Adrenal-Specific Scavenger Receptor BI Deficiency Induces Glucocorticoid Insufficiency and Lowers Plasma Very-Low-Density and Low-Density Lipoprotein Levels in Mice

Menno Hoekstra, Ronald J. van der Sluis, Miranda Van Eck, Theo J.C. Van Berkel

Objective—We determined the physiological consequences of adrenocortical-specific deletion of scavenger receptor BI (SR-BI) function in C57BL/6 wild-type mice.

Methods and Results—One adrenal from 10-day-old SR-BI knockout (KO) mice or wild-type controls was transplanted under the renal capsule of adrenalectomized C57BL/6 recipient mice. The fasting plasma corticosterone level increased over time in transplanted mice. Corticosterone values in SR-BI KO transplanted mice remained ≈50% lower (P<0.001) as compared with wild-type transplanted mice, which coincided with adrenocortical lipid depletion. A 6.5-fold higher (P<0.01) plasma adrenocorticotropic hormone level was present in SR-BI KO transplanted mice reminiscent of primary glucocorticoid insufficiency. On feeding with cholic acid-containing high cholesterol/high fat diet, SR-BI KO transplanted mice exhibited a 26% (P<0.05) reduction in their liver triglyceride level. Hepatic myosin regulatory light chain interacting protein/inducible degrader of the low-density lipoprotein receptor mRNA expression was 48% (P<0.01) decreased in adrenal-specific SR-BI KO mice, which was paralleled by a marked decrease (−46%; P<0.01) in proatherogenic very-low-density and low-density lipoprotein levels.

Conclusion—Adrenal-specific disruption of SR-BI function induces glucocorticoid insufficiency and lowers plasma very-low-density and low-density lipoprotein levels in atherogenic diet-fed C57BL/6 mice. These findings further highlight the interaction between adrenal high-density lipoprotein-cholesterol uptake by SR-BI, adrenal steroidogenesis, and the regulation of hepatic lipid metabolism. (Arterioscler Thromb Vasc Biol. 2013;33:e39-46.)

Key Words: adrenal transplantation ■ glucocorticoid ■ liver ■ scavenger receptor BI ■ SR-BI knockout mice

Reverse cholesterol transport, the transport of cholesterol from peripheral cells back to the liver for subsequent excretion into the bile is considered to be an important process to inhibit the development of atherosclerotic lesions (reviewed by Van Eck et al).1 Scavenger receptor BI (SR-BI) is a key player in the reverse cholesterol transport process as it is the sole molecule involved in the selective uptake of cholesterol esters by the liver from antiatherogenic high-density lipoprotein (HDL) particles in the circulation.2–4 SR-BI knockout (KO) mice that lack functional SR-BI protein expression exhibit a complex phenotype, including female infertility5,6; an enhanced tissue oxidative stress7; a markedly increased susceptibility for the development of atherosclerotic lesions and occlusive coronary artery disease5,8–10; and disturbances in red blood cell and platelet morphology, number, and function.11–15

Interestingly, the highest protein expression of SR-BI in mice has not been found in the liver but rather in the adrenals.16 SR-BI protein expression is specifically localized to adrenocortical cells within the glucocorticoid hormone-producing zona fasciculata of the adrenals.17 Proof-of-principle studies by Temel et al14 for the first time showed that steroid production in adrenocortical cell cultures is highly dependent on the delivery of HDL-cholesteryl esters by SR-BI. In accordance with a possible prominent role for SR-BI in the generation of substrate for adrenal steroidogenesis also in vivo, we and others recently showed that SR-BI KO mice suffer from primary glucocorticoid insufficiency (ie, an impaired ability to increase adrenal corticosterone secretion in response to metabolic [fasting] or inflammatory [lipopolysaccharide exposure] steroidogenic stress triggers).18–21 SR-BI KO mice exhibit hyperlipidemia (ie, the presence of dysfunctional large HDL particles),2 which may contribute to the complex phenotype observed as a result of total body SR-BI deficiency. To dissociate between nonspecific effects of general SR-BI deficiency and the specific role of SR-BI located on adrenocortical cells, in the current study, we therefore determined the physiological changes of adrenocortical-specific deletion of SR-BI function in normolipidemic mice by means of adrenal transplantation.

Methods

Adrenal Transplantation

Total body SR-BI KO mice2 crossed back to the C57BL/6 background (>8 generations) and C57BL/6 wild-type (WT) mice were
bred in house. Adrenalectomy and subsequent adrenal transplantations were performed essentially as previously described by Karpac et al.2 At postnatal day 10, adrenal glands were isolated from donor SR-BI KO or C57BL/6 pups. Approximately 12-week-old recipient C57BL/6 mice were bilaterally adrenalectomized under isoflurane inhalation anesthesia through a dorsal midline skin incision and lateral retroperitoneal incisions. Subsequently, 1 donor adrenal per recipient was placed under the kidney capsule through a slit in the renal capsule made by tweezers. Skin wounds were closed using microl suture clips. During the complete study, a part of the bottom of each cage surface was heated by a heating mattress and all mice were given 0.9% NaCl and normal water ad libitum. Adrenal-transplanted C57BL/6 were fed an atherogenic semisynthetic cholic acid-containing high cholesterol/high fat diet (15% (wt/wt) cacao butter, 1% (wt/wt) cholesterol, and 0.5% (wt/wt) cholic acid; Hope Farms, Woerden, NL) for 10 weeks starting from 6 weeks after the adrenal transplantation to stimulate the general steroidogenic activity of the adrenals.23 On the basis of visual examination of the residual amount present at the time of cage changes, no major difference in the average food intake was noted between the 2 experimental groups while feeding the atherogenic diet. At the end of the study, 16 weeks postadrenal transplantation, mice were fasted overnight, anesthetized, and euthanized. Subsequently, the arterial tree was perfused in situ with PBS (with the pressure of 100 mm Hg) for 10 minutes via a cannula in the left-ventricular apex, and organs were harvested. No signs of endogenous adrenal regeneration were macroscopically visible in any of the transplanted mice on euthanization. Animal experiments were performed in a temperature and light cycle (12-hour light/12-hour dark) controlled room 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.

**Hematologic Analysis**

Hematologic parameters in whole blood samples with EDTA anticoagulant were routinely measured using an automated Sysmex XT-2000iV Veterinary Hematology analyzer (Sysmex Corporation).

**Plasma Measurements**

Corticosterone and adrenocorticotropic hormone levels were determined using the Corticosterone and Adrenocorticotropic Hormone Double Antibody 125I Radioimmunoassay (RIA) Kits from MP Biomedicals (Irvine, CA). Glucose was measured using ACCU-CHEK Aviva standardized glucose meter strips. Concentrations of total cholesterol and triglycerides were determined using enzymatic colorimetric assays (Roche Diagnostics). The distribution over the different lipoproteins was analyzed by fractionation of 30 μL of serum of each mouse using a Supersose 6 column (3.2x300 mm, Smart-system, Pharmacia).

**Adrenal Transplant Histology**

Formalin-fixed cryosections (10 μm/L) of adrenal transplants were prepared on a Leica CM3050-S cryostat. Cryosections were routinely stained with hematoxylin & eosin and Oil red O for neutral lipids. Additional samples were fixed in 4% formalin and prepared on a Leica CM3050-S cryostat. Cryosections were routinely stained with hematoxylin & eosin, and Oil red O for neutral lipids. Additionally,ISH was performed essentially as described.24

**Analysis of Gene Expression by Real-Time Quantitative PCR**

Quantitative gene expression analysis on snap-frozen liver was performed as described.24 Total RNA was isolated according to Chomczynski and Sacchi25 and reverse transcribed using RevertAid reverse transcriptase. Gene expression analysis was performed using real-time SYBR Green technology (Eurogentec). Primers were validated for identical efficiencies. Primer sequences can be found in Table I of the online-only Data Supplement. β-actin (ACTB), GAPDH, and acidic ribosomal phosphoprotein P0 (36B4) were used as the standard housekeeping genes.

**Immunoblotting**

Thirty micrograms of total liver protein was separated on 7.5% SDS-PAGE gels and electrophoretically transferred to Protran nitrocellulose membranes (Schleicher and Schuell). Immunlabeling was performed using goat polyclonal low-density lipoprotein (LDL) receptor (C-20; Santa Cruz Biotechnology) as primary antibody and donkey anti-goat immunoglobulin G (Jackson ImmunoResearch) as secondary antibody. Immunolabeling of mature and immature LDL receptor protein was detected by enhanced chemiluminescence (Amersham Biosciences). Changes in protein expression levels were quantified using ImageJ software.

**Data Analysis**

Statistical analysis was performed using Graphpad Instat software (San Diego, http://www.graphpad.com). Normality testing of the experimental groups was performed using the Kolmogorov and Smirnov method. Significance was calculated using a 2-tailed Student t test or 2-way ANOVA with Bonferroni post test where appropriate. Probability values <0.05 were considered significant.

**Results**

**Adrenal SR-BI Deficiency Is Associated With Primary Glucocorticoid Insufficiency**

In the current study, we have determined the consequences of adrenocortical-specific deletion of SR-BI function in C57BL/6 WT mice that do exhibit normal SR-BI function in other organs involved in HDL metabolism such as the liver. For this purpose, we transplanted a donor adrenal of ~10-day-old female SR-BI KO or WT littermate pups under the renal capsule of mature (10–12 weeks old) female C57BL/6 mice that were subjected to bilateral adrenalectomy to delete their own adrenal function. To verify the success of the transplantation, we regularly measured plasma levels of corticosterone, the primary glucocorticoid circulating in fasted mice. In accordance with neovascularization and subsequent growth of the young adrenal transplants, a time-dependent increase in plasma corticosterone levels was detected in C57BL/6 mice transplanted with WT control adrenals both on the chow diet as well as on the atherogenic diet (Figure 1A; 2-way ANOVA P<0.001 for time), ultimately reaching levels at euthanization—16 weeks after transplantation with 10 weeks atherogenic diet—compared with those found in sham-operated controls containing 2 mature adrenals (366±81 ng/mL; n=5). Importantly, a markedly lower plasma corticosterone level was observed at all time points in C57BL/6/mice transplanted with adrenals from SR-BI KO pups (Figure 1A; 2-way ANOVA P<0.001 for genotype). In contrast, plasma adrenocorticotropic hormone levels were 6.5-fold higher (P<0.01; Figure 1B) at euthanization in C57BL/6/mice transplanted with SR-BI KO adrenals, indicative of primary glucocorticoid insufficiency.

**Adrenal SR-BI Deficiency Depletes Local Neutral Lipid Stores**

On euthanization of the mice, the kidneys and adrenal transplants were removed from the recipient mice for further analysis. Gross morphological examination already revealed a striking difference between the 2 transplant genotypes (Figure 2). SR-BI KO adrenal transplants seemed more redish in color compared with the WT transplants, which corresponds to previous findings by Rigotti et al26 on the change in gross morphology of adrenals in total body SR-BI KO mice. Histological analysis revealed that both WT and SR-BI
KO adrenal transplants primarily consisted of adrenocortical cells surrounding scar tissue on the medulla location. Adrenocortical cells in WT transplants stained positive for neutral lipids (ie, cholesteryl esters). In contrast, SR-BI KO adrenal transplants were virtually devoid of neutral lipids as judged by the nearly absent Oil red O staining (Figure 2), highlighting the importance of SR-BI for the supply of cholesterol to the intra-adrenal cholesteryl ester pool.

**Adrenal SR-BI Deficiency Does Not Affect General Hematologic Parameters**

Because total body SR-BI KO mice exhibit reticulocytosis and thrombocytopenia, we determined the effect of adrenal-specific SR-BI deletion on general hematologic parameters. As evident from Table II in the online-only Data Supplement, adrenal-specific SR-BI deletion did not significantly affect any of the parameters measured regarding red blood cell/platelet number or functionality, suggesting that the glucocorticoid insufficiency in total body SR-BI KO mice does not explain these pathologies.

**Adrenal SR-BI Deficiency Is Associated With Diminished Glucocorticoid Signaling**

In the mice transplanted with a SR-BI KO adrenal, we detected a marked increase compared with those transplanted with a WT adrenal in the hepatic mRNA levels of corticosteroid binding globulin (+159%; \( P<0.05 \)), whose gene expression level in hepatocytes is subject to negative feedback control by glucocorticoids.27 Because studies in corticosteroid binding globulin KO mice have suggested an active role for corticosteroid binding globulin in the biological response of glucocorticoids,28 this could theoretically serve as a compensatory response to overcome a defect in tissue glucocorticoid action. However, the expression of the glucocorticoid responsive-genes phosphoenolpyruvate carboxykinase (PEPCK),29 period homolog 1 (PER1),30 apolipoprotein A4 (APOA4),31 and FK506 binding protein 5 (FKBP5)32 was, respectively, 43% to 89% lower in livers of SR-BI KO adrenal transplanted-animals (\( P<0.05 \) for all; Figure 3A). Because PEPCK is the rate-limiting enzyme in the production of glucose (gluconeogenesis), the decrease in hepatic PEPCK expression was paralleled by a significant decrease in the fasting plasma glucose level in these mice (-31%; \( P<0.05 \); Figure 3B). The hepatic expression of the cytoprotective enzyme heme oxygenase 1 (HO-1) is repressed by glucocorticoids.33 A concomitant 2.5-fold higher (\( P<0.01 \)) expression level of HO-1 could be found in livers of adrenal-specific SR-BI KO mice (Figure 3A). Combined, these findings suggest that glucocorticoid signaling...
in liver was clearly diminished in adrenal-specific SR-BI KO mice despite a compensatory upregulation of the glucocorticoid carrier protein corticosteroid binding globulin.

**Adrenal SR-BI Deficiency Is Associated With a Lower Hepatic Triglyceride Level**

Previous findings have indicated that glucocorticoids—in addition to stimulating hepatic gluconeogenesis—can affect and the development of fatty liver disease.\(^3^4\) We therefore determined the effect of adrenal-specific deletion of SR-BI function and the associated glucocorticoid insufficiency on hepatic lipid levels (Table). Adrenal SR-BI KO mice displayed a decrease in the intrahepatic triglyceride levels (–26%; \(P<0.05\)). In contrast, hepatic-free cholesterol and cholesteryl ester contents were not significantly different between the 2 genotypes.

Previous reports have suggested that glucocorticoids alter the hepatic triglyceride content through modulation of de novo lipogenesis and fatty acid oxidation pathways.\(^3^5\),\(^3^6\) However, until now there is no clear consensus on the underlying mechanism of the glucocorticoid-mediated changes in triglyceride levels as it seems that cofounding factors (ie, plasma insulin levels), may be important.\(^3^6\) To acquire mechanistic insight in the significant decrease in liver triglycerides observed in the current study, we quantified expression levels of genes involved in hepatic fatty acid and triglyceride synthesis (Figure 4). Gene expression levels of fatty acid synthase and stearoyl-Coenzyme A desaturase 1 (SCD1) and the lipogenic transcription factor, sterol regulatory element binding transcription factor 1c (SREBP1c), were not significantly different between livers of C57BL/6 mice transplanted with either a SR-BI KO or WT adrenal. However, a striking 65% decrease (\(P<0.01\)) was noted in livers of adrenal-specific SR-BI KO mice in the expression of diacylglycerol acyltransferase-1 that catalyzes the final step in triglyceride synthesis.

**Adrenal SR-BI Deficiency Is Associated With a Less Atherogenic Lipoprotein Profile**

The liver plays a crucial role in the control of total lipid homeostasis because cholesterol can only leave the body through hepatobiliary excretion and the liver facilitates the peripheral delivery of fatty acids through their intrahepatic packaging and secretion in very-low-density lipoprotein (VLDL) particles. Because intrahepatic lipid levels were affected in adrenal-specific SR-BI KO mice, cholesterol and triglyceride levels were also measured in plasma of these mice collected at euthanization (ie, after an overnight fast on feeding a cholic acid–containing high-cholesterol/high-fat diet for 10 weeks). In accordance with the fact that cholic acid acquired from the diet markedly stimulates the lipoprotein lipase-mediated catabolism of triglycerides,\(^3^7\) plasma of both groups of transplanted mice contained little triglycerides (<5 mg/dL; Table). Strikingly, C57BL/6 mice that had received SR-BI KO adrenals exhibited a significantly lower plasma free (–26%; \(P<0.01\)) and total (–35%; \(P<0.05\)) cholesterol level compared with WT adrenal-transplanted controls (Table). Antiatherogenic HDL levels were unchanged in plasma (Figure 5). As shown in Figure 5, the decrease in plasma total cholesterol levels could be fully attributed to a 46% decrease (\(P<0.01\)) in the cholesterol level associated with proatherogenic apolipoprotein B containing VLDL.

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**Table. Hepatic and Plasma Lipid Levels in Different Adrenal Transplant Groups**

<table>
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<tr>
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<th>WT (n=7)</th>
<th>SR-BI KO (n=6)</th>
<th>(P) Value</th>
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<tr>
<td><strong>Liver, mg/g protein</strong></td>
<td></td>
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<td>Free cholesterol</td>
<td>4.5±0.3</td>
<td>4.1±0.1</td>
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<td>Cholesteryl esters</td>
<td>85±11</td>
<td>66±3</td>
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<td>Triglycerides</td>
<td>23±2</td>
<td>17±0.4</td>
<td>0.030</td>
</tr>
<tr>
<td><strong>Plasma, mg/dL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>60±4</td>
<td>45±3</td>
<td>0.007</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>173±18</td>
<td>113±7</td>
<td>0.015</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>4.7±0.7</td>
<td>4.3±0.4</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Data represent means±SEM. WT indicates wild-type mice; and SR-BI KO, scavenger receptor BI knockout mice.
and LDL. On their secretion from the liver into the plasma compartment, large triglyceride-rich VLDL particles are subject to lipolysis by hepatic lipase and lipoprotein lipase ultimately generating smaller cholesterol-rich LDLs that are primarily cleared from the circulation by the liver through receptor-mediated whole particle uptake. Dietary cholic acid effectively suppresses VLDL production by hepatocytes. Because we anticipated that the changes in VLDL/LDL levels are rather the result of changes in the catabolism and hepatic clearance of the particles, we measured the relative expression level of key genes involved in lipoprotein lipolysis and uptake in livers of our adrenal-specific SR-BI KO mice fed with cholic acid-containing diet. Hepatic lipase and lipoprotein lipase expression levels were unchanged (data not shown), suggestive of a similar lipolysis rate in the 2 groups of adrenal transplanted mice. Recent studies have shown a direct genomic action of glucocorticoids on the expression of the LDL receptor–related protein 1. In accordance with a diminished hepatic glucocorticoid signaling, we observed a trend toward lower liver LDL receptor–related protein 1 expression (–51%; P=0.081; Figure 6A) in response to adrenal SR-BI deficiency. A parallel decrease in the glucocorticoid-responsive transcript of the HDL receptor SR-BI was noted (–69%; P=0.055; Figure 6A). The transcript level of the cholesterol synthesis gene HMG-CoA reductase (HMGCR) was not significantly affected (Figure 6A). Because of the high variation between individual mice, the decrease in the LDL receptor transcript level associated with adrenal SR-BI deficiency failed to reach significance (Figure 6A). However, the LDL receptor protein expression was significantly decreased in livers of adrenal-specific SR-BI deficient mice (–42%; P<0.05; Figure 6B). Glycosylation of the LDL receptor is necessary to maintain its stability and execute its normal function. Importantly, Western blot analysis showed a marked shift in the ratio of mature (glycosylated) and immature LDL receptor protein expression, 11.1±3.8 for SR-BI KO versus 1.4±0.1 for C57BL/6 (P<0.05; Figure 6B). This suggests that the rate of maturation and catabolism of the LDL receptor in adrenal-specific SR-BI KO mice may be affected. No change was detected in the mRNA expression of proprotein convertase subtilisin/kexin type 9 (PCSK9) that degrades the LDL receptor protein (Figure 6C). In contrast, the hepatic mRNA expression of myosin regulatory light chain interacting protein (MYLIP) that modulates trafficking of the LDL receptor to the plasma membrane and LDL receptor degradation was decreased by a marked 2-fold (P<0.05; Figure 6C) in livers of adrenal-specific SR-BI KO mice. Combined, these findings suggest that the diminished glucocorticoid signaling attributable to adrenal SR-BI deficiency is probably associated with an altered maturation state of the LDL receptor, ultimately resulting in an enhanced clearance of apolipoprotein B–containing VLDL/LDL particles by the liver and an overall less atherogenic plasma lipoprotein profile.

**Figure 4.** Hepatic relative mRNA expression levels of lipogenic genes in C57BL/6 mice transplanted with C57BL/6 wild-type (WT; white bars; n=7) or scavenger receptor BI knockout mice (SR-BI KO; black bars; n=6) adrenals that were fed with atherogenic diet for 10 weeks. SREBP indicates sterol regulatory element binding transcription factor; FAS, fatty acid synthase; SCID, stearyl-Coenzyme A desaturase 1; and DGAT-1, diacylglycerol acyltransferase. Data represent means±SEM. **P<0.01 (t test).

**Figure 5.** The cholesterol distribution over the different lipoprotein fractions in C57BL/6 mice transplanted with C57BL/6 wild-type (WT; open circles/white bars) or scavenger receptor BI knockout mouse (SR-BI KO; closed circles/black bars) adrenals that were fed with atherogenic diet for 10 weeks. Data represent means±SEM. **P<0.01 (t test). VLDL indicates very-low-density lipoprotein; LDL, low-density lipoprotein; and HDL, high-density lipoprotein.

**Discussion**

In the current study, we show that adrenal SR-BI itself, rather than the accumulation of dysfunctional lipoproteins, is the bottleneck for cholesterol supply to adrenal steroidogenesis in SR-BI KO mice, because adrenal-specific SR-BI deficiency is also associated with adrenocortical cell neutral lipid depletion and primary adrenal glucocorticoid insufficiency in normolipidemic C57BL/6 mice. Our findings thus provide the final in vivo proof for the concept, originally derived from in vitro and in vivo studies by the groups of Dr David Williams and Dr Monty Krieger, that SR-BI–mediated uptake of cholesterol from HDL particles supplies cholesterol to intra-adrenal cholesterol stores that are efficiently coupled the steroidogenic pathway.

We did not observe a change in the platelet or red blood cell morphology or number in our adrenal-specific SR-BI–deficient C57BL/6 mice, suggesting that glucocorticoid insufficiency does not underlie the thrombocytopenia and reticulocytosis...
present in total body SR-BI KO mice. This is in accordance with findings by Dole et al,13 Ma et al,15 Hildebrand et al,44 and El Bouhassani et al, 45 which showed that the relatively high-plasma-free cholesterol levels/enhanced free cholesterol to total cholesterol ratio is primary responsible for the different blood cell abnormalities.

Excess endogenous glucocorticoid action or treatment with exogenous glucocorticoids is associated with the development of metabolic diseases, such as insulin resistance and type II diabetes mellitus.46–48 Because glucocorticoids are important anti-inflammatory agents, total blockage of glucocorticoid production and action, for instance through removal of the adrenals (adrenalectomy) or administration of a glucocorticoid receptor antagonist, is not considered a valuable approach to target metabolic disease because of a potential rise in the risk of inflammation-related mortality.49–51 However, inhibition of glucocorticoid action specifically in metabolic tissues could be theoretically applied to battle metabolic diseases. In the current study, we observed that adrenal-specific SR-BI KO mice exhibit both a lower fasting glucose level and decreased liver triglyceride content. High fasting glucose levels and liver triglyceride accumulation (hepatic steatosis) are hallmarks of 2 frequent metabolic pathologies (ie, type II diabetes mellitus and nonalcoholic fatty liver disease [NAFLD], respectively). The number of circulating white blood cells as well as the hepatic relative expression levels of the macrophage marker CD68 and proinflammatory cytokines interleukin-6 and tumor necrosis factor-α did not significantly differ between adrenal-specific SR-BI KO mice and their controls (data not shown). It can thus be concluded that mice lacking proper SR-BI function in their adrenals show a beneficial metabolic profile, whereas their general immune status is not affected. Importantly, human carriers of a functional mutation in the SR-BI gene do not show an apparent higher risk for the development of inflammation-related pathologies (Dr J.A. Kuivenhoven, personal communication), whereas their adrenal steroidogenesis rate is significantly attenuated.52 We therefore anticipate that interfering with adrenal SR-BI function may possibly represent an alternative therapeutic opportunity to treat glucocorticoid-induced metabolic pathologies.

The glucocorticoid insufficiency phenotype associated with adrenal-specific SR-BI deficiency was also accompanied by a decrease in the plasma level of cholesterol associated with proatherogenic apolipoprotein B-containing lipoproteins. In liver transplant patients53 as well as in a large cohort of SLE patients,54 exogenous corticosteroid therapy was independently associated with higher total/LDL-cholesterol levels, suggesting that glucocorticoids do also influence the levels of apolipoprotein B-containing lipoproteins in the clinical setting.

Our expression analysis revealed that the decrease in plasma cholesterol levels are accompanied by a decrease in plasma levels of cholesterol associated with proatherogenic apolipoprotein B-containing lipoproteins. In liver transplant patients and in a large cohort of SLE patients, exogenous corticosteroid therapy was independently associated with higher total/LDL-cholesterol levels, suggesting that glucocorticoids do also influence the levels of apolipoprotein B-containing lipoproteins in the clinical setting.

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VLDDL/LDL levels was probably secondary to a decrease in the expression of MYLIP. MYLIP is also known as E3 ubiquitin ligase-inducible degrader of the low-density lipoprotein receptor (IDOL). Initial studies by Zelcer et al. have indicated that shRNA-mediated targeting of MYLIP/IDOL mRNA is associated with increased LDL receptor protein expression and an enhanced LDL uptake in hepatocytes. Recent follow-up studies have shown that MYLIP/IDOL transcript levels can be modulated by fibroblast growth factor-21 and estrogen, suggesting that MYLIP/IDOL expression may be highly responsive to changes in the body’s hormonal balance. Although functional studies are necessary to prove a direct regulatory control of MYLIP/IDOL translocation by glucocorticoids, our studies at least hint to a potentially relevant link between the tissue glucocorticoid status and hepatic MYLIP/IDOL expression and thus indirectly the LDL receptor-mediated clearance of apolipoprotein B-containing lipoproteins.

In conclusion, we have shown that adrenal-specific disruption of SR-BI function induces primary glucocorticoid insufficiency and lowers plasma VLDDL/LDL levels in athrogenic diet-fed C57BL/6 mice. Our studies further highlight the link between adrenal HDL-cholesterol uptake by SR-BI, adrenal steroidogenesis, and the regulation of (hepatic) lipid metabolism, and indicate the value of the whole adrenal transplantation technique for studies regarding the contribution of adrenocortical cell-derived transcripts to glucocorticoid homeostasis and glucocorticoid-associated pathologies, including disturbances in lipid metabolism.

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Disclosures
None.

References
Apolipoprotein A-IV is regulated by nutritional and metabolic stress: period1 mRNA expression in mouse peripheral tissues via a glucocorticoid-binding globulin. Mol Cell Biol. 2006;8:5.


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### Supplemental Table I: Primers used for real-time quantitative PCR analysis

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<tr>
<td>Hematological Parameters</td>
<td>WT (n=7)</td>
<td>SR-BI KO (n=6)</td>
<td>P value</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>----------</td>
<td>----------------</td>
<td>---------</td>
</tr>
<tr>
<td>White blood cell # (10E9/L)</td>
<td>5.4 ± 0.4</td>
<td>6.6 ± 0.9</td>
<td>0.23</td>
</tr>
<tr>
<td>Red blood cell # (10E12/L)</td>
<td>8.9 ± 0.3</td>
<td>9.1 ± 0.5</td>
<td>0.77</td>
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<tr>
<td>Reticulocyte # (10E12/L)</td>
<td>0.50 ± 0.04</td>
<td>0.50 ± 0.07</td>
<td>0.97</td>
</tr>
<tr>
<td>Mean cellular volume (MCV; fL)</td>
<td>45.7 ± 0.6</td>
<td>46.3 ± 0.4</td>
<td>0.45</td>
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<tr>
<td>Hemoglobin (HGB; mM)</td>
<td>8.1 ± 0.2</td>
<td>8.4 ± 0.4</td>
<td>0.39</td>
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<tr>
<td>Hematocrit (HCT; %)</td>
<td>41 ± 1</td>
<td>42 ± 2</td>
<td>0.64</td>
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<tr>
<td>Platelet # (10E9/L)</td>
<td>1097 ± 205</td>
<td>1587 ± 143</td>
<td>0.085</td>
</tr>
<tr>
<td>Mean platelet volume (MPV; fL)</td>
<td>7.4 ± 0.1</td>
<td>7.3 ± 0.1</td>
<td>0.52</td>
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<tr>
<td>Plateletcrit (PCT; %)</td>
<td>0.81 ± 0.15</td>
<td>1.16 ± 0.11</td>
<td>0.097</td>
</tr>
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<td>Platelet distribution width (PDW; fL)</td>
<td>8.1 ± 0.2</td>
<td>8.0 ± 0.2</td>
<td>0.73</td>
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</tbody>
</table>

Data represent means±SEM.