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THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 278, No. 26, Issue of June 27, pp. 23699–23705, 2003
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Differential Effects of Scavenger Receptor BI Deficiency on Lipid Metabolism in Cells of the Arterial Wall and in the Liver*

Received for publication, November 4, 2002, and in revised form, February 19, 2003
Published, JBC Papers in Press, March 14, 2003, DOI 10.1074/jbc.M211233200

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Scavenger receptor class B, type I (SRBI) is a key regulator of high density lipoprotein (HDL) metabolism. It facilitates the efflux of cholesterol from cells in peripheral tissues to HDL and mediates the selective uptake of cholesteryl esters from HDL in the liver. We investigated the effects of SRBI deficiency in the arterial wall and in the liver using SRBI-deficient mice and wild-type littermates fed a Western-type diet. The SRBI-deficient mice showed massive accumulation of cholesterol-rich HDL in the circulation, reflecting impaired delivery to the liver. Strikingly, SRBI deficiency did not alter hepatic cholesterol (ester) content nor did it affect the expression of key regulators of hepatic cholesterol homeostasis, including HMG-CoA reductase, the low density lipoprotein receptor, and cholesterol 7α-hydroxylase. However, a ~40% reduction in biliary cholesterol content was observed, and the expression of ABCG8 and ABCG5, ATP half-transporters implicated in the transport of sterols from the liver to the bile, was attenuated by 70 and 35%, respectively. In contrast to the situation in the liver, SRBI deficiency *did* result in lipid deposition in the aorta and atherosclerosis. Vascular mRNA analysis showed increased expression of inflammatory markers as well as of genes involved in cellular cholesterol homeostasis. Our data show that, although hepatic cholesterol homeostasis is maintained upon feeding a Western-type diet, SRBI deficiency is associated with de-regulation of cholesterol homeostasis in the arterial wall that results in an increased susceptibility to atherosclerosis.

A low level of high density lipoprotein (HDL)[1] is an important risk factor for the development of atherosclerosis (1, 2). Stimulation of reverse transport of cholesterol from cells of the arterial wall to the liver is considered the mechanism by which HDL exerts its anti-atherogenic properties (3). In addition, interaction of HDL with the arterial wall directly protects against oxidative stress and vascular inflammation (4).

An important mediator of HDL metabolism is scavenger receptor class B, type I (SRBI) (5). SRBI is a multifunctional receptor capable of binding a wide array of native and modified lipoproteins. SRBI is abundantly expressed in liver and steroidogenic tissues, where it mediates the selective uptake of cholesteryl esters from HDL (6, 7). A definitive role for SRBI in HDL metabolism and reverse cholesterol transport *in vivo* has been demonstrated using different transgenic and knockout mouse models. Hepatic overexpression of SRBI is accompanied by decreased levels of HDL cholesterol (8, 9). Furthermore, targeted inactivation of SRBI induced a 2-fold increase in plasma cholesterol levels due to the accumulation of abnormally large, heterogeneous HDL (10). When cross-bred onto the apolipoprotein E knockout background, SRBI deficiency leads to severe cardiac dysfunction and premature death (11, 12). Hepatic overexpression of SRBI, on the other hand, protects against the development of atherosclerosis (13).

In addition to the liver, SRBI is also expressed by cells within the arterial wall, including endothelial cells, smooth muscle cells, and macrophages (14–16). At least in macrophages, SRBI has been implicated in the bi-directional flux of cholesterol and phospholipids between HDL and the cell membrane (17, 18). However, whether vascular SRBI plays a direct role in arterial lipid homeostasis or in HDL-mediated anti-atherogenic effects is currently unknown.

In this study, the differential effects of SRBI deficiency on lipid metabolism and related gene expression in cells of the liver and the arterial wall were evaluated in response to a challenge with an atherogenic diet. We show that, despite an impaired delivery of HDL cholesterol to the liver in SRBI−/− mice, hepatic cholesterol homeostasis is maintained by processes involving down-regulation of ABCG5/ABCG8 and up-regulation of ABCG1. In contrast, SRBI deficiency in the arterial wall results in de-regulation of vascular lipid homeostasis and induction of inflammation culminating in atherosclerosis.

EXPERIMENTAL PROCEDURES

Mice—SRBI-deficient mice were kindly provided by Dr. M. Krieger (10). Heterozygous SRBI-deficient mice were cross-bred to generate wild-type (SRBI+/+), heterozygous (SRBI+/−), and homozygous (SRBI−/−) progeny. The presence of the targeted and wild-type SRBI alleles was assessed by PCR amplification of DNA extracted from tail biopsies (primers 5′-GAT-GGG-ACA-TGG-GAC-ACG-AAG-CCA-TTC-T-3′ and 5′-TCT-GTC-TCC-GTC-TCC-TTC-AGG-TCC-TGA-3′). Mice were maintained on sterilized regular chow containing 4.3% (w/w) fat and no cholesterol (RM3, Special Diet Services, Witham, UK). At 12–16 weeks of age, the diet was switched to a semi-synthetic high fat/high cholesterol Western-type diet containing 15% (w/w) fat and 0.25% (w/w)

* This work was supported by Netherlands Heart Foundation Grant 2001D041 (to M. V. E.), an International HDL Research Program grant (to M. V. E.) (to I. S. T. B.), and Yamanouchi Europe BV (to J. T.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[1] The abbreviations used are: HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very LDL; SRBI, scavenger receptor class B, type I; CE, cholesteryl esters; HMG-CoA, hydroxymethylglutaryl-CoA; ABC, ATP-binding cassette.

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TABLE I
Primers for real-time PCR analysis

Gene	GenBank™ accession No.	Forward primer	Reverse primer	Amplicon size
SR-BI	NM_016741	GGCTGCTGTTTGCTGCG	GCTGCTTGATGAGGGAGGG	63
ABCA1	NM_013454	GGTTTGGAGATGGTTATACAATAGTTGT	TTCCCGGAAACGCAAGTC	96
ABCG1	NM_009593	AGGTCTCAGCCTTCTAAAGTTCCTC	TCTCTCGAAGTGAATGAAATTTATCG	85
ABCG5	NM_031884	TGGCCCTGCTCAGCATCT	ATTTTTAAAGGAATGGGCATCTCTT	81
ABCG8	NM_026180	CCGTCGTCAGATTTCCAATGA	GGCTTCCGACCCATGAATG	64
LRP[a]	NM_008512	TGGGTCTCCCGAAATCTGTT	ACCACCGCATTCTTGAAGGA	95
ApoE	NM_009696	AGCCAATAGTGGAAGACATGCA	GCAGGACAGGAGAAGGATACTCAT	130
LDL receptor	Z19521	CTGTGGGCTCCATAGGCTATCT	GCGGTCCAGGGTCATCTTC	68
CYP7A1	NM_007824	CTGTCATACCACAAAGTCTTATGTCA	ATGCTTCTGTGTCCAAATGCC	75
HMG-CoA reductase	M62766	TCTGGCAGTCAGTGGGAACTATT	CCTCGTCCTTCGATCCAATTT	69
CD36	L23108	GTTCTTCCAGCCAATGCCTTT	ATGTCTAGCACACCATAAGATGTACAGTT	110
CD68	NM_009853	CCTCCACCCTCGCCTAGTC	TTGGGTATAGGATTCGGATTTGA	118
VCAM-1[a]	X67783	ACAAAACGATCGCTCAAATCG	CGCGTTTAGTGGGCTGTCTATC	110
P-selectin	M87861	GGTATCCGAAAGATCAACAATAAGTG	GTTACTCTTGATGTAGATCTCCACACA	141
E-selectin	M87862	CCCTGCCCACGGTATCAG	CCCTTCCACACAGTCAAACGT	85
HPRT[a]	J00423	TTGCTCGAGATGTCATGAAGGA	AGCAGGTCAGCAAAGAACTTATAGC	91

[a] LRP, LDL receptor-related protein; HPRT, hypoxanthine-guanine phosphoribosyltransferase; VCAM-1, vascular cell adhesion molecule-1.

cholesterol (Diet W, Hope Farms, Woerden, The Netherlands). After 20 weeks of feeding the Western-type diet, the mice were anesthetized, and blood and gall bladder bile were collected for lipid analyses. Subsequently, a whole-body perfusion was performed using phosphate-buffered saline containing 1 mM EDTA (4 °C, 100 mm of Hg) for 20 min. After perfusion, the liver, aortic arch (from the base of the aorta to just below the left subclavian artery), and heart plus aortic root were excised. The aortic arch and liver were frozen in liquid N_2 and stored at −80 °C until RNA isolation. The heart plus aortic root were stored in 3.7% formalin. All experimental protocols were approved by the Ethics Committee for animal experiments of Leiden University.

In Vivo Analysis of HDL Cholesteryl Ester Delivery to the Liver—Human HDL was isolated by differential ultracentrifugation as described by Redgrave *et al.* (19) and subsequently labeled with [^{3}H]cholesterol oleate (Amersham Biosciences) by exchange from donor particles as reported previously (20). The radiolabeled HDL was injected via the vena cava inferior of anesthetized mice. After 4 h of circulation, blood was sampled, and the liver was excised. The associated radioactivity was determined in a Packard liquid scintillation counter after combustion in a Packard sample oxidizer.

Lipid Analyses—Serum concentrations of cholesterol, cholesteryl esters, and triglycerides were determined using enzymatic colorimetric assays (Roche Applied Science). The distribution of cholesterol over the different lipoproteins in serum was analyzed by fractionation of 30 μl of serum of each mouse using a Superose 6 column (3.2 × 30 mm, Smartsystem, Amersham Biosciences). Total cholesterol content of the effluent was determined using enzymatic colorimetric assays (Roche Applied Science), taking the efficiency of recovery from the column into account. HDL was isolated from pooled sera by density-gradient ultracentrifugation, and cholesteryl ester, free cholesterol, triglyceride, and phospholipid contents of the isolated HDL were determined as described. The protein content of the HDL fractions was determined according to Lowry *et al.* (21). Hepatic and biliary lipids were extracted according to Bligh and Dyer (22). After dissolving the lipids in 2% Triton X-100, the contents of cholesterol, cholesteryl ester, and triglycerides in liver tissue were determined as described above and expressed as nmol/mg of protein. Biliary bile salt and cholesterol concentrations were determined according to Setchell *et al.* (23) and Gamble *et al.* (24), respectively.

Assessment of Lesion Formation in the Aortic Root—Serial sections of the aortic root were cut using a Leica CM3050S cryostat. The atherosclerotic lesion area was quantified in oil red O-stained cryostat sections using the Leica image analysis system, consisting of a Leica DMRE microscope coupled to a video camera and Leica Qwin Imaging software (Leica Ltd., Cambridge, England). Mean lesion area (in μm^2) was calculated from 10 consecutive oil red O-stained sections, starting at the appearance of the tricuspid valves.

Analysis of Gene Expression by Real-time Quantitative PCR—Total RNA was extracted from liver and aortic arch by the acid guanidinium thiocyanate-phenol chloroform extraction method according to Chomczynski and Sacchi (25) in the presence of 0.2 mg/ml glycogen. cDNA was synthesized from 0.5–1 μg of total RNA using RevertAid™ Moloney murine leukemia virus reverse transcriptase. mRNA levels were quantitatively determined on an ABI Prism® 7700 sequence detection system (Applied Biosystems, Foster City, CA) using SYBR®-green technology according to manufacturer's instructions. PCR primers (Table I) were designed using Primer Express 1.5 Software using the manufacturer's default settings (Applied Biosystems).

The level of mRNA expression for each gene of interest was calculated using the threshold cycle (C_t) value, *i.e.* the number of PCR cycles at which the fluorescent signal during the PCR reaches a fixed threshold. For each sample, both the C_t for the gene of interest and for the housekeeping gene hypoxanthine-guanine phosphoribosyltransferase were determined to calculate $\Delta C_{t,\text{sample}}$ ($C_{t,\text{target gene}} - C_{t,\text{housekeeping gene}}$), thus normalizing the data and correcting for differences in amount and/or quality between the different RNA samples. The expression levels were related to an external calibrator consisting of a cDNA pool of different mouse organs (*i.e.* liver, spleen, lung, muscle, and aorta, each represented equally). Subsequently, $\Delta\Delta C_t$ ($\Delta C_{t,\text{sample}} - \Delta C_{t,\text{calibrator}}$) was determined, and the relative expression levels were calculated from $2^{-\Delta\Delta C_t}$, according to the manufacturer's instructions (Applied Biosystems). mRNA expression levels are, thus, indicated as arbitrary units ± S.E.

Immunoblot Analysis—Liver homogenate was prepared by lysis in 50 mM Tris-HCl, 1% Triton X-100, 0.5% deoxycholate, 1% SDS containing 0.02 μg/ml leupeptin, 0.02 μg/ml aprotinin, and 0.02 μg/ml trypsin inhibitor. Cell debris was removed by centrifugation at 10,000 rpm for 10 min, and the protein concentration was determined according to Lowry *et al.* (21). Equal amounts of protein were separated on 7.5% SDS-PAGE gels and electrophoretically transferred to Protran nitrocellulose membrane (Schleicher & Schnell). Immunolabeling was performed using either rabbit polyclonal αSRBI (anti-BI^{495} (26)) or murine monoclonal αABCA1 (AC-10 (27)) as primary antibody and goat-anti-rabbit IgG and goat-anti-mouse IgG (Jackson ImmunoResearch), respectively, as secondary antibodies. Finally, immunolabeling was detected by enhanced chemiluminescence (ECL, Amersham Biosciences).

Statistical Analyses—Statistical significant differences among the means of the different groups of mice were tested using analysis of variance and the Student-Newman-Keuls multicomparison test (Graphpad Instat software, San Diego, CA).

RESULTS

A High Cholesterol Western-type Diet Results in Accumulation of Cholesteryl Ester-rich HDL in Serum of SRBI-deficient Mice—On chow diet, containing 4.3% fat and no cholesterol, SRBI deficiency induced a 1.8-fold increase in total serum cholesterol levels ($p < 0.001$, Table II). Interestingly, this increase in total cholesterol levels was mainly a result of a 3.2-fold increase in free cholesterol ($p < 0.001$, Table II). As indicated in Fig. 1*a*, changes in serum cholesterol levels were caused by a marked increase in the amounts of cholesterol transported by HDL. So far these findings confirmed the role of SRBI in determining circulating HDL levels (10). Subsequently, we challenged the SRBI-deficient mice with a Western-type diet containing 15% fat and 0.25% cholesterol for a period of 20 weeks. The length of the dietary period was chosen such that effects on vascular function and atherosclerosis, if

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TABLE II
Effect of SRBI deficiency on serum lipid levels

Serum lipids were measured in SRBI+/+, SRBI+/−, and SRBI−/− mice maintained on a chow diet; and after feeding a high cholesterol Western-type diet (WTD) for 20 weeks. Data represent the means ± S.E. Statistically significant difference: compared to SRBI+/+ mice is shown in the table footnotes.

Mice	Time	Diet	n	Triglycerides	Total cholesterol	Free cholesterol	Cholesteryl esters	HDL cholesteryl esters
	weeks			*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*
SRBI+/+	0	Chow	6	108 ± 15	93 ± 7	24 ± 1	70 ± 6	49.1 ± 6
	20	WTD	6	98 ± 8	122 ± 12	28 ± 3	94 ± 10	61.5 ± 8
SRBI+/−	0	Chow	5	118 ± 20	139 ± 6[a]	36 ± 3[a]	103 ± 4[a]	81.2 ± 5[a]
	20	WTD	5	97 ± 12	185 ± 8[b]	47 ± 1[b]	138 ± 7[a]	86.8 ± 5[c]
SRBI−/−	0	Chow	5	99 ± 4	167 ± 10[b]	81 ± 3[b]	88 ± 7[a]	76 ± 7[a]
	20	WTD	5	96 ± 11	322 ± 20[b]	150 ± 20[b]	172 ± 9[b]	138 ± 10[b]

[a] $p < 0.01$.
[b] $p < 0.001$.
[c] $p < 0.05$.

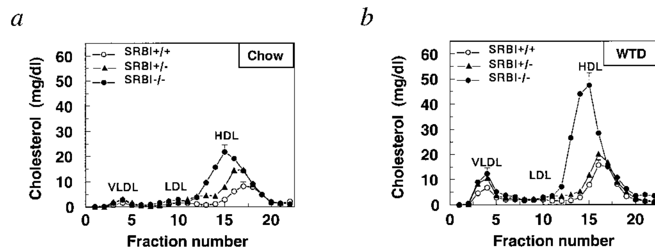

FIG. 1. **Effect of SRBI deficiency on the plasma cholesterol distribution.** Blood samples were drawn after an overnight fast while feeding regular chow diet (*a*) and after 20 weeks of feeding a high cholesterol Western-type diet (*WTD*) (*b*). Sera from individual mice were loaded onto a Superose 6 column, and fractions were collected. Fractions 3–7 represent VLDL; fractions 8–14 represent LDL; fractions 15–19 represent HDL. The distribution of cholesterol over the different lipoproteins in SRBI+/+ (○), SRBI+/− (▲), and SRBI−/− (●) is shown. Values represent the mean of 5–6 mice. S.E. are shown only for fractions containing the top of the VLDL, LDL, and HDL peaks.

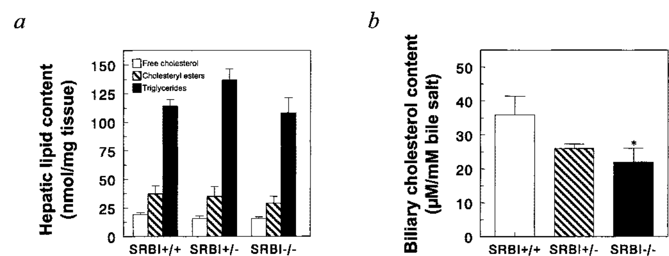

FIG. 2. **Effect of SRBI deficiency on hepatic and biliary cholesterol content.** Hepatic (*a*) and biliary (*b*) cholesterol content was analyzed in SRBI+/+, SRBI+/−, and SRBI−/− mice after 20 weeks feeding of a high cholesterol Western-type diet. Values represent the mean ± S.E. of 5–6 mice. *, statistically significant difference of $p < 0.05$ compared with SRBI+/+ mice.

present, could also be assessed. Remarkably, the atherogenic diet resulted in a dramatic further accumulation of HDL cholesterol, with only minor effects on VLDL cholesterol levels (Fig. 1*b*). Upon dietary induction, the cholesterol distribution in the heterozygous SRBI-deficient littermates was similar to that of wild-type controls, except for a tendency toward slightly increased VLDL cholesterol levels. Compositional lipid analysis of HDL, isolated after 20 weeks Western-type diet, showed that the free cholesterol content was 4.5% in HDL from wild types and 9.6% in HDL from SRBI knockouts. Cholesteryl ester contents were 18.8 and 32.7% in wild-type and SRBI-deficient mice, respectively, whereas the corresponding percentages for phospholipids amounted to 40.6 and 23.6%, respectively. There was no effect of SRBI deficiency on the protein content of the isolated HDL. Thus, SRBI deficiency in combination with diet-induced hypercholesterolemia resulted in the accumulation of large HDLs enriched in cholesteryl esters and free cholesterol and depleted of phospholipids. There was no effect of SRBI deficiency on serum triglyceride levels under both dietary conditions.

Maintenance of Hepatic Lipid Homeostasis in SRBI-deficient Mice—In wild-type mice, the liver is responsible for the removal of the majority of the cholesteryl esters (CEs) from HDL. When injected with ^{3}H-labeled HDL, 31.3 ± 4.5% of the injected dose was recovered from the liver in wild-type mice 4 h after administration. In the absence of SRBI, uptake of cholesteryl esters was reduced by ~90% to only 3.3 ± 0.8% of the injected dose ($p < 0.05$). A similar value was found for ^{125}I-labeled tyramine cellobiose-labeled HDL, indicative for whole particle uptake (data not shown), suggesting that in SRBI knockout mice, the selective uptake is completely abolished. Correction for the HDL-CE pool size indicated the HDL-CE mass influx into the liver was reduced from 22.0 ± 3.8 μg of HDL-CE/hour in wild-type mice to 4.1 ± 0.7 μg of HDL-CE/h in SRBI knockout animals. Reduced delivery of HDL cholesteryl esters to the liver is, thus, most likely the cause of the accumulation of large cholesterol-enriched HDL particles in the circulation of the SRBI-deficient mice (Table II, Fig. 1). Interestingly, this did not affect the hepatic levels of free cholesterol, cholesteryl esters, and triglycerides (Fig. 2*a*).

Real-time PCR was subsequently used to determine the expression of genes involved in maintaining liver cholesterol homeostasis. In accordance with the observed similar hepatic lipid levels, there was no significant effect on the mRNA expression levels of hepatic HMG-CoA reductase and of the LDL receptor (Fig. 3), both of which are regulated by the hepatic cholesterol content. Similarly, the expression of cholesterol 7α-hydroxylase was not affected. Other genes involved in hepatic cholesterol metabolism, including CD36, scavenger receptor class A, LDL receptor-related protein, and apolipoprotein E (apoE) were also not affected by the absence of SRBI (Fig. 3). Collectively, the data indicate that the expression of key regulators in *de novo* cholesterol biosynthesis, cholesterol uptake from native and modified lipoproteins, and cholesterol breakdown (28) are not affected by SRBI deficiency and reduced delivery of HDL cholesterol to the liver.

SRBI deficiency, however, did coincide with an approximate 40% reduction in biliary cholesterol content normalized to bile salt content (Fig. 2*b*). Two members of the ABCG subfamily of ABC transporters that have recently been implicated in the efflux of sterols from the liver into the bile are the co-operating half-transporters ABCG5 and ABCG8 (29). Interestingly, ABCG5 and ABCG8 mRNA expression was attenuated by 35 and 70% ($p < 0.05$), respectively, in mice lacking SRBI as compared with wild-type mice (Fig. 3). Other members of the family of ABC transporters implicated in sterol trafficking are ABCA1 and ABCG1 (30, 31). SRBI deficiency did not affect ABCA1 mRNA expression. For most genes mRNA expression strongly correlates with protein expression. ABCA1 mRNA

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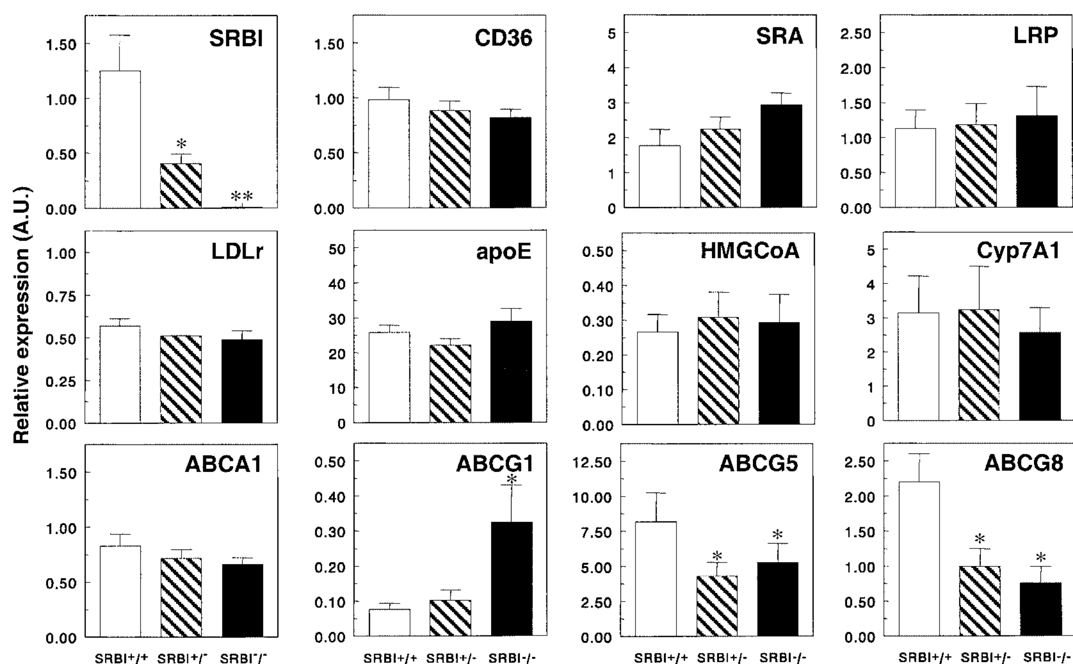

FIG. 3. **Effect of SRBI deficiency on hepatic mRNA expression of genes involved in lipid homeostasis.** mRNA levels of the indicated genes in livers of SRBI+/+, SRBI+/−, and SRBI−/− mice were quantified using real-time PCR with SYBR®-green detection after 20 weeks high cholesterol Western-type diet feeding. Values are in arbitrary units (*A.U.*) and represent the mean ± S.E. of 5–6 mice. *, statistically significant difference of $p < 0.05$ compared with SRBI+/+ mice. **, statistically significant difference of $p < 0.01$ compared with SRBI+/+ mice.

FIG. 4. **Protein expression of SRBI and ABCA1 in livers of SRBI+/+, SRBI+/−, and SRBI−/− mice after 20-week Western-type diet feeding.** Hepatic lysates (75 and 25 μg for detection of SRBI and ABCA1, respectively) were separated on non-reducing 7.5% SDS-PAGE and immunoblotted with anti-BI[495] or AC-10 for detection of SRBI or ABCA1, respectively.

levels, however, appear to be a poor predictor for protein expression levels (27). Therefore, in addition to ABCA1 mRNA analysis, the protein expression was determined by immunoblotting. In accordance with the mRNA data, SRBI deficiency did not influence the protein expression of ABCA1 (Fig. 4). Although ABCA1 expression was not affected, ABCG1 expression was stimulated 4.3-fold ($p < 0.05$) in the absence of SRBI (Fig. 3), suggesting a compensatory function of ABCG1 in maintaining cholesterol homeostasis in the livers of mice lacking SRBI.

Lipid Deposition and Vascular Inflammation in SRBI-deficient Mice—Although the liver has several established mechanisms to maintain lipid homeostasis, the arterial wall mainly relies on cholesterol efflux pathways to prevent its accumulation. Vascular tissue was analyzed after 20 weeks of feeding the mice a Western-type diet to examine the effects of SRBI disruption and hypercholesterolemia on arterial wall morphology and function. As shown in Fig. 5, SRBI deficiency led to a 72-fold ($p < 0.001$) increase in mean atherosclerotic lesion area, *i.e.* from $0.8 \pm 0.4 \times 10^3$ μm^2 in wild-type mice to $60 \pm 6 \times 10^3$ μm^2 in mice lacking SRBI. Lesions consisted primarily of fatty streaks with oil red O-positive lipid-laden macrophages (Fig. 5*b*). Heterozygous SRBI-deficient mice did not display any lipid deposition in the arterial wall, in line with the minor effects on serum HDL cholesterol levels when compared with wild-type littermates.

Both de-regulation of cholesterol homeostasis and inflammatory processes play a pivotal role in the pathogenesis of atherosclerosis (32). Therefore, the effect of SRBI deficiency on the mRNA expression of genes involved in these two processes was analyzed. As shown in Fig. 6*a*, SRBI deficiency led to a significant induction of genes implicated in adhesion and transendothelial migration of monocytes, including P-selectin (2-fold, $p < 0.05$), E-selectin (4-fold, $p < 0.05$), and vascular cell adhesion molecule-1 (2-fold, $p < 0.01$). The induction of these genes coincided with a 2-fold ($p < 0.05$) increase in CD68 expression, indicative of an increase in the amount of macrophages present within the arterial wall. The observed increase in inflammatory markers may, thus, be directly related to atherosclerotic lesion development. SRBI deficiency also led to a significant induction of genes implicated in cholesterol efflux from macrophages, including apoE (2-fold, $p < 0.05$), ABCA1 (3-fold, $p < 0.001$), and ABCG1 (7-fold, $p < 0.001$) (Fig. 6*b*). The increase in expression of these genes was more pronounced than the increase in CD68 expression, suggesting cellular activation in addition to infiltration. ApoE and ABCA1 as well as ABCG1 are cholesterol-responsive genes that show increased expression upon cholesterol loading of macrophages. Importantly, despite the up-regulation of apoE, ABCA1, and ABCG1, vascular inflammation and atherosclerotic lesion development could not be prevented in SRBI-deficient mice challenged with an atherogenic diet.

DISCUSSION

SRBI is an important mediator of HDL metabolism. The availability of gene-targeted mice that lack functional SRBI expression enabled us to study its role in the maintenance of cellular cholesterol homeostasis in the liver, the site of cholesterol disposal, and in the arterial wall, the site where pathological cholesterol accumulation occurs.

As previously shown by Rigotti *et al.* (10), SRBI deficiency resulted in elevated circulating levels of HDL cholesterol. When injected with ^{3}H-labeled HDL as a tracer of HDL metabolism, SRBI-deficient mice showed a dramatic reduction of the

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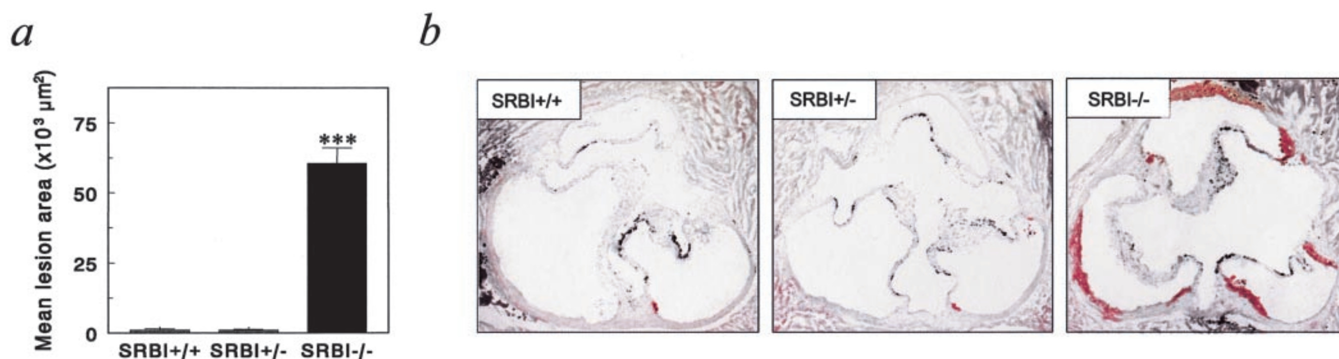

FIG. 5. **SRBI deficiency induces atherosclerotic lesion development.** Formation of atherosclerotic lesions was determined at the aortic root in SRBI+/+, SRBI+/−, and SRBI−/− mice after 20 weeks of feeding a high cholesterol Western-type diet. *a*, quantification of the mean lesion area. Values represent the mean ± S.E. of 5–6 mice. *b*, representative photomicrographs of oil red O-stained cross-sections of the aortic root at the level of the tricuspid valves. Original magnification ×50. ***, statistically significant difference of $p < 0.001$ compared with SRBI+/+ mice.

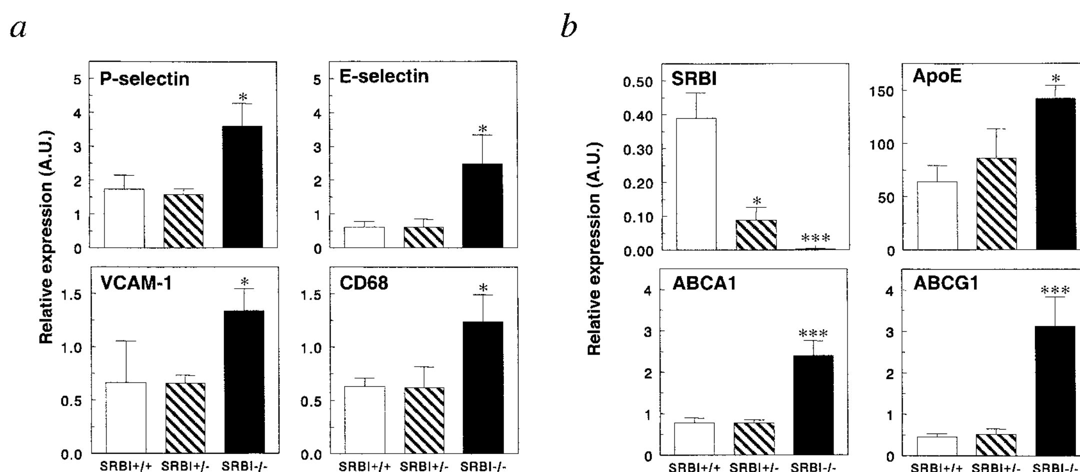

FIG. 6. **SRBI deficiency induces mRNA expression of inflammatory markers (*a*) and of genes involved in lipid homeostasis (*b*) in the aortic arch.** mRNA levels of the indicated genes in aortae of SRBI+/+, SRBI+/−, and SRBI−/− mice were quantified using real-time PCR with SYBR®-green detection after 20 weeks of high cholesterol Western-type diet feeding. Values represent the mean ± S.E. of 5–6 mice. *, statistically significant difference of $p < 0.05$ compared with SRBI+/+ mice; **, $p < 0.01$; ***, $p < 0.001$.

hepatic uptake of HDL cholesteryl esters, indicating that the accumulation of HDL cholesterol within the circulation is a direct consequence of an impaired delivery to the liver. Furthermore, we show that SRBI deficiency combined with a high fat/high cholesterol Western-type diet resulted in an even more dramatic accumulation of HDL cholesterol in the circulation. The amount of cholesterol (ester) in HDL was increased relative to the phospholipid content. This increase in core-surface lipid ratio would indicate the presence of larger particles rather than an increase in particle number, which is supported by the fast protein liquid chromatography data (Fig. 1). Interestingly, not only the amount of cholesteryl esters in HDL increased but also that of free cholesterol. The conversion of cholesterol in HDL into cholesteryl esters is mediated by the plasma enzyme lecithin cholesterol acyltransferase (33). Kosek *et al.* (34) have previously shown that lecithin cholesterol acyltransferase is subject to product inhibition. Because HDL from SRBI-deficient mice is highly enriched in cholesteryl esters, reactivity with lecithin cholesterol acyltransferase might be reduced because the cholesteryl ester products cannot be effectively removed from the active site of the enzyme, hence, resulting in the accumulation of free cholesterol.

Although the delivery of cholesteryl esters to the liver is reduced in the absence of SRBI, there was no effect on hepatic lipid content in SRBI-deficient mice challenged with a Western-type diet. Mardones *et al.* (35) have previously shown that on a regular chow diet, hepatic cholesterol levels in SRBI-deficient mice are even slightly increased. We show that on a Western-type diet SRBI deficiency also did not alter the hepatic expression of HMG-CoA reductase, cholesterol 7α-hydroxylase, LDL receptor, CD36, scavenger receptor class A, LDL receptor-related protein, and apoE. Thus, the decreased influx of cholesterol due to absence of SRBI was not compensated for by changes in expression of key regulators of hepatic cholesterol synthesis, cholesterol conversion into bile acids, or liver uptake of native or modified lipoproteins. This is in accordance with the recent observation that a total absence of HDL in mice due to ABCA1 deficiency is also not associated with altered hepatic cholesterol metabolism (36). In contrast to ABCA1-deficient mice, however, we found that SRBI-deficient mice did exhibit a reduction in biliary cholesterol. SRBI-deficient mice on chow diet also display reduced biliary cholesterol secretion rates (35), whereas mice overexpressing SRBI have increased secretion rates (13, 37).

Although the hepatic SRBI expression level appears to be associated with the level of biliary cholesterol secretion, the exact nature of this relationship is unknown. HDL has been suggested to provide an important source of biliary cholesterol (20, 38), and impaired delivery of HDL cholesterol to the liver might, thus, affect the availability of cholesterol for excretion into the bile. However, recent studies have unequivocally demonstrated that the absence of HDL does not influence biliary cholesterol secretion rates in mice (36, 39). Alternatively, an increasing amount of evidence suggests a direct role for hepatic

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SRBI in the targeting of (HDL-derived) cholesterol into pathways of biliary secretion. In polarized hepatocytes, SRBI is localized to both the sinusoidal membrane and the canalicular membrane (13, 40). Thus, SRBI may mediate the transfer of HDL cholesterol from the basal to the apical membrane under normal conditions. In addition, we found that mRNA expression levels of two half-size ATP binding cassette transporters, ABCG5 and ABCG8, were decreased in livers of SRBI-deficient mice fed a Western-type diet. Their relevance in sterol transport has been established in β-sitosterolemia, a rare autosomal disorder characterized by mutations in ABCG5 and ABCG8 (41, 42), that results in highly elevated plasma levels of plant sterols and cholesterol as a consequence of intestinal hyperabsorption and impaired biliary secretion. In a very recent study, Yu *et al.* (29) demonstrate that simultaneous overexpression of ABCG5 and ABCG8 strongly promotes biliary cholesterol secretion in mice. Furthermore, Berge *et al.* (42) show that feeding wild-type mice a 2% cholesterol diet results in a compensatory up-regulation of hepatic ABCG5 and ABCG8, probably to allow rapid secretion of dietary cholesterol into the bile. In view of this, it is possible that the impaired biliary cholesterol secretion in SRBI-deficient mice is a result of attenuated hepatic expression of ABCG5 and ABCG8.

In addition to ABCG5 and ABCG8, other ABC transporters including ABCG1 and ABCA1 may participate in maintaining cholesterol homeostasis in the liver. We did not observe any effect of SRBI deficiency on hepatic ABCA1 expression. ABCG1 expression, however, was markedly increased in SRBI-deficient mice. The physiological function of ABCG1 in the liver is not well defined. In macrophages, the expression of ABCG1 is induced by cholesterol-loading and LXR agonists, which in turn is associated with enhanced cholesterol efflux (43). Conversely, blockade of the ABCG1 expression using antisense oligonucleotides inhibits cholesterol efflux. Because ABCG1 is primarily localized intracellularly (43), it is thought that it facilitates the translocation of phospholipids and cholesterol from intracellular compartments to the plasma membrane (30). Adenovirus-mediated overexpression of ABCG1 in mice results in a significant reduction of serum HDL-cholesterol levels, indicating an important role for ABCG1 in HDL cholesterol metabolism (44). Increased expression of ABCG1 in livers of SRBI-deficient mice might offer an alternative route for removal of HDL cholesterol.

Although the liver is able to maintain lipid homeostasis in the absence of SRBI by reducing cholesterol excretion into the bile, SRBI deficiency did result in a dramatic de-regulation of lipid homeostasis and induction of vascular inflammation in the arterial wall and in atherosclerotic lesion development. Remarkably, no lesions were observed in heterozygous SRBI-deficient mice. This would indicate that the single allele is sufficient to prevent atherosclerosis and, thus, that agents that establish a mild increase in SRBI expression might have the same effect. In what respect does vascular tissue differ from liver tissue, in that the absence of SRBI shows such deleterious effects in the former and not in the latter? First, the liver has multiple pathways at its disposal (uptake, endogenous synthesis, regulated storage, re-secretion, and excretion) to stabilize cellular lipid homeostasis. In contrast, vascular tissue lipid homeostasis is dependent solely on its efflux capacity. Cellular cholesterol efflux rates correlate with the level of SRBI expression (17, 18, 45), suggesting that vascular SRBI may be directly involved in this process. The absence of SRBI from macrophages and smooth muscle cells of the arterial wall might, therefore, have resulted in impairment of the cholesterol efflux capacity and induction of foam cell formation. Remarkably, increased expression of other genes involved in cholesterol efflux, including apoE, ABCA1, and ABCG1, failed to prevent the formation of lipid-laden macrophages in the arterial wall of SRBI-deficient mice, underscoring the total derailment of lipid homeostasis in this tissue. As for ABCA1, it is known that it binds large cholesteryl ester-rich HDL with low affinity and mainly effluxes lipid to lipid-free or poorly lipidated apolipoproteins (46, 47). Under the dietary conditions studied, the large cholesteryl ester-rich HDL that circulates in the SRBI-deficient mice was probably also unable to induce the efflux of cholesterol via alternative pathways.

Second, SRBI mediates the uptake of α-tocopherol, a cellular antioxidant (48), and binding of HDL to SRBI on endothelial cells stimulates endothelial nitric-oxide synthase, an enzyme that is critical for vascular function (49). The absence of SRBI locally in the arterial wall might, thus, also directly have affected vascular function and the availability of cellular antioxidants.

Finally, the large cholesteryl ester-rich HDL particles that accumulate in SRBI-deficient mice might exhibit reduced anti-oxidant and anti-inflammatory potential. The anti-oxidant and anti-inflammatory properties of HDL are primarily dependent on its content of anti-inflammatory proteins such as platelet-activating factor acylhydrolase, paraoxonase, and apolipoprotein J and the pro-inflammatory proteins serum amyloid A and ceruloplasmin (4). Elevated levels of HDL prevent vascular inflammation in humans (3) and in mouse models of atherosclerosis (50). The observed susceptibility of SRBI-deficient mice to atherosclerosis, despite highly elevated levels of HDL, demonstrates that not only its concentration but also its specific composition are of importance for the anti-atherogenic properties of HDL. The fact that HDL isolated from patients with coronary artery disease but with normal blood lipid levels also displays reduced anti-oxidant potential illustrates the clinical relevance of this finding (4).

In conclusion, SRBI deficiency differentially affects lipid metabolism in cells of the liver and those of the arterial wall; in liver, cholesterol homeostasis is maintained by down-regulation of the capacity for cholesterol secretion into the bile, whereas in the arterial wall, SRBI deficiency is associated with a severe de-regulation of cholesterol homeostasis and induction of inflammation and atherosclerosis.

Acknowledgments—Anti-BI[495] and AC-10 antibodies were kindly provided by D. R. van der Westhuyzen (University of Kentucky Medical Center, Lexington, KY) and M. R. Hayden (University of British Columbia, Vancouver, British Columbia, Canada), respectively. Vincent W. Bloks is acknowledged for assistance with hepatic and biliary lipid analysis.

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Differential Effects of Scavenger Receptor BI Deficiency on Lipid Metabolism in Cells of the Arterial Wall and in the Liver

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J. Biol. Chem. 2003, 278:23699-23705.
doi: 10.1074/jbc.M211233200 originally published online March 14, 2003

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