Liver-specific Overexpression of Scavenger Receptor BI Decreases Levels of Very Low Density Lipoprotein ApoB, Low Density Lipoprotein ApoB, and High Density Lipoprotein in Transgenic Mice*

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Scavenger receptor BI (SR-BI) is known to mediate the selective uptake of high density lipoprotein (HDL) cholesterol ester (CE) in liver and steroidogenic tissues. To evaluate the role of SR-BI in plasma lipoprotein metabolism, we have generated transgenic mice with liver-specific overexpression of murine SR-BI. On a chow diet SR-BI transgenic (SR-BI Tg) mice have decreased HDL-CE, apoA-I, and apoA-II levels; plasma triglycerides, low density lipoprotein (LDL) cholesterol, and very low density lipoprotein (VLDL) and LDL apoB were also decreased, compared with control mice. Turner studies using non-degradable CE and protein labels showed markedly increased total and selective uptake of HDL-CE in the liver and increased HDL protein catabolism in both liver and kidney. To evaluate the changes in apoB further, mice were challenged with high fat, high cholesterol diets. In SR-BI Tg mice plasma apoB levels were only 3–15% of control levels, and the dietary increase in VLDL and LDL apoB was virtually abolished. These studies show that steady state overexpression of hepatic SR-BI reduces HDL levels and increases reverse cholesterol transport. They also indicate that SR-BI can play a role in the metabolism of apoB-containing lipoproteins. The dual effects of increased reverse cholesterol transport and lowering of apoB-containing lipoproteins that result from hepatic SR-BI overexpression could have anti-atherogenic consequences.

The risk of coronary heart disease is inversely correlated with the levels of plasma high density lipoproteins (HDL) (1, 2). HDL appears to transport cholesterol from peripheral tissues to the liver for catabolism and secretion (reverse cholesterol transport) (3, 4). A putative cell-surface receptor for this process has been identified (5). This receptor, scavenger receptor BI (SR-BI), mediates high affinity binding of HDL and the selective uptake of HDL cholesterol ester (CE) (5), a process for delivery of cholesterol ester into cells without degradation of HDL proteins (6). Furthermore, SR-BI mRNA and protein levels are highest in adrenal gland, ovary, testis, and liver, tissues that display greatest selective cholesterol ester uptake from HDL (7–9). SR-BI expression in steroidogenic cells is regulated by hormones and mutations that alter cholesterol supply or metabolism in those tissues in vivo (8–11). More recently, strong support for the role of SR-BI in HDL metabolism has been provided by studies of mice with a targeted mutation resulting in decreased SR-BI gene expression (12, 13). These mice demonstrate increased plasma HDL cholesterol, decreased adrenal cholesterol content (12, 13), and decreased hepatic fractional clearance rate (FCR) for HDL-CE (13), suggesting that SR-BI is the major molecule mediating HDL-CE-selective uptake in the liver. By contrast, adenovirus-mediated, hepatic overexpression of SR-BI in mice results in depletion of plasma HDL and an increase in biliary cholesterol concentration (14). Although these studies nicely demonstrate the effect of acute overexpression of SR-BI on HDL levels (14), they do not necessarily demonstrate plasma lipoprotein changes that would accompany steady state overexpression of SR-BI.

In this paper we report an in depth study of transgenic mice with hepatic overexpression of murine SR-BI. These studies were designed to understand better the role of SR-BI in HDL metabolism and reverse cholesterol transport. During the initial characterization of these animals on a chow diet, we observed decreased LDL cholesterol and apoB levels. Whereas the mouse model studies to date have focused on HDL changes, SR-BI was originally identified as a receptor recognizing both native and modified LDL (15). Thus, further studies were performed on high fat, high cholesterol diets in order to delineate the effects of SR-BI on plasma apoB levels.

MATERIALS AND METHODS

Generation of Transgenic Mice—A 1.5-kilobase cDNA fragment of murine SR-BI (16) was cloned into the HpaI site of the pLIV-7 plasmid (17), kindly provided by Dr. John M. Taylor (Gladstone Institute of Cardiovascular Disease, University of California, San Francisco). A linearized fragment of the construct containing the promoter, first exon, first intron, and part of the second exon of the human apoE gene, the murine SR-BI cDNA, and the polyadenylation sequence, and hepatic control region of the apoE/C-I gene locus was used to generate transgenic mice by standard procedures. Founder animals were backcrossed to C57Bl/6J mice and two transgenic mouse lines, SR-BI Tg(1) and SR-BI Tg(2), were established. Studies in this paper were performed using 8–10-week-old SR-BI Tg(1) or SR-BI Tg(2) N2 or N3 mice positive for both SR-BI transgene genotype and phenotype (decreased plasma total cholesterol) versus control littermates negative for the SR-BI transgene.

For studies of responses to high fat diets, mice were fed either a Western type diet containing 20% hydrogenated coconut oil and 0.15% cholesterol (Research Diets, Inc.) or a very high cholesterol diet containing 1.25% cholesterol, 7.5% cocoa butter, 7.5% casein, and 0.5% sodium cholate for 2 weeks.

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The abbreviations used are: FC, free cholesterol; CE, cholesteryl ester; Cet, cholesteryl oleyl ether; HDL-CE, HDL cholesteryl ester; FCR, fractional catabolic rate; LCAT, lecithin:cholesterol acyltransferase; SR-BI, scavenger receptor class B type I; Tg, transgenic; apoA-I, apolipoprotein A-I; apoA-II, apolipoprotein A-II; apoB, apolipoprotein B; apoE, apolipoprotein E; HDL, high density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; VLDL, very low density lipoprotein; PL, phospholipids; 12H-NMTC, 12H-N-methyltyramine cellobiose; PAGE, polyacrylamide gel electrophoresis; FPLC, fast protein liquid chromatography.
Plasma Lipoprotein Analysis—Total plasma cholesterol, free cholesterol, phospholipids, and triglycerides were determined using commercial enzymatic assays (Wako, Japan) (8). Determination of plasma apoB levels was carried out using an enzyme-linked immunosorbent immunoassay with an affinity purified polyclonal antibody against murine apoB-100. For SDS-PAGE, VLDL (d < 1.006 g/ml), IDL + LDL (d = 1.006–1.055 g/ml), and HDL (d = 1.055–1.21 g/ml) were separated by sequential density ultracentrifugation of pooled mouse plasma. In some experiments, VLDL + LDL (d < 1.006–1.019) and LDL (d = 1.019–1.055) were separated as indicated. Denaturing polyacrylamide gel analysis of isolated lipoproteins was performed using 4–20% SDS-PAGE gradient gels from Bio-Rad. Gels were stained with Coomassie Brilliant Blue R, and the identity of individual apolipoproteins was confirmed by Western analysis.

HDL Catabolism—HDL was prepared in the density range 1.063–1.21 g/ml from plasma of C57BL/6 wild type mice, dialed against phosphate-buffered saline containing 0.3 mM EDTA and 0.02% NaN₃, and radiolabeled in the protein moiety with [3H]-N-methyltyramine cellulose (3H-NMTC) (18), and thereby with [3H]cholesterol oleyl ether ([3H]CEt, Amersham Pharmacia Biotech) (19). [3H]CEt was introduced in a liposomal preparation and exchanged (6 h, 37 °C) into 125I-NMTC-labeled HDL using purified recombinant human plasma cholesteryl ester transfer protein. The donor liposomes were separated from labeled HDL by ultracentrifugation at d = 1.063 g/ml, followed by another spin at d = 1.21 g/ml to remove cholesteryl ester transfer protein from the labeled HDL preparation. Then the doubly labeled HDL was dialyzed against phosphate-buffered saline containing 0.3 mM EDTA.

Experiments to determine plasma decay of both HDL tracers and their tissue sites of uptake were carried out (19, 20). Food was removed from five female control and SR-BI Tg mice 4 h before tracer injection, and animals were fasted throughout the 24-h study period but had free access to water. Doubly radiolabeled HDL was injected at 10:00 a.m. in an iliac vein, and blood samples were drawn from the tail vein of each animal at 0.08, 0.5, 2.0, 5.0, 9.0, and 24.0 h post-injection. Plasma samples were directly radioassayed for 125I and analyzed for [3H] after lipid extraction (19). 24 h after tracer injection the animals were anesthetized and perfused with saline (50 ml per animal), and organs were collected, weighed, homogenized, and radioassayed. Tissue content of 125I radioactivity was directly assayed and that of [3H] was analyzed after lipid extraction. Based on plasma decay of both HDL tracers, plasma FCRs were calculated using a two-compartment model (21). Organ FCRs, representing the fraction of the plasma pool of the traced HDL component cleared per h by an organ, were calculated as the plasma FCR × fraction of total tracer (%) recovered in a specific organ (19, 20).

Miscellaneous—Western blot analysis for SR-BI and Southern and Northern analyses were performed as described (8, 22). Dot blot was carried out with a 900-base pair cDNA fragment of mouse apoB to determine hepatic apoB mRNA levels. Hepatic and adrenal cholesterol levels was carried out using an enzyme-linked immunosorbent immunoassay (Wako, Japan) (8). Determination of plasma apoB levels was carried out using an enzyme-linked immunosorbent immunoassay with an affinity purified polyclonal antibody against murine apoB-100.

RESULTS

SR-BI Expression in SR-BI Tg Mice—Two separate lines of SR-BI Tg mice, SR-BI Tg(1) and SR-BI Tg(2), were established. The SR-BI Tg(1) mice demonstrated a marked liver-specific expression of SR-BI mRNA (Fig. 1A); there was no appreciable expression in the kidney (Fig. 1A, lane 1). Western analysis showed a 12-fold increase in hepatic membrane SR-BI levels in transgenic mice (Fig. 1, B and C). Similar levels of expression were observed for both lines of SR-BI Tg mice.

Plasma Lipids, Lipoproteins, and Apolipoproteins of Mice on Chow Diet—Analysis of plasma lipids on a chow diet revealed that female SR-BI Tg mice (both lines) had a profound 92–94% decrease of plasma total cholesterol (TC) (Table I), with decreases in both free cholesterol (FC) (∼80%) and cholesteryl ester (CE) (96%). There was also a significant but less pronounced decrease in plasma phospholipids (PL) (∼75%) and triglycerides (TG) (∼45–58%) (Table I). Similar results were obtained for male mice (not shown).

When plasma was analyzed by fast protein liquid chromatography (FPLC), most of the CE and FC were in the HDL fraction in the control mice on the chow diet (Fig. 2A and B). By contrast HDL-CE and FC were almost undetectable in SR-BI Tg mice. HDL phospholipids were also markedly decreased (Fig. 2C). There was also a decrease in lipids in VLDL and LDL region, although these were also low in control mice.

Assessment of apolipoprotein composition of centrifugally isolated lipoproteins by reducing SDS-PAGE gels revealed a marked decrease of HDL apoA-I, apoA-II, and apoE levels in SR-BI Tg(1) mice (Fig. 3A). The VLDL and LDL apoB and apoE levels also were decreased. The results were confirmed by Western analysis using antisera specific for murine apoA-I, apoA-II, and apoE. Similar results were obtained in four separate analyses of pooled plasma from a total of 9 SR-BI Tg(1) mice and 10 control mice and were also confirmed in the SR-BI Tg(2) line (data not shown).

HDL Metabolism—The changes in HDL in SR-BI Tg mice resemble these occurring 3 days after adenovirus-mediated expression of SR-BI (14) where HDL turnover was evaluated using 125I and DiI labels (14). Next we carried out HDL turnover studies using non-degradable radiolabels (18, 19). In the control mice, the higher rate of removal from plasma of the lipid ([3H]CEt), relative to protein (125I-NMTC), represents whole body selective uptake of HDL-CE (Fig. 4A). There was a...
significantly accelerated rate of clearance for both tracers in SR-BI Tg mice. The plasma FCRs calculated from these decay curves showed a 370% increase in protein catabolism and 330% increase in lipid tracer catabolism (Fig. 4B). The selective removal of HDL-CE from plasma, calculated as the difference between CE and protein FCRs, was increased by 260% in SR-BI Tg mice.

Tissue sites of tracer uptake from doubly radiolabeled HDL were determined, and results are expressed as the organ FCRs (Table II). The liver was the predominant organ for both HDL lipid and protein catabolism (19, 20). The higher liver FCR for lipid, relative to protein, indicates selective uptake of HDL [3H]CEt in the liver. In contrast, a negative value of 3H minus 125I was derived for kidney FCR (Table II), indicating this organ is a major site for selective HDL protein catabolism (20). The higher liver FCR for HDL protein and lipid were increased 10.5- and 6.3-fold, respectively, and the adrenal-selective uptake of HDL-CE FCRs for HDL protein and lipid were increased 260% in SR-BI Tg mice, whereas this organ contributed little to the clearance of HDL lipid in wild type or SR-BI Tg mice. Adrenal FCRs for HDL protein and lipid were increased 10.5- and 6.3-fold, respectively, and the adrenal-selective uptake of HDL-CE was increased 5.6-fold. This finding may have reflected an up-regulation of endogenous adrenal SR-BI expression secondary to reduced HDL levels and depletion of adrenal cholesterol stores (see below). Other organs (heart, spleen, and stomach), with minor contribution to HDL lipid and protein uptake, did not display any major changes in SR-BI Tg mice.

The HDL turnover studies demonstrated that the reduced plasma HDL lipids and apolipoproteins were at least in part due to accelerated HDL catabolism in SR-BI Tg mice. We also measured hepatic apoAI mRNA levels and found no difference between the control and SR-BI Tg animals (not shown). Hepatic free cholesterol was increased by 43% ($p < 0.001$) in SR-BI Tg mice (Table III). By contrast adrenal cholesteryl ester virtually disappeared in SR-BI Tg mice, and free cholesterol was also decreased (Table III), probably reflecting the decreased plasma HDL-CE levels that result from hepatic overexpression of SR-BI (8). There was a 3.5-fold increase in adrenal SR-BI protein (not shown), probably secondary to decreased adrenal cholesterol content (8). The hepatic LDL receptor mRNA levels in SR-BI Tg mice were not altered, as determined by Northern analysis using poly(A)⁺ RNA from liver (data not shown).

**Plasma Lipoprotein Responses to High Fat, High Cholesterol Diets—**An unexpected finding in SR-BI Tg mice on the chow diet was the marked decreases in LDL apoB levels (Fig. 3A). To evaluate apoB changes further, mice were challenged with a Western type high fat diet (20% hydrogenated coconut oil and 0.15% cholesterol) or a very high cholesterol diet (1.25% cholesterol, 7.5% cocoa butter, 7.5% casein, 0.5% sodium cholate) for 2 weeks. In response to these diets VLDL and LDL apoB levels were increased in control mice, but changes in apoB were much smaller in SR-BI Tg mice (Fig. 3, B and C). The decrease in apoB was more pronounced for SR-BI Tg(2) mice than SR-BI Tg(1) mice. Quantitative determination of plasma apoB levels by immunoassay revealed that plasma apoB levels were markedly reduced by 89–94% on the chow diet and 85–97% on the high fat, high cholesterol diets in SR-BI Tg mice compared with control mice (Table IV). The decreases in apoB appeared to include both apoB100 and apoB48 in VLDL and LDL (Fig. 3). Hepatic apoB mRNA levels were not significantly altered in SR-BI Tg mice relative to the control mice on the chow diet (not shown). Similar to the chow diet, apoA-I levels were much lower in SR-BI Tg mice than the control mice on high fat, high cholesterol diets (Fig. 3, B and C). However, apoE levels in HDL were not decreased on the high fat, high cholesterol diets.

On the Western type diet plasma TC was decreased by 27–36% and CE by 77–80% in SR-BI Tg mice compared with control mice (Table I). Surprisingly, plasma FC was approximately 2-fold higher in SR-BI Tg mice than in the control mice, and phospholipids were only slightly decreased (Table I). Plasma triglycerides were moderately decreased (36–37%) in SR-BI Tg mice. On the very high cholesterol diet plasma TC, CE, FC, and PL were all decreased, but the changes were less pronounced than on the chow diet in SR-BI Tg mice (Table I).

FPLC analysis of plasma lipoprotein lipids of mice on the Western type diet showed that HDL-CE, FC, and PL were substantially depressed in SR-BI Tg mice (Fig. 2, A–C). Free cholesterol eluting in the VLDL region, however, was markedly increased, and free cholesterol eluting in the IDL/LDL region was moderately increased and shifted toward larger particle size. CE eluting in the VLDL region was only slightly increased in SR-BI Tg mice. VLDL-eluting free cholesterol accounted for >90% of total VLDL cholesterol, whereas IDL/LDL fractions contained ~70% total cholesterol as free cholesterol. Consistent with these alterations, phospholipids eluting in the VLDL and IDL/LDL region were also markedly increased in SR-BI Tg mice (Fig. 2C). The high content of free cholesterol and phospholipids, low cholesteryl ester, and low apoB (Fig. 2B) suggested that the VLDL/LDL fractions might contain lipoproteins consisting of apoB-free lamellar-free cholesterol/phospholipid particles, i.e. lipoprotein X-like particles of vesicular structure (24). Subsequently, SR-BI Tg mice were found to have functional LCAT deficiency (see below) in which lipoprotein X accumulates especially on a high fat diet (25, 26).

The percentage composition of HDL fractions isolated from plasma of mice on Western type diet is shown in Table V. In

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**Table I**

**Plasma lipid concentrations of control and SR-BI Tg mice**

| Diet                    | Animal | TC   | FC   | CE   | PL   | TG   |
|-------------------------|--------|------|------|------|------|------|
| **Chow**                |        |      |      |      |      |      |
| Control                 | 67 ± 11| 15 ± 5| 52 ± 10| 185 ± 18| 77 ± 37|
| SR-BI Tg(1)             | 5 ± 2  | 3 ± 2| 2 ± 1| 47 ± 6| 32 ± 6|
| SR-BI Tg(2)             | 4 ± 2  | 2 ± 1| 2 ± 1| 45 ± 8| 42 ± 16|
| **Western**             |        |      |      |      |      |      |
| Control                 | 107 ± 13| 24 ± 5| 82 ± 11| 255 ± 30| 148 ± 30|
| SR-BI Tg(1)             | 78 ± 13| 56 ± 5| 16 ± 7| 213 ± 24| 93 ± 37|
| SR-BI Tg(2)             | 68 ± 20| 51 ± 13| 19 ± 8| 211 ± 33| 95 ± 27|
| **Very high cholesterol** |        |      |      |      |      |      |
| Control                 | 114 ± 11| 41 ± 6| 73 ± 8| 207 ± 40| 85 ± 30|
| SR-BI Tg(1)             | 54 ± 25| 18 ± 8| 24 ± 3| 132 ± 44| 61 ± 24|
| SR-BI Tg(2)             | 16 ± 8| 8 ± 5| 8 ± 4| 82 ± 32| 67 ± 37|

[\(^a\) Statistical significance, \(p < 0.01\) between the control and SR-BI Tg animals on the same diet as determined by two-tailed Student’s \(t\) test for unpaired data.

[\(^b\) \(p < 0.05\).]
SR-BI Tg mice, HDL was essentially devoid of cholesteryl ester, whereas triglyceride was increased and became the major neutral lipid. Phospholipids were moderately increased and so was free cholesterol.

**LCAT Activity—** The pronounced accumulation of FC and PL in VLDL and LDL on the Western type diet suggested that SR-BI Tg mice might have a defect in plasma CE formation secondary to the markedly decreased HDL levels, as has been shown previously in apoA-I knock-out mice (27). Therefore, we determined the plasma cholesteryl ester formation (Fig. 5). SR-BI Tg mice displayed a markedly depressed fractional plasma CE formation rate as compared with the control animals on both chow and Western type diets. In SR-BI Tg mice, the marked depression of plasma apoA-I might affect LCAT activity, since apoA-I activates LCAT (28). To evaluate further the basis of decreased CE formation, LCAT levels were assessed, using addition of exogenous discoidal PL/apoA-I substrates to small amount of plasma. This revealed similar levels of LCAT in the control and SR-BI Tg plasma (for control 0.42, 0.74, and 1.20% of FC to CE conversion; for SR-BI Tg 0.39, 0.65, and 1.31% of FC to CE conversion by incubating with 0.1, 0.5, and 2 μl of plasma, respectively), suggesting that the defect in plasma cholesterol esterification is related to the reduced levels of apoA-I. Thus, SR-BI Tg mice appear to have functional LCAT deficiency secondary to depletion of plasma apoA-I.

**DISCUSSION**

Kozarsky et al. (14) showed that the acute adenovirus-mediated overexpression of SR-BI in the liver resulted in a marked decrease in HDL cholesterol and apoA-I levels, enhanced clearance of HDL protein from plasma, increased hepatic uptake of DiI label from HDL, and increased hepatic cholesterol levels. Our studies show a major decrease in HDL cholesterol, apoA-I, and apoA-II as a result of sustained hepatic overexpression of SR-BI in a transgenic mouse model. They further demonstrate increased selective uptake of HDL-CE in the liver, and increased uptake of HDL protein in both liver and kidney. Moreover, we observed a profound decrease in VLDL and LDL CE formation. This suggests a defect in plasma CE formation secondary to depletion of plasma apoA-I.
and apoB levels in SR-BI Tg mice compared with controls, and in one line of mice a failure to increase plasma apoB levels when challenged with high fat, high cholesterol diets. This provides the first in vivo evidence that SR-BI can play a role in the determination of plasma lipoprotein apoB levels, and this suggests that there will be important consequences of hepatic SR-BI overexpression on VLDL and LDL metabolism that may influence the outcome of atherosclerosis studies.

SR-BI was originally described as a receptor that bound both native LDL and acetyl-LDL with high affinity (15). CLA-1, the human homolog of SR-BI, has been shown to bind VLDL in addition to HDL and LDL (29). The ability of SR-BI to mediate the cellular uptake and degradation of LDL and VLDL has not been reported, and its role in apoB metabolism in vivo is unknown. One explanation for the decrease in VLDL and LDL apoB in SR-BI Tg mice is that hepatic SR-BI directly mediates the removal of apoB-containing lipoproteins from plasma. However, there are several alternative explanations. For example, SR-BI overexpression could mediate increased binding of VLDL and LDL to hepatocytes, and this might lead to increased particle catabolism via the LDL receptor or proteoglycan pathways (35). Another explanation is that SR-BI overexpression leads to decreased secretion of apoB from liver cells. However, the transgenic mice have increased uptake of HDL cholesterol and esterified fatty acids into the liver which would be more likely to increase apoB secretion (30). Another factor involved in reduced apoB levels could be the state of partial LCAT deficiency, since LCAT knock-out mice have reduced apoB levels (31). However, the moderate 30% reduction of apoB levels in knock-out mice with complete LCAT deficiency on a very high cholesterol diet (31) is unlikely to explain the profound 85–97% decrease of apoB levels in SR-BI Tg mice (Table IV). The changes in apoB were observed in the context of about 12-fold overexpression of SR-BI in the liver. In contrast, mice with decreased SR-BI expression in the liver, as a result of disruption of SR-BI gene expression, do not display increased apoB in plasma lipoproteins compared with wild type mice on...
TABLE II
Organ fractional catabolic rates for $^{125}\text{I}-\text{NMTC}/^{3}\text{H}\text{CEt}$ double-labeled HDL in mice

| Organ          | Mice          | $^{125}\text{I}-\text{NMTC}$ | $^{3}\text{H}\text{CEt}$ | $^{3}\text{H}^{125}\text{I}$ |
|---------------|---------------|--------------------------------|--------------------------|-------------------------------|
| Liver         | Control       | 16.7 ± 1.5                     | 54 ± 5.6                 | 38 ± 5.7                      |
|               | SR-BI Tg      | 124 ± 18.1*                   | 346 ± 74*                | 221 ± 57*                     |
| Kidney        | Control       | 3.5 ± 0.4                      | 0.5 ± 0.05               | -3.1 ± 0.4                    |
|               | SR-BI Tg      | 23.3 ± 1.2*                   | 1.8 ± 0.1*               | -21.3 ± 1.1*                  |
| Adrenal       | Control       | 0.1 ± 0.01                     | 0.4 ± 0.2                | 0.3 ± 0.2                     |
|               | SR-BI Tg      | 0.6 ± 0.3*                    | 2.5 ± 0.9*               | 1.9 ± 0.7*                    |
| Heart         | Control       | 0.2 ± 0.02                     | 0.3 ± 0.03               | 0.02 ± 0.03                   |
|               | SR-BI Tg      | 0.6 ± 0.12*                   | 0.3 ± 0.1                | -0.3 ± 0.04                   |
| Spleen        | Control       | 0.4 ± 0.04                     | 0.7 ± 0.1                | 0.3 ± 0.06                    |
|               | SR-BI Tg      | 1.1 ± 0.06*                   | 1.3 ± 0.1*               | 0.2 ± 0.2                     |
| Stomach       | Control       | 0.2 ± 0.1                      | 0.2 ± 0.1                | 0.01 ± 0.02                   |
|               | SR-BI Tg      | 0.4 ± 0.2                      | 0.4 ± 0.1*               | 0.02 ± 0.2                    |

*Statistical significance $p < 0.01$ between the control and SR-BI Tg animals as determined by two-tailed Student's $t$ test for unpaired data.

TABLE III
Hepatic and adrenal cholesterol content

| Organ          | Mice          | Hepatic CE | Hepatic FC | Adrenal CE | Adrenal FC |
|---------------|---------------|------------|------------|------------|------------|
| Control       | 0.5 ± 0.1     | 2.0 ± 0.4  | 77.3 ± 21.2| 4.4 ± 0.9  |
| SR-BI Tg      | 0.9 ± 0.5     | 2.9 ± 0.2* | -0.2 ± 0.3*| 1.6 ± 1.0* |

*Statistical significance $p < 0.001$ between the control and SR-BI Tg animals as determined by two-tailed Student's $t$ test for unpaired data, $n = 5$.

$p < 0.01$

TABLE IV
Plasma apoB levels

| Diet         | Animal | ApoB (mg/ml) | $p$ value* |
|--------------|--------|--------------|------------|
| Chow         | Control| 89 ± 65      | 0.05       |
|              | SR-BI Tg(1) | 10 ± 3       | 0.04       |
|              | SR-BI Tg(2)| 5 ± 4        | 0.04       |
| Western      | Control| 192 ± 88     | 0.01       |
|              | SR-BI Tg(1) | 29 ± 18      | 0.01       |
|              | SR-BI Tg(2)| 5 ± 4        | 0.01       |
| Very high cholesterol | Control| 227 ± 119    | 0.02       |
|              | SR-BI Tg(1) | 35 ± 10      | 0.02       |
|              | SR-BI Tg(2) | 6 ± 6        | 0.01       |

* $p$ value* indicates the significance level of the $t$ test for unpaired data, comparing the control and SR-BI Tg mice.

A two-tailed Student's $t$ test for unpaired data was used to compare the SR-BI Tg mice with the control mice on the same diet.

Findings were provided by our discovery that mice with hepatic SR-BI overexpression have impaired plasma cholesteryl ester formation (total and fractional). On the Western type diet the particles accumulating in VLDL were found to contain free cholesteryl and phospholipids and to be almost devoid of cholesteryl esters and apoB. Thus they are likely to represent lipoprotein X-like particles that are non-apoB-containing vesicular lipoproteins that accumulate in animals with LCAT deficiency, especially in response to high fat diets, where they probably represent surface remnants of triglyceride-rich lipoproteins (25, 26). It is not clear why these particles were more prominent on the Western type diet than the very high cholesterol diet, although it could be related to a more pronounced rise in plasma Tg levels on the Western type diet.
An important feature of our study was the quantitative measurements of organ uptake of HDL cholesteryl esters and protein, using non-degradable radiolabeled lipid and protein. One potential caveat to the turnover data is that the results could reflect the markedly decreased pool size of HDL in the SR-BI Tg mice. However, comparably reduced pool size of LDL in apoA-I knock-out mice does not affect the fractional catabolism of HDL-CE (23). We found that 12-fold overexpression of SR-BI in the liver led to a 6-fold increase in selective uptake of HDL-CE in the same organ, showing a remarkably increased selective uptake capacity in the liver (13). The SR-BI transgenic mice also have increased biliary free cholesterol content and decreased dietary cholesterol absorption.2 Interestingly, the SR-BI transgenic mice also have increased biliary free cholesterol content and decreased dietary cholesterol absorption.2 Interestingly, we also observed an increase in HDL protein uptake in both liver and kidney (Table II). In the kidney where there was negligible expression of SR-BI (Fig. 1), these changes must reflect modifications of HDL size or composition that result from overexpression of SR-BI in the liver.3 These results contrast with decreased expression of SR-BI where there were no changes in HDL protein uptake in the liver (13). Although SR-BI overexpression could be directly responsible for increased uptake of HDL proteins in the liver, we hypothesize that the marked modification of HDL secondary to increased SR-BI activity leads to entry of HDL into distinct HDL protein catabolic pathways that are active in the liver and kidney.

Our studies show that in addition to stimulating reverse cholesterol transport, SR-BI overexpression leads to marked decreases in VLDL and LDL CE and apoB levels. While an elucidation of the physiological role of SR-BI in the removal of apoB from plasma must await further studies with SR-BI knock-out mice, pharmacological overexpression of SR-BI is likely to have similar consequences to transgenic overexpression of SR-BI. The results of stimulating reverse cholesterol transport by SR-BI overexpression are uncertain, because they will also lead to reduced HDL levels. However, it seems likely that reductions in plasma apoB levels (and the associated decreases in VLDL and LDL cholesterol) will have anti-atherogenic consequences (33).

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* S. Ephraim, N. Wang, A. R. Tall, and J. L. Breslow, unpublished observations.
3 In SR-BI Tg mice there was too little HDL to allow a reliable analysis of HDL size changes by native PAGE.
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