Overexpression of SR-BI by adenoviral vector promotes clearance of apoA-I, but not apoB, in human apoB transgenic mice

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Abstract **Scavenger receptor BI (SR-BI) is a multi-ligand lipoprotein receptor that mediates selective lipid uptake from HDL, and plays a central role in hepatic HDL metabolism. In this report, we investigated the extent to which SR-BI selective lipid uptake contributes to LDL metabolism. As has been reported for human LDL, mouse SR-BI expressed in transfected cells mediated selective lipid uptake from mouse LDL. However, LDL-cholesteryl oleoyl ester (CE) transfer relative to LDL-CE bound to the cell surface (fractional transfer) was ~18-fold lower compared with HDL-CE. Adenoviral vector-mediated SR-BI overexpression in livers of human apoB transgenic mice (~10-fold increased expression) reduced plasma HDL-cholesterol (HDL-C) and apolipoprotein (apo)A-I concentrations to nearly undetectable levels 3 days after adenovirus infusion. Increased hepatic SR-BI expression resulted in only a modest depletion in LDL-C that was restricted to large LDL particles, and no change in steady-state concentrations of human apoB. Kinetic studies showed a 19% increase in the clearance rate of LDL-CE in mice with increased SR-BI expression, but no change in LDL apolipoprotein clearance. Quantification of hepatic uptake of LDL-CE and LDL-apolipoprotein showed selective uptake of LDL-CE in livers of human apo B transgenic mice. However, such uptake was not significantly increased in mice over-expressing SR-BI. We conclude that SR-BI-mediated selective uptake from LDL plays a minor role in LDL metabolism in vivo.**—Webb, N. R., M. C. de Beer, J. Yu, M. S. Kindy, A. Daugherty, D. R. van der Westhuyzen, and F. C. de Beer. **Overexpression of SR-BI by adenoviral vector promotes clearance of apoA-I, but not apoB, in human apoB transgenic mice.** ***J. Lipid Res.*** **2002.** 43: **1421–1428.**

Supplementary key words scavenger receptor BI • selective uptake • apolipoprotein B • transfected cells • low density lipoprotein receptor • transgenic mice • adenoviral vector

Scavenger receptor class B type I (SR-BI) is a cell-surface receptor that mediates selective lipid uptake from HDL (1). During this process, cholesteryl oleoyl esters (CEs) from the core of the HDL particle are transferred to the cell without degradation of the protein moiety. In rodents, selective lipid uptake represents the major mechanism by which HDL-CE is delivered to the liver (2) and steroidogenic cells (3, 4). Selective uptake of HDL-cholesterol (HDL-C) is also likely to be substantial in humans (5). SR-BI is now considered to be the principal receptor for the HDL selective lipid uptake pathway (6). Targeted mutations in the SR-BI gene in mice that prevent or reduce SR-BI expression result in increased plasma HDL-C concentrations (7, 8) and decreased HDL-CE uptake by the liver (8). Conversely, transgenic mice expressing high levels of SR-BI in the liver have decreased plasma HDL-CE, apoA-I, and apoA-II concentrations (9, 10). Overexpression of SR-BI by adenoviral vectors produces a similar reduction in plasma HDL-C concentrations (11, 12), and increased cholesterol secretion into the bile (11). It is notable that transgene or adenoviral vector-induced overexpression of SR-BI results in accelerated catabolism of HDL apoA-I, despite the fact that SR-BI mediates only CE uptake from HDL (9, 11). A major site of apoA-I catabolism is the kidney, leading to the concept that increased hepatic SR-BI activity leads to the production of lipid-depleted forms of apoA-I that are subject to glomerular filtration (9, 13).

Analyses in cultured cells have demonstrated that SR-BI binds other classes of lipoproteins in addition to HDL. Hamster SR-BI was originally identified through its ability to bind modified human LDL (14). CLA-I, the human ho-

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Abbreviations: CE, cholesteryl oleoyl ester; CEt, cholesteryl oleoyl ether; CHO, Chinese hamster ovary; SR-BI, scavenger receptor class B type I.

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molog of SR-BI, binds VLDL in addition to LDL and HDL (15). In addition to mediating high affinity LDL binding, SR-BI expressed in transfected cells or Y1 adrenocortical cells promotes selective lipid uptake from LDL (16, 17). It is notable that in two separate reports, LDL appeared to be a less effective donor for SR-BI selective uptake when compared with HDL. The possibility exists, however, that the difference in SR-BI activity toward LDL and HDL in these studies may be due to species-specific differences in lipoproteins and receptor, since selective uptake was assessed using cells expressing mouse SR-BI and radiolabeled human HDL and LDL as ligands. It remains to be established whether the LDL binding domain of mouse and human SR-BI is functionally distinct, as has been reported previously for the LDL receptor (LDLR) (10, 18).

In studies in vivo, alterations in hepatic SR-BI expression have been associated with changes in plasma concentrations of apoB-containing lipoproteins. Sustained, high-level expression of SR-BI in livers of transgenic mice results in reduced plasma concentrations of LDL-C and apoB (9, 10), as well as decreased VLDL and IDL/LDL particle size (10). Transgenic SR-BI overexpression also results in decreased concentrations of apoB-containing lipoproteins that accumulate in LDLR-deficient mice, and the degree of VLDL + LDL (but not HDL) lowering was strongly correlated with the extent of atherosclerosis in the aortic root of these mice (19). Increased SR-BI expression is also associated with decreased non-HDL-C in SR-BI/human apoB double-transgenic mice fed a chow diet (20). This ability of SR-BI in transgenic mice to influence plasma non-HDL-C and apoB concentrations is variable, however, and may be influenced by genotype as well as diet (19, 20). Kozarsky and colleagues investigated the effect of adenoviral vector-mediated SR-BI overexpression on lipoprotein profiles and atherogenesis in LDLR$^{-/-}$ mice (21). Modest but significant decreases in non-HDL cholesterol and apoB were observed in LDLR$^{-/-}$ mice fed a diet enriched in saturated fat 14 to 21 days after recombinant adenovirus injection. In this study, effects on atherosclerotic lesion size were significantly correlated with HDL-C levels, but not non-HDL-C. Thus, the ability of SR-BI to modulate non-HDL-C levels in vivo and the consequence of such effects on atherogenesis remain to be clarified.

An unresolved question is whether alterations in apoB-containing lipoproteins in mice with constitutive SR-BI overexpression are a direct result of SR-BI-mediated particle clearance, or to secondary effects due to other perturbations in lipoprotein metabolism. Huszar et al. reported that attenuated SR-BI expression (produced by a mutation in the SR-BI promoter) results in increased LDL-C and apoB concentrations in LDLR$^{-/-}$ mice. However, this effect was attributed to increased LDL production rather than reduced LDL catabolism (22). Ueda et al. reported an accelerated clearance of radioiodinated human LDL in SR-BI transgenic mice, suggesting a direct role for SR-BI in non-HDL metabolism (10). Since LDL particles were not traced in the lipid component, it was not established whether selective lipid uptake, or perhaps some other pathway, underlies this enhanced rate of clearance.

In this study, we set out to establish whether SR-BI-mediated selective uptake from LDL in vivo leads to LDL particle and apoB clearance, as occurs with HDL and apoA-I (9, 13). We assessed the effect of acute (1–3 day) SR-BI overexpression on steady-state concentrations of apoB-containing particles that accumulate in the plasma of human apoB transgenic mice. In kinetic studies using non-degradable radiolabels, we assessed whether a 10-fold increase in hepatic SR-BI expression alters liver uptake of LDL-CE or apolipoprotein. To determine whether there are species-specific differences in the interaction of SR-BI and LDL, we compared mouse SR-BI activity toward human and mouse LDL in assays in vitro.

EXPERIMENTAL PROCEDURES

Mice

Human apoB transgenic mice were obtained from Taconic (Germantown, NY). LDLR-deficient in a C57BL/6 background and wild-type C57BL/6 mice were from Jackson Labs. All animals were maintained in a pathogen-free facility with equal light/dark cycle and free access to regular mouse chow and water, unless otherwise indicated. All procedures were approved by the Veterans Administration Medical Center Institutional Animal Use and Care Committee.

Isolation and radiolabeling of lipoproteins

LDL (d = 1.019 to 1.063 g/ml) and HDL (d =1.063 to 1.21 g/ml) were isolated from mouse or human plasma by density gradient ultracentrifugation as described previously (23). All isolated fractions were dialyzed against 150 mM NaCl, 0.01% EDTA, and stored under nitrogen gas at 4°C. Protein concentrations were determined by the method of Lowry et al. (24) and total and free cholesterol concentrations were determined enzymatically (Wako Chemicals). The difference between total and free cholesterol concentrations was used to determine CE concentrations. LDL and HDL fractions were doubly radiolabeled with the intracellularly trapped radiolabels dilactitol ^{125}I-tyramine (DLT) (25) and 1α,2α (n) [^{3}H]cholesteryl oleoyl ether (CEt) (26). The specific radioactivity of the ^{125}I-[^{3}H]HDL ranged from 10 to 28 cpm/ng protein for ^{125}I and from 10 to 22 dpm/ng protein for ^{3}H. The specific activity of the ^{125}I-[^{3}H]LDL ranged from 4 to10 cpm/ng protein for ^{125}I and from 4 to 20 dpm/ng protein for ^{3}H. For degradation assays, lipoproteins were radioiodinated using iodine monochloride (27) to a specific radioactivity of 500–800 cpm/ng.

Ligand binding, uptake, and degradation assays

The production and maintenance of a Chinese hamster ovary (CHO) line stably transfected with mouse SR-BI cDNA was described previously (12). This line, derived from CHO ldlA (clone 7) cells, is deficient in the LDLR (28). SR-BI-expressing CHO cells and control CHO cells were seeded in 6-well plates 48 h prior to assays (2.5×10^5 cells per well). Plasma from LDLR-deficient mice was used as the source for the mouse LDL ligand. Cell-association assays were performed at 37°C in Ham's F-12 media containing 100 U/ml penicillin, 100 U/ml streptomycin, 2 mM glutamine, 0.5% essentially fatty acid free BSA, and HDL or LDL radiolabeled with ^{125}I-DLT and [^{3}H]CEt. After 2 h incubations, unbound ligand was removed from cells by washing four times with 50 mM Tris-HCl buffer (pH 7.4) containing 150 mM NaCl and 2 mg/ml fatty acid free BSA, followed by two washes with 50 mM Tris-HCl, 150 mM NaCl (pH 7.4). All washes were per-

formed at 4°C with pre-chilled solutions. Cells were solubilized in 0.1 N NaOH for 60 min at room temperature prior to protein and radioactivity quantification. Samples were radioassayed directly for ^{125}I determinations and after lipid extraction (29) for ^{3}H. The amount of ^{125}I and ^{3}H cell-associated radioactivity was expressed as ng LDL or HDL-CE equivalents, which was calculated from the known specific activities and CE-protein ratio for each of the lipoprotein ligands. By expressing results in "CE equivalents", surface binding and lipid uptake can be directly compared for different lipoprotein fractions, since any differences in ^{3}H specific activity (in terms of lipoprotein CE content) have been taken into account. Selective CE uptake was calculated by subtracting ^{125}I cell-associated radioactivity from lipid extractable ^{3}H cell-associated radioactivity. For degradation assays, cell-free supernatants were precipitated with trichloroacetic acid at a final concentration of 14% (w/v). The trichloroacetic acid-soluble material was oxidized with H_2O_2 and extracted with $CHCl_3$ to remove inorganic iodide, and counted (30).

Adenovirus treatments and analysis of plasma lipids

The production of a replication-defective adenoviral vector expressing mouse SR-BI was described previously (12). Adnull (provided by Dr. D. J. Rader, University of Pennsylvania) is a recombinant adenovirus containing no transgene. Human apoB transgenic mice weighing at least 25 g were injected in the tail vein with 1×10^{11} particles of either AdSR-BI or Adnull in 100 μl PBS. Liver expression of SR-BI was assessed by quantitative immunoblotting as previously described (12). Plasma was collected from mice after a 10 h fast. Aliquots (200 μl) were clarified by centrifugation and resolved by size exclusion chromatography with a Superose 6 column (Pharmacia LKB Biotechnology Inc.). The column was eluted at a flow rate of 0.5 ml/min in buffer containing 150 mM NaCl, 10 mM Tris/HCl pH 7.4, 0.01% sodium azide. The cholesterol content of fractions (0.5 ml) or whole plasma was determined enzymatically (Wako Chemicals).

Quantification of plasma apolipoproteins

Aliquots from mouse plasma (5 μl) were separated by reducing SDS-PAGE (5–20% acrylamide gradient), electroblotted onto 0.2 μM pore-size PVDF membrane (Schleicher and Schuell, Keene, NH), and immunoblotted using rabbit anti-mouse apoA-I (gift of J. Lusis, UCLA). Antibody binding was visualized by chemiluminescence detection (ECL, Amersham Corp.). Human apoB in human apoB transgenic mouse serum was quantified by an immunoturbidimetric assay using a Sigma Diagnostics® kit.

LDL turnover studies

LDL, isolated from human apoB transgenic mouse plasma and radiolabeled with ^{125}I-DLT and [^{3}H]CEt, was injected via the jugular vein into human apoB transgenic mice 3 days after treatment with 1×10^{11} particles AdSR-BI or Adnull (five mice per group). Blood samples were collected 3 min, 1 h, 2 h, 4 h, 6 h, 11 h, and 24 h after tracer injection by retro-orbital bleeding. At 24 h after tracer injection, animals were anesthetized, exsanguinated, and perfused with saline (30 ml per animal). Tissue and plasma samples were radioassayed directly for ^{125}I content and after lipid extraction (3, 29) for ^{3}H. Tracers in the gut were attributed to uptake by the liver (3, 31). Radiotracer clearance curves were generated by expressing the radioactivity at each time point as a percentage of the radioactivity determined 3 min after tracer injection. To calculate plasma fractional catabolic rates (FCR) for both LDL tracers, a non-compartmental analysis was performed using WinNonlinin (v 2.1, Pharsight Corp., Palo Alto, CA). Estimates of the terminal slope were made using at least the last three observations. Liver FCRs for both LDL tracers were calculated by multiplying the plasma FCR of the tracer with the fraction of the injected tracer recovered in the liver and gut.

Statistical Analysis

Statistical analyses were performed using a parametric unpaired Student's *t*-test.

RESULTS

Studies in vitro have demonstrated that LDL can serve as a substrate for selective uptake by SR-BI (16, 17). However, relative to the amount of CE bound to the cell surface, lipid transfer mediated by SR-BI from LDL particles appeared to be less efficient when compared with HDL (16, 17). These experiments were carried out using cells expressing mouse SR-BI and human LDL and HDL as ligands, raising the possibility that more optimal lipid transfer could occur between mouse SR-BI and mouse LDL. Accordingly, we set out to assess SR-BI activity toward LDL and HDL using a homologous system, and to determine whether there are species differences in selective uptake by mouse SR-BI from human and mouse LDL. LDLR-deficient CHO cells (28) and LDLR-deficient CHO cells that stably express high levels of mouse SR-BI (CHO-SRBI cells) (32) were incubated with either mouse LDL, human LDL, or mouse HDL doubly radiolabeled with ^{125}I-DLT and [^{3}H]CEt. To allow for a direct comparison of different lipoprotein fractions, cell-associated radioactivity was expressed as CE equivalents, which is calculated from the known specific activities (cpm or dpm per ng protein) and the CE-protein ratio for each of the lipoprotein ligands (**Fig. 1A–C**). The differences in the absolute amount of CE associated to cells for the different lipoprotein ligands (Fig. 1) are likely accounted for in part by differences in particle content of CE (see Discussion).

Compared with non-transfected cells, CHO-SR-BI cells exhibited a 4- and 6-fold increase in the amount of mouse and human ^{125}I-DLT LDL associated to cells, respectively (Fig. 1A, B). However, this substantial increase in ^{125}I cell association by SR-BI expressing cells was accompanied by only a 30–40% increase in selective CE uptake (defined as the difference between ^{3}H and ^{125}I cell-associated radioactivity). In contrast, in the case of HDL, a 3.5-fold increase in ^{125}I cell association resulted in a 4.8-fold increase in selective CE uptake by CHO-SRBI cells compared with the parental line (Fig. 1C). In multiple experiments encompassing a range of ligand concentrations, SR-BI-dependent cell association of [^{3}H]CEt relative to the amount of ^{125}I-DLT was consistently greater for mouse HDL compared with mouse and human LDL (data not shown).

To determine whether the increased ^{125}I-DLT LDL association by CHO-SR-BI cells could be attributable in part to SR-BI-mediated whole particle uptake, ldlA7 and CHO-SRBI cells were also incubated with radioiodinated mouse and human LDL. Minimal ^{125}I-labeled degradation products (less than 10% of the total ^{125}I associated with the cells) were detected after a 2 h incubation period (data not shown), indicating that mouse and human LDL, like HDL, is not internalized by mouse SR-BI and that the

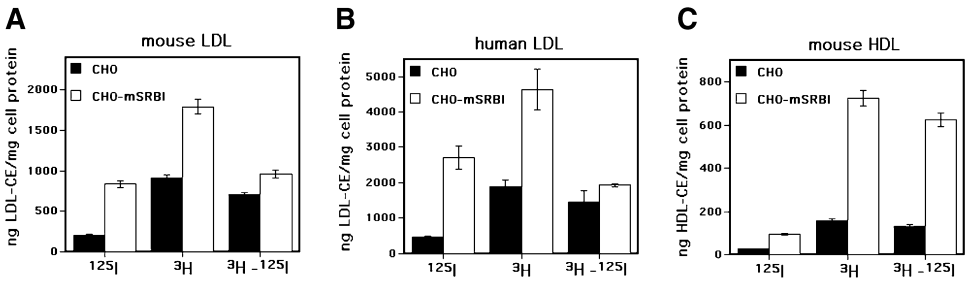

Fig. 1. Scavenger receptor class B type I (SR-BI)-mediated LDL and HDL selective lipid uptake. Non-transfected LDL receptor (LDLR)-deficient Chinese hamster ovary (CHO) cells or cells transfected with mouse SR-BI cDNA were incubated at 37°C for 2 h with 10 μg/ml $LDLR^{-/-}$ mouse LDL (A), human LDL (B), or C57BL/6 mouse HDL (C) radiolabeled with dilactitol ^{125}I-tyramine (DLT) and [^{3}H]cholesteryl oleoyl ether (CEt). Cell-associated radiolabel, expressed as cholesteryl oleoyl ester (CE) equivalents, was quantified as described in "Experimental Procedures".

large majority of ^{125}I-DLT radioactivity associated to both cell lines represents surface-bound LDL. We conclude from these in vitro experiments that mouse SR-BI activity toward mouse and human LDL is similar. Although SR-BI is capable of mediating selective transport of CE from both mouse and human LDL, these ligands deliver a much smaller fraction of core lipid to cells compared with HDL. When expressed as a fractional delivery, (defined as the ratio of the amount of SR-BI-dependent selective uptake relative to the amount of SR-BI-dependent LDL-CE bound to the cell surface) the capacity of mouse SR-BI to transfer CE from human and mouse LDL (0.4 and 0.2, respectively) was markedly less than from mouse HDL (7.2) as shown in the representative experiment in Fig. 1. In three separate experiments, the mean (±SD) fractional transfer of CE from mouse LDL in 2 h (10 μg/ml ligand) was 0.36 ± 0.26. The corresponding fractional transfer of mouse HDL was 6.3 ± 0.78.

Studies in transgenic mice have indicated that constitutive overexpression of SR-BI results in reduced plasma LDL-C and apoB concentrations (9, 10). One possible explanation for these findings is that SR-BI activity results in the removal of apoB-containing lipoproteins from the circulation. To further investigate this possibility, we transiently overexpressed the receptor in vivo using an adenoviral vector. We assessed the acute effects of hepatic SR-BI overexpression in human apoB transgenic mice that have high plasma concentrations of LDL due to hepatic production of human apoB (33, 34). For these experiments, mice were administered a dose of AdSR-BI (1×10^{11} particles) to produce an ~10-fold increase in hepatic SR-BI expression, which corresponds to the amount of receptor constitutively expressed in SR-BI transgenic mouse models (9, 10) (**Fig. 2A**; note 10-fold difference in protein loading). We have shown that this dose of adenovirus does not generate a general acute phase response in mice (35). As depicted in Fig. 2B, increased hepatic SR-BI expression resulted in a significant reduction in plasma HDL-C concentrations as early as 24 h after adenovirus infusion. By 72 h post-treatment, HDL-C was reduced to only 3% of baseline values. Plasma total cholesterol was also significantly lower 72 h after AdSR-BI-treatment compared with untreated mice. However, the magnitude of the decrease in total cholesterol appeared to be accounted for by the reduction in HDL-C.

Analyses of plasma lipoprotein fractions by size exclusion chromatography confirmed that transient hepatic SR-BI overexpression predominantly affects HDL particles in human apoB transgenic mice (**Fig. 3**). Whereas HDL-C (eluting in fractions 27–31) was reduced to virtually undetectable concentrations 72 h after AdSR-BI infusion, the effect on the LDL peak (fractions 20–25) was much less pronounced. Compared with mice infused with a control adenoviral vector (Adnull), AdSR-BI-treated mice had a modest, albeit significant, reduction in the amount of

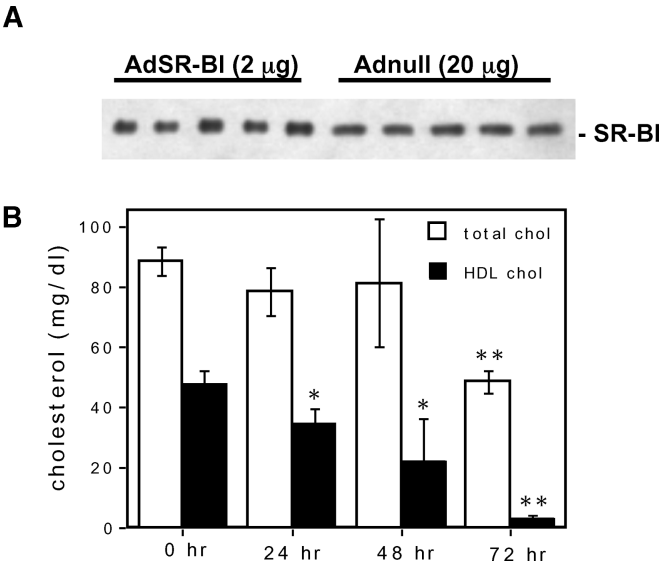

Fig. 2. Adenoviral-vector mediated SR-BI overexpression in livers of human apoB transgenic mice. A: Livers were collected from mice 72 h after infusion of 1×10^{11} particles Adnull or AdSR-BI and homogenates prepared. Aliquots from individual livers corresponding to 20 μg (Adnull) or 2 μg (AdSR-BI) protein were separated by non-reducing SDS-PAGE and immunoblotted with anti-BI^{495} (12). B: Plasma was collected at the indicated intervals after AdSR-BI infusion and total and HDL-cholesterol (HDL-C) concentrations were determined as described in "Experimental Procedures". The values shown represent the mean (± SD) of three to five mice. * $P < 0.01$; ** $P < 0.0001$.

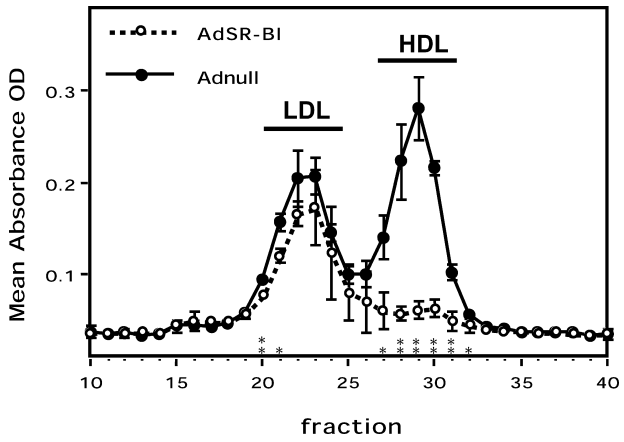

Fig. 3. Lipoprotein-C distributions of control and AdSR-BI-treated human apoB transgenic mice. Plasma was collected 72 h after infusion with 1×10^{11} particles AdSR-BI or Adnull. Aliquots (200 μl) were fractionated by size exclusion chromatography, and the cholesterol content of 0.5 ml fractions was determined. Values for each fraction are the mean absorbance (± SD) from the analysis of 3 individual mice after adenovirus treatment. * $P < 0.05$; ** $P < 0.005$

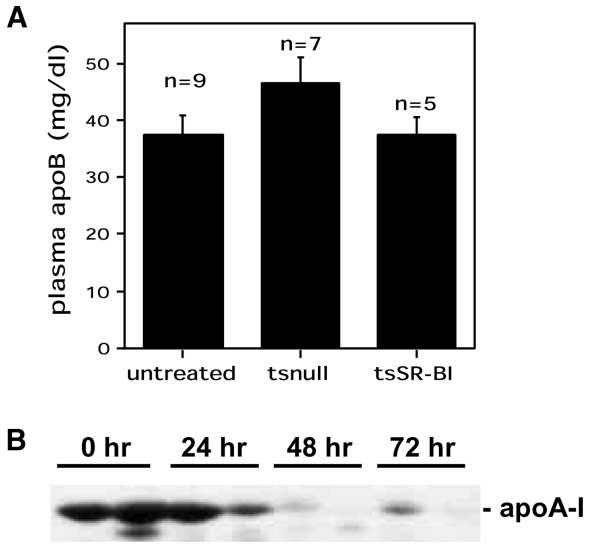

Fig. 4. Plasma apolipoprotein content after adenovirus treatments. A: Plasma human apoB concentration was determined as described in Experimental Procedures for mice before and 72 h after infusion with 1×10^{11} particles AdSR-BI or Adnull. Histograms represent the mean (± SD) of values from the indicated number of mice. B: Aliquots (2.5 μl) of individuals mouse plasmas collected at the indicated interval after infusion with 1×10^{11} particles AdSR-BI were separated by reducing SDS PAGE and immunoblotted using rabbit anti-mouse apoA-I.

cholesterol in fractions containing large LDL particles (fractions 20 and 21).

We investigated whether the modest decrease in LDL-C in AdSR-BI-treated mice was accompanied by any alterations in plasma apoB concentrations. As shown in **Fig. 4A**, concentrations of human apoB were not significantly different in control mice (37.5 ± 3.4 mg/dl; n = 9) and mice 3 days after treatment with AdSR-BI (37.5 ± 3.2 mg/dl; n = 5) or a control adenoviral vector (46.4 ± 4.5 mg/dl; n = 7) (Fig. 4B). In contrast, immunoblot analysis of plasma samples showed that HDL apoA-I was markedly reduced as a result of hepatic SR-BI overexpression (Fig. 4B). The time course of apoA-I depletion corresponded to the reduction in HDL-C (Fig. 2B).

Our data indicate that transient liver-specific SR-BI overexpression results in a small reduction in steady-state concentrations of LDL-C, but no change in apoB in human apoB transgenic mice. To assess directly whether increased hepatic SR-BI activity affects the rate of LDL clearance, we measured the plasma clearance and liver uptake of LDL-CE and apolipoprotein in human apoB transgenic mice after AdSR-BI or Adnull infusion. For these experiments, LDL isolated from human apoB transgenic mice was doubly radiolabeled with the residualizing tracers ^{125}I-DLT and [^{3}H]CEt. In both groups of mice, [^{3}H]CEt cleared from the plasma at a faster rate compared with ^{125}I-DLT, indicating whole body selective uptake of LDL-CE. The plasma FCRs calculated from the plasma decay curves showed no difference in the clearance of ^{125}I-DLT for the two groups of mice (**Table 1**). In AdSR-BI-treated mice, there was a modestly (19%) higher rate of removal of [^{3}H]CEt compared with control. The selective clearance of LDL-CE, defined as the difference between [^{3}H]CEt and ^{125}I-DLT FCRs, was ~24% greater in AdSR-BI-treated mice.

To determine whether a 10-fold increase in hepatic SR-BI expression leads to enhanced liver LDL uptake, we measured the amount of [^{3}H]CEt and ^{125}I-DLT uptake 24 h after tracer injection as described in Experimental Procedures. The calculation of liver FCRs showed no significant effect of SR-BI overexpression on LDL apolipoprotein uptake (**Fig. 5**). Although there was a modest (18.6%) increase in LDL-CE uptake in livers of mice treated with AdSR-BI, this not did reach statistical significance.

DISCUSSION

Chronic over-expression of SR-BI in livers of transgenic mice produces a marked reduction in VLDL and LDL apoB concentrations (9, 10). ApoB concentrations are also reduced in heterozygous human apoB transgenic

TABLE 1. Fractional catabolic rates for dilactitol ^{125}I-tyramine, [^{3}H]cholesteryl oleoyl ether LDL from plasma in human apoB transgenic mice with and without adenovirus-mediated SR-BI overexpression

Treatment	^{125}I-DLT LDL	[^{3}H]CEt LDL	[^{3}H]^{125}I-DLT
	Pools/h	*Pools/h*	*Pools/h*
Adnull	0.068 ± 0.009	0.131 ± 0.013	0.063 ± 0.011
AdSR-BI	0.077 ± 0.011	0.156 ± 0.015	0.078 ± 0.009
	($P = 0.17$)	($P = 0.023$)	($P = 0.045$)

Human apoB transgenic mice were infused with 1×10^{11} particles Adnull or AdSR-BI (five mice per goup). Three days after adenovirus infusion, mice were injected with homologous LDL traced with ^{125}I-DLT and [^{3}H]CEt. Blood was collected at selected intervals, and radioassayed as described in Experimental Procedures. CEt, cholesteryl oleoyl ether, DLT, dilactitol ^{125}I-tyramine. Values are mean ± SD.

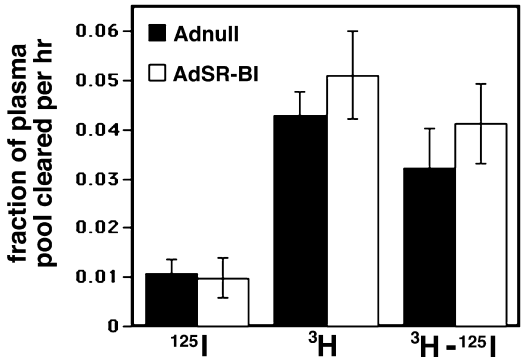

Fig. 5. Liver uptake of ^{125}I-DLT and [^{3}H]CEt from ^{125}I-[^{3}H]labeled mouse LDL. Double-labeled LDL isolated from human apoB transgenic mice was injected via the jugular vein into homologous mice 2 days after treatment with 1×10^{11} particles AdSR-BI or Adnull. At 24 h after tracer injection, animals were humanely killed and the liver content of ^{125}I and ^{3}H tracers was determined as described in "Experimental Procedures." Values represent the mean (± SD) from five individual mice.

mice and LDLR$^{-/-}$ mice containing the SR-BI transgene (20, 36). Given the known ability of SR-BI to bind apoB-containing lipoproteins, it is possible that SR-BI is exerting these effects through direct clearance of apoB-containing particles. Since it is generally accepted that SR-BI mediates selective lipid uptake from lipoproteins (and not endocytic uptake), it would follow that selective lipid uptake from VLDL and LDL can impact plasma concentrations of apoB-containing particles. To date, the effect of increased SR-BI activity in vivo on plasma clearance rates of VLDL-CE or LDL-CE, and the potential impact of this on LDL particle catabolism, have not been assessed. In the current study we investigated whether SR-BI-mediated selective lipid uptake from LDL leads to LDL particle and apoB clearance. For these studies, we used a recombinant adenoviral vector to produce a 10–15-fold increase in hepatic SR-BI expression. This increase in expression corresponds to the amount of receptor constitutively expressed in SR-BI transgenic mouse models (9, 10). The major finding from our studies is that although SR-BI has a modest capacity to mediate CE uptake from LDL in vivo, this activity does not lead to increased apoB catabolism. Our data also shows that a 10-fold increase in hepatic SR-BI expression does not result in significant alterations in LDL-CE or apolipoprotein uptake in the liver.

In our studies, increased hepatic SR-BI expression in human apoB transgenic mice resulted in only a modest depletion in LDL-C, which mainly affected large LDL particles, and no change in steady-state concentrations of human apoB. This contrasts to the marked depletion of HDL-C and apoA-I that occurred in these mice. The lack of a major effect of acute (1–3 day) hepatic SR-BI overexpression on plasma LDL-C and apoB concentrations (Figs. 3 and 4) differs from what occurs in SR-BI transgenic mice, where increased SR-BI expression resulted in markedly decreased plasma VLDL, LDL, and apoB concentrations in addition to reduced HDL levels (9, 10). Kozarsky et al. assessed the effect of longer-term (1–4 week) adenoviral vector-mediated SR-BI overexpression in LDLR$^{-/-}$ mice fed a diet enriched in fat and cholesterol (21). Such overexpression resulted in a modest reduction in plasma apoB concentrations. This reduction, which was not accompanied by a significant decrease in non-HDL-C, only occurred at day 14 after treatment, when plasma HDL-C had returned to values similar to those of control animals. Taken together, our results and the results of others indicate that effects on steady-state non-HDL concentrations occur only after constitutive or long-term (greater than 2 week) SR-BI overexpression. At these longer time intervals, a number of adaptive mechanisms could be operative.

Kinetic studies showed a modest (19%) increase in the plasma clearance rate of LDL-CE in mice with high level SR-BI expression, and no change in LDL-apolipoprotein clearance. There was also an apparent increase, although not statistically significant, in the amount of [^{3}H]CEt taken up by the liver in mice over-expressing SR-BI compared with control. The mean liver FCR for ^{3}H uptake was .043 (±.005) pools per hour in control mice, and .051 (±.009) pools per hour in AdSR-BI-treated mice. This increase would represent an 18.6% difference in the mean rate of liver uptake in the two groups of mice, which is similar to the difference in plasma clearance rates. We conclude from these results that SR-BI plays only a minor role in LDL catabolism in vivo. In a previously reported study, the impact of reduced hepatic SR-BI expression on [^{3}H]CEt, ^{125}I-LDL clearance was investigated in LDLR$^{-/-}$ mice maintained on a high-fat diet (22). In these studies, attenuated SR-BI expression had no effect on LDL-apolipoprotein or lipid clearance rates. In addition, they found no evidence that SR-BI mediates LDL selective lipid uptake in vivo. Conflicting results were reported by Ueda et al., who measured a significantly increased rate of LDL-apolipoprotein clearance in transgenic mice with constitutive SR-BI overexpression (10). Clearance rates of LDL-CE were not measured in these experiments, however. Non-HDL-C concentrations in the transgenic mice were markedly lower (reduced 90%) compared with non-transgenic mice, providing the possibility that differences in lipoprotein pool size may contribute to the differences in apoB clearance rates. In our studies, the pool size of non-HDL-cholesterol in AdSR-BI-treated mice and control mice was similar.

It is notable that we detected a substantial amount of LDL-CE accumulation in livers of human apoB transgenic mice that could not be accounted for by whole particle uptake, and that was not enhanced by a 10-fold increase in hepatic SR-BI expression. This suggests that a mechanism for LDL selective lipid uptake that is independent of SR-BI may be operative in vivo. Assays in vitro revealed a substantial amount of selective uptake from double-radiolabeled mouse LDL in non-transfected CHO cells. Only a relatively small amount of HDL selective uptake was measured in these cells. Stangl et al. also reported a large amount of [^{3}H]CEt associated with CHO cells after incubations with human LDL, but not HDL (17). It seems unlikely that the large amount of LDL selective uptake in CHO cells is mediated by the small amount of endogenous SR-BI in these cells, given the modest increase in

LDL selective uptake measured in CHO-SR-BI cells that have highly elevated SR-BI expression (12). Interestingly, COS-7 cells that have no detectable SR-BI also mediate substantial amounts of selective lipid uptake from LDL (16). Thus, there appears to be a mechanism for LDL selective lipid uptake in both CHO and COS cells that is independent of SR-BI expression. A SR-BI-independent, apoE-dependent pathway for selective LDL-CE uptake has been described in mouse adrenocortical cells (37, 38). Lipoprotein lipase has been shown in vitro and in vivo to promote LDL selective lipid uptake via a pathway that is independent of SR-BI (39). Additional studies are required to further characterize these SR-BI-independent LDL-CE selective uptake pathways and the extent to which they contribute to LDL metabolism in vivo.

Consistent with the in vivo data, studies in vitro demonstrated that SR-BI metabolizes LDL particles to a lesser extent than HDL. To our knowledge, our in vitro data provides the first published report whereby SR-BI activity toward apoB-containing lipoproteins was measured in a homologous system (i.e., mouse receptor and mouse LDL). The results show that selective lipid uptake mediated by mouse SR-BI from human and mouse LDL is similar. Although mouse SR-BI mediates the uptake of CE from both human and mouse LDL, it is clear that the amount of CE selectively taken up relative to the amount of CE bound to the cell surface is considerably less when compared with mouse HDL. Our results are qualitatively similar to two published reports, where the ability of mouse SR-BI to metabolize apoB-containing lipoproteins was assessed using human LDL (16, 17). Swarnakar et al. (16) analyzed selective uptake in COS-7 cells transiently transfected with mouse SR-BI and found a 6.1-fold greater fractional delivery of human HDL-CE compared with human LDL-CE. Interestingly, subclones of the murine adrenocortical Y1 cell line exhibited a similar (6–7-fold) difference in HDL-CE and LDL-CE fractional delivery. It should be noted that LDL contains ~40-fold more CE molecules on a per particle basis compared with HDL (16). Thus, SR-BI has the potential to deliver a significant amount of CE from LDL to cells even though only a small fraction of core lipid is transferred. Nevertheless, irrespective of the total mass of lipoprotein CE that is transferred to cells, LDL particles would be considerably less lipid-depleted compared with HDL as a result of SR-BI-mediated lipid transfer. This has important implications with respect to SR-BI's ability to promote HDL versus LDL particle clearance in vivo. Transgene or adenoviral-induced overexpression of SR-BI results in the production of lipid-depleted forms of apoA-I that are susceptible to clearance in the kidney (9, 13). The lack of effect on apoB concentrations in mice with adenovirus-induced SR-BI expression likely reflects the fact that non-HDL particles are less significantly depleted of lipid by SR-BI, as well as the unlikelihood that apoB would ever be filtered by the glomerulus.

In summary, we have shown in studies in vitro and in vivo that SR-BI metabolizes LDL particles to a much lesser extent than HDL. Whereas adenoviral vector-mediated SR-BI overexpression in the livers of human apoB transgenic mice (~10-fold increase) reduced HDL-C and apoA-I concentrations to nearly undetectable concentrations, such over-expression had no effect on plasma apoB concentrations, and only modest effects on steady state LDL-C. Although selective uptake represents a significant fraction of total LDL-CE uptake in livers of human apoB transgenic mice, this uptake pathway was not enhanced by increased SR-BI expression. We conclude that the reduced capacity of SR-BI to delipidate LDL particles relative to HDL accounts for the lack of influence of SR-BI on LDL particle catabolism.

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