ApoB-containing lipoproteins in apoE-deficient mice are not metabolized by the class B scavenger receptor BI

Nancy R. Webb,[1] Maria C. de Beer, Frederick C. de Beer, and Deneys R. van der Westhuyzen

Department of Internal Medicine, University of Kentucky Medical Center, Lexington, KY 40536; and Department of Veterans Affairs Medical Center, Lexington, KY 40511

Abstract **The scavenger receptor class B type I (SR-BI) recognizes a broad variety of lipoprotein ligands, including HDL, LDL, and oxidized LDL. In this study, we investigated whether SR-BI plays a role in the metabolism of cholesterol-rich lipoprotein remnants that accumulate in apolipoprotein E (apoE)$^{-/-}$ mice. These particles have an unusual apolipoprotein composition compared with conventional VLDL and LDL, containing mostly apoB-48 as well as substantial amounts of apoA-I and apoA-IV. To study SR-BI activity in vivo, the receptor was overexpressed in apoE$^{-/-}$ mice by adenoviral vector-mediated gene transfer. An ~10-fold increase in liver SR-BI expression resulted in no detectable alterations in VLDL-sized particles and a modest depletion of cholesterol in intermediate density lipoprotein/LDL-sized lipoprotein particles. This decrease was not attributable to altered secretion of apoB-containing lipoproteins in SR-BI-overexpressing mice. To directly assess whether SR-BI metabolizes apoE$^{-/-}$ mouse lipoprotein remnants, in vitro assays were performed in both CHO cells and primary hepatocytes expressing high levels of SR-BI. This analysis showed a remarkable deficiency of these particles to serve as substrates for selective lipid uptake, despite high-affinity, high-capacity binding to SR-BI. Taken together, these data establish that SR-BI does not play a direct role in the metabolism of apoE$^{-/-}$ mouse lipoprotein remnants.**—Webb, N. R., M. C. de Beer, F. C. de Beer, and D. R. van der Westhuyzen. **Apolipoprotein B-containing lipoproteins in apolipoprotein E-deficient mice are not metabolized by the class B scavenger receptor BI. *J. Lipid Res.* 2004.** 45: **272–280.**

Supplementary key words selective lipid uptake • lipoprotein metabolism • adenoviral vector • hepatocytes • apolipoprotein B • apolipoprotein E

The scavenger receptor class B type I (SR-BI) is an HDL receptor that mediates selective cholesteryl ester (CE) uptake, a process by which CEs from the core of the HDL particle are transferred to the cell without intracellular accumulation of the protein moiety (1). In the liver, SR-BI carries out a critical step in the reverse cholesterol transport pathway, whereby HDL-cholesterol is delivered to the liver for excretion in the bile [as reviewed in ref. (2)]. In addition to binding HDL, SR-BI also binds LDL, VLDL, and oxidized LDL (3, 4). The ability of SR-BI to mediate selective lipid uptake from mouse and human LDL has been investigated (5, 6). These studies have shown that although SR-BI mediates selective lipid uptake from LDL, the amount of CE transferred to cells relative to the amount of LDL-CE bound to the cell surface is significantly lower compared with that of HDL-CE. In addition, studies in human apolipoprotein B (apoB) transgenic mice with adenoviral vector overexpression of SR-BI indicate that this receptor does not contribute significantly to LDL metabolism in vivo (6).

In this study, we investigated the role of SR-BI in the metabolism of non-HDL lipoproteins in apoE$^{-/-}$ mice. Mice lacking apoE accumulate cholesterol-rich lipoprotein remnants and develop atherosclerosis spontaneously on a low-fat diet and in an accelerated manner on a high-fat, high-cholesterol diet (7, 8). SR-BI deficiency in apoE$^{-/-}$ mice results in even higher concentrations of plasma total cholesterol as well as dramatically accelerated atherosclerosis (9, 10). The increased cholesterol, which is mainly distributed to VLDL-sized particles, appears to be at least partly attributable to the accumulation of abnormally large HDL-like particles containing apoA-I and lacking apoB (9). In the case of attenuated SR-BI expression (brought about by a mutation in the SR-BI promoter), lipoprotein profiles in apoE$^{-/-}$ mice remain unchanged (11). To date, there are no published reports directly assessing whether SR-BI mediates the uptake of apoE$^{-/-}$ lipoprotein remnants. Studies of the metabolism

Manuscript received 22 July 2003 and in revised form 29 September 2003.

Published, JLR Papers in Press, October 16, 2003.
DOI 10.1194/jlr.M300319-JLR200

Abbreviations: apoE, apolipoprotein E; CE, cholesteryl ester; CEt, cholesteryl ether; LDLR, LDL receptor; SR-BI, scavenger receptor class B type I.

[1] To whom correspondence should be addressed.
e-mail: nrwebb1@uky.edu

of apoB-containing lipoproteins in apoE$^{-/-}$ mice with respect to SR-BI may be particularly relevant in light of the unusual apolipoprotein composition of these particles (7). Both the VLDL and LDL density remnants in apoE$^{-/-}$ mice contain apoB-48 as the major apoB species as well as substantial amounts of apoA-I and apoA-IV. Given the important, but not exclusive, role of apoA-I in selective CE uptake (12–14), this apolipoprotein could impart a different characteristic to apoE$^{-/-}$ mouse LDL compared with LDL derived from normal human plasma or apoE$^{+/+}$ mice.

To assess whether SR-BI plays a role in the metabolism of lipoprotein remnants that accumulate in apoE$^{-/-}$ mice, we analyzed plasma lipoproteins before and after acute overexpression of this receptor by adenoviral vector. In addition, the ability of SR-BI to metabolize these particles was directly measured in assays in vitro using transfected CHO cells and primary hepatocytes from apoE$^{-/-}$ mice.

EXPERIMENTAL PROCEDURES

Animals

LDL receptor-deficient (LDLR$^{-/-}$) and apoE$^{-/-}$ mice (C57BL/6 background) were obtained from Jackson Laboratories (Bar Harbor, ME). The animals were housed in a pathogen-free facility with equal light/dark periods and free access to water and regular rodent chow, unless otherwise indicated. All procedures were approved by the Veterans Administration Medical Center Institutional Animal Use and Care Committee.

Adenoviral vector treatments and plasma lipid analyses

The production of a replication-defective adenoviral vector expressing mouse SR-BI (AdSR-BI) has been described (15). Adnull is a recombinant adenovirus containing no transgene. Mice weighing at least 25 g were injected into the tail vein with the indicated dose of either AdSR-BI or Adnull in 100 μl of PBS. Plasma was collected from mice after a 10 h fast. Aliquots (200 μl) were separated by size exclusion chromatography with a Superose 6 column (Pharmacia LKB Biotechnology, Inc., Piscataway, NJ). 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, and 0.01% sodium azide. The cholesterol content of fractions (0.5 ml) and plasma was determined enzymatically (Wako Chemicals). Triglyceride analysis was performed using enzymatic assay kits (Sigma Chemical Co., St. Louis, MO).

Immunoblot analysis

The preparation of anti-mouse SR-BI^{495} and immunoblot analysis of mouse liver have been described (15).

Isolation and radiolabeling of lipoproteins

VLDL (d = 1.006–1.019 g/ml), LDL (d = 1.019–1.063 g/ml), and HDL (d = 1.063–1.21 g/ml) were isolated from fresh mouse or human plasma by density gradient ultracentrifugation as described previously (16). All isolated fractions were dialyzed against 150 mM NaCl and 0.01% EDTA, pH 7.4, sterile filtered, and stored under nitrogen gas at 4°C. Protein concentrations were determined by the method of Lowry et al. (17). Lipoprotein fractions were traced with nonhydrolyzable, intracellularly trapped 1α,2α(n)-[^{3}H]cholesteryl ether (CEt) according to the methods of Gwynne and Mahaffee (18). Apolipoproteins were radioiodinated by the iodine monochloride method (19).

Ligand binding and uptake assays in SR-BI-transfected cells

The production and maintenance of a CHO cell line stably transfected with murine SR-BI cDNA (CHO-SRBI) was described previously (15). This line, derived from CHO ldlA (clone 7) cells, is deficient in the LDLR (20). Cells were seeded in six-well plates 48 h before assays (2.5×10^5 cells per well). Cell-association assays were performed at 37°C in Ham's F-12 medium containing 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, 0.5% essentially fatty acid-free BSA, and radiolabeled lipoprotein. After incubating for the indicated times, unbound ligand was removed from cells by washing three times with 50 mM Tris-HCl buffer, pH 7.4, containing 150 mM NaCl and 0.2% fatty acid-free BSA, followed by two washes with 50 mM Tris-HCl and 150 mM NaCl, pH 7.4. All washes were performed at 4°C with prechilled solutions. Cells were solubilized in 0.1 N NaOH for 60 min at room temperature before protein and radioactivity determinations. For apolipoprotein degradation assays, cell-free supernatants were analyzed for trichloroacetic acid-soluble, noniodide radioactivity (21). To allow for a direct comparison of different radiolabeled tracers, ^{3}H uptake was expressed as apparent uptake of lipoprotein protein, assuming whole particle uptake (22). Binding parameters were calculated using GraphPad Prism 3.0.

Selective uptake assays in primary hepatocytes

Primary hepatocytes were isolated from apoE$^{-/-}$ mice 3 days after treatment with 1×10^{11} particles of AdSR-BI or Adnull by collagenase perfusion (fraction IV, 1 mg/ml in Hanks balanced salt solution supplemented with 10 mM HEPES, pH 7.4, 0.5 μM pig insulin, 5.6 mM glucose, and 1.3 mM $CaCl_2$) and repeated low-speed centrifugations (60 g). Immediately after harvesting, cells were suspended in prewarmed Williams' E medium (Life Technologies) containing 0.5% BSA (2.5×10^6 cells/ml). Selective uptake experiments were performed in 12-well cluster dishes containing 1×10^6 cells. Cells were incubated at 37°C for 1 h with ^{125}I- and ^{3}H-labeled lipoproteins with slow shaking. After the incubation period, cells were washed once with Williams' E medium containing 0.5% BSA and then twice with Williams' E medium at 4°C. Cells were solubilized in 0.1 N NaOH for 20 min at room temperature before protein and radioactivity determinations. The viability of cells used in the experiments was greater than 90%.

In vivo VLDL secretion assays

The effect of SR-BI overexpression on hepatic triglyceride and apoB secretion was assessed using a previously described method (23). Age-matched male apoE$^{-/-}$ mice were injected in the tail vein with either 5×10^{10} or 1.5×10^{11} particles of AdSR-BI or Adnull. Three days after injection of viral vectors, mice were placed on a fat-free diet 4 h before intravenous injection of 20 mg of Triton WR1339 (tyloxapol; Sigma) in 100 μl. Blood was drawn from the retro-orbital sinus just before and 1, 3, and 5 h after injection. Plasma triglyceride concentrations were quantified as described above. Plasma apoB content was assessed by immunoblot analysis using rabbit anti-mouse apoB (Biodesign International).

Statistical analysis

Statistical analysis was performed using two-tailed Student's t-test for unpaired data.

RESULTS

Apolipoprotein composition of apoE$^{-/-}$ mouse VLDL and LDL is distinct from that of LDLR$^{-/-}$ mouse and human VLDL and LDL

Triglyceride-rich lipoprotein particles (chylomicrons and VLDL) are hydrolyzed in the peripheral circulation by

lipoprotein lipase to form cholesterol-enriched lipoprotein remnants. Normally, these lipoprotein remnants are efficiently cleared from the plasma by the liver. Mice deficient in apoE accumulate large amounts of cholesterol-rich lipoprotein remnants, demonstrating the importance of apoE in the clearance of these particles. The apolipoprotein composition of lipoprotein remnants in apoE$^{-/-}$ mice is markedly distinct from the light lipoprotein fractions isolated from human or LDLR$^{-/-}$ mouse plasma (**Fig. 1**). Whereas human and LDLR$^{-/-}$ mouse VLDL (d = 1.006–1.019 g/ml) and LDL (d = 1.019–1.063 g/ml) contain apoB-100 as the major apolipoprotein, the VLDL and LDL fractions from apoE$^{-/-}$ mice contain primarily apoB-48. ApoE$^{-/-}$ mouse VLDL and LDL also contain large amounts of apoA-I and apoA-IV, both of which are present in negligible amounts in human and LDLR$^{-/-}$ mouse VLDL and LDL.

Adenoviral vector-mediated SR-BI overexpression in apoE$^{-/-}$ mice results in a modest depletion of LDL-cholesterol

SR-BI is a class B scavenger receptor that binds a broad variety of lipoprotein ligands. The distinctive apolipoprotein composition of VLDL and LDL in apoE$^{-/-}$ mice prompted us to investigate whether SR-BI plays a role in the metabolism of these lipoprotein remnants. To assess SR-BI activity toward these lipoproteins in vivo, the receptor was overexpressed in livers of apoE$^{-/-}$ mice using a replication-deficient adenoviral vector, AdSR-BI (15). Mice were infused with a dose of AdSR-BI (3×10^{10} particles) that resulted in a 5- to 10-fold increase in hepatic SR-BI expression (**Fig. 2A**). Three days after AdSR-BI infusion, mice showed a nonsignificant decrease in plasma total cholesterol concentration (190 ± 30.3 mg/dl; n = 4) compared with mice treated with a control adenovirus (290 ± 108 mg/dl; n = 4). Plasma triglyceride concentrations in AdSR-BI-treated mice (57.0 ± 8.3 mg/dl; n = 4) were similar to those of controls (55.5 ± 28 mg/dl; n = 4). Analysis of plasma lipoproteins by size exclusion chromatography revealed a depletion of LDL-cholesterol in AdSR-BI-treated mice but no alteration in VLDL-cholesterol (Fig. 2B).

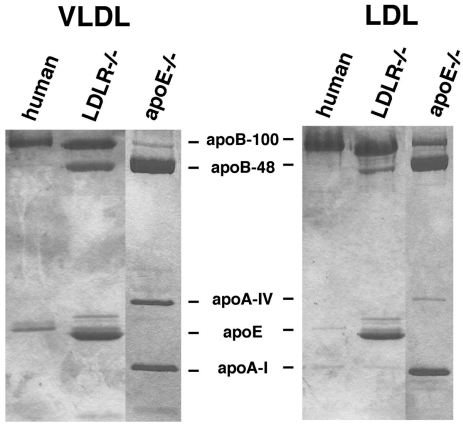

Fig. 1. Apolipoprotein content of human, LDL receptor-deficient (LDLR$^{-/-}$) mouse, and apoE$^{-/-}$ mouse lipoproteins. VLDL and LDL fractions were isolated from human and mouse plasma by density gradient ultracentrifugation as described in Experimental Procedures. Aliquots corresponding to 7 μg of protein were separated by SDS-PAGE (5–20% acrylamide gradient) and stained with Coomassie blue. The migration of apolipoproteins is indicated.

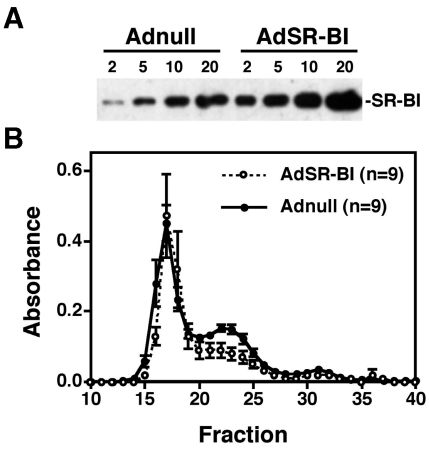

Fig. 2. Effect of adenoviral vector-mediated scavenger receptor class B type I (SR-BI) expression on plasma lipoprotein profiles in apoE$^{-/-}$ mice. A: Liver SR-BI content in mice at 3 days after infusion of 3×10^{10} particles of a recombinant adenovirus containing no transgene (Adnull) or a replication-defective adenoviral vector expressing mouse SR-BI (AdSR-BI). Aliquots of total liver homogenate from a representative individual mouse were separated by nonreducing SDS-PAGE (5–20% acrylamide gradient) and immunoblotted using rabbit anti-mouse SR-BI[495]. The amount of protein (micrograms) analyzed in each lane is indicated. B: Lipoprotein cholesterol profiles of mice at 3 days after infusion of 3×10^{10} particles of AdSR-BI or Adnull. Plasma from individual mice (200 μl) was fractionated by fast-protein liquid chromatography, and the cholesterol content of 0.5 ml fractions was determined. Values for each fraction indicate mean absorbance (±SEM) from the analysis of nine individual mice after adenovirus treatment.

SR-BI expressed in transfected CHO cells does not mediate selective lipid uptake from apoE$^{-/-}$ VLDL or LDL

To assess the possibility that SR-BI may directly metabolize apoB-containing lipoproteins that accumulate in apoE$^{-/-}$ mice, we performed in vitro selective lipid uptake assays using CHO cells stably transfected with mouse SR-BI (15). This allowed us to quantify SR-BI-dependent LDL uptake, which is defined as the difference between the association of SR-BI-transfected and control nontransfected cells. For these studies, lipoprotein particles were separated by sequential density ultracentrifugation into two density ranges conventionally defined as VLDL and LDL. These lipoprotein fractions correspond to the fractions analyzed by SDS-PAGE in Fig. 1. For comparison, HDL isolated from C57BL/6 mouse plasma was also analyzed. SR-BI-transfected cells exhibited SR-BI-dependent ^{125}I-lipoprotein cell association for apoE$^{-/-}$ VLDL and LDL as well as normal mouse HDL (**Fig. 3A**, C, E, hatched symbols). Very little SR-BI-dependent ^{125}I-labeled degradation products were detected in the medium during the

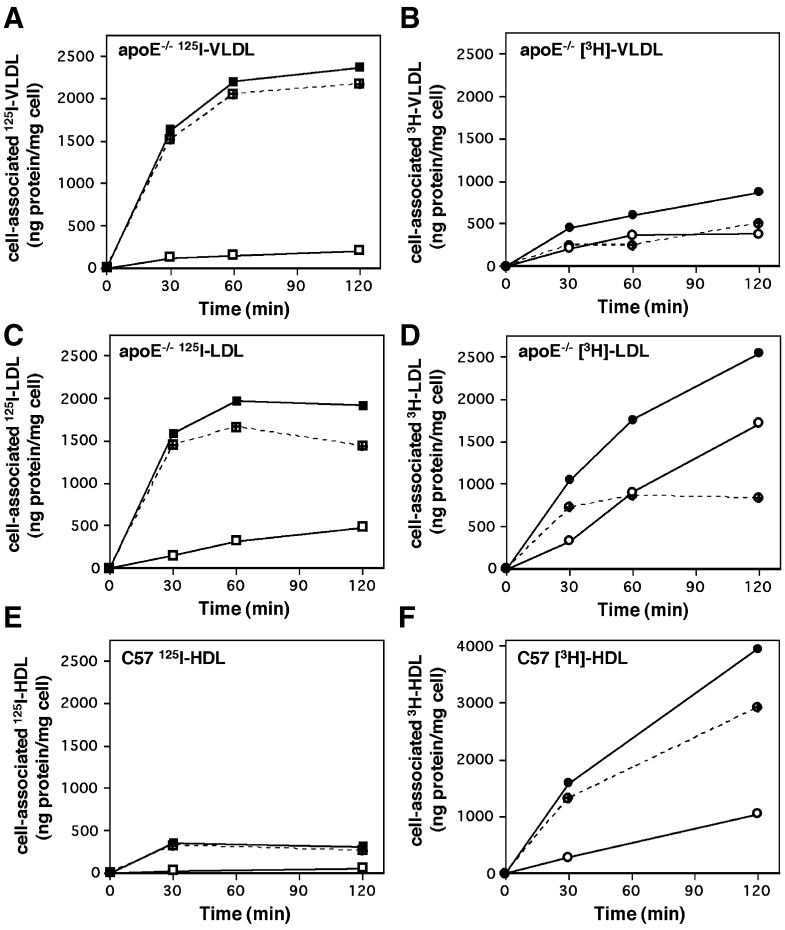

Fig. 3. Time-dependent association of apoE$^{-/-}$ VLDL and LDL and C57BL/6 HDL with nontransfected (open symbols) and mouse SR-BI-transfected (closed symbols) CHO cells. SR-BI-dependent cell association (hatched symbols) is defined as the difference between the association with transfected cells and with control nontransfected cells. Cells were incubated at 37°C for the indicated period of time with 10 μg/ml lipoprotein ligand radiolabeled with either ^{125}I- (A, C, E) or [^{3}H]cholesteryl ether (B, D, F), and cell-associated radiolabel was quantified as described in Experimental Procedures. ^{3}H radioactivity associated with the cells is expressed as apparent uptake of lipoprotein protein, assuming whole particle uptake. Values represent means of duplicate determinations. Results similar to those depicted in A and B were obtained in an additional experiment with a separate batch of radiolabeled ligand. The data shown in C–F are representative of at least four experiments.

2 h incubation period for any of the mouse ligands (representing less than 10% of SR-BI-dependent ^{125}I cell association), indicating that these lipoprotein particles were not internalized and degraded by SR-BI-expressing cells (data not shown).

To determine whether SR-BI mediates selective lipid uptake from apoE$^{-/-}$ remnant lipoproteins, the amount of cell-associated VLDL and LDL traced with nonhydrolyzable [^{3}H]CEt was quantified. To allow for a direct assessment of selective lipid uptake, [^{3}H]CEt cell association was expressed as the amount of apparent lipoprotein protein uptake by the cells, assuming whole particle uptake (22). Incubation of untransfected CHO cells with [^{3}H]VLDL resulted in the association of increasing amounts of ^{3}H label during the 2 h incubation period that was approximately 2-fold greater than the amount that could be accounted for by ^{125}I-VLDL cell association (Fig. 3A, B, open symbols). This result indicates SR-BI-independent selective lipid uptake from this lipoprotein fraction. Although CHO-SRBI cells showed a greater than 2-fold increase in the amount of cell-associated [^{3}H]VLDL compared with control CHO cells (Fig. 3B, closed symbols), the amount of SR-BI-dependent [^{3}H]VLDL cell association was far less than the corresponding amount of SR-BI-dependent ^{125}I-VLDL cell association (Fig. 3A, B, hatched symbols). Thus, binding of apoE$^{-/-}$ VLDL to the transfected cells did not lead to selective lipid uptake by SR-BI.

A lack of SR-BI-dependent selective lipid uptake was also observed from apoE$^{-/-}$ mouse LDL (Fig. 3C, D). Control CHO cells took up approximately 3.5-fold more [^{3}H]LDL over the 2 h incubation period than ^{125}I-LDL, indicating SR-BI-independent selective lipid uptake. During the same incubation period, CHO-SRBI cells accumulated 30% more [^{3}H]LDL than ^{125}I-LDL. However, the SR-BI-dependent component of [^{3}H]LDL uptake in the transfected cells (Fig. 3D) was more than offset by the SR-BI-dependent component of ^{125}I-LDL binding (Fig. 3C). These data indicate that LDL isolated from apoE$^{-/-}$ mice, like VLDL, does not serve as a substrate for SR-BI-mediated selective lipid uptake in CHO-SRBI cells. Thus, SR-BI activity toward apoB-containing lipoproteins that accumulate in apoE$^{-/-}$ mice contrasts markedly with its activity toward normal mouse HDL. As depicted in Fig. 3E, F, CHO-SRBI cells accumulated ~4-fold more [^{3}H]HDL in 2 h compared with control CHO cells, and this ^{3}H uptake appeared to be selective, because SR-BI-dependent [^{3}H]HDL association exceeded SR-BI-dependent ^{125}I-HDL association more than 12-fold in the transfected cells (Fig. 3E, F; note difference in scales).

SR-BI-mediated metabolism of apoE$^{-/-}$ and LDLR$^{-/-}$ mouse LDLs are distinct

We reported previously that SR-BI mediates selective lipid uptake from LDLR$^{-/-}$ mouse LDL (6). Thus, the

current data indicate that the interaction of SR-BI with LDL isolated from apoE$^{-/-}$ and LDLR$^{-/-}$ mice is distinct. To confirm this result directly, selective lipid uptake experiments were performed in vitro using doubly radiolabeled LDL (d = 1.019–1.063 g/ml) isolated from either LDLR$^{-/-}$ or apoE$^{-/-}$ mouse plasma. The lipoprotein fractions analyzed in these experiments correspond to the LDLR$^{-/-}$ and apoE$^{-/-}$ LDL shown in Fig. 1. As depicted in **Fig. 4A**, CHO-SRBI cells exhibited high-affinity, SR-BI-dependent ^{125}I-lipoprotein cell association for both mouse ligands (apoE$^{-/-}$ LDL: apparent K_d = 4,600 ± 700 ng/ml, B_{max} = 3,300 ± 130 ng/mg cell protein; LDLR$^{-/-}$ LDL: apparent K_d = 5,200 ± 600 ng/ml, B_{max} = 1,600 ± 50 ng/mg cell protein). Very few SR-BI-dependent ^{125}I-labeled degradation products were detected in the medium during the 2 h incubation period for either ligand (<7% of SR-BI-dependent ^{125}I cell association). To assess the extent to which SR-BI mediates selective lipid uptake from the respective LDLs, [^{3}H]CEt accumulation in cells was quantified (Fig. 4B). In the case of LDLR$^{-/-}$ LDL, approximately 1.2- to 1.8-fold more [^{3}H]CEt was associated with cells in an SR-BI-dependent manner compared with ^{125}I, indicating a modest amount of selective lipid uptake from this lipoprotein ligand. This result is in agreement with earlier reports, in which it has been shown using similar in vitro assays that the amount of CE taken up by SR-BI from human and mouse LDL represents only a fraction of the amount of LDL-CE bound to the cell surface (5, 6). However, in the case of apoE$^{-/-}$ LDL, there was no evidence of SR-BI-dependent selective lipid uptake. We conclude from these in vitro studies that, unlike normal human and mouse LDLs, SR-BI expressed in transfected CHO cells does not mediate the metabolism of apoB-containing lipoproteins from apoE$^{-/-}$ mice.

SR-BI expressed in primary hepatocytes does not mediate selective lipid uptake from apoE$^{-/-}$ LDL

The results depicted in Figs. 3 and 4 clearly demonstrate that SR-BI expressed in transfected CHO cells does not mediate selective lipid uptake from apoB-containing lipoproteins from apoE$^{-/-}$ mice. To address the possibility that SR-BI expressed in a more physiological setting could promote the metabolism of apoE$^{-/-}$ lipoprotein remnants, selective lipid uptake assays were carried out using primary hepatocytes isolated from apoE$^{-/-}$ mice. For these experiments, hepatocytes were isolated from apoE$^{-/-}$ mice at 3 days after infusion of 1×10^{11} particles of AdSR-BI or the control virus, Adnull. Quantitative immunoblot analysis showed that SR-BI expression was ~60-fold higher in hepatocytes isolated from AdSR-BI-treated mice compared with control mice (data not shown). As depicted in **Fig. 5A**, cells isolated from Adnull-treated mice exhibited selective lipid uptake when incubated with doubly radiolabeled normal mouse HDL. SR-BI overexpression enhanced selective lipid uptake 2- to 4-fold for the range of HDL concentrations tested. In contrast, in the case of [^{125}I,^{3}H]apoE$^{-/-}$ mouse LDL, primary hepatocytes isolated from Adnull-treated mice did not accumulate [^{3}H]LDL in excess of the amount of cell-associated ^{125}I-LDL, indicating that this ligand is not a substrate for selective lipid uptake by these cells (Fig. 5B). Overexpression of SR-BI in hepatocytes resulted in a 3- to 4-fold increase in both cell-associated ^{125}I-LDL and [^{3}H]LDL. Thus, although high-level SR-BI expression in apoE$^{-/-}$ hepatocytes enhanced the cell association of apoE$^{-/-}$ LDL, such expression did not promote selective lipid uptake from these particles.

To assess whether apoE$^{-/-}$ LDL was being internalized by primary hepatocytes expressing high levels of SR-BI, liver cells isolated from AdSR-BI-treated apoE$^{-/-}$ mice were incubated at 37°C with radioiodinated ligand (5 μg/ml) for 1–4 h. During this time period, little degradation of ^{125}I-LDL occurred, as indicated by the relatively small amount of degradation products detected in the medium at 4 h (degradation products accounted for <4% of the cell-associated ligand). This observation, coupled with the fact that the cell association of apoE$^{-/-}$ mouse LDL approached a maximum by 1 h of incubation (data not

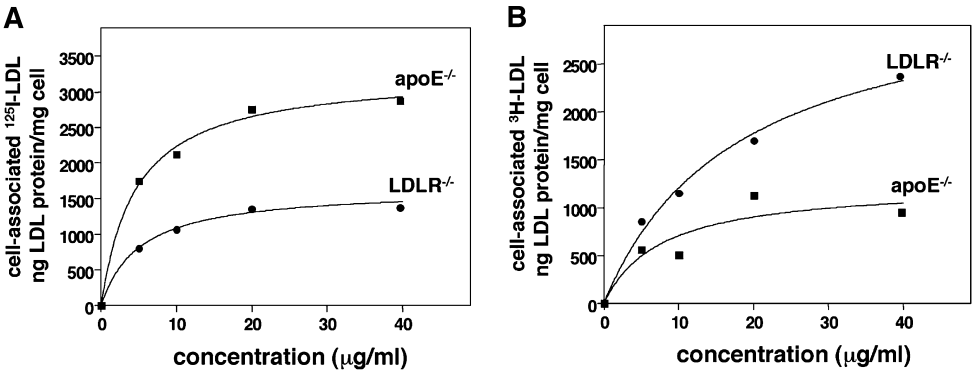

Fig. 4. Concentration-dependent association of apoE$^{-/-}$ LDL and LDLR$^{-/-}$ LDL with CHO-SRBI cells. Cells were incubated for 2 h with the indicated concentrations of ^{125}I-labeled (A) and ^{3}H-labeled (B) lipoproteins, and cell-associated radiolabel was quantified as described in Experimental Procedures. Shown are SR-BI-specific values, which were calculated as the difference between values for SR-BI-transfected cells and non-transfected CHO cells. Values represent means of duplicate determinations. Similar results were obtained in three experiments performed with two batches of radiolabeled ligands.

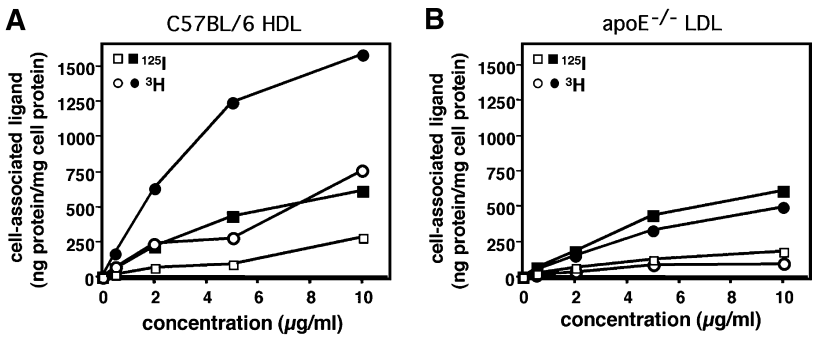

Fig. 5. Concentration-dependent association of ^{125}I- and ^{3}H-labeled C57BL/6 HDL and apoE$^{-/-}$ LDL with apoE$^{-/-}$ mouse primary hepatocytes. Hepatocytes were isolated from apoE$^{-/-}$ mice at 3 days after infusion with Adnull (open symbols) or AdSR-BI (closed symbols) as described in Experimental Procedures. Freshly prepared cells were incubated at 37°C for 1 h with either doubly radiolabeled C57BL/6 HDL (A) or apoE$^{-/-}$ LDL (B), and cell-associated radiolabel was quantified. Values represent means of duplicate determinations. Results similar to those depicted here were obtained from a repeat experiment using a separate batch of hepatocytes.

shown), provides evidence that these particles do not undergo receptor-mediated endocytosis and subsequent intracellular degradation.

Hepatic SR-BI overexpression does not alter VLDL secretion in apoE$^{-/-}$ mice

Although our data from in vitro assays clearly showed that SR-BI does not metabolize lipoprotein remnants that accumulate in apoE$^{-/-}$ mice, transient overexpression of SR-BI in the livers of these mice produced a modest decrease in LDL-cholesterol (Fig. 2). It is possible that LDL-cholesterol is cleared from the plasma in AdSR-BI-treated mice through a "trapping" mechanism, whereby LDL particles are extracellularly bound on the surface of liver cells expressing high levels of SR-BI. We assessed the alternative possibility that increased hepatic SR-BI expression results in decreased secretion of apoB-containing lipoproteins. The apoE$^{-/-}$ mice were infused with 5×10^{10} particles of AdSR-BI or Adnull. Three days after adenovirus injections, mice were placed on a fat-free diet to prevent intestinal secretion of chylomicrons. Four hours after the initiation of the fat-free diet, mice were injected with Triton WR1339, which is used to acutely inhibit the hydrolysis and subsequent removal from the plasma of triglyceride-rich lipoproteins (23, 24). As shown in **Fig. 6**, the rate of accumulation of triglycerides in the plasma of AdSR-BI- and Adnull-treated mice was similar after Triton WR1339 injection. In addition, there was no detectable difference in the amount of plasma apoB-48 between the two groups of mice (data not shown). Immunoblot analysis of livers showed that SR-BI expression was ~10-fold higher in AdSR-BI-treated mice compared with Adnull-treated mice (data not shown). To assess whether an even greater induction of AdSR-BI expression could alter hepatic lipoprotein secretion rates, we repeated the experiment in mice infused with 1.5×10^{11} particles of AdSR-BI (Fig. 6). The results from this analysis demonstrated that a 50-fold increase in hepatic SR-BI expression (data not shown) does not alter the rate of accumulation of plasma triglycerides compared with control mice. We conclude from these experiments that high-level expression of SR-BI does not impede hepatic VLDL production and secretion in apoE$^{-/-}$ mice.

DISCUSSION

ApoE$^{-/-}$ mice accumulate cholesterol-rich lipoprotein remnants that contain apoB-48 as the major apolipoprotein, as well as substantial amounts of apoA-I and apoA-IV (7) (Fig. 1). These mice have served as valuable animal models for studies of lipoprotein metabolism and atherosclerosis (25, 26). In this study, we assessed the role of SR-BI in the metabolism of remnant particles that accumulate in apoE$^{-/-}$ mice. These investigations were motivated by two previous observations. First, apoE$^{-/-}$ mice that are also deficient in SR-BI have significantly increased plasma cholesterol compared with apoE$^{-/-}$ mice that is mostly con-

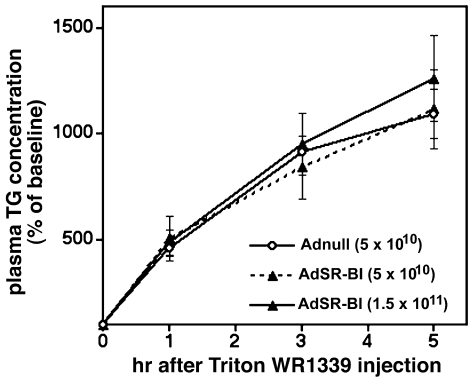

Fig. 6. Plasma triglyceride (TG) concentrations in apoE$^{-/-}$ mice after Triton WR1339 injection. Three days before Triton WR1339 injection, mice were injected with the indicated number of particles of Adnull or AdSR-BI. Plasma triglyceride values represent means ± SEM from five mice and are expressed as percentages of the baseline values.

tained within VLDL-sized particles (9). This alteration in plasma lipoproteins may contribute to the accelerated atherosclerosis, myocardial infarction, and early death seen in these mice (9, 10). Although it appears that this increase in large lipoproteins is primarily attributable to the presence of abnormally large HDL-like particles, the possibility that SR-BI may also play a role in the clearance of apoB-containing particles in apoE$^{-/-}$ mice has not been directly tested. The second rationale for this study is the unusual apolipoprotein composition of apoE$^{-/-}$ mouse VLDL and LDL. Recent evidence indicates that HDL binding to SR-BI may be driven by a direct interaction of the receptor with amphipathic α-helices on apoA-I (12). In addition, apoA-I likely plays a role in the formation of HDL particles with high capacity for SR-BI-selective CE uptake (14). Given the significant amounts of apoA-I in the LDL and VLDL fractions of apoE$^{-/-}$ mice, and the fact that the uptake pathways for these lipoprotein fractions have not been well delineated, we set out to directly assess whether or not these lipoproteins are metabolized by SR-BI.

For studies in vitro, lipoprotein remnants isolated from apoE$^{-/-}$ mice were separated into two density ranges conventionally defined as VLDL and LDL and used as ligands in selective uptake experiments. Selective uptake assays were performed using LDLR$^{-/-}$ CHO cells stably transfected with mouse SR-BI cDNA, which allowed us to define SR-BI-specific lipoprotein binding and uptake. Transfected CHO cells consistently showed more SR-BI-dependent ^{125}I-radiolabeled apolipoprotein associated with cells than can be accounted for by the apparent cell-associated [^{3}H]lipoprotein, indicating that these fractions do not serve as substrates for SR-BI-selective lipid uptake. Although the basis for the difference between the amount of SR-BI-dependent ^{125}I and ^{3}H associated with cells is not clear, it is notable that this discrepancy was not observed in assays using primary hepatocytes. In the case of hepatocyte cells, the amount of [^{3}H]LDL associated with cells corresponded closely to the amount of ^{125}I-LDL associated with cells, even when SR-BI expression was increased by more than 60-fold over endogenous levels by adenoviral vector-mediated gene transfer. The absence of any evidence for uptake and/or degradation of apoE$^{-/-}$ mouse LDL by primary hepatocytes with or without SR-BI overexpression confirms the conclusion that this receptor does not directly metabolize these lipoprotein particles.

Our data demonstrate distinct differences in the interaction of SR-BI with apoB-containing lipoproteins from apoE$^{-/-}$ mice compared with LDL derived from LDLR$^{-/-}$ mice. Interestingly, SR-BI demonstrated an increased capacity for binding particles from apoE$^{-/-}$ mice, as indicated by an almost 2-fold greater apparent B_{max} compared with LDLR$^{-/-}$ mouse LDL. Recent studies by Thuahnai et al. (27) support the concept that there are multiple binding sites on SR-BI that can interact with a lipoprotein particle. These distinct sites may or may not be recognized, depending on features of the lipoprotein particle, including the lipidation state of apolipoproteins. The lack of cross-competition between HDL and LDL (1), along with the identification of an SR-BI mutant that binds LDL but not HDL (28), also supports the conclusion that SR-BI interacts with different lipoprotein fractions in a distinct manner.

As reported previously (6), the current data demonstrate that SR-BI mediates selective CE uptake, albeit inefficiently, from LDL isolated from LDLR$^{-/-}$ mice. In contrast, non-HDL lipoproteins isolated from apoE$^{-/-}$ mice were completely deficient in selective uptake activity, despite high-affinity binding to SR-BI. Interestingly, Arai et al. (11) reported that SR-BI-mediated lipid uptake from apoE$^{-/-}$ mouse HDL is also severely impaired. We have confirmed this finding (data not shown). Thus, lipoprotein particles that accumulate in apoE$^{-/-}$ mice are clearly altered in the manner in which they interact with SR-BI. Whether apoE deficiency per se or other structural alterations in apoE$^{-/-}$ mouse lipoproteins are responsible for reduced lipid transfer activity remains to be determined. It is possible that differences in apolipoprotein composition other than the presence or absence of apoE account for the disparity in CE uptake from LDLR$^{-/-}$ and apoE$^{-/-}$ mouse LDLs. Whereas LDLR$^{-/-}$ mouse LDL contains primarily apoB-100 and apoE, the corresponding fraction from apoE$^{-/-}$ mice is enriched in apoB-48 and apoA-I. One interpretation of our data is that SR-BI is capable of mediating a modest amount of lipid transfer from apoB-100-containing lipoproteins, but selective lipid uptake is impeded in the case of apoB-48-containing particles, which constitute the vast majority of apoE$^{-/-}$ LDL. Other differences in particle composition and/or structure could influence SR-BI interaction. Lipoproteins from apoE$^{-/-}$ mice are uniquely enriched in sphingomyelin (29, 30), and it is possible that a high sphingomyelin-to-phospholipid ratio in LDL decreases selective uptake. Alternatively, possible oxidative modification of apoB-containing lipoproteins in apoE$^{-/-}$ mice (31) could influence CE transfer by SR-BI.

The inability of the apoE$^{-/-}$ remnant particles to serve as substrates for SR-BI-mediated selective lipid uptake is notable, given the abundance of apoA-I on these lipoprotein particles. Interestingly, recent data indicate that although apoA-I plays a role in SR-BI-dependent HDL binding (12) and efficient selective lipid uptake (14), the mere presence of apoA-I on HDL is not sufficient to confer selective uptake activity (32). HDL isolated from apoA-I$^{-/-}$ mice is deficient in its ability to serve as a substrate for SR-BI-selective lipid uptake compared with normal mouse HDL, and this deficiency is attributable to the absence of apoA-I. However, the addition of apoA-I to apoA-I$^{-/-}$ HDL does not restore efficient selective lipid uptake unless the particles are also exposed to lecithin:cholesterol acyltransferase, which appears to reorganize the HDL particle to enhance lipid transfer activity (32). Thus, it is clear from the analysis of both HDL and non-HDL lipoproteins that apoA-I by itself is not sufficient to promote CE transfer by SR-BI.

Using adenoviral vector-mediated gene transfer, we showed that a 5- to 10-fold increase in hepatic SR-BI expression reduces plasma concentrations of LDL/interme-

diate density lipoprotein-sized particles in apoE$^{-/-}$ mice. We ruled out the possibility that high SR-BI expression hinders the production and/or secretion of apoB-containing lipoproteins by apoE$^{-/-}$ mouse liver in a manner analogous to LDLR modulation of apoB-100 secretion (33). Given the capacity of SR-BI to bind apoE$^{-/-}$ mouse LDL, it is possible that cell surface expression of SR-BI effectively traps these particles in the liver, thereby removing them from the circulation. Although assays in vitro indicated a complete lack of selective lipid uptake from apoE$^{-/-}$ mouse LDL, we cannot rule out the possibility that SR-BI mediates a modest amount of lipid transfer from the small subset of apoB-100-containing particles in this lipoprotein fraction. The reduction in LDL-cholesterol in AdSR-BI-treated mice may reflect lipid depletion of apoB-100-containing LDLs. Alternatively, it is possible that lipid from the LDL fraction is transferred to a pool that is rapidly turned over and cleared by SR-BI.

In summary, our data clearly show that SR-BI is deficient in mediating the uptake of apoB-containing lipoproteins isolated from apoE$^{-/-}$ mice despite high-affinity, high-capacity binding. In addition, SR-BI does not play a role in the rate of secretion of these particles by the liver. These findings provide important new information about a mouse strain used extensively for studies in cardiovascular disease and lipoprotein metabolism.

The authors thank Dr. I. Deaciuc for assistance in hepatocyte isolations and Jin Yu, Ryan Mulligan, and Nathan Whitaker for valuable technical assistance. This work was supported by American Heart Association Award 0130020N (to N.R.W.) and National Institutes of Health Grants HL-65730 (to D.R.v.d.W.) and AG-17237 (to F.C.d.B.).

REFERENCES

1. 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.
2. Krieger, M. 1999. Charting the fate of the "good cholesterol": identification and characterization of the high-density lipoprotein receptor SR-BI. *Annu. Rev. Biochem.* **68:** 523–558.
3. Acton, S. L., P. E. Scherer, H. F. Lodish, and M. Krieger. 1994. Expression cloning of SR-BI, a CD36-related class B scavenger receptor. *J. Biol. Chem.* **269:** 21003–21009.
4. Calvo, D., D. Gomez-Coronado, M. A. Lasuncion, and M. A. Vega. 1997. CLA-1 is an 85-kD plasma membrane glycoprotein that acts as a high-affinity receptor for both native (HDL, LDL, and VLDL) and modified (OxLDL and AcLDL) lipoproteins. *Arterioscler. Thromb. Vasc. Biol.* **17:** 2341–2349.
5. Swarnakar, S., R. E. Temel, M. A. Connelly, S. Azhar, and D. L. Williams. 1999. Scavenger receptor class B, type I, mediates selective uptake of low density lipoprotein cholesteryl ester. *J. Biol. Chem.* **274:** 29733–29739.
6. Webb, N. R., M. C. de Beer, J. Yu, M. S. Kindy, A. Daugherty, D. R. van der Westhuyzen, and F. C. de Beer. 2002. Overexpression of SR-BI by adenoviral vector promotes clearance of apoA-I, but not apoB, in human apoB transgenic mice. *J. Lipid Res.* **43:** 1421–1428.
7. Zhang, S. H., R. L. Reddick, J. A. Piedrahita, and N. Maeda. 1992. Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science.* **258:** 468–471.
8. Plump, A. S., J. D. Smith, T. Hayek, K. Aalto-Setala, A. Walsh, J. G. Verstuyft, E. M. Rubin, and J. L. Breslow. 1992. Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell.* **71:** 343–353.
9. Trigatti, B., H. Rayburn, M. Vinals, A. Braun, H. Miettinen, M. Penman, M. Hertz, M. Schrenzel, L. Amigo, A. Rigotti, and M. Krieger. 1999. Influence of the high density lipoprotein receptor SR-BI on reproductive and cardiovascular pathophysiology. *Proc. Natl. Acad. Sci. USA.* **96:** 9322–9327.
10. Braun, A., B. L. Trigatti, M. J. Post, K. Sato, M. Simons, J. M. Edelberg, R. D. Rosenberg, M. Schrenzel, and M. Krieger. 2002. Loss of SR-BI expression leads to the early onset of occlusive atherosclerotic coronary artery disease, spontaneous myocardial infarctions, severe cardiac dysfunction, and premature death in apolipoprotein E-deficient mice. *Circ. Res.* **90:** 270–276.
11. Arai, T., F. Rinninger, L. Varban, V. Fairchild-Huntress, C. P. Liang, W. Chen, T. Seo, R. Deckelbaum, D. Huszar, and A. R. Tall. 1999. Decreased selective uptake of high density lipoprotein cholesteryl esters in apolipoprotein E knock-out mice. *Proc. Natl. Acad. Sci. USA.* **96:** 12050–12055.
12. Williams, D. L., M. de la Llera-Moya, S. T. Thuahnai, S. Lund-Katz, M. A. Connelly, S. Azhar, G. M. Anantharamaiah, and M. C. Phillips. 2000. Binding and cross-linking studies show that scavenger receptor BI interacts with multiple sites in apolipoprotein A-I and identify the class A amphipathic α-helix as a recognition motif. *J. Biol. Chem.* **275:** 18897–18904.
13. de Beer, M. C., D. M. Durbin, L. Cai, A. Jonas, F. C. de Beer, and D. R. van der Westhuyzen. 2001. Apolipoprotein A-I conformation markedly influences HDL interaction with scavenger receptor BI. *J. Lipid Res.* **42:** 309–313.
14. Temel, R. E., R. L. Walzem, C. L. Banka, and D. L. Williams. 2002. Apolipoprotein A-I is necessary for the in vivo formation of high density lipoprotein competent for scavenger receptor BI-mediated cholesteryl ester-selective uptake. *J. Biol. Chem.* **277:** 26565–26572.
15. 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.
16. Strachan, A. F., F. C. de Beer, D. R. van der Westhuyzen, and G. A. Coetzee. 1988. Identification of three isoform patterns of human serum amyloid A protein. *Biochem. J.* **250:** 203–207.
17. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and B. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
18. Gwynne, J. T., and D. D. Mahaffee. 1989. Rat adrenal uptake and metabolism of high density lipoprotein cholesteryl ester. *J. Biol. Chem.* **264:** 8141–8150.
19. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoproteins. *Biochim. Biophys. Acta.* **260:** 212–221.
20. Kingsley, D. M., and M. Krieger. 1984. Receptor-mediated endocytosis of low density lipoprotein: somatic cell mutants define multiple genes required for expression of surface-receptor activity. *Proc. Natl. Acad. Sci. USA.* **81:** 5454–5458.
21. Bierman, E. L., O. Stein, and Y. Stein. 1974. Lipoprotein uptake and metabolism by rat aortic smooth muscle cells in tissue culture. *Circ. Res.* **35:** 136–150.
22. Glass, C., R. C. Pittman, D. W. Weinstein, and D. Steinberg. 1983. Dissociation of tissue uptake of cholesterol ester from that of ApoA-I of rat plasma high density lipoprotein: selective delivery of cholesterol ester to liver, adrenal, and gonad. *Proc. Natl. Acad. Sci. USA.* **80:** 5435–5439.
23. Li, X., F. Catalina, S. M. Grundy, and S. Patel. 1996. Method to measure apolipoprotein B-48 and B-100 secretion rates in an individual mouse: evidence for a very rapid turnover of VLDL and preferential removal of B-48- relative to B-100-containing lipoproteins. *J. Lipid Res.* **37:** 210–220.
24. Tsukamoto, K., C. Maugeais, J. M. Glick, and D. J. Rader. 2000. Markedly increased secretion of VLDL triglycerides induced by gene transfer of apolipoprotein E isoforms in apoE-deficient mice. *J. Lipid Res.* **41:** 253–259.
25. Breslow, J. L. 1996. Mouse models of atherosclerosis. *Science.* **272:** 685–688.
26. Daugherty, A. 2002. Mouse models of atherosclerosis. *Am. J. Med. Sci.* **323:** 3–10.
27. Thuahnai, S. T., S. Lund-Katz, G. M. Anantharamaiah, D. L. Williams, and M. C. Phillips. 2003. A quantitative analysis of apolipo-

protein binding to scavenger receptor class B, type I (SR-BI): multiple binding sites for lipid-free and lipid-associated apolipoproteins. *J. Lipid Res.* **44:** 1132–1142.
28. Gu, X., R. Lawrence, and M. Krieger. 2000. Dissociation of the high density lipoprotein and low density lipoprotein binding activities of murine scavenger receptor class B type I (mSR-BI) using retrovirus library-based activity dissection. *J. Biol. Chem.* **275:** 9120–9130.
29. Jeong, T., S. L. Schissel, I. Tabas, H. J. Pownall, A. R. Tall, and X. Jiang. 1998. Increased sphingomyelin content of plasma lipoproteins in apoE knockout mice reflects combined production and catabolic defects and enhances reactivity with mammalian sphingomyelinase. *J. Clin. Invest.* **101:** 905–912.
30. Jiang, X. C., F. Paultre, T. A. Pearson, R. G. Reed, C. K. Francis, M. Lin, L. Berglund, and A. R. Tall. 2000. Plasma sphingomyelin level as a risk factor for coronary artery disease. *Arterioscler. Thromb. Vasc. Biol.* **20:** 2614–2618.
31. Aviram, M., I. Maor, S. Keidar, T. Hayek, J. Oiknine, Y. Bar-El, Z. Adler, V. Kertzman, and S. Milo. 1995. Lesioned low density lipoprotein in atherosclerotic apolipoprotein E-deficient transgenic mice and in humans is oxidized and aggregated. *Biochem. Biophys. Res. Commun.* **216:** 501–513.
32. Temel, R. E., J. S. Parks, and D. L. Williams. 2003. Enhancement of scavenger receptor class B type I-mediated selective cholesteryl ester uptake from apoA-I−/− high density lipoprotein (HDL) by apolipoprotein A-I requires HDL reorganization by lecithin cholesterol acyltransferase. *J. Biol. Chem.* **278:** 4792–4799.
33. Twisk, J., D. L. Gillian-Daniel, A. Tebon, L. Wang, P. H. Barrett, and A. D. Attie. 2000. The role of the LDL receptor in apolipoprotein B secretion. *J. Clin. Invest.* **105:** 521–532.