Absence of HDL cholesteryl ester uptake in mice via SR-BI impairs an adequate adrenal glucocorticoid-mediated stress response to fasting

Menno Hoekstra,[1] Illiana Meurs, Mieke Koenders, Ruud Out, Reeni B. Hildebrand, J. Kar Kruijt, Miranda Van Eck, and Theo J. C. Van Berkel

Division of Biopharmaceutics, Leiden/Amsterdam Center for Drug Research, Gorlaeus Laboratories, 2300RA Leiden, The Netherlands

Abstract **Receptor-mediated cholesterol uptake has been suggested to play a role in maintaining the adrenal intracellular free cholesterol pool and the ability to produce hormones. Therefore, in the current study, we evaluated the importance of scavenger receptor class B type I (SR-BI)-mediated cholesteryl ester uptake from HDL for adrenal glucocorticoid hormone synthesis in vivo. No difference was observed in the plasma level of corticosterone between SR-BI-deficient and wild-type mice under ad libitum feeding conditions. Overnight fasting (~16 h) stimulated the plasma level of corticosterone by 2-fold in wild-type mice. In contrast, no effect of fasting on plasma corticosterone levels was observed in SR-BI-deficient mice, leading to a 44% lower plasma corticosterone level compared with their wild-type littermate controls. In parallel, an almost complete depletion of lipid stores in the adrenal cortex of fasted SR-BI-deficient mice was observed. Plasma adrenocorticotropic hormone levels were increased by 5-fold in fasted SR-BI-deficient mice. SR-BI deficiency induced marked changes in the hepatic expression of the glucocorticoid-responsive genes cholesterol 7α-hydroxylase, HMG-CoA synthase, apolipoprotein A-IV, corticosteroid binding globulin, interleukin-6, and tumor necrosis factor-α, which coincided with a 42% decreased plasma glucose level under fasting conditions. In conclusion, we show that the absence of adrenal HDL cholesteryl ester uptake in SR-BI-deficient mice impairs the adrenal glucocorticoid-mediated stress response to fasting as a result of adrenal glucocorticoid insufficiency and attenuated liver glucocorticoid receptor signaling, leading to hypoglycemia under fasting conditions.**—Hoekstra, M., I. Meurs, M. Koenders, R. Out, R. B. Hildebrand, J. K. Kruijt, M. Van Eck, and T. J. C. Van Berkel. **Absence of HDL cholesteryl ester uptake in mice via SR-BI impairs an adequate adrenal glucocorticoid-mediated stress response to fasting.** ***J. Lipid Res.*** **2008.** 49: **738–745.**

Supplementary key words scavenger receptor class B type I • high density lipoprotein • hormones

Hormones are generally considered to be substances secreted into the blood in very small amounts by specialized cells or glands and carried by the bloodstream or by diffusion to other parts of the body, where they interact with specific receptors in target tissue cells to produce a biological response. Hormones can be derived from amino acids, peptides and proteins, or cholesterol. This latter class of hormones is known as the steroid hormones. The adrenals predominantly secrete two types of steroid hormones: mineralocorticoids and glucocorticoids (also called "stress hormones"). Mineralocorticoids such as aldosterone are able to modulate the body's salt balance via the action of the nuclear receptor mineralocorticoid receptor (1). Glucocorticoids (i.e., cortisol in human and corticosterone in rodents) play an essential role in the regulation of total body glucose metabolism and the inflammatory status under conditions of physiological or psychological stress through their interaction with the glucocorticoid receptor (2, 3).

Within the adrenals, a constant supply of free cholesterol is required to serve as precursor for the synthesis of mineralocorticoids and glucocorticoids. The rate-limiting step for steroid hormone synthesis is the transport of free cholesterol into mitochondria by steroidogenic acute regulatory protein. In the mitochondria, free cholesterol is rapidly metabolized by the cholesterol side chain cleavage enzyme cytochrome P450scc and other enzymes in the steroidogenic pathway to generate the hormones. The cholesterol needed for optimal steroid synthesis in the adrenals can be acquired from *1*) intracellular de novo synthesis of free cholesterol by the enzyme HMG-CoA reductase, *2*) intracellular catabolism of stored cholesteryl esters to free cholesterol by neutral cholesteryl ester hydrolase, or *3*) receptor-mediated uptake and subsequent intracellular catabolism of cholesteryl esters transported in circulating LDL and HDL [reviewed by Kraemer (4)].

Manuscript received 19 October 2007 and in revised form 18 January 2008.

Published, JLR Papers in Press, January 19, 2008.
DOI 10.1194/jlr.M700475-JLR200

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

Copyright © 2008 by the American Society for Biochemistry and Molecular Biology, Inc.

This article is available online at http://www.jlr.org

Downloaded from www.jlr.org by guest, on June 14, 2012

The scavenger receptor class B type I (SR-BI) is a cell surface receptor expressed predominantly in liver and steroidogenic tissues (i.e., testis, ovaries, and adrenals) (5–8) that mediates the selective uptake of cholesteryl esters from HDL without the concomitant uptake and degradation of the HDL particle (6). Studies by Temel et al. (9) have shown that SR-BI serves as the major route for the selective uptake of HDL cholesteryl esters and for the delivery of HDL cholesterol to the steroidogenic pathway in cultured adrenal cells in vitro. Previously, using double labeled [^{3}H]cholesterol ether-HDL/[^{125}I]tyramine cellobiose-HDL, we showed that SR-BI is the primary molecule involved in the selective uptake of cholesteryl esters from HDL in the liver and adrenals of mice in vivo. The selective uptake of cholesteryl esters in the liver was completely diminished in SR-BI-deficient mice compared with wild-type mice at 4 h after injection of the double labeled [^{3}H]cholesterol ether-HDL/[^{125}I]tyramine cellobiose-HDL, whereas the absence of SR-BI also resulted in a marked 87% decrease in the selective uptake of HDL cholesteryl esters in the adrenals (10). As receptor-mediated cholesterol uptake has been suggested to play a role in maintaining the adrenal intracellular free cholesterol pool, and thus the ability to produce hormones, in the current study we evaluated the importance of SR-BI-mediated cholesteryl ester uptake from HDL for adrenal glucocorticoid hormone synthesis in vivo.

MATERIALS AND METHODS

Animals

SR-BI-deficient mice were kindly provided by Dr. M. Krieger (11). Heterozygous SR-BI-deficient mice were cross-bred to generate wild-type and homozygous progeny. The offspring of the mice were analyzed for the presence of targeted or wild-type SR-BI alleles by PCR, as described by Van Eck et al. (12). Male homozygous SR-BI-deficient mice (generated on a 129Sv background and back-crossed five times to C57Bl/6) and wild-type littermate controls were maintained on a sterilized regular chow (RM3; Special Diet Services, Witham, UK). At 12–16 weeks of age, overnight-fasted (~16 h) mice were anesthetized and blood was collected between 9:00 and 11:00 AM for lipid, hormone, and glucose analyses. Subsequently, a whole-body perfusion was performed using phosphate-buffered saline (4°C, 100 mm Hg) for 10 min. After perfusion, livers and adrenals were excised and frozen in liquid nitrogen and stored at −80°C. Animal experiments were performed at the Gorlaeus Laboratories of the Leiden/Amsterdam Center for Drug Research in accordance with national laws. All experimental protocols were approved by the Ethics Committee for Animal Experiments of Leiden University.

Plasma lipid analyses

Plasma concentrations of free cholesterol, cholesteryl esters, triglycerides, phospholipids, and free fatty acids were determined using enzymatic colorimetric assays. The cholesterol distribution over the different lipoproteins in plasma was analyzed by fractionation of 30 μl of plasma of each mouse using a Superose 6 column (3.2 × 30 mm, Smart system; Pharmacia). Total cholesterol content of the effluent was determined using enzymatic colorimetric assays, taking the efficiency of recovery from the column into account.

Tissue lipid composition and histology

Lipids from adrenals were extracted using the method of Bligh and Dyer (13). After dissolving the lipids in 1% Triton X-100, contents of phospholipids, triglycerides, free cholesterol, and cholesteryl esters were determined as described above and expressed as micrograms per milligram of protein. Seven micrometer cryosections were prepared on a Leica CM3050-S cryostat. Cryosections were routinely stained with hematoxylin (Sigma) and Oil Red O (Sigma) for lipid visualization.

Plasma hormone analysis

Corticosterone and adrenocorticotropic hormone (ACTH) levels in plasma were determined using the corticosterone and ACTH ^{3}H RIA kits from ICN Biomedicals according to the protocols from the supplier.

Plasma glucose analysis

Plasma glucose levels were determined essentially as described (14). In short, 200 μl of coloring reagent, containing 2,2′-azino-di(3-ethylbenzthiazoline-6-sulfonate), glucose oxidase, and peroxidase, was added to 5 μl of 25× diluted plasma of SR-BI-deficient and wild-type mice. After a 30 min incubation at 37°C, the absorbance was measured at 405 nm and the glucose concentration was determined.

Analysis of gene expression by real-time quantitative PCR

Quantitative gene expression analysis on livers of SR-BI-deficient mice and their wild-type littermates was performed as described (15). In short, total RNA was isolated according to Chomczynski and Sacchi (16) and reverse-transcribed using RevertAid™ reverse transcriptase. Gene expression analysis was performed using real-time SYBR Green technology (Eurogentec) with the primers listed in **Table 1**, which were validated for identical efficiencies [slope = −3.3 for a plot of threshold cycle number (Ct) versus log ng cDNA]. Hypoxanthine guanine phosphoribosyl transferase (HPRT), β-actin, GAPDH, acidic ribosomal phosphoprotein P0 (36B4), and cyclophilin were used as the standard housekeeping genes. Relative gene expression numbers were calculated by subtracting the Ct value of the target gene from the average Ct of HPRT, β-actin, GAPDH, 36B4, and cyclophilin (Ct housekeeping) and raising 2 to the power of this difference. The average Ct of five housekeeping genes was used to exclude the possibility that changes in the relative expression were caused by variations in the expression of the separate housekeeping genes.

Data analysis

Statistical analysis was performed using Graphpad Instat software (San Diego, CA; http://www.graphpad.com). Normality testing of the experimental groups was performed using the method of Kolmogorov and Smirnov (Graphpad Instat Software, San Diego, CA; http://www.graphpad.com). The significance of differences was calculated using a two-tailed unpaired *t*-test or one-way ANOVA. $P < 0.05$ was considered significant.

RESULTS

Previous studies by Rigotti, Miettinen, and Krieger (17) have shown that mice that do not contain a functional

Downloaded from www.jlr.org by guest, on June 14, 2012

TABLE 1. Primers used for real-time PCR analysis

Gene	GenBank Accession Number	Forward Primer	Reverse Primer
36B4	X15267	GGACCCGAGAAGACCTCCTT	GCACATCACTCAGAATTTCAATGG
Apolipoprotein A-IV	NM007468	CAGCTGACCCCATACATCCAG	TCATCGAGGTGTGCAGGTTG
β-Actin	X03672	AACCGTGAAAAGATGACCCAGAT	CACAGCCTGGATGGCTACGTA
Corticosteroid binding globulin	X70533	GAATGAGACAAGCACAGTGAAGGT	CGCCGAATCACGAAAGTAACT
Cyclophilin	AK010338	CCATTTCAAGAAGCAGCGTTT	ATTTTGTCTTAACTGGTGGT
Cholesterol 7α-hydroxylase	NM007824	CTGTCATACCACACAAAGTCTTATGTCA	ATGCTTCTGTGTCCAAATGCC
GAPDH	NM008084	TCCATGACAACTTTGGCATTG	TCACGCCACAGCTTTCCA
Hypoxanthine guanine phosphoribosyl transferase	J00423	TTGCTCGAGATGTCATGAAGGA	AGCAGGTCAGCAAAGAACTTATAG
HMG-CoA synthase	NM008256	TTTCATTCCGAGTGTCCAAGG	CTGACACACTAGACACCAGTTTCTCC
Interleukin-6	M20572	GAAGAATTTCTAAAAGTCACTTTGAGATCTAC	CACAGTGAGGAATGTCCACAAAC
Phosphoenolpyruvate carboxykinase	NM011044	TTGAACTGACAGACTCGCCCT	GATATGCCCATCCGAGTCATG
Pyruvate kinase	NM013631	AAGACAGTGTGGGTGGACTACCA	CGTCAATGTAGATGCGGCC
Tumor necrosis factor-α	X02611	GCCAGCCGATGGGTTGTA	AGGTTGACTTTCTCCTGGTATGAGA

SR-BI protein (SR-BI-deficient mice) show no gross phenotypic indications of adrenal insufficiency under standard feeding conditions, suggesting that SR-BI-mediated cholesteryl ester uptake does not play an essential role in the basal adrenal hormone synthesis. In accordance, we also did not observe a difference in the plasma level of the primary glucocorticoid circulating in mice, corticosterone, between SR-BI-deficient and wild-type mice under ad libitum feeding conditions (**Fig. 1**). Importantly, the adrenals produce relatively low amounts of glucocorticoids under nonstressed conditions, whereas the production and secretion of glucocorticoid increases rapidly upon some form of biological stress, because glucocorticoids are crucially involved in the fine-tuning of the response to stress. Therefore, to define the quantitative importance of SR-BI-mediated cholesteryl ester uptake from HDL for adrenal hormone synthesis, we determined the effect of SR-BI deficiency in mice on plasma corticosterone levels under conditions of high glucocorticoid demand, caused by a commonly used form of physiological stress, food deprivation.

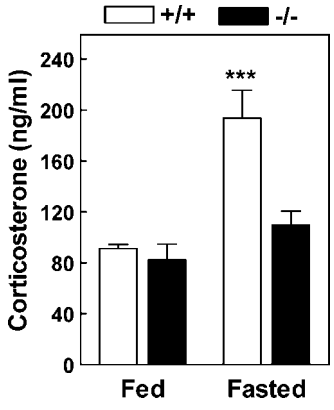

Fig. 1. Plasma corticosterone levels in scavenger receptor class B type I (SR-BI)-deficient ($^{-/-}$) mice (black bars) and wild-type ($^{+/+}$) mice (white bars) that were nonfasted (fed) or subjected to ~16 h of fasting (fasted). Values represent means ± SEM of four to six mice per group. *** $P < 0.001$ compared with the three other experimental groups (ANOVA).

Overnight fasting (~16 h) significantly stimulated the plasma level of corticosterone by 2-fold ($P < 0.001$) in wild-type mice. In contrast, no effect of fasting on plasma corticosterone levels was observed in SR-BI-deficient mice, leading to a 44% ($P < 0.001$) lower plasma corticosterone level compared with their wild-type littermate controls under fasting conditions (Fig. 1). This suggests that SR-BI-mediated cholesteryl ester uptake from HDL is crucial to maintain optimal adrenal hormone synthesis under fasting ("stressed") conditions. Under conditions of stress, the pituitary synthesizes ACTH, which upon its release into the circulation binds to cell surface ACTH receptors on adrenocortical cells and thereby stimulates the cortex of the adrenal gland, leading to a boost in the synthesis of glucocorticoids. To rule out the possibility that the decrease in plasma corticosterone levels in fasted SR-BI-deficient mice was secondary to a decrease in ACTH levels, we determined the effect of the absence of SR-BI on plasma ACTH levels. As shown in **Fig. 2**, under fasting

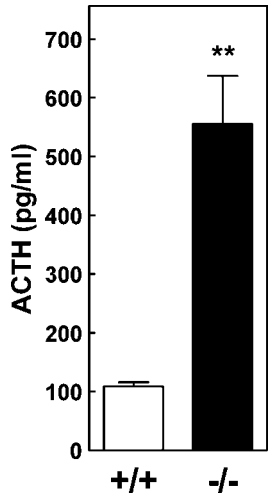

Fig. 2. Plasma adrenocorticotropic hormone (ACTH) levels in SR-BI-deficient ($^{-/-}$) mice (black bars) and wild-type ($^{+/+}$) mice (white bars) that were subjected to ~16 h of fasting. Values represent means ± SEM of four to six mice per group. ** $P < 0.01$ compared with SR-BI-deficient mice (*t*-test).

Downloaded from www.jlr.org by guest, on June 14, 2012

conditions, plasma ACTH levels were increased by 5-fold ($P < 0.001$) in SR-BI-deficient mice compared with controls. The finding that SR-BI-deficient mice produce significantly less corticosterone while ACTH levels are relatively high compared with wild-type mice indicates that SR-BI-deficient mice suffer from adrenal glucocorticoid insufficiency.

Because ACTH is a potent stimulator of the adrenal cortex, we investigated the effect of the high circulating ACTH levels caused by SR-BI deficiency on adrenal morphology under fasting conditions. Macroscopically, the adrenals of SR-BI-deficient mice are darker and have a significantly increased weight compared with those of wild-type mice (**Fig. 3A, B**). Oil Red O staining for lipid of adrenal slides from wild-type mice showed intense staining in the cortex area, with no staining in the adrenal medulla. Strikingly, virtually no Oil Red O staining was seen in both the medulla and the cortex of adrenals from SR-BI-deficient mice (Fig. 3C), suggesting an almost complete depletion of lipid stores in the adrenal cortex of these mice. Quantification of adrenal lipid levels showed a significant decrease in both free cholesterol (−23%; $P = 0.025$) and cholesteryl esters (−52%; $P = 0.015$), probably because of a decrease specifically in the adrenal cortex, whereas adrenal phospholipid and triglyceride levels remained unchanged upon SR-BI deficiency (**Table 2**).

Glucocorticoids are important regulators of glucose metabolism and are vital to prevent (possibly fatal) depletion of plasma glucose stores (hypoglycemia) under fasting conditions. As glucocorticoids thus play an important role in the regulation of metabolism under fasting conditions, we analyzed the effect of SR-BI deficiency on plasma glucose levels. In accordance with the essential role for glucocorticoids in maintaining glucose homeostasis, overnight-fasted SR-BI-deficient mice suffered from hypoglycemia, as their plasma glucose levels were decreased by 42% ($P = 0.008$) compared with wild-type mice (**Fig. 4**). No change in plasma free fatty acid or phospholipid levels was seen as a result of SR-BI deficiency (**Table 3**). Plasma free cholesterol and cholesteryl ester levels were increased by 4-fold ($P < 0.001$) and 2.3-fold ($P < 0.001$) in SR-BI-deficient mice (Table 3) as a result of the accumulation of large cholesterol-rich HDL particles (data not shown), as first described by the Krieger group (11).

Under fasting conditions, the liver and, to a minor extent, muscles and adipose tissue are the primary target organs of glucocorticoids. To investigate whether the decrease in plasma corticosterone levels led to changes in

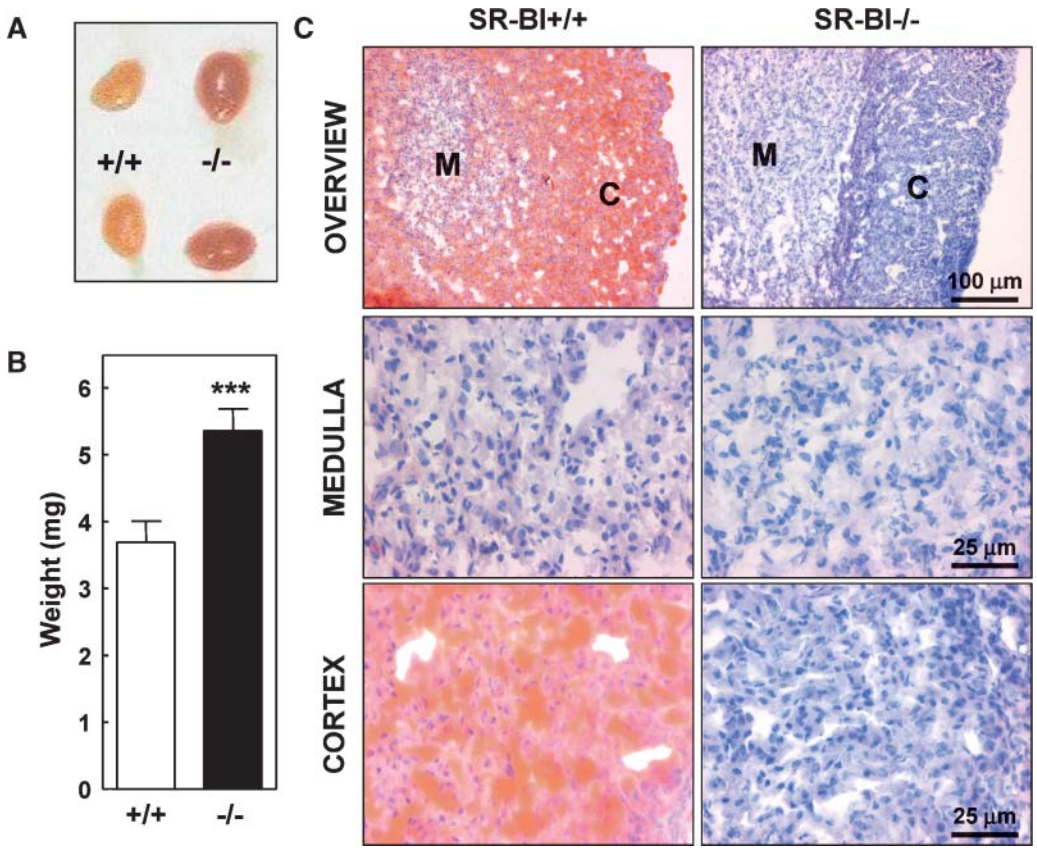

Fig. 3. A: Photograph of adrenals from SR-BI deficient ($^{-/-}$) and wild-type ($^{+/+}$) mice. A clear darkening of the adrenals can be seen as a result of SR-BI deficiency. B: Quantification of the weight of the adrenals of SR-BI deficient mice (black bars) and wild-type mice (white bars). Values represent means ± SEM of four to six mice per group. *** $P < 0.001$ compared with the wild-type mice (ANOVA). C: Cryosections of adrenals were stained with Oil Red O for neutral lipids and counterstained with hematoxylin for nuclei. C, cortex; M, medulla. A clear absence of lipid staining in the adrenal cortex of SR-BI-deficient mice is seen.

Downloaded from www.jlr.org by guest, on June 14, 2012

TABLE 2. Adrenal weights and lipid levels of SR-BI-deficient and wild-type mice on a regular chow diet that were subjected to ~16 h of fasting

Parameter	Wild Type	SR-BI-Deficient	*P*
Weight (mg)	3.7 ± 0.3	5.4 ± 0.3	<0.001
Free cholesterol (μg/mg protein)	83 ± 5	64 ± 5	<0.05
Cholesteryl ester (μg/mg protein)	78 ± 12	37 ± 6	<0.05
Triglycerides (μg/mg protein)	922 ± 113	769 ± 136	NS
Phospholipids (μg/mg protein)	125 ± 4	117 ± 6	NS

SR-BI, scavenger receptor class B type I. Data represent means ± SEM of six mice per group.

TABLE 3. Plasma lipid levels of SR-BI-deficient and wild-type mice on a regular chow diet that were subjected to ~16 h of fasting

Parameter	Wild Type	SR-BI-Deficient	*P*
Free cholesterol (mg/dl)	20 ± 1	85 ± 3	<0.001
Cholesteryl ester (mg/dl)	71 ± 10	164 ± 11	<0.001
Phospholipids (mg/dl)	172 ± 4	183 ± 4	NS
Triglycerides (mg/dl)	81 ± 4	108 ± 13	<0.05
Free fatty acids (mM)	0.69 ± 0.05	0.66 ± 0.09	NS

Data represent means ± SEM of six mice per group.

hepatic glucocorticoid signaling, the mRNA expression levels of genes known to be regulated by glucocorticoids were determined in livers of overnight-fasted SR-BI-deficient mice and wild-type mice. The mRNA expression of the corticosteroid binding globulin (CBG), the expression of which is inhibited by glucocorticoids via the action of the glucocorticoid receptor (18, 19), was increased by 2.5-fold ($P = 0.002$) in livers from SR-BI-deficient mice. This suggests that as a consequence of relatively low glucocorticoid levels, hepatic glucocorticoid receptor activity was decreased in SR-BI-deficient mice. Furthermore, a significant decrease (−43% to −65%) was observed in the hepatic mRNA expression of genes that are stimulated by the glucocorticoid receptor and are involved in hepatic lipid and glucose metabolism, such as cholesterol 7α-hydroxylase (CYP7A1) (20), HMG-CoA synthase (HMGCS) (21), and apolipoprotein A-IV (ApoA-IV) (22) (**Fig. 5**).

Glucocorticoids, via the action of the glucocorticoid receptor, are able to repress the expression of the proinflammatory mediators interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) (23–25). In accordance with an impaired glucocorticoid receptor signaling, the hepatic mRNA expression of IL-6 was stimulated by 3.4-fold ($P = 0.006$) in SR-BI-deficient mice (Fig. 5), suggesting that the (hepatic) inflammatory status was increased in these mice. Moreover, a 2.0-fold increase in TNF-α expression was observed, but that failed to reach significance because of the large variation in both groups of mice (Fig. 5). No change in the expression of the macrophage marker CD68 was detected (data not shown), which excludes the possibility that the relative increase in IL-6 and TNF-α expression levels was attributable to an increase in the absolute amount of tissue macrophages (Kupffer cells) in the liver as a result of the absence of functional SR-BI. No effect on the mRNA expression of the key gluconeogenic enzymes phosphoenolpyruvate carboxykinase (PEPCK) and pyruvate kinase (PK) was observed in SR-BI-deficient mice (Fig. 5), which suggests that the hepatic gluconeogenesis rate was unaffected in SR-BI-deficient mice under fasting conditions. These combined data indicate that, in addition to a decreased circulating level of the primary glucocorticoid corticosterone, the hepatic activity of the glucocorticoid receptor also was impaired significantly in SR-BI-deficient mice under fasting conditions, ultimately leading to marked changes in the expression of genes involved in lipid metabolism and inflammation in the liver.

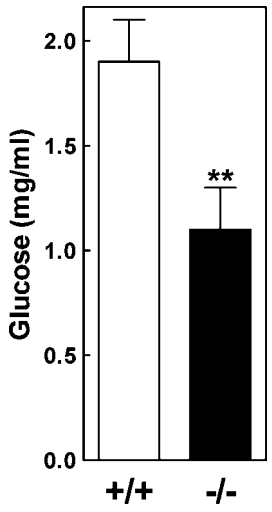

Fig. 4. Plasma glucose levels in SR-BI deficient ($^{-/-}$) mice (black bars) and wild-type ($^{+/+}$) mice (white bars) that were subjected to ~16 h of fasting. Values represent means ± SEM of six mice per group. ** $P < 0.01$ compared with SR-BI-deficient mice (*t*-test).

DISCUSSION

Glucocorticoids, an important class of cholesterol-derived (steroid) hormones, play an essential role in the regulation of total body glucose metabolism and the inflammatory status under conditions of physiological or psychological stress through their interaction with the glucocorticoid receptor (2, 3). Previous studies by Rigotti, Miettinen, and Krieger (17) have shown that mice that do not contain a functional SR-BI protein (SR-BI-deficient mice) show no gross phenotypic indications of adrenal insufficiency under standard feeding conditions, suggesting that cholesteryl ester uptake by SR-BI does not play an essential role in basal ("nonstressed") adrenal hormone synthesis. In the present study, however, we explored the possibility that SR-BI does play an essential role in physiological stress-induced adrenal glucocorticoid production, as SR-BI-deficient mice fail to induce plasma glucocorticoid levels upon overnight (~16 h) fasting, ultimately leading to a 43% decreased plasma corticosterone level in these mice compared with wild-type mice. In addition, the total adrenal cholesterol content was decreased by ~40% in SR-BI-deficient mice under fasting conditions, presumably causing the morphological darkening of the adrenals.

Downloaded from www.jlr.org by guest, on June 14, 2012

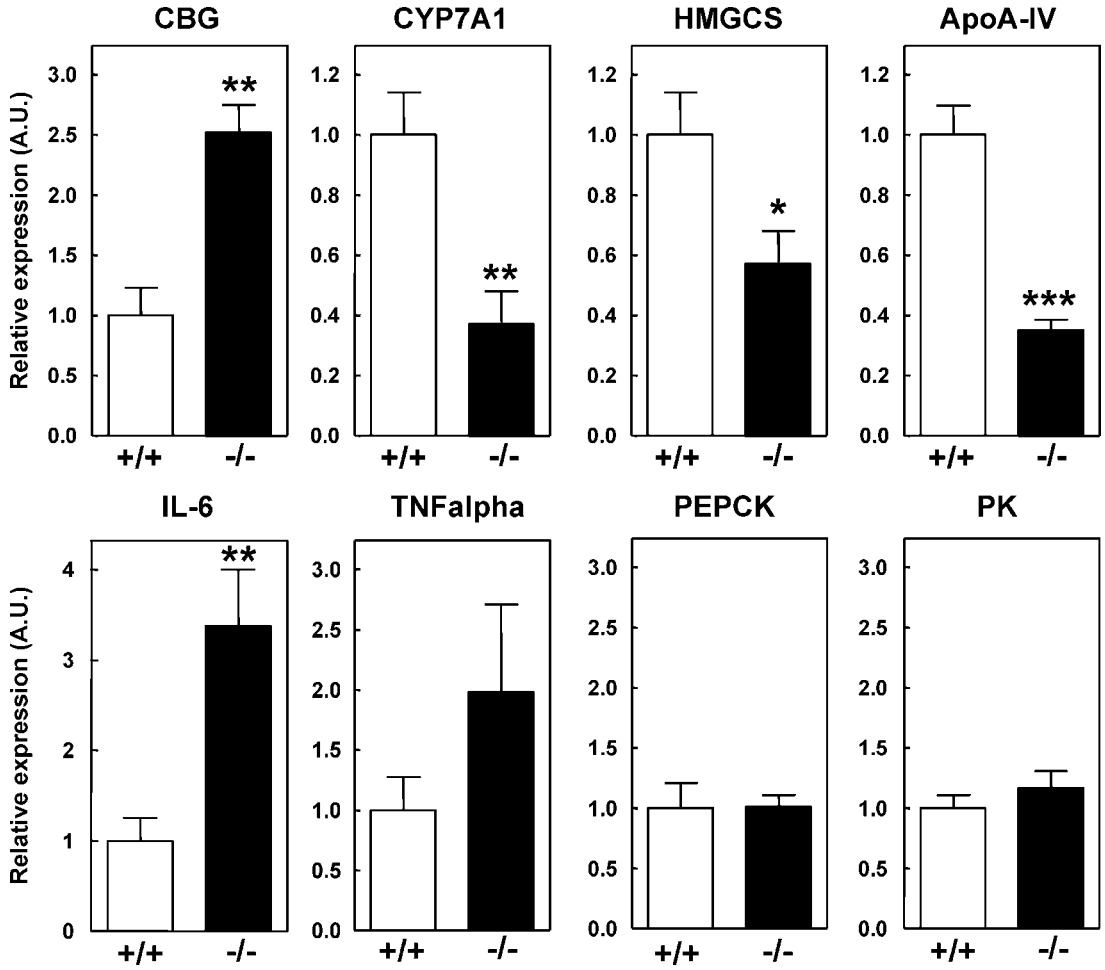

Fig. 5. The relative mRNA levels of corticosteroid binding globulin (CBG), cholesterol 7α-hydroxylase (CYP7A1), HMG-CoA synthase (HMGCS), apolipoprotein A-IV (ApoA-IV), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), phosphoenolpyruvate carboxykinase (PEPCK), and pyruvate kinase (PK) in livers of SR-BI deficient ($^{-/-}$) mice (black bars) and wild-type ($^{+/+}$) mice (white bars). Values represent means ± SEM of five to six mice per group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with SR-BI-deficient mice (*t*-test). A.U., arbitrary units.

As cholesterol is a precursor of steroid hormones (4), it is anticipated that the decreased level of corticosterone results directly from the decline in adrenal cholesterol attributable to the impaired HDL cholesteryl ester uptake via SR-BI. Brundert et al. (26) have shown that the large cholesteryl ester-rich HDL accumulating in SR-BI-deficient mice is less capable of delivering cholesterol for steroidogenesis. This may also contribute to the decrease in the plasma corticosterone level in SR-BI-deficient mice. Importantly, Trigatti et al. (27) have shown that female SR-BI-deficient mice are infertile, probably because of a similarly impaired steroidogenesis in the ovaries. Given the fact that SR-BI-deficient mice have very high circulating levels of ACTH, a potent activator of adrenal cortex glucocorticoid production (28), and a concomitant increase in adrenal weight under fasting conditions, it is suggested that SR-BI-deficient mice are unable to respond adequately to physiological stress impulses, as they suffer from adrenal glucocorticoid insufficiency. Probably as a consequence of the hampered response in plasma corticosterone levels, SR-BI deficiency also resulted in a significant attenuation of hepatic glucocorticoid signaling, as judged from the marked changes in the hepatic expression of the glucocorticoid-responsive genes CYP7A1, HMGCS, ApoA-IV, CBG, IL-6, and TNF-α.

Previous studies in mice have shown that loss of adrenal function as a result of the removal of the adrenals (adrenalectomy) led to reduced plasma glucose levels (29). In accordance with a hampered adrenal function, we observed that plasma glucose levels were decreased by 42% in SR-BI-deficient mice compared with wild-type littermates. Although an extended glucocorticoid regulatory unit has been detected in the key gluconeogenesis gene PEPCK (30), no change in the hepatic mRNA expression of PEPCK or PK was observed as a result of the impaired hepatic corticosteroid signaling in SR-BI-deficient mice, suggesting that the decrease in plasma glucose levels was not caused by a change in the hepatic gluconeogenesis rate. In parallel with our data, Friedman et al. (31) showed that adrenal removal in mice, leading to a markedly decreased corticosteroid level, did not affect hepatic PEPCK expression in the fasting state. Importantly, inhibition of

Downloaded from www.jlr.org by guest, on June 14, 2012

glucose uptake is a consistently reproducible effect of glucocorticoids added at physiological concentrations to intact fat tissue as well as isolated adipocytes (32). Therefore, it is suggested that SR-BI-deficient mice suffer from fasting hypoglycemia as a consequence of increased peripheral glucose usage secondary to impaired glucocorticoid signaling.

In conclusion, we have shown that the absence of HDL cholesteryl ester uptake via SR-BI impairs the adrenal glucocorticoid-mediated stress response to fasting in mice as a result of adrenal glucocorticoid insufficiency and attenuated glucocorticoid receptor signaling, leading to hypoglycemia under fasting conditions. Recent findings by Cai et al. (33) have indicated that SR-BI-deficient mice are incapable of adequately responding to another form of physiological stress, lipopolysaccharide-induced endotoxic shock, as a result of primary adrenal malfunctioning. Our combined findings highlight the importance of SR-BI-mediated HDL uptake in the adrenals for maintaining an appropriate response to stress. In light of the fact that functional mutations in the human SR-BI gene [also known as CD36 and lysosomal integral membrane protein-II analogous-1 (7)] have been detected that are associated with changes in HDL cholesterol levels (34–37), it will be interesting to study whether changes in SR-BI expression are also associated with a different response to psychological or physiological stress in the human situation.

The authors thank Maaike van de Mark and Vera Brinks (Division of Medical Pharmacology of the Leiden/Amsterdam Center for Drug Research) for their assistance with the plasma corticosterone measurements. This study was supported by Top Institute Pharma (TIPharma Project T2-110), by Grants 2001T041 (M.V.E.) and 2003B134 (R.O.) from the Netherlands Heart Foundation, and by VIDI Grant 917.66.301 from the Netherlands Organization for Scientific Research (I.M. and M.V.E.). M.V.E. is an Established Investigator of the Netherlands Heart Foundation (Grant 2007T056).

REFERENCES

1. de Kloet, E. R., W. Sutanto, D. T. van den Berg, M. P. Carey, A. D. van Haarst, C. D. Hornsby, O. C. Meijer, N. Y. Rots, and M. S. Oitzl. 1993. Brain mineralocorticoid receptor diversity: functional implications. *J. Steroid Biochem. Mol. Biol.* **47:** 183–190.
2. Webster, J. I., L. Tonelli, and E. M. Sternberg. 2002. Neuroendocrine regulation of immunity. *Annu. Rev. Immunol.* **20:** 125–163.
3. De Martino, M. U., S. Alesci, G. P. Chrousos, and T. Kino. 2004. Interaction of the glucocorticoid receptor and the chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII): implications for the actions of glucocorticoids on glucose, lipoprotein, and xenobiotic metabolism. *Ann. N. Y. Acad. Sci.* **1024:** 72–85.
4. Kraemer, F. B. 2007. Adrenal cholesterol utilization. *Mol. Cell. Endocrinol.* **265–266:** 42–45.
5. Acton, S. L., P. E. Scherer, H. F. Lodish, and M. Krieger. 1994. Expression cloning of SR-BI, a CD36-related class B scavenger receptor. *J. Biol. Chem.* **269:** 21003–21009.
6. Acton, S., A. Rigotti, K. T. Landschulz, S. Xu, H. H. Hobbs, and M. Krieger. 1996. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science.* **271:** 518–520.
7. Murao, K., V. Terpstra, S. R. Green, N. Kondratenko, D. Steinberg, and O. Quehenberger. 1997. Characterization of CLA-1, a human homologue of rodent scavenger receptor BI, as a receptor for high density lipoprotein and apoptotic thymocytes. *J. Biol. Chem.* **272:** 17551–17557.
8. Landschulz, K. T., R. K. Pathak, A. Rigotti, M. Krieger, and H. H. Hobbs. 1996. Regulation of scavenger receptor, class B, type I, a high density lipoprotein receptor, in liver and steroidogenic tissues of the rat. *J. Clin. Invest.* **98:** 984–995.
9. Temel, R. E., B. Trigatti, R. B. DeMattos, S. Azhar, M. Krieger, and D. L. Williams. 1997. Scavenger receptor class B, type I (SR-BI) is the major route for the delivery of high density lipoprotein cholesterol to the steroidogenic pathway in cultured mouse adrenocortical cells. *Proc. Natl. Acad. Sci. USA.* **94:** 13600–13605.
10. Out, R., M. Hoekstra, J. A. Spijkers, J. K. Kruijt, M. Van Eck, I. S. Bos, J. Twisk, and T. J. Van Berkel. 2004. Scavenger receptor class B type I is solely responsible for the selective uptake of cholesteryl esters from HDL by the liver and the adrenals in mice. *J. Lipid Res.* **45:** 2088–2095.
11. Rigotti, A., B. L. Trigatti, M. Penman, H. Rayburn, J. Herz, and M. Krieger. 1997. A targeted mutation in the murine gene encoding the high density lipoprotein (HDL) receptor scavenger receptor class B type I reveals its key role in HDL metabolism. *Proc. Natl. Acad. Sci. USA.* **94:** 12610–12615.
12. Van Eck, M., J. Twisk, M. Hoekstra, B. T. Van Rij, C. A. Van der Lans, I. S. Bos, J. K. Kruijt, F. Kuipers, and T. J. Van Berkel. 2003. Differential effects of scavenger receptor BI deficiency on lipid metabolism in cells of the arterial wall and in the liver. *J. Biol. Chem.* **278:** 23699–23705.
13. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911–917.
14. Reljic, R., M. Ries, N. Anic, and B. Ries. 1992. New chromogen for assay of glucose in serum. *Clin. Chem.* **38:** 522–525.
15. Hoekstra, M., J. K. Kruijt, M. Van Eck, and T. J. Van Berkel. 2003. Specific gene expression of ATP-binding cassette transporters and nuclear hormone receptors in rat liver parenchymal, endothelial, and Kupffer cells. *J. Biol. Chem.* **278:** 25448–25453.
16. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162:** 156–159.
17. Rigotti, A., H. E. Miettinen, and M. Krieger. 2003. The role of the high-density lipoprotein receptor SR-BI in the lipid metabolism of endocrine and other tissues. *Endocr. Rev.* **24:** 357–387.
18. Cole, T. J., H. J. Harris, I. Hoong, N. Solomon, R. Smith, Z. Krozowski, and M. J. Fullerton. 1999. The glucocorticoid receptor is essential for maintaining basal and dexamethasone-induced repression of the murine corticosteroid-binding globulin gene. *Mol. Cell. Endocrinol.* **154:** 29–36.
19. Smith, C. L., and G. L. Hammond. 1992. Hormonal regulation of corticosteroid-binding globulin biosynthesis in the male rat. *Endocrinology.* **130:** 2245–2251.
20. Mitropoulos, K. A., and S. Balasubramaniam. 1976. The role of glucocorticoids in the regulation of the diurnal rhythm of hepatic beta-hydroxy-beta-methylglutaryl-coenzyme A reductase and cholesterol 7 alpha-hydroxylase. *Biochem. J.* **160:** 49–55.
21. Casals, N., N. Roca, M. Guerrero, G. Gil-Gomez, J. Ayte, C. J. Ciudad, and F. G. Hegardt. 1992. Regulation of the expression of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene. Its role in the control of ketogenesis. *Biochem. J.* **283:** 261–264.
22. Hanniman, E. A., G. Lambert, Y. Inoue, F. J. Gonzalez, and C. J. Sinal. 2006. Apolipoprotein A-IV is regulated by nutritional and metabolic stress: involvement of glucocorticoids, HNF-4 alpha, and PGC-1 alpha. *J. Lipid Res.* **47:** 2503–2514.
23. Nakamura, A., T. Kohsaka, D. Jin, and N. Kobayashi. 1992. Influence of glucocorticoid, aspirin, and gamma globulin on interleukin-6 production in the hepatoma cell line. *Immunopharmacology.* **24:** 31–36.
24. Vanden Berghe, W., E. Francesconi, K. De Bosscher, M. Resche-Rigon, and G. Haegeman. 1999. Dissociated glucocorticoids with anti-inflammatory potential repress interleukin-6 gene expression by a nuclear factor-kappaB-dependent mechanism. *Mol. Pharmacol.* **56:** 797–806.
25. Elenkov, I. J., and G. P. Chrousos. 2002. Stress hormones, proinflammatory and antiinflammatory cytokines, and autoimmunity. *Ann. N. Y. Acad. Sci.* **966:** 290–303.
26. Brundert, M., A. Ewert, J. Heeren, B. Behrendt, R. Ramakrishnan, H. Greten, M. Merkel, and F. Rinninger. 2005. Scavenger receptor class B type I mediates the selective uptake of high-density lipoprotein-associated cholesteryl ester by the liver in mice. *Arterioscler. Thromb. Vasc. Biol.* **25:** 143–148.

Downloaded from www.jlr.org by guest, on June 14, 2012

27. Trigatti, B., H. Rayburn, M. Vinals, A. Braun, H. Miettinen, M. Penman, M. Hertz, M. Schrenzel, L. Amigo, A. Rigotti, et al. 1999. Influence of the high density lipoprotein receptor SR-BI on reproductive and cardiovascular pathophysiology. *Proc. Natl. Acad. Sci. USA.* **96:** 9322–9327.
28. Ho, M. M., and G. P. Vinson. 1997. Peptide growth factors and the adrenal cortex. *Microsc. Res. Tech.* **36:** 558–568.
29. Blair, S. C., I. D. Caterson, and G. J. Cooney. 1994. Effect of adrenalectomy on glucose tolerance and lipid metabolism in gold-thioglucose obese mice. *Am. J. Physiol.* **266:** E993–E1000.
30. Cassuto, H., K. Kochan, K. Chakravarty, H. Cohen, B. Blum, Y. Olswang, P. Hakimi, C. Xu, D. Massillon, R. W. Hanson, et al. 2005. Glucocorticoids regulate transcription of the gene for phosphoenolpyruvate carboxykinase in the liver via an extended glucocorticoid regulatory unit. *J. Biol. Chem.* **280:** 33873–33884.
31. Friedman, J. E., J. S. Yun, Y. M. Patel, M. M. McGrane, and R. W. Hanson. 1993. Glucocorticoids regulate the induction of phosphoenolpyruvate carboxykinase (GTP) gene transcription during diabetes. *J. Biol. Chem.* **268:** 12952–12957.
32. Leung, K., and A. Munck. 1975. Peripheral actions of glucocorticoids. *Annu. Rev. Physiol.* **37:** 245–272.
33. Cai, L., A. Ji, F. C. de Beer, L. R. Tannock, and D. R. van der Westhuyzen. 2008. SR-BI protects against endotoxemia in mice through its roles in glucocorticoid production and hepatic clearance. *J. Clin. Invest.* **118:** 364–375.
34. Hsu, L. A., Y. L. Ko, S. Wu, M. S. Teng, T. Y. Peng, C. F. Chen, C. F. Chen, and Y. S. Lee. 2003. Association between a novel 11-base pair deletion mutation in the promoter region of the scavenger receptor class B type I gene and plasma HDL cholesterol levels in Taiwanese Chinese. *Arterioscler. Thromb. Vasc. Biol.* **23:** 1869–1874.
35. Morabia, A., B. M. Ross, M. C. Costanza, E. Cayanis, M. S. Flaherty, G. B. Alvin, K. Das, R. James, A. S. Yang, O. Evagrafov, et al. 2004. Population-based study of SR-BI genetic variation and lipid profile. *Atherosclerosis.* **175:** 159–168.
36. Osgood, D., D. Corella, S. Demissie, L. A. Cupples, P. W. Wilson, J. B. Meigs, E. J. Schaefer, O. Coltell, and J. M. Ordovas. 2003. Genetic variation at the scavenger receptor class B type I gene locus determines plasma lipoprotein concentrations and particle size and interacts with type 2 diabetes: the Framingham Study. *J. Clin. Endocrinol. Metab.* **88:** 2869–2879.
37. Acton, S., D. Osgood, M. Donoghue, D. Corella, M. Pocovi, A. Cenarro, P. Mozas, J. Keilty, S. Squazzo, E. A. Woolf, et al. 1999. Association of polymorphisms at the SR-BI gene locus with plasma lipid levels and body mass index in a white population. *Arterioscler. Thromb. Vasc. Biol.* **19:** 1734–1743.

ASBMB JOURNAL OF LIPID RESEARCH

Downloaded from www.jlr.org by guest, on June 14, 2012