol and increases the catabolism of HDL-associated CE (45). These investigations suggest that LPL possibly modifies HDL metabolism in vivo. However, future experiments have to address the interaction between LPL and SR-BI in HDL-mediated reverse cholesterol transport in vivo.

This study was supported by research grant Gr 258/10-2 from Deutsche Forschungsgemeinschaft, Bonn, Germany. The expert assistance of Mrs. B. Schulz in these studies is gratefully acknowledged. Mrs. U. Beisiegel, Mrs. R. Kozyraki, I. J. Goldberg, J. Heeren, R. Morton, B. P. Schimmer, A. R. Tall, D. van der Westhuyzen, and H. Will donated materials that were used in these experiments. Contributions of these scientists are acknowledged.

Manuscript received 6 December 2000 and in revised form 29 May 2001.

REFERENCES

1. Pittman, R. C., T. P. Knecht, M. S. Rosenbaum, and C. A. Taylor, Jr. 1987. A nonendocytotic mechanism for the selective uptake of high density lipoprotein-associated cholesterol esters. *J. Biol. Chem.* **262:** 2443–2450.
2. 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.
3. Rigotti, A., B. L. Trigatti, M. Penman, H. Rayburn, J. Herz, and M. Krieger. 1997. A targeted mutation in the murine gene encoding the high density lipoprotein (HDL) receptor scavenger receptor class B type I reveals its key role in HDL metabolism. *Proc. Natl. Acad. Sci. USA.* **94:** 12610–12615.
4. Varban, M-L., F. Rinninger, N. Wang, V. Fairchild-Huntress, J. H. Dunmore, Q. Fang, M. J. Gosselin, K. L. Dixon, J. D. Deeds, S. L. Acton, A. R. Tall, and D. Huszar. 1998. Targeted mutation reveals a central role for SR-BI in hepatic selective uptake of high density lipoprotein cholesterol. *Proc. Natl. Acad. Sci. USA.* **95:** 4619–4624.
5. Goldberg, I. J. 1996. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *J. Lipid Res.* **37:** 693–707.
6. Jansen, H., C. Kalkman, A. J. Zonneveld, and W. C. Huelsmann. 1979. Secretion of triacylglycerol hydolase activity by isolated parenchymal rat liver cells. *FEBS Lett.* **98:** 299–302.
7. Kuusi, T., E. A. Nikkilae, I. Virtanen, and P. K. J. Kinnunen. 1979. Localization of the heparin-releasable lipase in situ in the rat liver. *Biochem. J.* **181:** 245–246.
8. Thuren, T. 2000. Hepatic lipase and HDL metabolism. *Curr. Opin. Lipidol.* **11:** 277–283.
9. Eisenberg, S., E. Sehayek, T. Olivecrona, and I. Vlodavsky. 1992. Lipoprotein lipase enhances binding of lipoproteins to heparan sulfate on cell surfaces and extracellular matrix. *J. Clin. Invest.* **90:** 2013–2021.
10. Rumsey, S. C., J. C. Obunike, Y. Arad, R. J. Deckelbaum, and I. J. Goldberg. 1992. Lipoprotein lipase-mediated uptake and degradation of low density lipoproteins by fibroblasts and macrophages. *J. Clin. Invest.* **90:** 1504–1512.
11. Beisiegel, U., W. Weber, and G. Bengtsson-Olivecrona. 1991. Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein. *Proc. Natl. Acad. Sci. USA.* **88:** 8342–8346.
12. Mulder, M., P. Lombardi, H. Jansen, T. J. C. van Berkel, R. R. Frants, and L. M. Havekes. 1993. Low density lipoprotein receptor internalizes low density and very low density lipoproteins that are bound to heparan sulfate proteoglycans via lipoprotein lipase. *J. Biol. Chem.* **268:** 9369–9375.
13. Ji, Z-S., H. L. Dichek, R. D. Miranda, and R. W. Mahley. 1997. Heparan sulfate proteoglycans participate in hepatic lipase- and apolipoprotein E-mediated binding and uptake of plasma lipoproteins, including high density lipoproteins. *J. Biol. Chem.* **272:** 31285–31292.
14. Obunike, J. C., I. J. Edwards, S. C. Rumsey, L. K. Curtiss, W. D. Wagner, R. J. Deckelbaum, and I. J. Goldberg. 1994. Cellular differences in lipoprotein lipase-mediated uptake of low density lipoproteins. *J. Biol. Chem.* **269:** 13129–13135.
15. Panzenboeck, U., A. Wintersberger, S. Levak-Frank, R. Zimmermann, R. Zechner, G. M. Kostner, E. Malle, and W. Sattler. 1997. Implications of endogenous and exogenous lipoprotein lipase for the selective uptake of HDL_3-associated cholesteryl esters by mouse peritoneal macrophages. *J. Lipid Res.* **38:** 239–253.
16. Rinninger, F., T. Kaiser, W. A. Mann, N. Meyer, H. Greten, and U. Beisiegel. 1998. Lipoprotein lipase mediates and increase in the selective uptake of high density lipoprotein-associated cholesteryl esters by hepatic cells in culture. *J. Lipid Res.* **39:** 1335–1348.
17. Rinninger, F., W. A. Mann, T. Kaiser, S. Ahle, N. Meyer, and H. Greten. 1998. Hepatic lipase mediates an increase in selective uptake of high-density lipoprotein-associated cholesteryl esters by human Hep 3B hepatoma cells in culture. *Atherosclerosis.* **141:** 273–285.
18. Seo, T., M. Al-Haideri, E. Treskova, T. S. Worgall, Y. Kako, I. J. Goldberg, and R. J. Deckelbaum. 2000. Lipoprotein lipase-mediated selective uptake from low density lipoprotein requires cell surface proteoglycans and is independent from scavenger receptor class B type 1. *J. Biol. Chem.* **275:** 30355–30362.
19. Lambert, G., M. B. Chase, K. Dugi, A. Bensadoun, H. B. Brewer, Jr., and S. Santamarina-Fojo. 1999. Hepatic lipase promotes the selective uptake of high density lipoprotein-cholesteryl esters via the scavenger receptor B1. *J. Lipid Res.* **40:** 1294–1303.
20. Schimmer, B. P. 1979. Adrenocortical Y1 cells. *Methods Enzymol.* **52:** 570–574.
21. Pittman, R. C., and C. A. Taylor, Jr. 1986. Methods for assessment of tissue sites of lipoprotein degradation. *Methods Enzymol.* **129:** 612–628.
22. Havel, R. J., H. A. Eder, and H. Bragdon. 1955. The distribution

and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
23. Rinninger, F., M. Brundert, S. Jaeckle, P. R. Galle, C. Busch, J. R. Izbicki, X. Rogiers, D. Henne-Bruns, B. Kremer, C. E. Broelsch, and H. Greten. 1994. Selective uptake of high-density lipoprotein-associated cholesteryl esters by human hepatocytes in primary culture. *Hepatology.* **19:** 1100–1114.
24. Weisgraber, K. H., and R. W. Mahley. 1980. Subfractionation of human high density lipoproteins by heparin-sepharose affinity chromatography. *J. Lipid Res.* **21:** 316–325.
25. Saxena, U., L. R. Witte, and I. J. Goldberg. 1989. Release of endothelial cell lipoprotein lipase by plasma lipoproteins and free fatty acids. *J. Biol. Chem.* **264:** 4349–4355.
26. Hadvary, P., W. Sidler, W. Meister, W. Vetter, and H. Wolfer. 1991. The lipase inhibitor tetrahydrolipstatin binds covalently to the putative active site serine of pancreatic lipase. *J. Biol. Chem.* **266:** 2021–2027.
27. Skottova, N., R. Savonen, A. Lookene, M. Hultin, and G. Olivecrona. 1995. Lipoprotein lipase removal of chylomicrons and chylomicron remnants by the perfused rat liver. *J. Lipid Res.* **36:** 1334–1344.
28. Calvo, D., and M. A. Vega. 1993. Identification, primary structure, and distribution of CLA-1, a novel member of the CD36/LIMPII gene family. *J. Biol. Chem.* **268:** 18929–18935.
29. Hawley-Nelson, P., V. Ciccarone, G. Gebeyehu, J. Jessee, and P. Felgner. 1993. Lipofectamine reagent: a new, higher efficiency polycationic liposome transfection reagent. *Focus.* **15:** 73–79.
30. Chang, S-H., H. J. Netter, E. Hildt, R. Schuster, S. Schaefer, Y-C. Hsu, A. Rang, and H. Will. 2001. Duck hepatitis B virus expresses a regulatory HBx-like protein from a hidden open reading frame. *J. Virol.* **75:** 161–170.
31. Temel, R. E., B. Trigatti, R. B. DeMattos, S. Azhar, M. Krieger, and D. L. Williams. 1997. Scavenger receptor class B, type I (SR-BI) is the major route for the delivery of high density lipoprotein cholesterol to the steroidogenic pathway in cultured mouse adrenocortical cells. *Proc. Natl. Acad. Sci. USA.* **94:** 13600–13605.
32. Rigotti, A., E. R. Edelman, P. Seifert, S. N. Iqbal, R. B. DeMattos, R. E. Temel, M. Krieger, and D. L. Williams. 1996. Regulation by adrenocorticotropic hormone of the in vivo expression of scavenger receptor class B type I (SR-BI), a high density lipoprotein receptor, in steroidogenic cells of the murine adrenal gland. *J. Biol. Chem.* **271:** 33545–33549.
33. Kozyraki, R., J. Fyfe, M. Kristiansen, C. Gerdes, C. Jacobsen, S. Cui, E. I. Christensen, M. Aminoff, A. de la Chapelle, R. Krahe, P. J. Verroust, and S. K. Moestrup. 1999. The intrinsic factor-vitamin B12 receptor, cubilin, is a high-affinity apolipoprotein A-I receptor facilitating endocytosis of high-density lipoprotein. *Nat. Med.* **5:** 656–661.
34. Rinninger, F., S. Jaeckle, and R. C. Pittman. 1993. A pool of reversibly cell-associated cholesteryl esters involved in the selective uptake of cholesteryl esters from high-density lipoproteins by Hep G2 hepatoma cells. *Biochim. Biophys. Acta.* **1166:** 275–283.
35. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1957. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
36. 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 depeletion of cholesterol stores. *J. Biol. Chem.* **271:** 21001–21004.
37. Webb, N. R., P. M. Connell, G. A. Graf, E. J. Smart, W. J. S. de Villiers, F. C. de Beer, and D. R. van der Westhuyzen. 1998. SR-BII, an isoform of the scavenger receptor BI containing an alternate cytoplasmic tail, mediates lipid transfer between high density lipoprotein and cells. *J. Biol. Chem.* **273:** 15241–15248.
38. 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.
39. Rodrigueza, W. V., S. T. Thuahnai, R. E. Temel, S. Lund-Katz, M. C. Phillips, and D. L. Williams. 1999. Mechanism of scavenger receptor class B type I-mediated selective uptake of cholesteryl esters from high density lipoprotein to adrenal cells. *J. Biol. Chem.* **274:** 20344–20350.
40. Rinninger, F., and R. C. Pittman. 1988. Regulation of the selective uptake of high-density lipoprotein-associated cholesteryl esters by human fibroblasts and Hep G2 hepatoma cells. *J. Lipid Res.* **29:** 1179–1194.
41. Hammad, S. M., S. Stefansson, W. O. Twal, C. J. Drake, P. Fleming, A. Remaley, H. B. Brewer, Jr., and W. S. Argraves. 2000. Cubilin, the endocytic receptor for intrinsic factor-vitamin B12 complex, mediates high-density lipoprotein holoparticle endocytosis. *Proc. Natl. Acad. Sci. USA.* **96:** 10158–10163.
42. Dugi, K. A., M. J. A. Amar, C. C. Haudenschild, R. D. Shamburek, A. Bensadoun, R. F. Hoyt, Jr., J. Fruchart-Najib, Z. Madj, H. B. Brewer, Jr., and S. Santamarina-Fojo. 2000. In vivo evidence for both lipolytic and nonlipolytic function of hepatic lipase in the metabolism of HDL. *Arterioscler. Thromb. Vasc. Biol.* **20:** 793–800.
43. Connelly, M. A., S. M. Klein, S. Azhar, N. A. Abumrad, and D. L. Williams. 1999. Comparison of class B scavenger receptors, CD36 and scavenger receptor BI (SR-BI), shows that both receptors mediate high density lipoprotein-cholesteryl ester selective uptake but SR-BI exhibits a unique enhancement of cholesteryl ester uptake. *J. Biol. Chem.* **274:** 41–47.
44. Morrison, J. R., M. J. Silvestre, and R. C. Pittman. 1994. Cholesteryl ester transfer between high density lipoprotein and phospholipid bilayers. *J. Biol. Chem.* **269:** 13911–13918.
45. Levak-Frank, S., P. H. Weinstock, T. Hayek, R. Verdery, W. Hofmann, R. Ramakrishnan, W. Sattler, J. L. Breslow, and R. Zechner. 1997. Induced mutant mice expressing lipoprotein lipase exclusively in muscle have subnormal triglycerides yet reduced high density lipoprotein cholesterol levels in plasma. *J. Biol. Chem.* **272:** 17182–17190.