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Hoekstra, M.; Ouweneel, A.B.; Price, J.; Geest, R. van der; Sluis, R.J. van der; Geerling, J.J.; ... ; Eck, M. van

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ORIGINAL STUDY

SR-BI deficiency disassociates obesity from hepatic steatosis and glucose intolerance development in high fat diet-fed mice[☆]

Menno Hoekstra^{*}, Amber B. Ouweneel, Juliet Price, Rick van der Geest, Ronald J. van der Sluis, Janine J. Geerling, Joya E. Nahon, Miranda Van Eck

Division of BioTherapeutics, Leiden Academic Centre for Drug Research, Leiden University, Gorlaeus Laboratories, Einsteinweg 55, 2333CC Leiden, The Netherlands

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Abstract

Scavenger receptor BI (SR-BI) has been suggested to modulate adipocyte function. To uncover the potential relevance of SR-BI for the development of obesity and associated metabolic complications, we compared the metabolic phenotype of wild-type and SR-BI deficient mice fed an obesogenic diet enriched in fat. Both male and female SR-BI knockout mice gained significantly more weight as compared to their wild-type counterparts in response to 12 weeks high fat diet feeding (1.5-fold; $P < .01$ for genotype). Plasma free cholesterol levels were ~2-fold higher ($P < .001$) in SR-BI knockout mice of both genders, whilst plasma cholesteryl ester and triglyceride concentrations were only significantly elevated in males. Strikingly, the exacerbated obesity in SR-BI knockout mice was paralleled by a better glucose handling. In contrast, only SR-BI knockout mice developed atherosclerotic lesions in the aortic root, with a higher predisposition in females. Biochemical and histological studies in male mice revealed that SR-BI deficiency was associated with a reduced hepatic steatosis degree as evident from the 29% lower ($P < .05$) liver triglyceride levels. Relative mRNA expression levels of the glucose uptake transporter GLUT4 were increased (+47%; $P < .05$), whilst expression levels of the metabolic PPARgamma target genes CD36, HSL, ADIPOQ and ATGL were reduced 39%–58% ($P < .01$) in the context of unchanged PPARgamma expression levels in SR-BI knockout gonadal white adipose tissue. In conclusion, we have shown that SR-BI deficiency is associated with a decrease in adipocyte PPARgamma activity and a concomitant uncoupling of obesity development from hepatic steatosis and glucose intolerance development in high fat diet-fed mice.

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Keywords: scavenger receptor BI; obesity; glucose intolerance; hepatic steatosis; adipocyte; PPARgamma.

1. Introduction

Scavenger receptor class B type I (SR-BI) is a membrane-associated glycoprotein that can bind native lipoprotein species such as high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoproteins (VLDL), and chylomicrons as well as their modified forms [1]. SR-BI is highly expressed

in hepatocytes and steroidogenic tissues, where it mediates the selective uptake of cholesteryl esters from HDL [2]. Exemplary for the important physiological role for SR-BI in total body HDL metabolism are the observations that SR-BI deficiency in mice is associated with the accumulation of cholesterol-enriched HDL particles in the circulation [3] and a decrease in biliary cholesterol secretion [4]. The function of SR-BI appears to be largely conserved since human subjects carrying a functional mutation in the SR-BI gene similarly display an increase in plasma HDL-cholesterol levels [5,6]. Lack of proper SR-BI functionality is also consistently associated with glucocorticoid insufficiency, that is, a diminished ability of the adrenals to produce respectively cortisol in humans and corticosterone in mice [5,7–9]. Furthermore, SR-BI deficiency markedly stimulates the development of atherosclerotic lesions in mice [10] and several rare variants are associated with a significantly higher (atherosclerotic) cardiovascular disease frequency in humans [6,11].

White adipose tissue adipocytes are essential in the bodies response to overconsumption and the associated protection against lipotoxicity due to their ability to store excess energy. A defect in white adipocyte functionality can therefore predispose to obesity

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^{*} Corresponding author at: Division of BioTherapeutics, Leiden Academic Centre for Drug Research, Leiden University, Gorlaeus Laboratories, Einsteinweg 55, 2333CC Leiden, The Netherlands, Tel.: +31-71-5276582.

E-mail address: hoekstra@lacdr.leidenuniv.nl (M. Hoekstra).

and metabolic pathologies such as type 2 diabetes, nonalcoholic fatty liver disease, and cardiovascular disease. Interestingly, SR-BI has also been suggested to impact adipocyte functionality. Relatively high mRNA expression levels of SR-BI have been detected in the human 3T3-L1 adipocyte cell line as well as in murine white adipose tissue [12], with the expression of SR-BI increasing during adipocyte differentiation [12,13]. Moreover, SR-BI mRNA and protein expression levels in murine white adipose tissue are significantly higher in the ad libitum fed (high glucose / high insulin) state than in the fasted (low glucose / low insulin) state [14]. In line with a previously proposed function of SR-BI in cellular cholesterol efflux, a higher expression of SR-BI in adipocytes also translates into a higher rate of cholesterol efflux to HDL [15], whilst SR-BI knockout adipocytes display an attenuated cholesterol efflux capacity as compared to wild-type adipocytes [16]. In line, Yvan Charvet et al. found that basal SR-BI mRNA expression levels correlate significantly with the intracellular cholesterol content of the 3T3-L1 adipocyte cell line [14]. In addition to their function in adipocyte cholesterol homeostasis, HDL and SR-BI have also been implicated in glucose and triglyceride metabolism. In vitro studies have shown that blockade of SR-BI function is associated with a diminished HDL-induced increase in glucose uptake and the extent of cellular triglyceride accumulation by adipocytes [14,17]. In further support of a potential link between adipocyte SR-BI functionality and glucose homeostasis, treatment of adipocytes in culture with insulin induces the translocation of SR-BI protein from intracellular stores towards the cell surface [13]. Thus, several lines of evidence suggest that SR-BI is an important player in total body lipid and glucose metabolism. To uncover the potential relevance of SR-BI function for the development of obesity and associated metabolic complications, we compared the metabolic phenotype of wild-type and SR-BI deficient mice fed an obesogenic diet enriched in fat.

2. Materials and methods

2.1. Mice

All animal work was approved by the Leiden University Animal Ethics committee and performed in compliance with the Dutch government guidelines and the Directive 2010/63/EU of the European Parliament. Age-matched 8- to 11-week old male and female C57BL/6 wild-type mice (N = 10 and N = 9) and SR-BI knockout mice (N = 10 and N = 9) were provided ad libitum with high fat diet #12451 from Research Diet Service BV, The Netherlands containing the fat component lard (45% of total energy), the protein components casein and cysteine (20% of total energy) and the carbohydrate sources sucrose, Iodex 10, and corn starch (35% of total energy). For a more detailed view on the diet composition please visit: <https://www.researchdiets.com/formulas/d12451>. Body weight was monitored on a weekly basis. No significant effect of the genotype on food intake was detected. After 4 weeks of feeding, 1 male wild-type mouse had to be taken out of the experiment as a result of severe fighting wounds. From this point onwards, the 19 remaining male mice were kept single-housed. After 10 weeks of diet feeding, all mice were fasted overnight and subsequently given an oral 2 g/kg glucose bolus in water. Tail blood samples were taken from 6 randomly chosen mice per group before and 15, 30, 45, 60, 90, and 120 minutes after glucose administration for determination of blood glucose levels using an Accu-Check Compact monitor (Roche Diagnostics, Almere, The Netherlands). After respectively 12 and 13 weeks of high fat diet feeding, the male and female mice were fasted overnight, anesthetized by subcutaneous injection with a mix of 70 mg/kg body weight xylazine, 1.8 mg/kg bodyweight atropine and 350 mg/kg body weight ketamine, bled via retro-orbital bleeding for plasma lipid and hormone measurements, sacrificed, and subjected to whole body perfusion with PBS. Organs were collected, weighed, and stored at -20°C or fixed overnight in 3.7% neutral-buffered formalin solution (Formalfix; Shandon Scientific Ltd, United Kingdom).

2.2. Plasma measurements

Concentrations of free cholesterol and cholesteryl esters were measured using standard colorimetric assays as described by Out et al. [18]. The cholesterol distribution over the different lipoproteins was analyzed by fractionation of 30 μ l plasma using a Superose 6 column (3.2 \times 30 mm, Smart-system, Pharmacia). Total

cholesterol content of the effluent was determined using the same colorimetric assays. Triglycerides were detected using a colorimetric assay from Roche Diagnostics. Adipose tissue-derived hormones resistin and leptin were measured using a Bio-Plex Pro mouse diabetes 8-plex immunoassay (Bio-Rad). Adiponectin levels were quantified in plasma using the mouse adiponectin/Acrp30 DuoSet ELISA from R&D systems.

2.3. Tissue lipid extraction and quantification

The liver triglyceride content was determined through the use of a Nonidet P 40 Substitute-based extraction protocol, essentially as described by Nahon et al. [19]. Triglyceride levels were measured with an enzymatic colorimetric assay (Roche) and corrected for the tissue weight. Cholesterol was extracted from liver tissue using the Folch extraction method [20]. The concentration of free cholesterol and cholesteryl esters was determined using the enzymatic colorimetric assays [18]. Free cholesterol and cholesteryl ester levels were corrected for the protein input, determined using a Pierce BCA Protein Assay Kit (ThermoFisher Diagnostics, Waltham, MA, USA).

2.4. Histological analysis

Gonadal white adipose tissue and livers were embedded in paraffin and sectioned at 5 μ m thickness. Paraffin sections were stained with hematoxylin and eosin for imaging on a Leica DMRE microscope. Leica QWin imaging software (Leica Ltd., Cambridge, England) was used to measure the number of adipocytes per area from 2 sections per mouse and calculate the average adipocyte size. The aortic root of the heart was cut into 10 μ m thick serial sections starting from the three valve area using a Leica CM3050S cryostat. Cryosections were routinely stained for neutral lipids using Oil red O for blinded quantification with the Leica QWin imaging software. Mean lesion area of each individual mouse was calculated from 5 cryosections.

2.5. Gene expression analysis

Total RNA was isolated by the acid guanidinium thiocyanate-phenol chloroform extraction method and reverse-transcribed using RevertAid reverse transcriptase. Quantitative gene expression analysis was performed on an ABI-PRISM 7500 Fast machine (Applied Biosystems) using SYBR Green technology. Hypoxanthine guanine phosphoribosyl transferase, β -actin, glyceraldehyde 3-phosphate dehydrogenase, peptidylprolyl isomerase, acidic ribosomal phosphoprotein P0, and 60S ribosomal protein were used as housekeeping genes.

2.6. Data analysis

Statistical analysis was performed using Graphpad Instat software (San Diego, USA, <http://www.graphpad.com>). Normality of the experimental groups was confirmed using the method of Kolmogorov and Smirnov. The significance of differences was calculated using a two-tailed unpaired t-test or two-way analysis of variance (ANOVA) with Bonferroni post-test where appropriate. Probability values less than 0.05 were considered significant.

3. Results

Age-matched SR-BI knockout mice and C57BL/6 wild-type control mice of both genders were fed a commonly used high fat (45% of energy) lard diet for 12–13 weeks to stimulate the development of obesity [21–23]. Both the wild-type and SR-BI knockout male mice weighed more than their female counterparts at baseline, with no significant baseline effect of the SR-BI genotype being present (Fig. 1A). As can be appreciated from Figs. 1A and 1B, male wild-type mice as compared to female wild-type controls also were generally more prone to become obese in response to the high fat diet challenge. Wild-type male mice gained on average $33 \pm 5\%$ of weight versus $24 \pm 3\%$ in wild-type females (Fig. 1A). Importantly, as also evident from Figs. 1A and 1B, SR-BI deficiency was associated with an exacerbated body weight gain in both male and female mice (1.5-fold; two-way ANOVA $P < .01$ for genotype). The weight gain stimulating effect of SR-BI deficiency was independent of the gender of mice (two-way ANOVA: $P > .05$ for interaction). As a result, SR-BI knockout male and female mice had gained $51 \pm 4\%$ and $38 \pm 4\%$ of weight at the end of the high fat diet challenge (Fig. 1A). This translated into a respective final body weight of 32.6 ± 1.8 and 37.1 ± 1.4 grams in male wild-type and

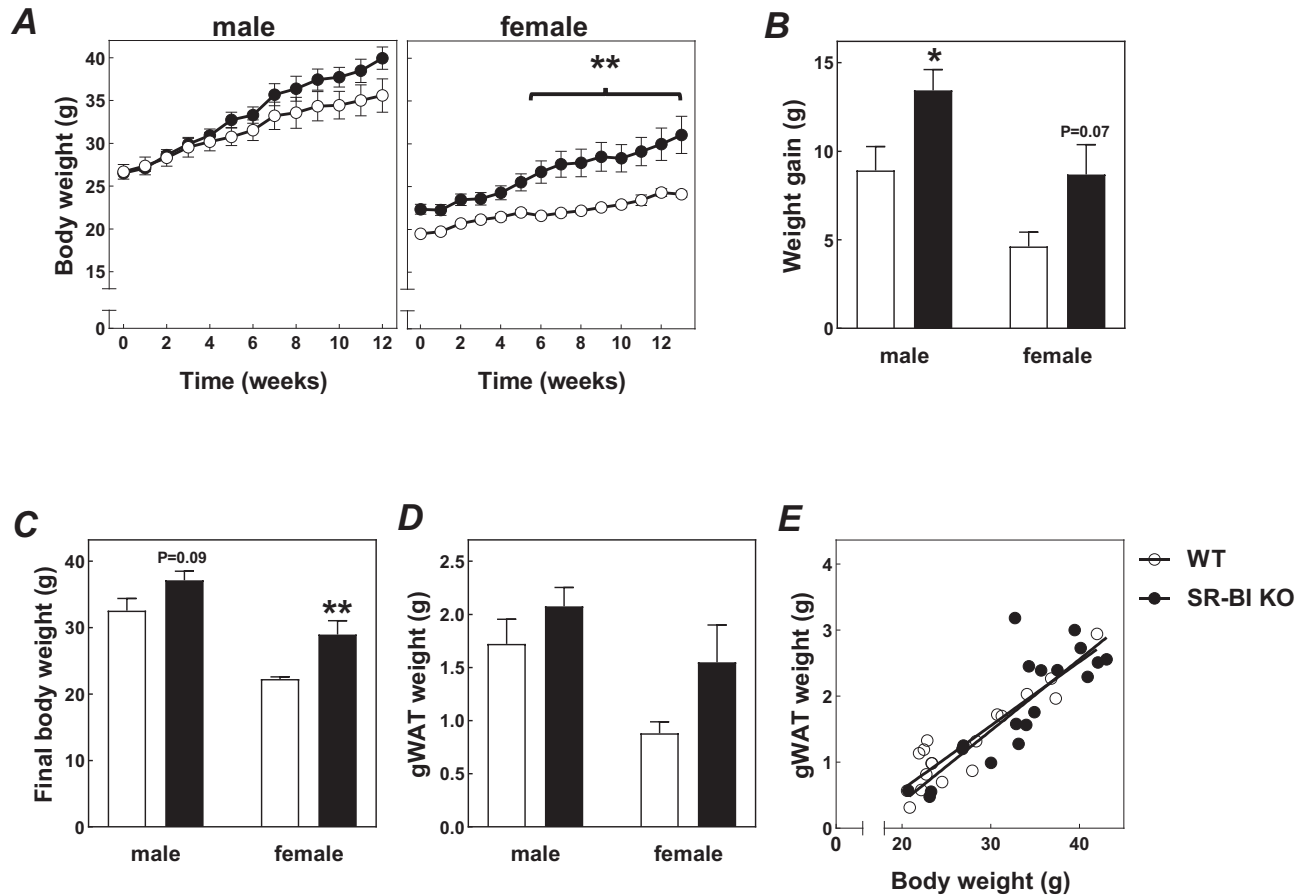


Fig. 1. Effect of total body SR-BI deficiency on body composition in high fat diet-fed mice. (A) Body weight development upon high fat diet feeding. (B) High fat diet-induced absolute weight gain. Absolute body weights (C) and gonadal white adipose (gWAT) tissue weights (D) measured at sacrifice. (E) Correlations between body weights and gWAT weights of individual mice. White bars & dots represent C57BL/6 wild-type (WT) mice and black bars & dots represent SR-BI knockout (SR-BI KO) mice. Data represent means \pm SEM of 9–10 mice per group. Two-way ANOVA Bonferroni post-test: * $P < .05$, ** $P < .01$.

SR-BI knockout mice and 22.3 ± 0.3 and 29.0 ± 2.1 grams in female wild-type and SR-BI knockout mice (Fig. 1C). The differences in total body weights were paralleled by similar changes in the gonadal white adipose tissue weight profile (two-way ANOVA: $P < .05$ for genotype; $P < .01$ for gender; Fig. 1D). In further support of the notion that enhanced adiposity was the major driver behind the exacerbated obesity, a highly significant correlation between gonadal white adipose tissue weights and respective body weights was detected in mice of both genotypes ($R^2 = 0.85$ for C57BL/6 mice and $R^2 = 0.71$ for SR-BI KO mice; $P < .001$; Fig. 1E). Linear regression analysis suggested that the SR-BI genotype did not significantly change the relationship between gonadal white adipose tissue and total body weights, as can be appreciated from the almost identical slopes of the two regression lines displayed in Fig. 1E.

In humans, obesity predisposes to the development of dyslipidemia, typically characterized by increased plasma triglyceride levels and decreased HDL-cholesterol [24]. Plasma levels of three major lipoprotein lipid classes—free cholesterol, cholesteryl esters and triglycerides—were measured in the experimental groups of mice at the day of sacrifice, that is, at 12–13 weeks on high fat diet after overnight fasting. Plasma total cholesterol levels were elevated as a result of the SR-BI deficiency (2.02 ± 0.05 mg/mL vs 1.01 ± 0.07 mg/mL for males and 1.30 ± 0.06 mg/mL vs 0.68 ± 0.02 mg/mL for females). As can be seen in Fig. 2A, plasma free cholesterol levels were increased to a similar extent (~2-fold) in both male and female mice in response to the genetic lack of SR-BI

(two-way ANOVA: $P < .001$ for genotype; $P < .001$ for gender; $P > .05$ for interaction). Plasma cholesteryl ester levels were also markedly increased (+84%; Bonferroni post-test: $P < .05$) in male mice, while the less extensive elevation in plasma cholesteryl esters in females (+39%) failed to reach significance (Fig. 2A). Fractionation of pooled plasma from male SR-BI knockout and C57BL/6 wild-type mice revealed that the SR-BI deficiency-associated increase in plasma cholesterol levels could primarily be attributed to a ≥ 2 -fold increase in LDL-cholesterol and HDL-cholesterol levels (Fig. 2B). Two-way ANOVA analysis suggested that the SR-BI genotype also significantly influenced plasma triglyceride levels ($P < .05$ for genotype). This overall effect was, however, fully driven by a triglyceride-raising effect of SR-BI deficiency in male mice only (+41%; Bonferroni post-test: $P < .01$).

Obesity and dyslipidemia constitute a risk factor for the development of glucose intolerance and type 2 diabetes in both humans and mice. Previous studies have indicated that feeding an obesogenic high fat diet to mice reduces glucose tolerance, in a gender-dependent manner—with male mice being more susceptible to develop glucose intolerance in response to the high fat diet challenge [25,26]. In agreement, our male wild-type mice as compared to our wild-type female mice appeared more glucose intolerant in response to the high fat diet feeding challenge as evidenced by their failure to return to baseline values within the 120-minute test period (Fig. 3A). Strikingly, in contrast to the general assumption that exacerbated obesity will translate into an impaired glucose

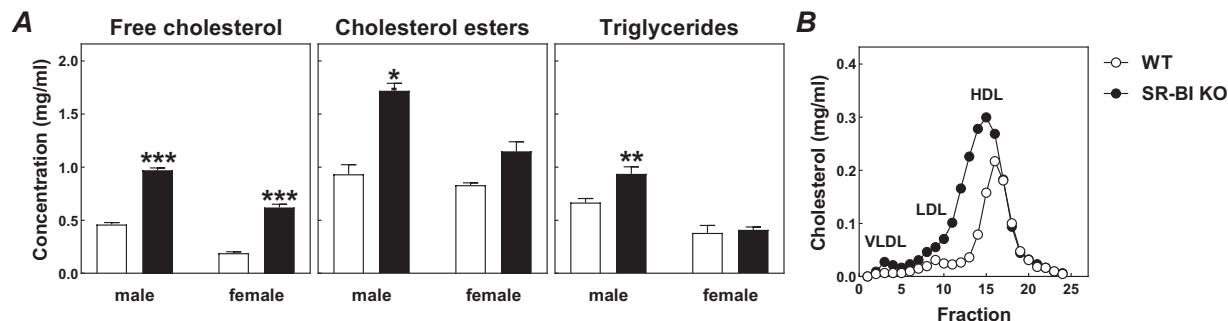


Fig. 2. Effect of total body SR-BI deficiency on plasma lipid levels in high fat diet-fed mice. (A) Plasma lipid levels measured at sacrifice after overnight fasting, i.e. 12–13 weeks on high fat diet. (B) Representative lipoprotein profile generated from pooled plasma of male mice. White bars & dots represent C57BL/6 wild-type (WT) mice and black bars & dots represent SR-BI knockout (SR-BI KO) mice. Data represent means+SEM of 9–10 mice per group. Two-way ANOVA Bonferroni post-test: * $P < .05$, ** $P < .01$, *** $P < .001$.

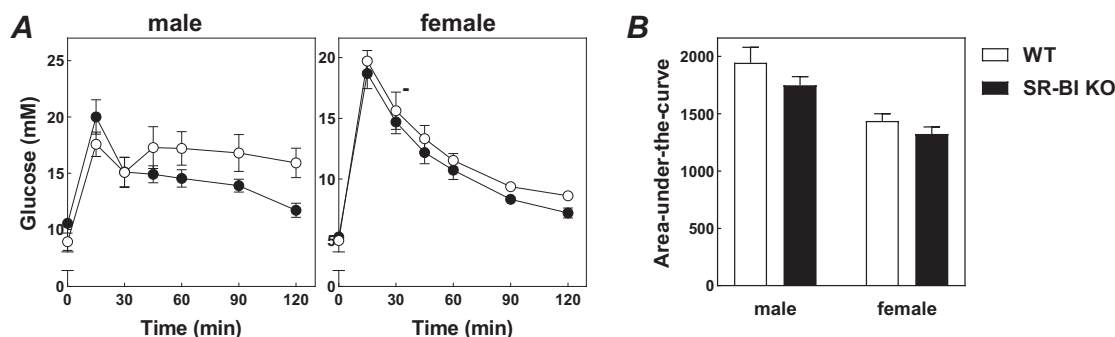


Fig. 3. Effect of total body SR-BI deficiency on the glucose tolerance in high fat diet-fed mice after overnight fasting. (A) Blood glucose levels were measured in response to an oral glucose bolus after 10 weeks on the high fat diet. White dots represent C57BL/6 wild-type (WT) mice and black dots represent SR-BI knockout (SR-BI KO) mice. (B) Quantification of the area-under-the-curves of the data presented in panel A. Data represent means+SEM of 6 mice per group.

tolerance, the genetic absence of SR-BI had a beneficial impact on the glucose tolerance. The positive effect of SR-BI deficiency on glucose tolerance was most apparent in male mice, probably because of the fact that the female wild-type mice were much less obese as compared to their male counterparts and therefore still able to respond very well to the glucose challenge. As evident from Fig. 3A, blood glucose levels did almost return to baseline values in male SR-BI knockout mice within the 120-minute test period. Furthermore, glucose levels returned back to baseline values slightly faster in female SR-BI knockout mice than in female wild-type mice. Quantification of the area-under-the-curve further showed the relative impact of the SR-BI genotype (two-way ANOVA $P = .095$; 6% of total variation) and gender (two-way ANOVA $P < .001$; 54% of total variation) on the blood glucose responses to the oral glucose challenge in our high fat diet-fed mice (Fig. 3B).

Previous studies showed that total body SR-BI knockout mice exhibit a relatively high susceptibility for the development of atherosclerotic lesions when fed a Western-type diet enriched in both cholesterol and fat [10,27]. To uncover whether this phenotype is also evident when mice are challenged with the obesogenic diet specifically enriched in fat, cryosections of the aortic root of our experimental mice were stained with Oil red O to visualize neutral lipid deposition and potentially identify atherosclerotic lesions. As can be acknowledged from the representative images in Fig. 4A and the plaque size quantification in Fig. 4B, wild-type mice were resistant to the development of atherosclerotic lesions upon high fat diet feeding. No atherosclerotic lesions were seen in the aortic root of any of the male and female wild-type mice. In contrast, small atherosclerotic lesions could be detected in the aortic root of all SR-BI knockout mice (Figs. 4A & 4B; $P < .001$ vs WT for both genders). In line with earlier findings [27], female SR-BI knockout mice displayed a relatively higher atherosclerosis

susceptibility compared to male SR-BI knockout mice ($11.1 \pm 1.7 \times 10^3 \mu\text{m}^2$ for females versus $6.8 \pm 1.5 \times 10^3 \mu\text{m}^2$ for males; Fig. 4B). A chronic fat challenge alone (without cholesterol additive) is thus apparently sufficient to stimulate the development of small atherosclerotic lesions in SR-BI knockout mice.

The effect of SR-BI deficiency on body weight development, plasma cholesterol and triglyceride levels and the glucose tolerance extent (but not atherosclerosis susceptibility) appeared to be larger in males than in females under high fat diet feeding conditions. We therefore further analyzed plasma specimens and organs from male mice, collected in the overnight fasted state, to uncover why the relatively higher obesity extent was not paralleled by a greater degree of glucose intolerance in high fat diet-fed SR-BI knockout mice. Humans suffering from non-alcoholic fatty liver disease are more likely to develop type 2 diabetes [28]. We therefore investigated whether a change in hepatic metabolic status could have contributed to the genotype-associated difference in glucose tolerance. No difference in hepatic free cholesterol or cholesteryl ester content was detected between male SR-BI knockout mice and wild-type mice after an overnight fasting period (Fig. 5A). In contrast, SR-BI deficiency was associated with reduced liver triglyceride stores (-29% ; $P < .05$; Fig. 5A). Histological analysis verified that the hepatic steatosis extent was reduced in SR-BI knockout mice as compared to wild-type mice (Fig. 5B). In further support, relative mRNA expression levels of the adiposity marker ABCD2 were markedly lower in livers of SR-BI knockout mice (-68% ; $P < .01$; Fig. 5C). Fatty acids stored in the hepatocyte triglyceride pool can theoretically be derived from extracellular sources, that is, from intestinally secreted chylomicrons and via flux from adipose tissue or acquired endogenously through de novo synthesis (lipogenesis). Hepatic mRNA expression levels of the lipoprotein receptors LDL receptor (LDLR) and LDL receptor-related protein 1 (LRP1) as

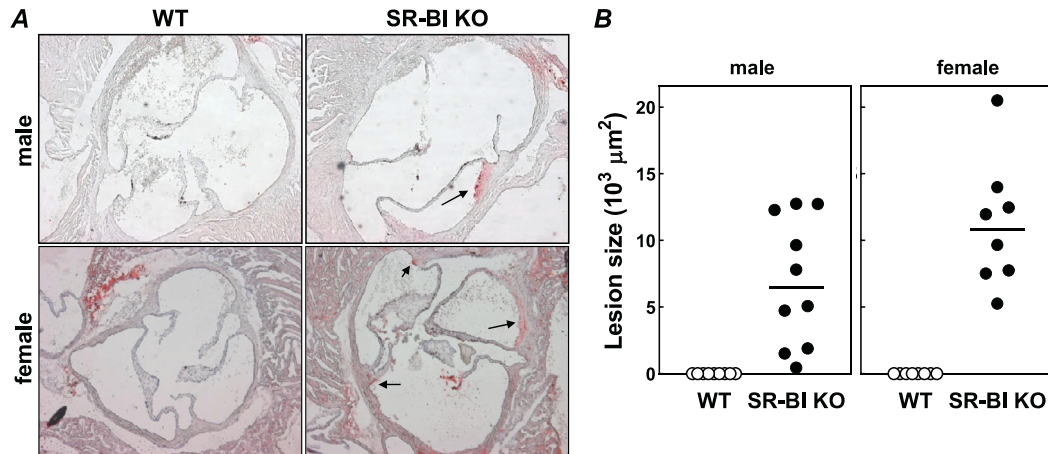


Fig. 4. Effect of total body SR-BI deficiency on atherosclerosis susceptibility in high fat diet-fed mice. (A) Representative images of Oil red O-stained aortic root cryosections. The arrows points towards Oil red O-positive atherosclerotic lesions. (B) Aortic root atherosclerotic lesion sizes. White dots represent C57BL/6 wild-type (WT) mice and black dots represent SR-BI knockout (SR-BI KO) mice.

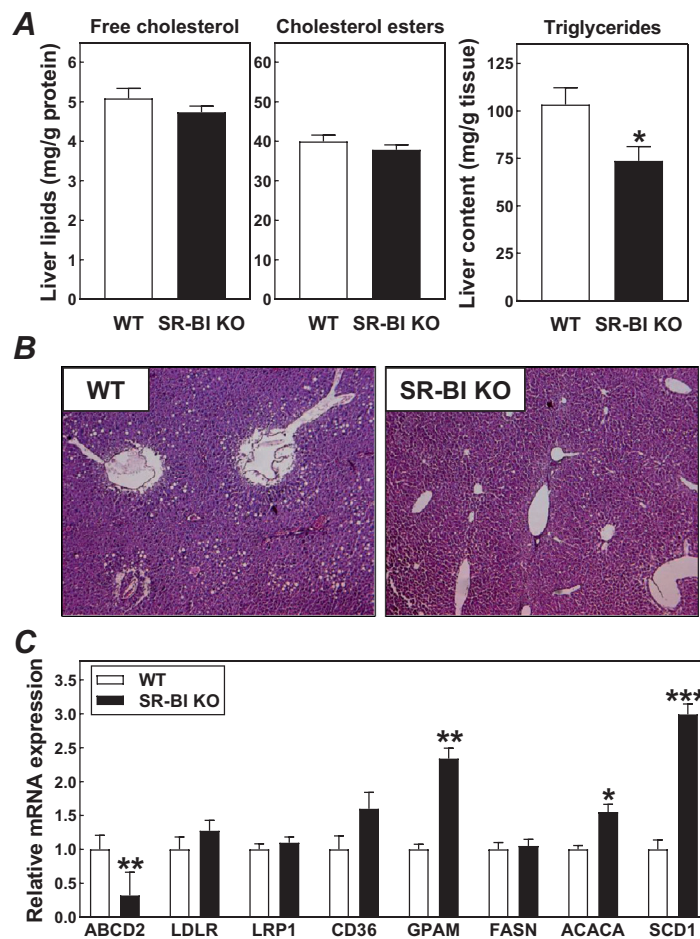


Fig. 5. Effect of total body SR-BI deficiency on hepatic lipid stores and relative expression levels of genes involved in lipid metabolism in high fat diet-fed male mice after overnight fasting. (A) Hepatic lipid levels. (B) Representative images of hematoxylin & eosin-stained liver paraffin sections. (C) Hepatic relative mRNA expression levels of genes involved in lipid mobilization and synthesis. White bars represent C57BL/6 wild-type (WT) mice and black bars represent SR-BI knockout (SR-BI KO) mice. Data represent means+SEM of 9-10 mice per group. T-test: * $P < .05$, ** $P < .01$, *** $P < .001$.

well as the fatty acid translocase CD36 were not different in the two types of mice (Fig. 5C), which argues against an important role for these specific lipid transport molecules in the effect of SR-BI deficiency on hepatic triglyceride levels. Relative mRNA expression levels of the key lipogenic gene fatty acid synthase

(FASN) were also not changed (Fig. 5C). In contrast, significantly higher relative mRNA expression levels of the other lipogenesis genes GPAM (+134%; $P < .01$), ACACA (+55%; $P < .05$), and SCD1 (+199%; $P < .001$) were found in livers of SR-BI knockout mice as compared to wild-type mice (Fig. 5C). Combined, these

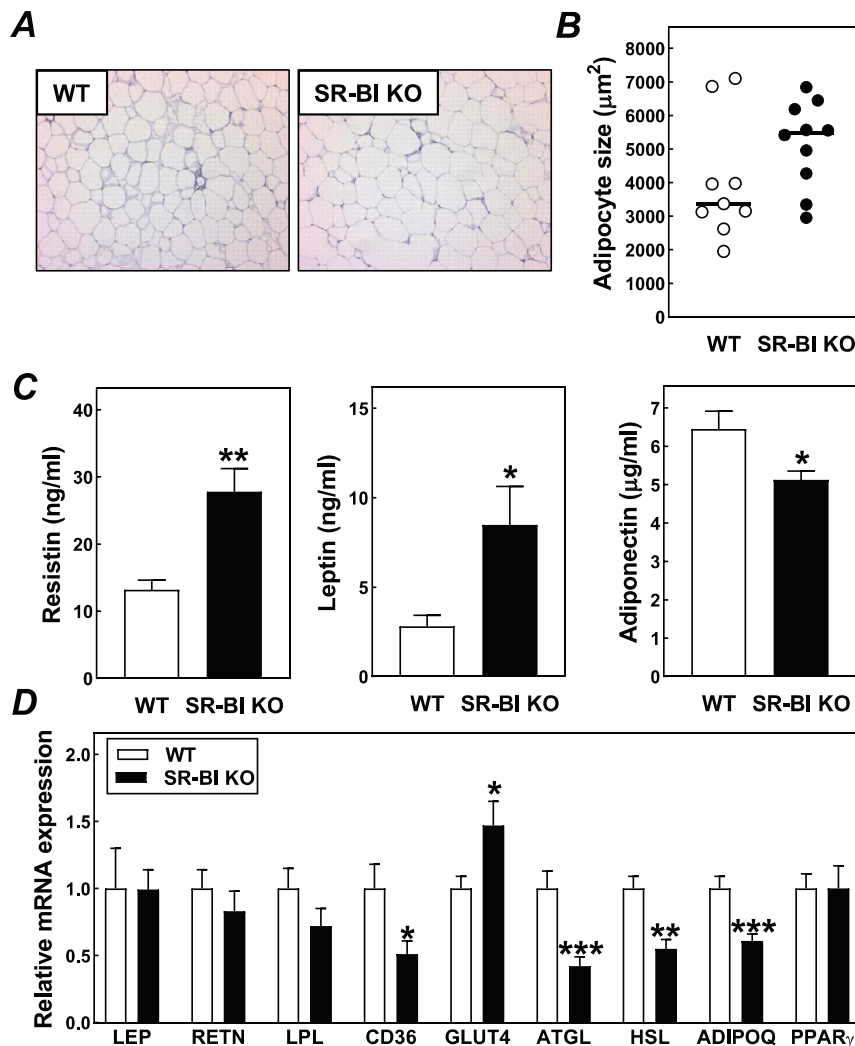


Fig. 6. Effect of total body SR-BI deficiency on the gonadal white adipose tissue phenotype and plasma adipokine levels in high fat diet-fed male mice after overnight fasting. (A) Representative images of hematoxylin and eosin-stained adipose tissue paraffin sections. (B) White adipocyte sizes of individual mice. (C) Plasma levels of the adipokines resistin and leptin. (D) Adipose tissue relative mRNA expression levels of adipokines, proteins involved in lipid and glucose mobilization and PPARgamma and its target genes. White bars & dots represent C57BL/6 wild-type (WT) mice and black bars & dots represent SR-BI knockout (SR-BI KO) mice. Data represent means+SEM of 9-10 mice per group. T-test: * $P < .05$, ** $P < .01$, *** $P < .001$.

findings indicate that the lowered hepatic triglyceride content was probably not secondary to a reduction in de novo lipogenesis. It is actually suggested that the lipogenesis rate was higher in SR-BI knockout mice, possibly as a (compensatory) response to the lowered hepatic influx of fatty acids into the triglyceride pool [29].

Since our study was based on the hypothesis that SR-BI deficiency can directly impact the metabolic function of adipocytes, we investigated the phenotype of the gonadal white adipose tissue depots. As can be appreciated from the representative images in Fig. 6A and the adipocyte cell area quantification in Fig. 6B, a clear trend towards an increase in the adipocyte size was detected in SR-BI knockout mice as compared to wild-type mice (+28%; $P = .13$). In humans, a higher obesity degree generally associates with relatively higher plasma levels of the adipose tissue-derived hormones resistin and leptin [30], whilst plasma levels of adiponectin are rather inversely correlated with body weight [31]. In accordance, plasma samples from SR-BI knockout mice contained relatively high levels of the adipokines resistin (+110%; $P < .01$) and leptin (+127%; $P < .05$) in the context of reduced adiponectin concentrations (-21%; $P < .014$) (Fig. 6C). Relative mRNA expression levels

of leptin (LEP) and resistin (RETN) were almost identical between gonadal white adipose tissue isolated from SR-BI knockout and wild-type mice (Fig. 6D), indicating that the increase in plasma adipokines levels was not resulting from a higher hormone production by individual adipocytes but rather due to a higher overall adipocyte number. Adipocytes can acquire fatty acids from the blood circulation through the combined action of lipoprotein lipase (LPL) that liberates fatty acids from triglyceride-rich lipoproteins and CD36 which mediates the actual cellular fatty acid uptake. Our gene expression analysis did not reveal a significant change in adipose tissue LPL transcript levels (Fig. 6D). However, CD36 mRNA expression levels were markedly lower in SR-BI knockout adipose tissue (-49%; $P < .05$; Fig. 6D). Notably, SR-BI deficiency was associated with a significant increase (+47%; $P < .05$) in GLUT4 expression levels (Fig. 6D), which suggests that the glucose uptake capacity of white adipocytes was higher in SR-BI knockout mice as compared to wild-type mice. The nuclear receptor peroxisome proliferator-activated receptor gamma (PPARgamma) is considered to be the driving force behind CD36 transcription in adipocytes [32], while data from Armoni et al. have suggested that

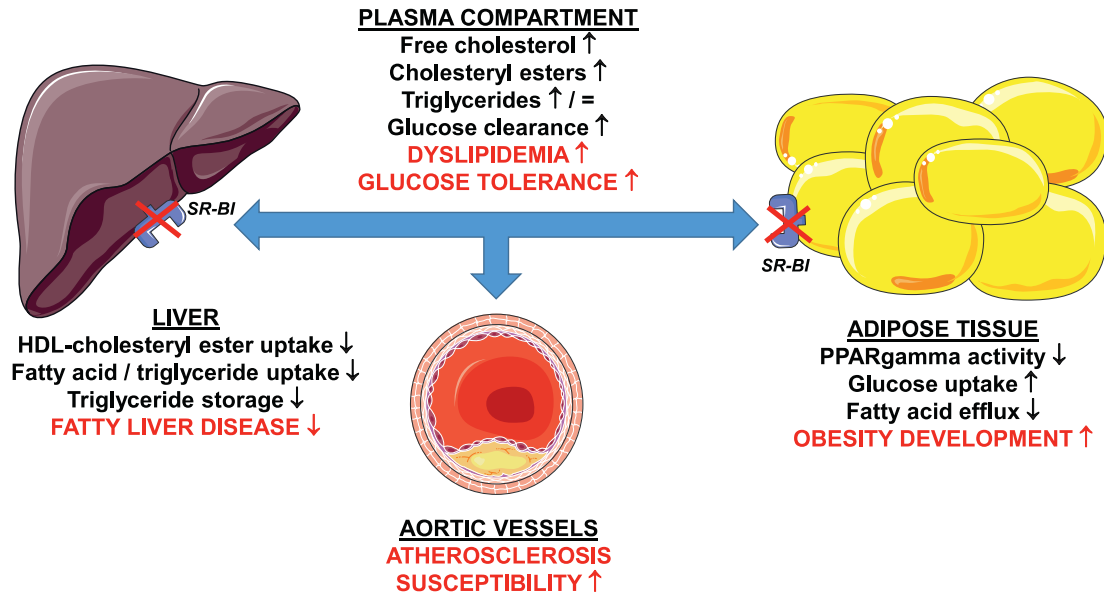


Fig. 7. Graphical overview of the effects of total body SR-BI deficiency on the metabolic profile in high fat diet-fed mice. The absence of SR-BI in adipocytes reduces the activity of the nuclear receptor PPARgamma which (1) increases cellular glucose uptake and improves the overall glucose tolerance and (2) reduces the flux of fatty acids from adipose tissue to the liver, thereby enhancing the predisposition to obesity. The parallel absence of SR-BI in hepatocytes further disrupts the flux adipocyte-derived fatty acids into the liver and impairs the uptake of HDL-cholesteryl esters. As a result, hepatic triglyceride stores are reduced. In contrast, plasma free cholesterol and cholesteryl ester levels are increased, resulting in a higher atherosclerosis susceptibility.

PPARgamma represses the transcriptional activity of the GLUT4 promoter [33]. Importantly, although PPARgamma gene expression levels were unaltered, mRNA expression levels of the established adipocyte PPARgamma target genes adipose triglyceride lipase / patatin like phospholipase domain containing 2 (ATGL/PNPLA2), hormone-sensitive lipase (HSL), and adiponectin were reduced by respectively 58% ($P < .001$), 46% ($P < .01$), and 39% ($P < .001$) in comparison to wild-type adipose tissue (Fig. 6D). Collectively, these data suggest that SR-BI deficiency is associated with a decrease in adipocyte PPARgamma activity that probably underlies the parallel shift in the energy mobilization status of adipocytes, that is, reduced fatty acid uptake by CD36 and excess storage of glucose obtained from the circulation through GLUT4.

4. Discussion

Here we have shown that SR-BI deficiency in high fat diet-fed mice is associated with a decrease in PPARgamma regulated genes in adipocytes, a higher predisposition to obesity and atherosclerotic lesion formation, and protection against the development of fatty liver disease and glucose intolerance (Fig. 7).

An interesting finding of our studies was that the reduced hepatic triglyceride content in SR-BI knockout mice after overnight fasting was not secondary to a decrease in de novo lipogenesis as the expression of key lipogenic genes was increased in liver as a result of SR-BI deficiency. Fasting liver triglyceride stores—that can also be used for VLDL production—are not primarily derived from de novo lipogenesis but rather generated via the flux of fatty acids from adipose tissue to the liver upon liberation during adipocyte triglyceride hydrolysis [34,35]. Notably, the SR-BI deficiency-associated decrease in extent of hepatic steatosis was paralleled by a reduction in adipocyte PPARgamma activity—as evidenced by the lower white adipose tissue PPARgamma target gene expression, including ATGL and HSL (two PPAR gamma target genes crucially involved in adipocyte triglyceride lipolysis). To our knowledge, no reports have been published on the specific effect of

adipocyte PPARgamma deficiency on fasting metabolism in mice. However, previous studies have validated that a deficiency in either ATGL or HSL can essentially recapitulate the fasting metabolic phenotype seen in our current study. HSL knockout mice exhibit reduced fasting liver triglycerides [36,37], whilst total body ATGL deficiency is associated with an increased fat mass and protection against high fat diet-induced glucose intolerance [38]. Furthermore, liver triglycerides are significantly lower in the context of a higher adipose tissue weight in adipocyte-specific ATGL knockout mice as compared to wild-type controls under fasting conditions [39]. Although the aforementioned studies have shown that HSL or ATGL deficiency is also associated with a reduction in fasting plasma triglycerides, triglyceride levels were normal in plasma samples of our overnight fasted female SR-BI knockout mice and even elevated in male SR-BI knockout mice as compared to their male wild-type controls. This discrepancy can—however—be explained by the fact that hepatocyte SR-BI participates in the clearance of triglyceride-rich lipoproteins [40,41]. More specifically, the impaired uptake of triglycerides by the liver can partially (in highly obese SR-BI knockout male mice) or fully (in mildly obese female SR-BI knockout mice) nullify the potential plasma triglyceride lowering effect associated with the relatively reduced PPARgamma-mediated flux of triglycerides from adipose tissue. We assume that the metabolic / obesogenic effects of SR-BI deficiency seen under high fat diet feeding conditions are primarily resulting from a change in adipocyte functioning. It is therefore not surprising that no reports have described a difference in body weight development or glucose tolerance between low fat, nonadipogenic / nonobesogenic (regular chow) diet-fed SR-BI knockout and wild-type mice.

An important question remains as to why SR-BI deficiency is associated with a reduction in adipocyte PPARgamma activity. Potent ligands for PPARgamma include various polyunsaturated fatty acids like arachidonic acid and arachidonic acid metabolites such as 15-d-PGJ2, 5-oxo-15-OH-ETE, and 5-oxo-ETE [42,43]. Recent in vitro studies using adipocytes isolated from wild-type and SR-BI knockout mice have suggested that SR-BI directly mediates the

cellular uptake of free fatty acids [44]. However, SR-BI deficiency can theoretically also diminish the cellular influx of fatty acids due to SR-BI's function in the selective uptake of cholesteryl esters from HDL, since mature HDL particles contain many cholesteryl ester species that are composed of arachidonic acid [45–47]. In this context it will be of interest to examine whether the large HDL particles that accumulate in the plasma compartment of SR-BI knockout mice and human carriers of a functional mutation in the SR-BI gene are relatively enriched in arachidonic acid-based cholesteryl ester species.

In conclusion, we have shown that high fat diet-fed SR-BI knockout mice exhibit an interesting metabolic phenotype that is characterized by enhanced adiposity, hypercholesterolemia, and atherosclerotic lesion development in the context of a reduced hepatic steatosis and glucose intolerance extent. In light of the fact that Koumanis et al. detected a higher frequency of mutations in the SR-BI gene in severely obese as compared to non-obese humans [48], our novel in vivo data clearly identify SR-BI as a potential therapeutic target to overcome (morbid) obesity. Our current findings suggest that SR-BI deficiency directly modulates in vivo adipocyte functioning, thereby impacting significantly on total body lipid and glucose metabolism under obesogenic high fat diet feeding conditions. However, follow-up studies in for instance hepatocyte or adipocyte-specific SR-BI knockout mice will be needed to uncover the exact mechanism(s) driving the SR-BI deficiency-associated metabolic changes and increased adiposity in the context of an unchanged food intake.

Authors contribution

Menno Hoekstra – Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – Original Draft, Visualization, Supervision.

Amber B. Ouweneel – Investigation, Writing – Review & Editing, Juliet Price – Formal analysis, Investigation.

Rick van der Geest – Investigation.

Ronald J. van der Sluis – Investigation.

Janine J. Geerling – Conceptualization, Methodology, Investigation, Writing – Review & Editing.

Joya E. Nahon – Conceptualization, Methodology, Investigation.

Miranda Van Eck – Writing – Review & Editing, Funding acquisition.

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