Adenovirus-mediated hepatic overexpression of scavenger receptor class B type I accelerates chylomicron metabolism in C57BL/6J mice

Ruud Out,[1,*] Menno Hoekstra,* Saskia C. A. de Jager,* Paula de Vos,* Deneys R. van der Westhuyzen,[†] Nancy R. Webb,[†] Miranda Van Eck,* Eric A. L. Biessen,* and Theo J. C. Van Berkel*

Division of Biopharmaceutics,* Leiden/Amsterdam Center for Drug Research, Gorlaeus Laboratories, 2300 RA Leiden, The Netherlands; and Department of Internal Medicine,[†] University of Kentucky Medical Center, Lexington, KY 40536

Abstract **The function of scavenger receptor class B type I (SR-BI) in mediating the selective uptake of HDL cholesteryl esters is well established. In SR-BI-deficient mice, we recently observed a delayed postprandial triglyceride (TG) response, suggesting an additional role for SR-BI in facilitating chylomicron (CM) metabolism. Here, we assessed the effect of adenovirus-mediated hepatic overexpression of SR-BI (Ad.SR-BI) in C57BL/6J mice on serum lipids and CM metabolism. Infection of 5×10^8 plaque-forming units per mouse of Ad.SR-BI significantly decreases serum cholesterol (>90%), phospholipids (>90%), and TG levels (50%), accompanied by a 41.4% reduction ($P < 0.01$) in apolipoprotein B-100 levels. The postprandial TG response is 2-fold lower in mice treated with Ad.SR-BI compared with control mice (area under the curve = 31.4 ± 2.4 versus 17.7 ± 3.2; $P < 0.05$). Hepatic mRNA expression levels of genes known to be involved in serum cholesterol and TG clearance are unchanged and thus could not account for the decreased plasma TG levels and the change in postprandial response. We conclude that overexpression of SR-BI accelerates CM metabolism, possibly by mediating the initial capture of CM remnants by the liver, whereby the subsequent internalization can be exerted by additional receptor systems such as the LDL receptor (LDLr) and LDLr-related protein 1.**—Out, R., M. Hoekstra, S. C. A. de Jager, P. de Vos, D. R. van der Westhuyzen, N. R. Webb, M. Van Eck, E. A. L. Biessen, and T. J. C. Van Berkel. **Adenovirus-mediated hepatic overexpression of scavenger receptor class B type I accelerates chylomicron metabolism in C57BL/6J mice.** ***J. Lipid Res.*** **2005.** 46: **1172–1181.**

Supplementary key words liver • triglyceride • postprandial response • gene expression

Scavenger receptor class B type I (SR-BI) binds HDLs and mediates the selective uptake of cholesteryl esters (CEs) from HDL without concomitant uptake of HDL protein (1). The major apolipoproteins from HDL [apolipoprotein A-I (apoA-I), apoA-II, and apoC-III] mediate the binding of HDL to SR-BI (2). Recently, it was shown that lipid-free apoE also binds to SR-BI and enhances CE uptake from lipoproteins (3). In addition to HDL, SR-BI was found to bind a broad spectrum of ligands, including maleylated BSA, anionic phospholipids (PLs), modified lipoproteins (acetylated LDL, oxidized LDL, and hypochlorite-modified LDL), and native lipoproteins (HDL, LDL, and VLDL) (4–7). In contrast, SR-BI does not bind polyanions (e.g., fucoidin and polyinosinic acid), which are well-known ligands for scavenger class A receptors. In addition to CE, SR-BI selectively takes up a variety of other molecules, such as lipoprotein-associated PL (8, 9), HDL-associated CE hydroxides (10), and triglycerides (TGs) (9, 11).

The importance of SR-BI in HDL cholesterol metabolism is readily observed in genetically altered mice. SR-BI-deficient mice are characterized by an increase in serum cholesterol levels, reflected in enlarged, cholesterol-rich HDL particles and impaired HDL cholesterol clearance (12). Conversely, adenoviral hepatic SR-BI overexpression results in decreased serum HDL cholesterol content as well as increased liver uptake and subsequent delivery of HDL cholesterol to the bile (13). In contrast with HDL cholesterol metabolism and despite several studies in both SR-BI transgenic mice (14–17) and in mice with adenovirus-mediated overexpression of SR-BI (18–20), the role

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Abbreviations: apoA-I, apolipoprotein A-I; BSEP, bile salt export pump; CE, cholesteryl ester; CM, chylomicron; HPRT, hypoxanthine guanine phosphoribosyl transferase; LDLr, low density lipoprotein receptor; LRP1, LDLr-related protein 1; MTP, microsomal triglyceride transfer protein; pfu, plaque-forming units; PL, phospholipid; SR-BI, scavenger receptor class B type I; TG, triglyceride; 36B4, acidic ribosomal phosphoprotein PO.

[1] To whom correspondence should be addressed.
e-mail: r.out@lacdr.leidenuniv.nl

of SR-BI in the metabolism of apoB-containing lipoproteins is still under discussion.

Chylomicrons (CMs) are TG-rich lipoproteins that transport dietary lipids from the intestine to the liver (21). In the intestines, CMs are formed by the addition of lipids to apoB-48, the structural protein of CM, which is mediated by microsomal triglyceride transfer protein (MTP). Upon entering the circulation, CMs are converted to remnants by the TG-hydrolyzing action of LPL and the acquisition of apolipoproteins such as apoE. CM remnants are subsequently taken up by the liver by an apoE-mediated process (reviewed in 22–24). The essential role of apoE in remnant clearance was indicated by the accumulation of remnants in apoE-deficient mice (25). Several apoE-dependent recognition sites have been suggested to contribute to the removal of remnants, including the (apoB and apoE) LDL receptor (LDLr) (25–30) and the LDLr-related protein/α2-macroglobulin receptor 1 (LRP1) (29, 31, 32). However, it is generally accepted that for the initial liver recognition of remnants, the so-called "capture step," additional systems are needed. The initial sequestration step was suggested to involve heparan sulfate proteoglycans (26, 33), the lipolysis-stimulated receptor (34–36), a TG-rich lipoprotein receptor (37, 38), the asialoglycoprotein receptor (39), LPL (40) and/or HL (41), and a specific remnant receptor (42–44). We recently observed a reduced recognition of 160 nm TG-rich CM-like emulsion particles to freshly isolated hepatocytes from SR-BI-deficient mice (45). Furthermore, the postprandial TG response to an intragastric fat load is 2-fold higher in SR-BI-deficient mice compared with wild-type littermates. These data suggest that SR-BI facilitates CM-remnant metabolism possibly by mediating the initial binding/capture of remnants by the liver (45). However, it remains to be established to what extent this facilitating role is critically dependent on SR-BI protein levels.

The aim of the present study was to further substantiate the role of SR-BI in CM metabolism by assessing the effect of adenovirus-mediated hepatic overexpression of SR-BI. It appears that adenovirus-mediated hepatic overexpression of SR-BI in C57BL/6J mice results in a decrease in plasma TG, a decrease in VLDL/CM-associated TG, and a modified postprandial TG response. In addition, a tendency to an increase in MTP expression was observed, suggesting increased VLDL production. These data support our earlier suggestion and indicate that besides its role in HDL metabolism, SR-BI levels modulate the kinetics of CM (remnant) metabolism.

MATERIALS AND METHODS

Animals

In all experiments, 10–12 week old male C57BL/6J mice (Broekman Institute BV, Someren, The Netherlands), weighing ~25 g were used. Mice were fed a regular chow diet containing 4.7% fat and no cholesterol (SDS, Whitham, UK). Animal experiments were performed at the Gorlaeus Laboratories of the Leiden/Amsterdam Center for Drug Research in accordance with the national laws. All experimental protocols were approved by the Ethics Committee for Animal Experiments of Leiden University.

Treatment with recombinant adenovirus

Construction of a recombinant replication-deficient adenoviral vector expressing mouse SR-BI (Ad.SR-BI) has been described previously (46). A total of 5×10^8 plaque-forming units (pfu) of Ad.SR-BI or Ad.LacZ (control) was injected into the tail vein of the mice (n = 4–5 per group) at 3 h after injection of Ad.LacZ (5×10^8 pfu) to saturate the uptake of viral particles by Kupffer cells (47). Before injection and 5 days after injection, mice were fasted overnight and a blood sample for lipid determination was collected by tail bleeding. Subsequently, mice were anesthetized [subcutaneous injection of ketamine (60 mg/kg; Eurovet Animal Health), fentanyl citrate, and fluanisone (1.26 and 2 mg/kg, respectively; Janssen Animal Health)] and exsanguinated by eye bleeding. A whole body perfusion was performed using phosphate-buffered saline containing 1 mM EDTA (4°C, 100 mm Hg) for 15min. After perfusion, liver lobules were excised and either kept in 3.7% formalin overnight, embedded in OCT compound (Tissue-Tec), and frozen in liquid nitrogen for histological analysis or snap frozen in liquid nitrogen and stored at −80°C until RNA isolation, Western blotting, or hepatic lipid composition analysis.

Analysis of gene expression by real-time quantitative PCR

mRNA analysis was performed as described previously (48, 49). Total RNA was extracted from the liver by the acid guanidinium thiocyanate-phenol chloroform extraction method according to Chomczynski and Sacchi (50). cDNA was synthesized from 2 μg of total RNA using RevertAid M-MuLV reverse transcriptase according to the protocols supplied by the manufacturer. Quantitative gene expression analysis was performed on an ABI PRISM 7700 machine (Applied Biosystems, Foster City, CA) using SYBR-green technology (Eurogentec) with the primers listed in **Table 1**. Hypoxanthine guanine phosphoribosyl transferase (HPRT), β-actin, and acidic ribosomal phosphoprotein PO (36B4) were used as the standard housekeeping genes. Relative gene expression was calculated by subtracting the threshold cycle number of the target gene from the average threshold cycle number of HPRT, β-actin, and 36B4 and raising 2 to the power of this difference. The average threshold cycle number of three housekeeping genes was used to exclude the possibility that changes in the relative expression were caused by variations in the separate housekeeping gene expressions.

Western blotting

Immunoblotting on protein from total liver was performed as described previously (49). In short, after running equal amounts of total liver protein (25 μg) on a 7.5% SDS-PAGE gel, SR-BI was detected using rabbit polyclonal anti-SR-BI peptide (496–509) IgG (Abcam, Cambridge, UK) as a primary antibody and goat-anti-rabbit IgG (Jackson ImmunoResearch) as a secondary antibody. LDLr and LRP1 were detected using goat anti-LDLr (C-20) IgG and goat anti-LRP (N-20) IgG (Santa Cruz Biotechnology, Inc.) as primary antibodies, respectively. As a secondary antibody, mouse anti-goat IgG was used (Jackson ImmunoResearch). Finally, immunolabeling was detected by enhanced chemiluminescence (Biosciences). For quantitation, ImageQuant 5.2 software was used.

Lipid analysis

Serum concentrations of total cholesterol, free cholesterol, PL, and TG were determined using enzymatic colorimetric assays (Roche Diagnostics, Mannheim, Germany). Precipath l was used as an internal standard. The distribution of total cholesterol, PL, and TG over the different lipoproteins in serum was analyzed by fractionation of 30 μl of pooled serum using a Superose 6 column

TABLE 1. Primers for quantitative real-time PCR analysis

Gene	GenBank Accession Number	Forward Primer	Reverse Primer	Amplicon Size
SR-BI	U76205	GTTGGTCACCATGGGCCA	CGTAGCCCCACAGGATCTCA	65
LDLr	Z19521	CTGTGGGCTCCATAGGCTATCT	GCGGTCCAGGGTCATCTTC	68
LRP	NM008512	TGGGTCTCCCGAAATCTGTT	ACCACCGCATTCTTGAAGGA	95
MTP	L47970	AGCTTTGTCACCGCTGTGC	TCCTGCTATGGTTTGTTGGAAGT	50
ABCA1	NM013454	GGTTTGGAGATGGTTATACAATAGTTGT	TTCCCGGAAACGCAAGTC	96
HMG-CoA receptor	M62766	TCTGGCAGTCAGTGGGAACTATT	CCTCGTCCTTCGATCCAATTT	69
ABCG1	NM053502	AGGTCTCAGCCTTCTAAAGTTCCTC	TCTCTCGAAGTGAATGAAATTTATCG	85
ABCG5	NM053754	CGCAGGAACCGCATTGTAA	TGTCGAAGTGGTGGAAGAGCT	67
ABCG8	NM130414	GATGCTGGCTATCATAGGGAGC	TCTCTGCCTGTGATAACGTCGA	69
CYP7A1	NM012942	CTGTCATACCACAAAGTCTTATGTCA	ATGCTTCTGTGTCCAAATGCC	75
CYP27	M38566	GTGTCCCGGGATCCCAGTGT	CTTCCTCAGCCATCGGTGA	66
BSEP	NM021022	TGGAAAGGAATGGTGATGGG	CAGAAGGCCAGTGCATAACAGA	76
HL	NM008280	CAGCCTGGGAGCGCAC	CAATCTTGTTCTTCCCGTCCA	62
LPL	NM008509	CCAGCAACATTATCCAGTGCTAG	CAGTTGATGAATCTGGCCACA	72
HPRT	X62085	TTGCTCGAGATGTCATGAAGGA	AGCAGGTCAGCAAAGAACTTATAG	91
36B4	X1526775	GGACCCGAGAAGACCTCCTT	GCACATCACTCAGAATTTCAATGG	85
β-Actin	X03672	AACCGTGAAAAGATGACCCAGAT	CACAGCCTGGATGGCTACGTA	75

BSEP, bile salt export pump; HPRT, hypoxanthine guanine phosphoribosyl transferase; LDLr, LDL receptor; LRP, LDLr-related protein; MTP, microsomal triglyceride transfer protein; SR-BI, scavenger receptor class B type I; 36B4, acidic ribosomal phosphoprotein PO.

(3.2 × 30 mm, Smart-system; Pharmacia, Uppsula, Sweden) and determination of total cholesterol, PL, and TG as described above.

ApoB-100 ELISA

Determination of plasma apoB-100 levels was carried out using an enzyme-linked immunosorbent assay with a monoclonal antibody against murine apoB-100 (LF3) essentially as described by Zlot et al. (51), who kindly provided LF3 and rabbit antisera against mouse apoB (rabbit865).

Hepatic lipid composition/liver histology

Hepatic lipids were extracted according to Bligh and Dyer (52). After dissolving the lipids in 2% Triton X-100, contents of cholesterol, CE, PL, and TG in liver tissue were determined as described above and expressed as micrograms per milligram of protein. Five micrometer cryosections were prepared on a Leica CM3050-S cryostat. Cryostat sections were routinely stained with hematoxylin (Sigma Diagnostics, St. Louis, MO) and Oil Red O (Sigma Diagnostics) for lipid visualization.

Intragastric fat load-induced postprandial TG response

Groups of five mice were fasted overnight. For basal TG and cholesterol levels, 50 μl blood samples were drawn just before 9:00 AM by tail bleeding into heparinized capillary tubes (time 0). At 9:00 AM, animals received an intragastric load of 400 μl of olive oil. After gavage, blood collection was performed every hour for 4 h. Plasma TG levels were measured at the various time points using enzymatic kits as described above. The distribution of TG over the different lipoproteins in plasma was analyzed by fractionation of 30 μl of pooled plasma using a Superose 6 column (3.2 × 30 mm, Smart-system; Amersham Biosciences) and determination of the TG content of the eluted fractions as described above.

RESULTS

Adenovirus-mediated SR-BI overexpression in C57BL/6J mice results in decreased plasma VLDL/CM-associated TG levels and plasma apo-B100 levels

SR-BI is a class B scavenger receptor that binds a broad variety of lipoprotein ligands. Recently, we showed that SR-BI is able to facilitate CM metabolism (45). To further assess the role of SR-BI in CM metabolism, the receptor was overexpressed in livers of C57BL/6J mice by infusion with a dose of Ad.SR-BI (5×10^8 pfu), which resulted in

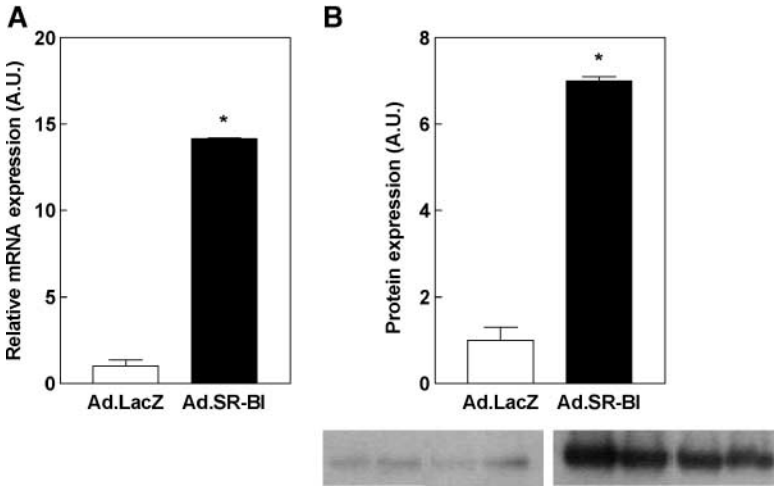

Fig. 1. Hepatic scavenger receptor class B type I (SR-BI) expression in Ad.SR-BI-treated mice and control mice. A: Analysis of SR-BI expression at 5 days after adenoviral administration by real-time quantitative PCR in C57BL/6J mice treated with Ad.LacZ [5×10^8 plaque-forming units (pfu)] or Ad.SR-BI (5×10^8 pfu) (n = 5 per group). B: Quantitation of Western blot analysis of SR-BI expression in C57BL/6J mice treated with Ad.LacZ (5×10^8 pfu) or Ad.SR-BI (5×10^8 pfu). Below the quantitation histogram, a representative immunoblot of four samples per group is shown. A.U., arbitrary units. Values shown are means ± SEM. * $P <$ 0.001.

TABLE 2. Effect of SR-BI overexpression on plasma lipid levels

Day	Treatment	n	Total Cholesterol	Free Cholesterol	PL	TG
				mg/dl		
−3	Ad.LacZ	5	62.0 ± 3.7	13.5 ± 0.6	26.5 ± 0.8	112.9 ± 8.7
5			67.6 ± 6.0	10.5 ± 1.4	23.6 ± 1.2	94.9 ± 6.1
−3	Ad.SR-BI	5	61.8 ± 2.3	14.3 ± 1.1	26.4 ± 1.0	122.8 ± 13.5
5			<5.0[a]	<1.0[a]	1.8 ± 0.3[a]	62.5 ± 12.1[b]

PL, phospholipid; TG, triglyceride. C57BL/6J mice (n = 5 per group) were injected with Ad.LacZ (5×10^8 plaque-forming units) or Ad.SR-BI (5×10^8 plaque-forming units). Three days before injection and at 5 days after injection, overnight-fasted plasma was collected from individual mice and assayed for total cholesterol, free cholesterol, PL, and TG. Values are means ± SEM.

[a] $P < 0.001$.

[b] $P < 0.01$.

14-fold and 7-fold increases in hepatic SR-BI mRNA (**Fig. 1A**) and protein expression (Fig. 1B), respectively, at 5 days after infusion. Five days after Ad.SR-BI infusion, mice showed highly significant decreases in plasma total cholesterol and free cholesterol (>90%) and plasma PL (>90%) compared with mice treated with control adenovirus (**Table 2**). In addition, plasma TG levels were decreased significantly (2-fold) in Ad.SR-BI-treated mice compared with mice treated with control adenovirus (Table 2). Analysis of lipoprotein profiles revealed a depletion of both HDL and LDL-cholesterol (**Fig. 2A**) and HDL and LDL-PL (Fig. 2B) in Ad.SR-BI-treated mice compared with control virus-treated mice. Interestingly, Ad.SR-BI-treated mice also showed a significant decrease in VLDL/CM-associated TG compared with control virus-treated mice (Fig. 2C).

Subsequently, apoB-100 levels in serum of Ad.SR-BI- and Ad.LacZ-treated mice were determined using an enzyme-linked immunosorbent assay with a monoclonal antibody against murine apoB-100 (LF3). ApoB-100 levels in serum of Ad.SR-BI-treated animals were significantly reduced compared with Ad.LacZ-treated mice (41.4% reduction; $P < 0.001$) (**Fig. 3**).

Influence of Ad.SR-BI on hepatic lipid metabolism, hepatic lipid composition, and liver morphology

Because adenovirus-mediated hepatic overexpression of SR-BI results in a substantial decrease of plasma cholesterol, PL, and TG, we next analyzed the effect of SR-BI overexpression at 5 days after infusion on hepatic genes involved in the uptake, metabolism, and efflux of cholesterol and TG. In addition to SR-BI, the uptake of cholesterol in the liver can be mediated by receptors such as the LDLr and LRP1. The decrease in LDL-cholesterol and VLDL/CM-TG observed in Ad.SR-BI-treated mice was not attributable to an increase in expression of the LDLr or LRP1. Compared with control mice, the mRNA level of the LDLr was similar and that of LRP1 was decreased significantly (**Fig. 4A**, **B**). In agreement with the mRNA levels, LDLr and LRP1 protein levels were similar and significantly decreased, respectively, in Ad.SR-BI-treated mice compared with control mice (Fig. 4C, D).

The liver has three well-known routes of cholesterol disposal. Cholesterol can be used for the synthesis of VLDL and HDL. The key process in VLDL synthesis is the intracellular association of apoB-48/apoB-100 with lipids in which the MTP is crucially involved. MTP mRNA levels have the tendency to be increased in Ad.SR-BI-treated mice ($P = 0.07$) (**Fig. 5**). Recently, it was shown that ABCA1 plays an essential role in the formation of HDL (53). ABCA1 mRNA levels are not affected by hepatic overexpression of SR-BI (Fig. 5). Hepatic cholesterol levels are the consequence of lipoprotein uptake and de novo synthesis of cholesterol by the enzyme HMG-CoA reductase. HMG-CoA reductase mRNA expression was not changed (Fig. 5). As a heterodimer, ABCG5 and ABCG8 mediate biliary cholesterol efflux from the liver to the bile duct (54). Recently, ABCG1 also has been proposed to have a role in the intracellular trafficking and efflux of cholesterol in the liver (48). Neither ABCG5 and ABCG8 nor ABCG1 mRNA expression was changed in Ad.SR-BI-treated

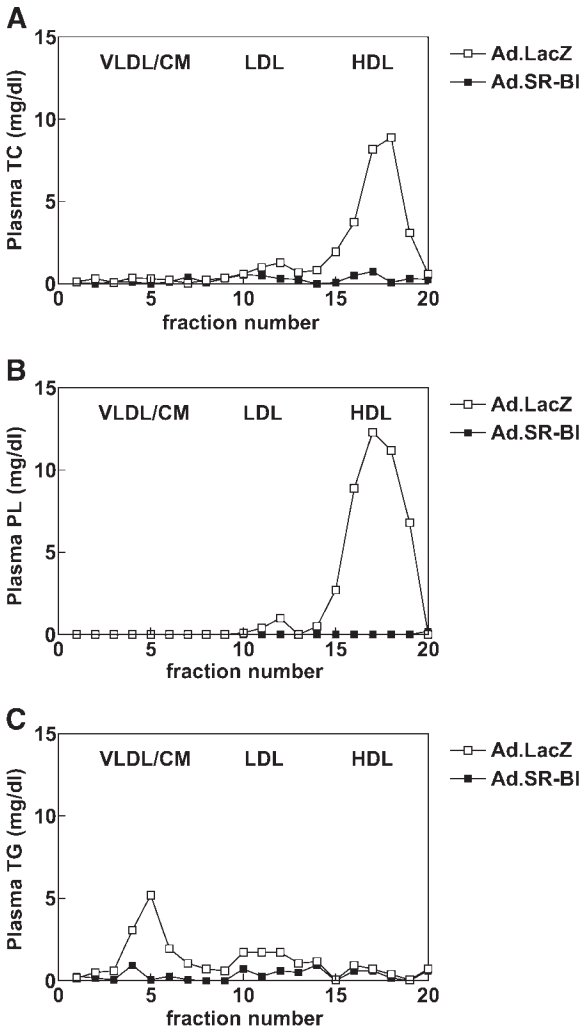

Fig. 2. Lipoprotein profiles in Ad.SR-BI-treated mice and control mice. C57BL/6J mice (n = 5 per group) were injected with Ad.LacZ (5×10^8 pfu) or Ad.SR-BI (5×10^8 pfu). After 5 days, overnight-fasted plasma was collected and total cholesterol (TC; A), phospholipid (PL; B), and triglyceride (TG; C) levels in the lipoprotein profiles of pooled plasma of Ad.SR-BI mice (closed squares) and control mice (open squares) were determined. CM, chylomicron.

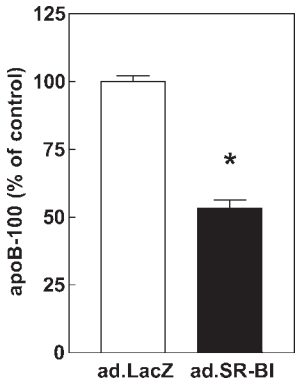

Fig. 3. Plasma apolipoprotein B-100 (apoB-100) levels. C57BL/6J mice (n = 5 per group) were injected with Ad.LacZ (5×10^8 pfu) or Ad.SR-BI (5×10^8 pfu). After 5 days, apoB-100 levels in serum of Ad.SR-BI- and Ad.LacZ-treated mice were determined using an enzyme-linked immunosorbent assay with a monoclonal antibody against murine apoB-100 (LF3). Values shown are means ± SEM for five mice per group. * $P < 0.001$.

animals (Fig. 5). Finally, CYP7A1 and CYP27 are responsible for the conversion of cholesterol into bile acids, which can be secreted from the liver into the bile via the bile salt export pump (BSEP). Whereas the level of CYP27 mRNA expression was significantly lower in mice overexpressing SR-BI, CYP7A1 and BSEP expression was not different (Fig. 5). LPL and HL regulate plasma TG levels by their TG-hydrolyzing action. The decrease in VLDL/CM-TG observed in Ad.SR-BI-treated mice was not attributable to an increase in the expression of these two enzymes, because HL was significantly lower in Ad.SR-BI-treated mice and LPL was unchanged (Fig. 5).

Hepatic lipid content in Ad.SR-BI-treated mice was not changed, as the hepatic levels of PL, TG (**Fig. 6A**), free cholesterol, or CE (Fig. 6B) are all similar. In accordance with the above hepatic lipid composition data, Oil Red O staining revealed no differences in lipid depots in Ad.SR-BI-treated and control mice (Fig. 6C).

Effect of SR-BI overexpression on CM metabolism in vivo

We next investigated the effect of SR-BI overexpression on the postprandial TG response upon an intragastric fat load, which is an established procedure to study the kinetics of CM metabolism. After an intragastric load of olive oil, plasma TG levels were determined over a period of 4 h in Ad.SR-BI-treated and control virus-treated mice. Before gavage, Ad.SR-BI-treated mice had significantly lower basal levels of plasma TG (Table 2). At 2 h after olive oil administration, control virus-treated animals showed a postprandial increase in plasma TG (3.1-fold) (**Fig. 7A**), which de-

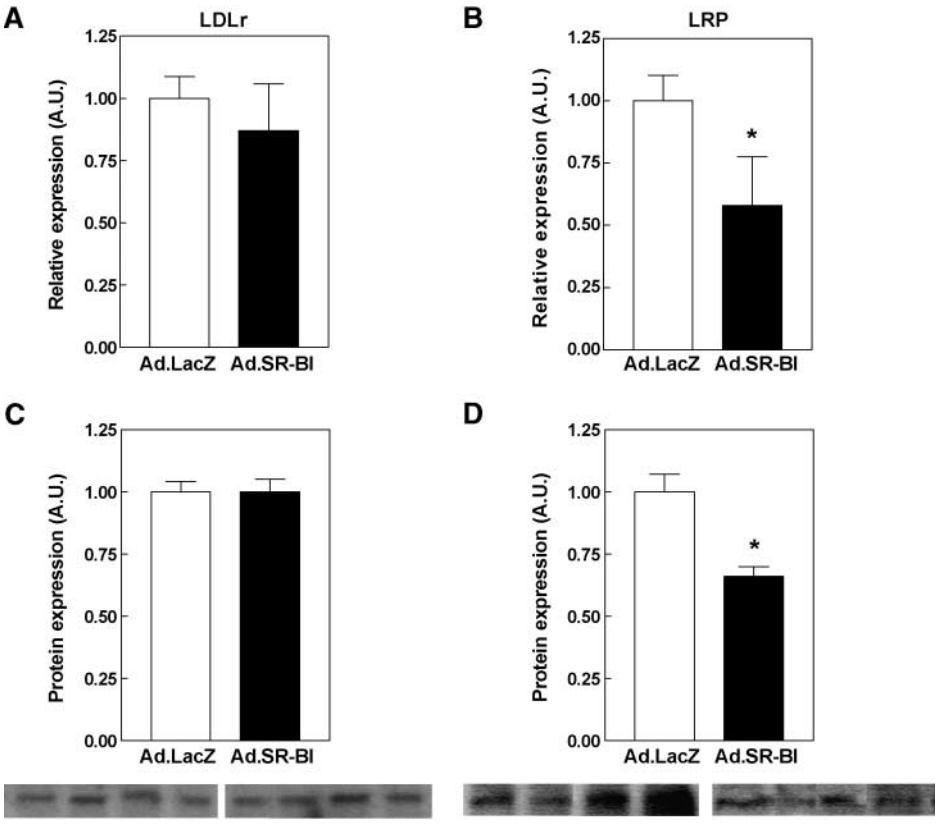

Fig. 4. Effect of SR-BI overexpression on hepatic LDL receptor (LDLr) and LDLr-related protein 1 (LRP1) expression. At 5 days after adenoviral administration, LDLr (A) and LRP1 (B) expression was analyzed by real-time quantitative PCR in C57BL/6J mice treated with Ad.LacZ (5×10^8 pfu) or Ad.SR-BI (5×10^8 pfu) (n = 5 per group). Also shown is the quantitation of Western blot analysis of LDLr (C) and LRP1 (D) expression in C57BL/6J mice treated with Ad.LacZ (5×10^8 pfu) or Ad.SR-BI (5×10^8 pfu). A.U., arbitrary units. Values shown are means ± SEM. * $P < 0.05$.

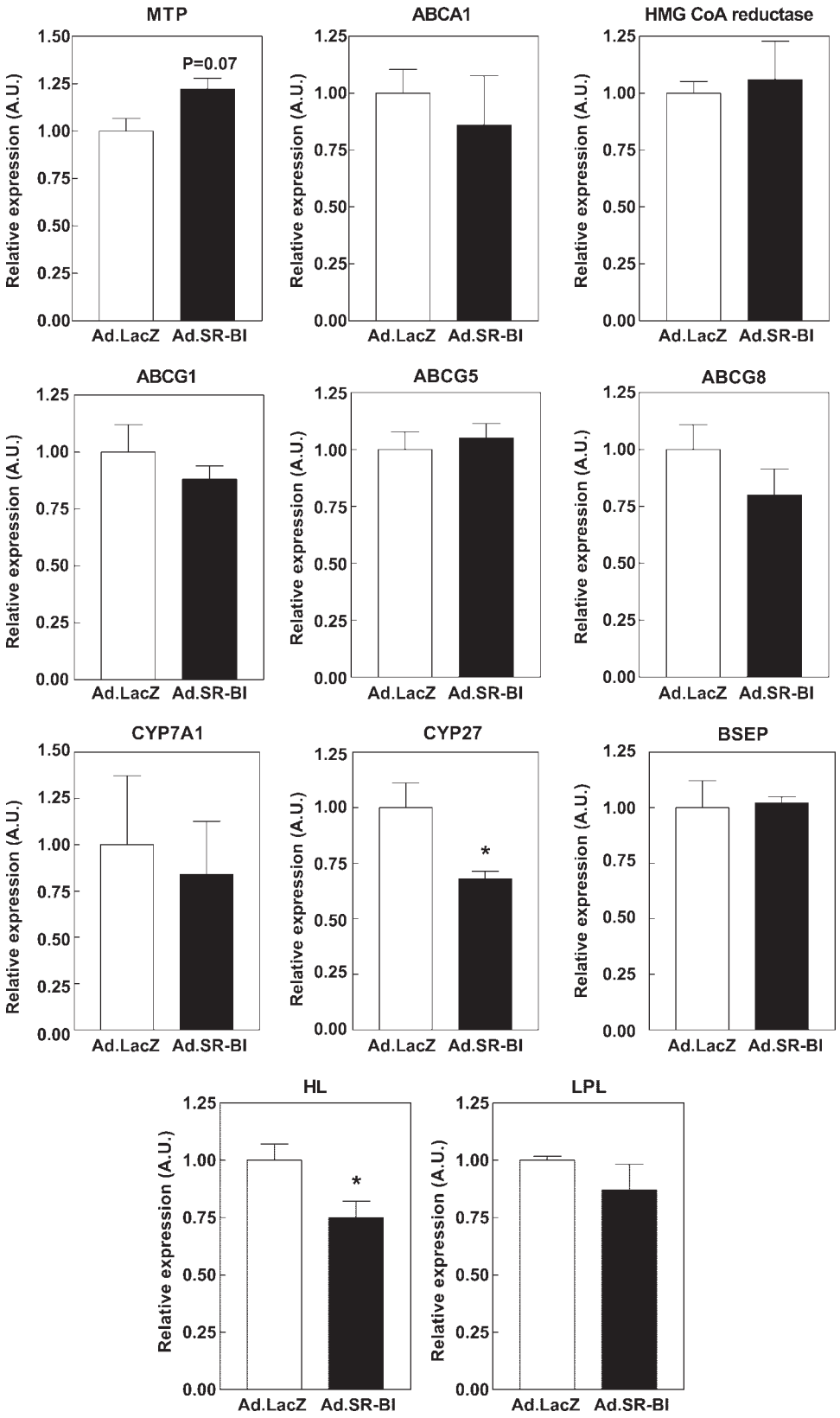

Fig. 5. Effect of SR-BI overexpression on genes involved in hepatic cholesterol metabolism. C57BL/6J mice (n = 5 per group) were injected with Ad.LacZ (5×10^8 pfu) or Ad.SR-BI (5×10^8 pfu). After 5 days, hepatic mRNA levels of the indicated genes were determined by quantitative real-time PCR. A.U., arbitrary units; BSEP, bile salt export pump; MTP, microsomal triglyceride transfer protein. Values shown are means ± SEM. * $P < 0.05$.

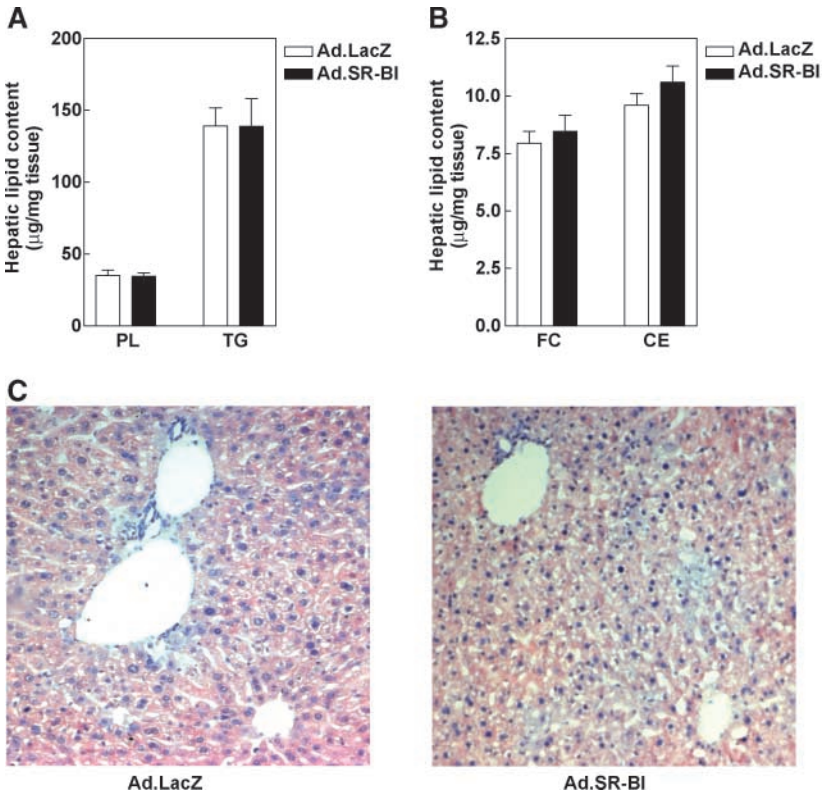

Fig. 6. Hepatic lipid content in Ad.SR-BI-treated mice and control mice. C57BL/6J mice (n = 5 per group) were injected with Ad.LacZ (5×10^8 pfu) or Ad.SR-BI (5×10^8 pfu). After 5 days, hepatic PL and TG (A), and free cholesterol (FC) and cholesteryl ester (CE; B) levels were analyzed. Five micrometer cryosections of livers of Ad.LacZ- and Ad.SR-BI-treated mice were stained with Oil Red O for lipid visualization and counterstained with hematoxylin. Values shown are means ± SEM.

creased again at 4 h after administration. In contrast, Ad.SR-BI-treated mice showed a 2-fold decreased TG response compared with control virus-treated mice (area under the curve = 31.4 ± 2.4 vs. 17.7 ± 3.2; $P < 0.05$). Specifically, the increase in plasma TG was significantly lower at 1, 2, and 3 h after gavage (Fig. 7A). Analysis of lipoprotein profiles at 3 h after gavage (Fig. 7B) showed that plasma TG in the VLDL/CM fraction was lower in Ad.SR-BI-treated mice compared with control virus-treated mice, and the reduction was mainly attributable to a decrease in CM-associated TG.

DISCUSSION

SR-BI is a multiligand cell surface receptor capable of binding HDL, LDL, VLDL, modified LDL and BSA, and liposomes containing anionic PL (4–7). Although the function of SR-BI in the selective uptake of CE from HDL is undisputable (12), conflicting information on a potential role of SR-BI in the metabolism of apoB-containing lipoproteins exists (14–20).

Using SR-BI-deficient mice, we recently showed that SR-BI can facilitate CM (remnant) metabolism (45). In the present study, we investigated to what extent the role of SR-BI in VLDL/CM (remnant) metabolism is critically dependent on SR-BI protein levels by assessing the effect of adenovirus-mediated hepatic overexpression of SR-BI in C57BL/6J mice. Adenovirus-mediated overexpression of SR-BI led to a significant decrease in HDL-cholesterol and was accompanied by a decrease in the main apolipoprotein constituent of HDL (apoA-I) (data not shown), as also observed in other studies (13, 15, 16, 46), and a substantial increase in biliary cholesterol (13). Strikingly, plasma TG levels, VLDL/CM-associated TG, and plasma apoB-100 levels were all significantly reduced: findings that correlate with a potential role for SR-BI in VLDL/CM metabolism. In the present work, we analyzed the effect of SR-BI protein level on endogenous CM metabolism by giving an intragastric fat load to Ad.SR-BI-treated mice and control mice. After administration of olive oil, the maximum level of TG reached in the blood circulation was 2-fold lower, corresponding with a significant decrease in the area under the curve (31.4 ± 2.4 vs. 17.7 ± 3.2; $P < 0.05$) in the Ad.SR-BI-treated mice compared with control mice. Both the decreased plasma TG levels and the decreased postprandial response could have been attributed to other processes, such as decreased VLDL production, and/or indirect effects of overexpression of SR-BI on the expression of other hepatic genes involved in cholesterol and/or CM metabolism. For this reason, we assessed both the mRNA and protein levels of the LDLr and LRP1, which are believed to be responsible for the internalization of VLDL and CM remnants by the liver. The expression of

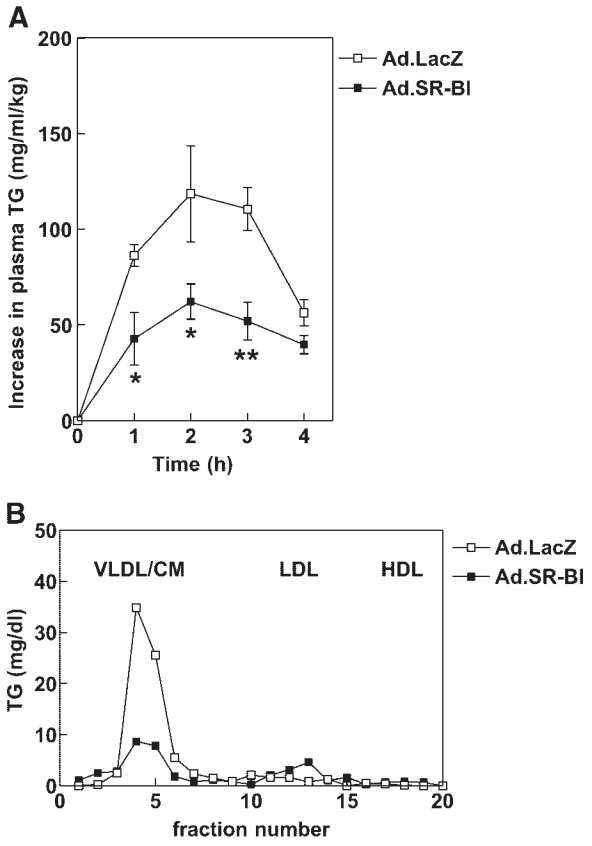

Fig. 7. Effect of SR-BI overexpression on the postprandial TG response upon an intragastric fat load. C57BL/6J mice (n = 5 per group) were injected with Ad.LacZ (5×10^8 pfu; open squares) or Ad.SR-BI (5×10^8 pfu; closed squares). After 5 days, overnight-fasted mice received an intragastric load of 400 μl of olive oil at time 0. A: Subsequently, plasma TG levels were determined at the indicated times, and the data are expressed as increases in TG levels relative to time 0. B: At 3 h after olive oil administration, TG levels in the lipoprotein profiles of pooled plasma were determined. Values shown are means ± SEM. * $P < 0.05$; ** $P < 0.01$.

the LDLr appeared unchanged, whereas the LRP level was actually lower by Ad.SR-BI administration. Thus, the decreased plasma TG levels and the change in postprandial response cannot be caused by an increased expression of these two receptors. Hepatic expression of proteins involved in cholesterol transport and/or metabolism, such as ABCA1, ABCG1, ABCG8, CYP7A1, CYP27, and BSEP, were not increased by Ad.SR-BI administration, indicating that the observed change in serum lipoproteins is not related to these proteins. Actually, only MTP had the tendency to be increased ($P = 0.07$), which suggests a compensatory mechanism to resecrete the SR-BI-mediated hepatic uptake of TG in the form of VLDL. LPL and HL regulate plasma TG levels by their TG-hydrolyzing action. The decrease in VLDL/CM-TG observed in Ad.SR-BI-treated mice was not attributable to an increase in the expression of these two enzymes, because HL was significantly lower in Ad.SR-BI-treated mice and LPL was unchanged.

Combined with our earlier data in SR-BI-deficient mice, the present experiments using transient adenovirus-mediated overexpression of SR-BI in C57BL/6J indicate that SR-BI levels are important for the kinetics of postprandial lipemia. Previous studies have suggested that postprandial remnant particles may predict the onset of atherosclerosis. Consistent with our present findings, it was suggested by Arai et al. (55) that in heterozygous LDLr-deficient mice, the transgene expression of SR-BI leads to decreased atherosclerosis, which correlated with decreased VLDL and LDL-cholesterol levels (55). Also, Wang et al. (15) and Ueda et al. (16, 17) observed in SR-BI transgenic mice decreased levels of VLDL-apoB and LDL-apoB. Kozarsky et al. (56) have shown that adenovirus-mediated hepatic overexpression of SR-BI in fat-fed LDLr-deficient mice leads to a marked decrease in HDL cholesterol and a modest decrease in intermediate density lipoprotein/LDL cholesterol. The modest reduction in non-HDL-cholesterol in these studies can be explained by the absent activity of the LDLr needed for the internalization of the remnants (30, 57). Furthermore, Fu, Kozarsky, and Borensztajn (20) recently observed that fibrate-induced hypercholesterolemia in apoE-deficient mice can be normalized by the overexpression of SR-BI. It was suggested that SR-BI can function as a remnant receptor responsible for the clearance of remnant particles from the circulation of apoE-deficient mice. Together with our recent observation in SR-BI-deficient mice, our present experiments suggest that SR-BI can indeed function as an initial recognition site for VLDL/CM not only in apoE-deficient mice but also under normal metabolic conditions.

The mechanism responsible for the initial liver capture of CM remnants has been a point of continuous dispute (22–24). In mice without apoE-recognizing internalizing receptor (LRP1/LDLr double-deficient mice), the initial association of lipoprotein remnants (30) and large emulsion particles (P. C. N. Rensen, J. K. Kruijt, and T. J. C. van Berkel, unpublished results) with the liver is not affected, indicating that another molecular structure is responsible for the initial liver recognition. SR-BI fulfills the requirements as an initial recognition site in that it is a multiligand cell surface receptor with a limited substrate specificity, which includes not only apolipoproteins but also lipids such as phosphatidylserine, a remnant surface component. The present experiments are consistent with SR-BI serving as an initial remnant recognition site. The locally available apoE (58) may subsequently be acquired and trigger internalization. Although apoB-100 levels were decreased by Ad.SR-BI, our data do not necessarily implicate SR-BI as an internalizing receptor, because studies with LRP1/LDLr double-deficient mice have clearly shown the decisive role of this combined system for the internalization and further catabolism of remnants (30, 57) and large emulsion particles (P. C. N. Rensen, J. K. Kruijt, and T. J. C. van Berkel, unpublished results) by the liver.

Very interesting and consistent with our data, Pérez-Martinez et al. (59) recently suggested a role for SR-BI in postprandial CM metabolism in humans. They observed that a polymorphism in the exon 1 variant at the SR-BI gene locus called genotype 1/2 was associated with a lower postprandial response compared with that in individuals with a 1/1 genotype (59). The expression levels of hepatic

SR-BI in both genotypes were not analyzed, but this knowledge could shed additional light on the role of SR-BI as a remnant receptor in humans.

In summary, we have further substantiated the proposed role of SR-BI in CM metabolism by assessing the effect of adenovirus-mediated hepatic overexpression of SR-BI. Adenovirus-mediated hepatic overexpression of SR-BI in C57BL/6J mice resulted in a decrease in plasma TG, a decrease in VLDL/CM-associated TG, and a changed postprandial TG response. These data support our earlier suggestion that SR-BI is involved in facilitating CM remnant metabolism, and the present study strengthens the notion that besides its role in HDL metabolism, SR-BI is crucially involved in facilitating CM (remnant) metabolism. We conclude that overexpression of SR-BI accelerates CM metabolism possibly by mediating the initial capture of CM remnants by the liver, leading to subsequent internalization by receptor systems such as the LDLr and LRP1.

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