Physiological importance of SR-BI in the in vivo metabolism of human HDL and LDL in male and female mice

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Abstract **The physiological role of murine scavenger receptor class B type I (SR-BI) was evaluated by in vivo clearances of human HDL_3 and LDL in normal and SR-BI knockout (KO) mice. In normal mice, cholesteryl esters (CEs) were removed faster than proteins, indicating a selective uptake process from both HDL_3 and LDL. SR-BI KO mice showed 80% losses of HDL-CE selective uptake and the complete loss of LDL-CE selective uptake in the first phase of clearance. However, the second phase was characterized by an acceleration of CE disappearance in SR-BI KO mice. Thus, SR-BI is the only murine receptor mediating HDL-CE selective uptake, whereas a SR-BI-independent pathway specific to LDL can rescue SR-BI deficiency. The analysis of LDL recovered 3 h after injection in mice from different genotypes revealed that LDLs are significantly depleted in CE (reduction from 19% to 50% of the CE/protein ratios). A smaller LDL size in comparison with that of noninjected LDL was also detectable but was more evident for LDL recovered from normal mice. All LDL preparations migrate faster than noninjected LDL on agarose-barbital gels. Thus, both SR-BI-dependent and -independent pathways lead to substantial changes in LDL.—** Brodeur, M. R., V. Luangrath, G. Bourret, L. Falstrault, and L. Brissette. **Physiological importance of SR-BI in the in vivo metabolism of human HDL and LDL in male and female mice.** ***J. Lipid Res.*** **2005.** 46: **687–696.**

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Numerous epidemiological studies have demonstrated that the risk of developing coronary artery diseases is directly related to plasma concentrations of LDL cholesterol (1) and inversely associated with plasma levels of HDL (2). Plasma levels of LDL cholesterol are in large part regulated via the LDL receptor (LDLr), which mediates the clearance of LDL through a well-defined process involving endocytosis and degradation of the entire LDL particle (3, 4). In contrast, clearance of HDL cholesterol seems to be accomplished by another pathway called selective uptake, which involves the extraction of cholesteryl esters (CEs) from lipoproteins without concomitant degradation of its apolipoproteins (5). Although selective uptake is usually associated with HDL cholesterol, evidence suggests that this pathway may also act on other lipoproteins, such as LDL (6–9).

The scavenger receptor class B type I (SR-BI) is a cell surface receptor that was initially found as a receptor for modified LDL (oxidized or acetylated) and maleylated BSA and was later shown to bind native lipoproteins (5, 10, 11). Although SR-BI can bind LDL with high affinity, attention has mainly been devoted to the relation between SR-BI and HDL, and SR-BI was demonstrated to mediate the selective uptake of HDL-CE in transfected cells (5, 12, 13). The abundance of SR-BI in steroidogenic organs and liver, the principal sites of cholesteryl ester selective uptake in vivo, has also been cited as an indication of SR-BI involvement in HDL metabolism (5, 14). However, the most convincing evidence for a role of SR-BI in HDL metabolism has come from studies using genetically manipulated mice. These studies demonstrated that a disruption of the SR-BI gene generates an increase in total plasma cholesterol levels, which is mainly associated with the appearance of large HDLs enriched in CE and containing less apolipoprotein A-II (apoA-II) and more apoE than normal mouse HDL (15). Similar results were obtained using mice having attenuated hepatic SR-BI expression attributable to a promoter mutation (SR-BIatt mice) (16). Recently Out et al. (17) and Brundert et al. (18) showed that SR-BI is solely responsible for the selective uptake of CE from HDL by the liver and the adrenals in mice. However, these studies did not address the issue of whether SR-BI has a similar importance in male and female mice. This is an important issue because hepatic SR-BI expression has been shown to be weaker among female than male rats; inversely, SR-BII, an alternatively spliced product of the SR-BI gene that only differs in the C-terminal cytoplasmic domain, was higher in female rats (19). Also, injections

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of high concentrations of estrogens were shown to decrease rat SR-BI expression in parenchymal cells, whereas increasing SR-BI expression in Kupffer cells (20) and administration of pharmacological levels of estrogens produced a decrease in CE selective uptake in rats (21).

The physiological role of SR-BI in the metabolism of apoB-containing particles is not yet established. However, it has been suggested by in vitro studies of SR-BI-overexpressing cells (22, 23) and HepG2 cells deficient in SR-BI as well as primary cultures of hepatic cells from SR-BI knockout (KO) male mice (24). The involvement of SR-BI in non-HDL lipoprotein metabolism is also supported by in vivo studies using mice expressing different levels of SR-BI. Transgenic mice overexpressing hepatic SR-BI show lower than normal levels of plasma LDL-cholesterol and apoB and a reduction in VLDL and intermediate density lipoprotein/LDL sizes (25–27). This last finding can be explained by a selective lipid uptake of LDL-CE by SR-BI. In view of the results obtained by Ueda et al. (26) showing an accelerated clearance of LDL-protein in SR-BI transgenic mice, it can be suggested that SR-BI leads to some LDL holoparticle uptake and degradation either directly or indirectly. This is supported by the study of Murao et al. (28), which showed a greater degradation of LDL in HEK293 cells overexpressing SR-BI. On the other hand, a recent study using human apoB transgenic mice overexpressing SR-BI demonstrated that plasma apoB concentration was not affected, and a turnover study showed a very small increase of LDL-CE clearance with no difference in LDL-protein disappearance (29). Thus, the physiological impact of SR-BI in vivo on LDL metabolism needs to be further investigated.

Our overall goal was to determine in vivo the role of SR-BI in HDL and LDL metabolism in male and female mice. To achieve this, we compared HDL- and LDL-protein and -CE disappearances between normal mice and SR-BI KO mice of both genders. We found that selective uptake from HDL is not influenced by mouse gender and that SR-BI has the same importance in the two genders and is fully responsible for HDL-CE selective uptake. We show for the first time that LDL-CE selective uptake occurs in mice of both genders. Furthermore, our results demonstrate the involvement of normal mouse SR-BI in this pathway. However, the loss of this receptor can be rescued by another pathway in both mouse genders. We also show that LDLs injected in mice are depleted in CE and that they are smaller and show changes in their charges compared with noninjected LDL.

EXPERIMENTAL PROCEDURES

Materials

Newborn calf serum and gentamycin were purchased from Life Technologies (Burlington, Ontario, Canada). 1,2-[^{3}H]Cholesteryl ether oleate (50 mCi/mmol) was bought from Amersham Pharmacia Biotech (Laval, Quebec, Canada), and ^{125}I (as sodium iodide; 100 mCi/mmol) was bought from ICN Canada (Montreal, Quebec, Canada). Anti-SR-BI polyclonal antibodies were obtained from Novus Biologicals (Littleton, CO), anti-LDLr antibodies were from Research Diagnostics (Flanders, NJ), and goat anti-rabbit IgG coupled to horseradish peroxidase was from Chemicon (Temecula, CA). Enhanced chemiluminescence substrate and Complete Protease Inhibitor Cocktail tablets were from Roche Diagnostics (Laval, Quebec, Canada). Nondenaturing polyacrylamide gradient gels were from Alamo Gels, Inc. (San Antonio, TX).

Animals

Three heterozygous B6/129S-*Srb1*^{tm1Kri} breeding pairs were obtained from Jackson Laboratories (Bar Harbor, ME) to generate a colony. These generated mice with a 1:2:0.46 ratio of normal, heterozygous, and homozygous KO mice as determined by the PCR method of Rigotti et al. (15). Because female SR-BI KO mice are infertile (30), male homozygous KO mice were crossed with heterozygous females. Normal mice were generated from the normal background. Animals were provided with a standard mouse chow diet and drinking water and were subjected to a 14 h light/10 h dark cycle. This study was conducted according to protocols approved by the Animal Care and Use Committee of the Université du Québec à Montréal (No. 0901-424-0904). Six to eight week old male and female mice were used.

Isolation and radiolabeling of lipoproteins

Lipoproteins were isolated from human plasma obtained from the Royal Victoria Hospital (Montreal, Quebec, Canada). Before isolation, plasma was adjusted to 0.01% EDTA, 0.02% sodium azide, 10 μM PMSF, and 10 μM Trolox. Human LDL (d = 1.025–1.063 g/ml) and HDL_3 (d = 1.125–1.21 g/ml) were prepared by ultracentrifugation as described by Brissette, Charest, and Falstrault (6). Both lipoproteins contained no detectable amount of apoE as assessed by immunoblotting. LDL and HDL_3 were iodinated by a modification (31) of the iodine monochloride method of McFarlane (32). One millicurie of sodium ^{125}I was used to iodinate 2.5 mg of LDL or HDL_3 in the presence of 30 nmol (10 nmol for HDL_3) of iodine monochloride in 0.5 M glycine-NaOH, pH 10. Free iodine was removed by gel filtration on Sephadex G-25 followed by dialysis in TBS. Specific radioactivity ranged from 100,000 to 250,000 cpm/μg protein. LDL and HDL_3 were labeled with [^{3}H]cholesteryl oleoyl ether (CEt) essentially as described by Roberts et al. (33). Thereafter, labeled lipoproteins were reisolated by ultracentrifugation. The specific activities of CE-labeled lipoproteins ranged from 6,800 to 11,900 cpm/μg protein.

Mildly oxidized LDL (OxLDL) was prepared as described previously (34). LDLs (200 μg protein/ml) were incubated with 5 μM $CuSO_4$ at 37°C for 4 h. Mildly OxLDL typically resulted in 1.5-fold increases in electrophoretic mobility relative to native LDL on 0.5% agarose-barbital gels.

Preparation of parenchymal and nonparenchymal cells from mouse livers

Hepatic cells were isolated from mouse liver as described previously (9, 35). Briefly, the portal vein was cannulated with a 23 gauge plastic cannula. The liver was perfused with calcium-free HBSS, pH 7.4, pregassed with 95% O_2/5% CO_2 at a flow rate of 5 ml/min. The liver was then perfused with collagenase solution (25 mg collagenase/100 ml HBSS containing 5 mM calcium) for 7 min. Hepatic cells were gently released from the Glisson capsule, and isolated hepatic cells were maintained in Williams' E medium (WME) containing 10% newborn calf serum and 0.5% gentamycin. The viability of hepatic cells was assessed by trypan blue exclusion immediately after isolation and was >85%. Differential centrifugations were used to separate parenchymal (hepatocytes) from nonparenchymal liver cells (liver endothelial and Kupffer cells). Briefly, parenchymal liver cells from total cells were sedimented by centrifugation for 5 min at 50 *g*, and the pellet

was washed three times in WME. The supernatants were centrifuged twice (5 min at 50 *g*) to eliminate any remaining hepatocytes, and nonparenchymal liver cells were sedimented by centrifugation for 10 min at 220 *g*. Finally, nonparenchymal cells were washed three times in WME.

Immunoblotting of SR-BI and LDLr

Total cell proteins of parenchymal and nonparenchymal liver cells from normal male or female mice were extracted by 1% Triton X-100 solubilization. Proteins (50 μg) were separated by 10% reducing SDS-PAGE and immunoblotted on nitrocellulose with anti-mouse SR-BI polyclonal antibody (1:5,000) or with anti-LDLr antibody (1:500) followed by enhanced chemiluminescence detection on Kodak Biomax ML film. Protein expression was measured by densitometric scanning and analyzed with ImageQuant 5.2 software (Molecular Dynamics, Sunnyvale, CA).

In vivo clearance of HDL_3 and LDL

Six to eight week old male and female mice (normal, heterozygous, and homozygous KO mice) were injected by the tail vein with a bolus of human HDL_3 or LDL containing 480 μg of nonradiolabeled lipoproteins and 20 μg of lipoproteins radiolabeled with either ^{125}I or [^{3}H]CEt in 150 μl of saline. At 2, 5, 10, 20, 30, 60, 180, 360, and 1,440 min, blood samples were collected in microvette tubes coated with heparin (Sarstedt, Laval, Quebec, Canada) from exposed saphenous veins and centrifuged at 10,000 *g* for 5 min at 4°C. [^{3}H]CEt was directly radioassayed from plasma, whereas ^{125}I was measured in the trichloroacetic acid-precipitable fraction of plasma to eliminate the contribution of protein degradation. The plasma disappearance curves were generated by dividing the plasma radioactivity at each point by the radioactivity determined 2 min after tracer injection. At 24 h after injection, animals were killed and livers and intestines were collected. Tissue [^{3}H]CEt was assessed after homogenization and lipid extraction. Radioactivity found in the gut was attributed to uptake by the liver (36, 37). With the help of plasma decay curves, plasma fractional catabolic rates (FCRs) were calculated using a two-compartment model according to the model of Matthews (38). Initial FCRs were calculated with the same model, and values expressed the catabolic rates in the first phase of the decay curve.

Injections into the vena cava were also made. Briefly, normal and KO mice were anesthetized, their abdomens opened, and 20 μg of LDL labeled with [^{3}H]CEt was injected into the inferior vena cava. One hour after injection, the livers were perfused and parenchymal liver cells were isolated. Then, the cells were solubilized and their protein contents and radioactivity were measured.

Other methods

Free cholesterol and CE were measured by the enzymatic protocol of Deacon and Dawson (39). Protein content was determined by the method of Lowry et al. (40). Student's *t*-test was used to obtain statistical comparisons of the data. Differences were considered significant at $P < 0.05$.

RESULTS

Western blot analysis of hepatic SR-BI and LDLr expression in normal male and female mice

Because physiological levels of estradiol are more increased in females than in males, we undertook to examine the expression of this receptor in different subtypes of hepatic cells from normal male and female mice. SR-BI expression was found to be 55% lower in female ($P < 0.01$) than in male parenchymal cells (**Fig. 1A**); inversely, it was 65% higher in female than in male nonparenchymal cells ($P < 0.01$) (Fig. 1B). Given that nonparenchymal cells represent less than 10% of the total amount of liver proteins, total hepatic SR-BI expression is therefore higher in males. LDLr levels were similar in female and male cells (data not shown).

Importance of gender-related differences in SR-BI expression in the in vivo catabolism of HDL

Lower SR-BI expression in female parenchymal cells could theoretically translate itself into gender differences in HDL metabolism. To verify this possibility, in vivo clearance of the protein or lipid moiety of human HDL_3 was examined in normal male and female mice. Plasma FCRs were calculated from plasma decay curves by the use of a two-compartment model (**Table 1**). Using this model, comparable FCR values for plasma clearance of ^{125}I-HDL_3 and [^{3}H]CEt-HDL_3 were obtained in male and female animals (Table 1). FCRs generated from the selective uptake data (the difference between [^{3}H]CEt and ^{125}I FCRs) show that a lower level of hepatic SR-BI expression in females did not affect the rate of selective uptake from human HDL_3 (Table 1). Experiments were also conducted in heterozygous and homozygous SR-BI KO male and female mice to establish whether the gender affects HDL fate more strongly when SR-BI is half than when it is normal or absent. The loss of SR-BI expression caused a complete loss of selective uptake, as demonstrated by FCR selective uptake data (Table 1). These data show that heterozygous SR-BI KO male mice lost 40% of their ability to selectively take up CE from HDL_3, whereas homozygous SR-BI KO male mice lost 80%. No significant differences were found between the FCRs of female and male mice. The only difference found between males and females was that 24 h after HDL injection, CE uptake by the liver of heterozygous SR-BI KO males was as affected as in homozygous SR-BI KO females (**Fig. 2A**).

Overall, our work on HDL confirms the loss of CE-selective uptake from HDL observed in SR-BI KO mice by Out et al. (17) and Brundert et al. (18) in male mice; in addi-

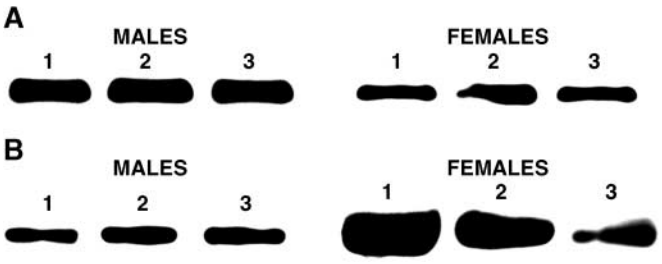

Fig. 1. Immunoblot analysis of scavenger receptor class B type I (SR-BI) expression in male and female mouse liver parenchymal and nonparenchymal cells. Liver cells were isolated and their proteins extracted as described in Experimental Procedures. Fifty micrograms of proteins from parenchymal cells (A) or from nonparenchymal cells (B) were separated by SDS-PAGE and immunoblotted with anti-mouse SR-BI polyclonal antibody followed by enhanced chemiluminescence detection. Three experiments gave essentially identical results, and differences in expression were quantified by densitometric scanning.

TABLE 1. FCRs for ^{125}I- and [^{3}H]CEt-labeled HDL$_3$ from plasma of normal, heterozygous, and homozygous SR-BI KO mice

	Males			Females		
Mice	^{125}I-HDL	[^{3}H]CEt-HDL	^{3}H − ^{125}I	^{125}I-HDL	[^{3}H]CEt-HDL	^{3}H − ^{125}I
+/+	0.078 ± 0.002	0.181 ± 0.012	0.104 ± 0.013	0.077 ± 0.002	0.182 ± 0.010	0.105 ± 0.010
+/−	0.077 ± 0.008	0.139 ± 0.008	0.062 ± 0.005[a]	0.073 ± 0.004	0.128 ± 0.003[a]	0.055 ± 0.002[a]
−/−	0.072 ± 0.002	0.093 ± 0.002[a,b]	0.021 ± 0.004[a,b]	0.098 ± 0.001[a,b,c]	0.108 ± 0.014[a]	0.010 ± 0.014[a,b]

CEt, cholesteryl oleoyl ether; FCR, fractional catabolic rate; KO, knockout; SR-BI, scavenger receptor class B type I; +/+, normal; +/−, heterozygous; −/−, homozygous. Male and female mice expressing various levels of SR-BI were injected via the tail vein with a bolus of human HDL$_3$ (480 μg of nonradiolabeled HDL$_3$ and 20 μg of lipoprotein HDL$_3$ radiolabeled with either ^{125}I or [^{3}H]CEt). Blood samples were taken at selected intervals and counted for radioactivity as described in Experimental Procedures. The FCRs were calculated using a two-compartment model and are expressed as pools per hour. All values are given as means ± SEM of three mice.

[a] Statistically different ($P < 0.05$) from +/+ mice.
[b] Statistically different ($P < 0.05$) from +/− mice.
[c] Statistically different ($P < 0.05$) from male mice.

tion, we show the same phenomenon in females. However, as the results can also reflect a competition by the endogenous HDLs of SR-BI KO mice that are larger and of different composition than those found in normal mice, we engineered in normal mice the in vivo disappearance of [^{3}H]CEt-HDL in the presence of a bolus of HDL from homozygous SR-BI KO and normal mice. The results presented in **Fig. 3A** show that both types of HDL similarly affect the disappearance of labeled HDL, indicating that HDLs from SR-BI KO mice are not responsible for the observed reduced clearance of HDL-CE in SR-BI KO mice. Thus, our work demonstrates conclusively the importance of SR-BI in HDL-CE selective uptake.

Role of SR-BI in the in vivo catabolism of LDL and importance of gender-related differences in SR-BI expression

The results presented in **Fig. 4A**, **B** and **Table 2** show that CEs from LDL are more rapidly cleared from the plasma than LDL protein in normal males. This clearly indicates the presence of an in vivo mechanism of CE selective uptake from LDL. In SR-BI KO male mice, the rate of removal of CE was also faster than the rate of removal of LDL-protein, indicating selective lipid uptake activity despite the absence of SR-BI expression (Fig. 4A, B). However, the disappearance of CE from LDL in homozygous SR-BI KO male mice was reduced in the first phase of clearance compared with normal mice (Fig. 4B). Indeed, the initial LDL-CE FCR was reduced by 63% in homozygous SR-BI KO male mice compared with normal male mice, and their CE selective uptake was completely abolished, as indicated by the identical removal rate of LDL-CE and proteins (Table 2). After that period, a curve inversion between normal and homozygous SR-BI KO mice was observed (Fig. 4B). This accelerated clearance of CE in homozygous SR-BI KO mice affected the FCR, which was 46% greater in homozygous SR-BI KO mice (Table 2). It is worth noting that curve inversion was not detectable

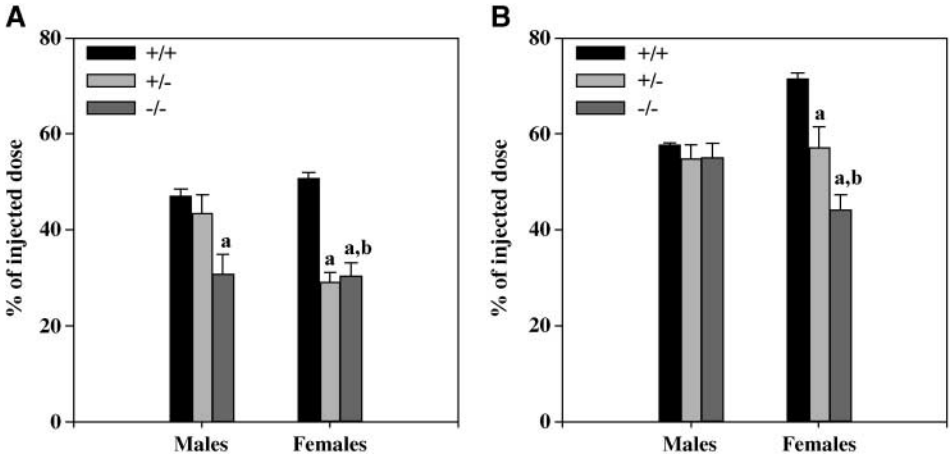

Fig. 2. Liver uptake of cholesteryl ester (CE) from HDL$_3$ or LDL in normal or SR-BI knockout (KO) male and female mice. Human [^{3}H]cholesteryl oleoyl ether ([^{3}H]CEt)-HDL$_3$ (A) or [^{3}H]CEt-LDL (B) (480 μg of nonradiolabeled lipoprotein and 20 μg of radiolabeled lipoprotein) was injected into wild-type (+/+), heterozygous (+/−), and homozygous (−/−) SR-BI KO male and female mice by the tail vein. At 24 h after injection, animals were humanely killed and the radioactivity in the liver was measured. Values represent means ± SEM from three mice. [a] Statistically different ($P < 0.05$) from +/+ mice; [b] statistically different ($P < 0.05$) from +/− mice from the same gender and injected with the same type of lipoprotein.

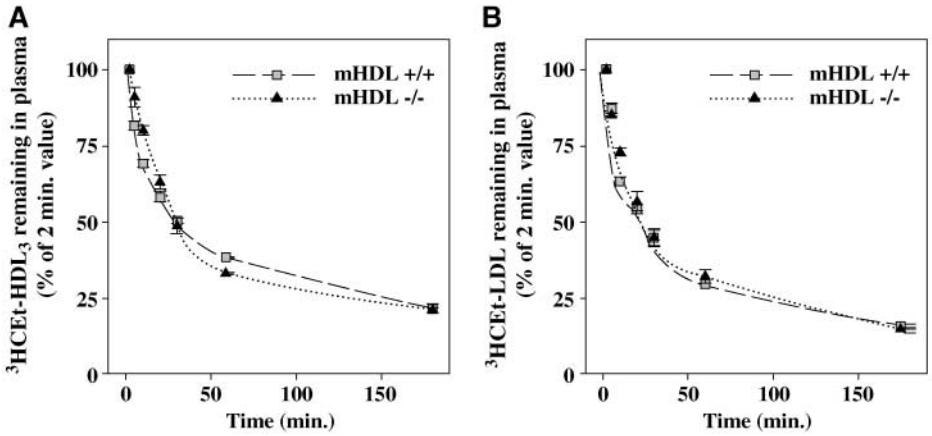

Fig. 3. Effect of coinjection of HDL from normal and SR-BI KO mice on HDL_3-CE and LDL-CE disappearance in normal mice. HDLs (d = 1.075–1.21 g/ml) were isolated from the plasma of normal and SR-BI KO mice. HDLs (480 μg of protein) from normal or SR-BI KO mice were injected together with [^{3}H]CEt-HDL_3 (20 μg of protein) (A) or [^{3}H]CEt-LDL (B) in normal male mice, and the disappearance of the labeled lipoproteins was followed in the blood. The value at 2 min after injection was defined as 100%. Each point represents the mean ± SEM derived from three mice.

in heterozygous mice (Table 2). Some differences were observed with females (Fig. 4C, D, Table 2). Thus, the initial and total LDL-CE FCRs were 49% and 20% lower, respectively, for normal females than for males. Also, the lack of SR-BI expression in females caused an accelerated elimination of LDL-protein in the first step of clearance (Fig. 4C), as demonstrated by a 60% higher initial FCR in homozygous SR-BI KO female mice compared with normal mice (Table 2). Conversely, the initial catabolism of LDL-CE was unaffected (Fig. 4D, Table 2). This difference in the initial FCR of protein and CE moieties of LDL between normal and homozygous SR-BI KO female mice resulted in the complete loss of CE selective uptake in the last mouse genotype during the first step of clearance. However, when total clearance was considered, homozygous SR-BI KO females showed 70% greater selective uptake activity than normal mice. This was caused by an important acceleration of the elimination of LDL-CE at 20 min after injection in SR-BI KO female mice. Although this inversion was observed in male SR-BI KO mice, it occurred earlier in SR-BI KO female mice. Also, in contrast to the heterozygous males, heterozygous females were not affected, at any time, by the lack of half SR-BI expression, as demonstrated by the FCR values (Table 2). Figure 3B shows that the endogenous pool of HDL in SR-BI KO or normal mice (LDLs are barely affected by the lack of SR-BI) (15) cannot explain our results, because labeled LDL-CEs disappear similarly in the absence or presence of a bolus of HDL from normal or SR-BI KO mice.

Liver uptake of [^{3}H]CEt-LDL was determined 24 h after LDL injection. The results shown in Fig. 2B demonstrate that the loss of SR-BI did not affect the uptake of LDL-CE. However, this was specific to male mice, because CE uptake was significantly reduced in heterozygous and homozygous SR-BI KO mice compared with normal female mice. Intrigued by the lack of effect of SR-BI in LDL-CE uptake by the liver of male mice and interested in defining the contribution of parenchymal and nonparenchymal cells in LDL-CE uptake, we injected LDLs into the vena cava of male mouse liver. One hour later, the liver was perfused, parenchymal and nonparenchymal cells were harvested, and the radioactivity associated with these cells was quantified. **Table 3** shows that both cell types were able to take LDL-CE, but when the contribution of each type of cell in the liver was considered, parenchymal cells were involved in 83% of LDL-CE uptake. We also found that 1 h after injection, LDL-CE uptake by liver parenchymal cells was 183% greater in normal mice than in SR-BI KO male mice, whereas SR-BI KO does not affect nonparenchymal activity. From these findings, we conclude that at early times, the lack of parenchymal SR-BI reduces LDL-CE uptake by male mouse liver, but not at later times.

Effect of CE selective uptake activity on LDL

As we demonstrated that CEs disappear faster than proteins from LDLs, LDLs were injected in normal and SR-BI KO male mice, recovered after 3 h, isolated, and analyzed. Notably, at most, one-tenth of these LDLs originate from endogenous LDLs, based on calculation from the blood of normal and SR-BI KO mice. Thus, those should not significantly affect our results. **Table 4** shows that LDLs that were injected in mice of all genders and genotypes were significantly reduced in their CE content; accordingly, their CE/protein ratios averaged 31% lower than those of noninjected LDLs. There seems to be no significant difference between the CE/protein ratios of LDLs injected into normal and SR-BI KO mice, suggesting that SR-BI-dependent and -independent pathways are as potent for the capture of CEs from LDLs when they are harvested after a 3 h period in mice. An increase in free cholesterol level and free cholesterol-protein ratio was also observed in the LDL preparations derived from SR-BI KO male mice. When analyzed by nondenaturing polyacrylamide gradient gel electrophoresis (NDGGE), we were able to detect a faster

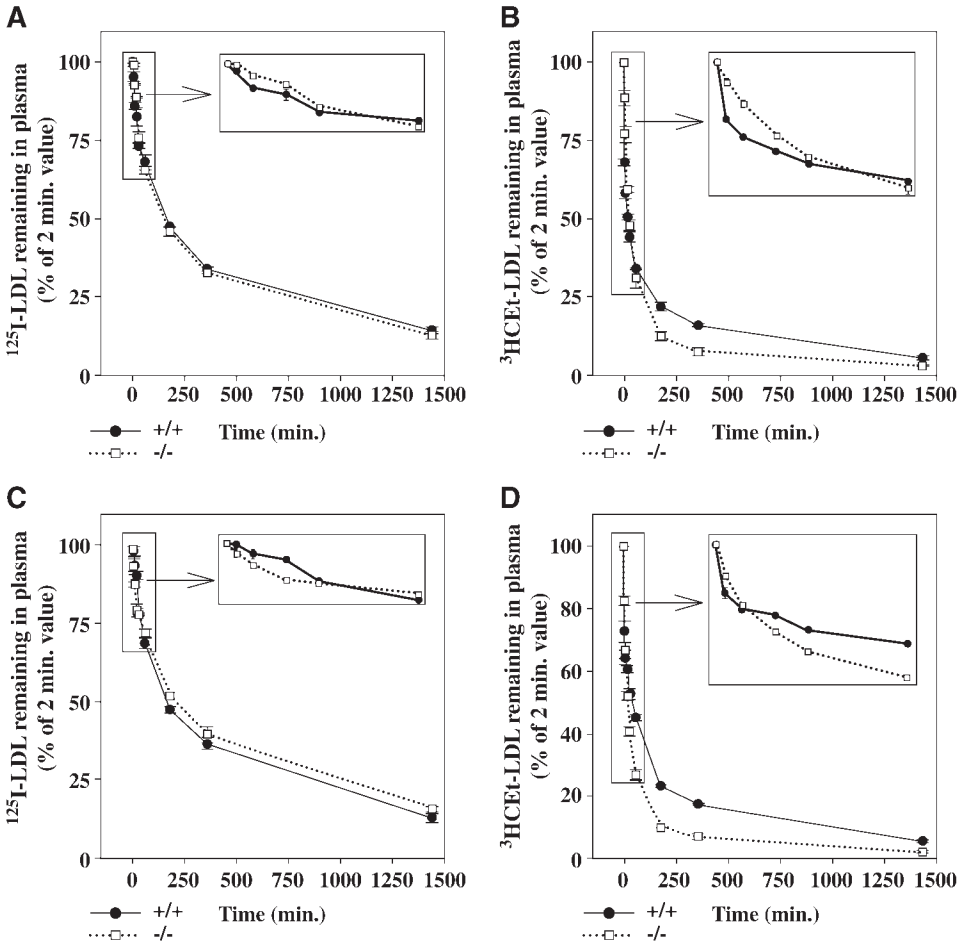

Fig. 4. Turnover studies of ^{125}I- and [^{3}H]CEt-labeled LDL in normal and SR-BI KO male and female mice. A bolus of 480 μg of nonradiolabeled human LDL and 20 μg of LDL labeled with ^{125}I (A, C) or with [^{3}H]CEt (B, D) was injected into the tail vein of either male (A, B) or female (C, D) +/+ or −/− SR-BI KO mice. At the indicated times, blood samples were collected from the saphenous vein into heparinized tubes, and the radioactivity remaining in the plasma was determined as described in Experimental Procedures. The value at 2 min after injection was defined as 100%. Each point represents the mean ± SEM derived from three mice.

migration for LDLs that had been injected in normal female or male mice than for noninjected LDLs (**Fig. 5A**). This reduction in size was not detectable for LDLs derived from SR-BI KO mice. LDL sizes were also determined as a function of time after the injection of ^{125}I-LDL in normal and SR-BI KO mice. This experimental setup excludes any contribution of mouse LDL in the electrophoretic pattern and eliminates the need for reisolating injected LDLs. Fig. 5B clearly shows that with time, LDLs injected in normal mice gradually become smaller. Similar results were obtained with SR-BI KO mice. Isolated LDLs were also subjected to electrophoresis on agarose-barbital gels to search for charge changes. The samples were run along with mildly OxLDL. As shown in **Fig. 6A**, independent of the genotypes or genders, all LDLs migrated between noninjected LDL and mildly OxLDL, including murine LDL. Fig. 6B shows that ^{125}I-LDL contained in the plasma of mice injected with this labeled LDL also migrated faster on agarose-barbital gels than did noninjected ^{125}I-LDL, indicating that the results shown in Fig. 6A are not a consequence of lipoprotein isolation from mouse plasma. Furthermore, Fig. 6B reveals that most of the change in the ability of LDL to migrate on the gel occurs soon after the injection. Thus, overall, these in vivo studies reveal that LDL-CE selective uptake being mediated or not by SR-BI has an impact on LDL composition and physiology.

DISCUSSION

In accordance with studies conducted with rats (14, 19, 20), we found that the level of expression of SR-BI is higher in male mouse hepatic parenchymal cells and lower in nonparenchymal cells compared with female cells (Fig. 1). Lower SR-BI expression in female parenchymal cells could theoretically translate into gender differences in HDL metabolism, but the similarity in fractional clearance rates of the HDL protein and CE observed between male and female mice clearly proves that this is not the case (Table 1). Our results also show similar levels of hepatic CE uptake

TABLE 2. FCRs for ^{125}I- and [^{3}H]CEt-labeled LDL from plasma of +/+, +/−, and −/− SR-BI KO mice

Mice	FCR	Males			Females		
		^{125}I-LDL	[^{3}H]CEt-LDL	^{3}H − ^{125}I	^{125}I-LDL	[^{3}H]CEt-LDL	^{3}H − ^{125}I
+/+	FCR	0.100 ± 0.007	0.249 ± 0.018	0.148 ± 0.017	0.104 ± 0.008	0.223 ± 0.011	0.119 ± 0.007
	Initial FCR	2.069 ± 0.566	5.257 ± 0.488	3.190 ± 0.226	1.855 ± 0.167	2.562 ± 0.029[a]	0.706 ± 0.196[a]
+/−	FCR	0.100 ± 0.005	0.161 ± 0.003[b]	0.061 ± 0.007[b]	0.086 ± 0.003	0.196 ± 0.020	0.110 ± 0.019
	Initial FCR	1.723 ± 0.186	5.442 ± 0.806	3.719 ± 0.629	1.221 ± 0.345	3.669 ± 0.082[b]	1.449 ± 0.406[a]
−/−	FCR	0.108 ± 0.009	0.383 ± 0.009[b,c]	0.275 ± 0.017[b,c]	0.090 ± 0.004	0.487 ± 0.057[b,c]	0.398 ± 0.054[b,c]
	Initial FCR	1.947 ± 0.061	1.960 ± 0.089[b,c]	0.011 ± 0.052[b,c]	4.590 ± 0.543[a,b,c]	2.588 ± 0.167[a,b,c]	−2.001 ± 0.625[a,b,c]

Male and female mice expressing various levels of SR-BI were injected via the tail vein with a bolus of human LDL (480 μg of nonradiolabeled LDL and 20 μg of lipoprotein LDL radiolabeled with either ^{125}I or [^{3}H]CEt). Blood samples were taken at selected intervals and counted for radioactivity as described in Experimental Procedures. The FCRs were calculated using a two-compartment model and are expressed as pools per hour. The initial FCRs were calculated during the first phase in the model. All values are given as means ± SEM of three mice.

[a] Statistically different ($P < 0.05$) from male mice.

[b] Statistically different ($P < 0.05$) from +/+ mice.

[c] Statistically different ($P < 0.05$) from +/− mice.

between sexes, which suggests either that the expression of SR-BI in female liver parenchymal cells is sufficient to achieve optimal levels of selective uptake or that the low levels of parenchymal SR-BI of female liver are counterbalanced by higher expression in nonparenchymal cells. The first possibility is more likely, given that Pieters et al. (21) previously observed selective uptake to be exerted only by parenchymal cells in the rat. Accordingly, the livers of heterozygous SR-BI KO males that have parenchymal SR-BI expression levels similar to those of normal female livers selectively take up as much CE as normal male livers. However, a minimum level of SR-BI expression appears to be required to ensure optimal liver HDL-CE uptake, because this pathway is reduced in heterozygous females. The nearly total loss of HDL-CE selective uptake observed in homozygous SR-BI KO mice suggests that SR-BI is the only efficient receptor mediating this function in vivo in both mouse genders. Alternatively, the loss of CE uptake that we observed in SR-BI KO mice could be attributed to HDL modifications in these mice (15). Indeed, HDLs were shown to be 30% and 125% richer in CE than those of normal mice in heterozygous and homozygous SR-BI KO mice, respectively (15). Disappearance assays of HDL-CE in normal mice in the presence of a 24-fold excess of HDL isolated from either normal mice or homozygous SR-BI KO mice showed no difference between curves (Fig. 2A). Consequently, we demonstrate for the first time that the endogenous pool of HDL in SR-BI KO mice does not influence the fate of injected human HDL; thus, the FCR data can be irrefutably attributed to the action of SR-BI toward injected HDL. Overall, our work on HDL confirms the recent findings of Out et al. (17) and Brundert et al. (18), extends the knowledge related to mouse genders, and clearly demonstrates that the encountered effect of SR-BI KO on HDL-CE fate is not a consequence of the endogenous HDLs of these mice.

TABLE 3. [^{3}H]CEt association of injected LDL with parenchymal and nonparenchymal cells of normal and SR-BI KO mice in vivo

Cell Type	SR-BI +/+ CEt Association	SR-BI −/− CEt Association
	μg/mg cell protein	
Parenchymal cells	0.017 ± 0.0003	0.006 ± 0.0008[a]
Nonparenchymal cells	0.036 ± 0.008	0.042 ± 0.007

CE, cholesteryl ester. Normal (SR-BI +/+) and SR-BI KO (SR-BI −/−) male mice were injected with 20 μg/ml radiolabeled LDL. The liver perfusion was started at 60 min. The [^{3}H]CEt associations (μg/mg cell protein) with the subsequently isolated parenchymal and nonparenchymal cells were determined. Results are shown as means ± SEM of three to four experiments.

[a] Statistically significant difference ($P < 0.05$) between the association of LDL with normal or SR-BI KO liver parenchymal or nonparenchymal cells.

The role of SR-BI in the in vivo metabolism of LDL-protein and -CE remains poorly understood. Our study demonstrates the existence of a CE selective uptake mechanism from LDL in normal male mice. Although this pathway is also present in females, the FCR data indicate that selective uptake is faster in males. The low level of SR-BI in female livers suggests that this receptor is indeed responsible for LDL-CE selective uptake. This is supported by kinetic experiments conducted with male heterozygous and homozygous SR-BI KO mice showing an important decrease in the initial plasma clearance of LDL-CE compared with normal mice. It is unlikely that SR-BI KO mouse lipoproteins could explain our results, as LDL concentrations are barely affected by SR-BI gene KO (15, 41), and we showed that the HDLs isolated from either normal or SR-BI KO mice when injected in normal mice in conjunction with LDL do not affect the fate of LDL-CE. Furthermore, the human LDLs that we injected can also be considered as a mouse LDL tracer, because Webb et al. (29) have shown that human and mouse LDLs behave similarly toward SR-BI.

Although there was no removal of LDL-CE in SR-BI KO male and female mice during the first phase of clearance, the selective uptake mechanism became visible when total FCRs were considered. This strongly suggests the existence of a SR-BI-independent pathway, at least in homozygous SR-BI KO mice. Although the SR-BI-independent pathway shows a low efficiency in taking up CE during the initial clearance, as seen in homozygous SR-BI KO mice, its ability becomes more important with time. Indeed, a complete inversion was observed between normal and ho-

TABLE 4. Lipid profiles of noninjected and injected LDLs into normal and SR-BI KO female and male mice

Lipid	Noninjected LDL (n = 3)	LDL Injected in Male Mice		LDL Injected in Female Mice	
		+/+ (n = 2)	−/− (n = 2)	+/+ (n = 3)	−/− (n = 1)
CE	81.3 ± 3.9	35.4 ± 2.8[a]	53.6 ± 5.1[a,b]	49.8 ± 1.3[a,c]	56.2
Free cholesterol	16.4 ± 1.1	12.5 ± 2.5[a]	28.1 ± 4.0[a,b]	14.5 ± 1.4	27.5
CE/protein	1.6 ± 0.1	0.8 ± 0.05[a]	1.1 ± 0.2[a]	1.3 ± 0.2	1.2
Free cholesterol/protein	0.3 ± 0.01	0.3 ± 0.05	0.5 ± 0.1	0.4 ± 0.1	0.6
CE/FC	5.0 ± 0.1	2.9 ± 0.4[a]	1.9 ± 0.1[a,b]	3.5 ± 0.3[a]	2.0

FC, free cholesterol. LDLs (1 mg of protein) were injected into normal and SR-BI KO female and male mice. Mice were bled 3 h after injection. Blood samples were pooled from five to six mice according to different genders and genotypes, and LDLs were reisolated by ultracentrifugation. Their protein and cholesterol (CE and free cholesterol) levels were measured in μg/ml LDL preparation. Results are shown as means ± SEM of the number of experiments indicated in parentheses.

[a] Statistically significant difference ($P < 0.05$) between the values of injected and noninjected LDLs.

[b] Statistically significant difference ($P < 0.05$) between the values of injected LDLs in normal versus SR-BI KO mice of the same gender.

[c] Statistically significant difference ($P < 0.05$) between the values of injected LDLs in normal male and female mice.

mozygous SR-BI KO mouse CE-disappearance curves. Such a phenomenon was not observed with HDLs (data not shown); thus, it is specific to LDL metabolism. This curve inversion suggests that the SR-BI-independent pathway has a greater capacity to deplete CEs from LDLs when those are not processed by SR-BI. SR-BI-independent pathways for LDL-CE selective uptake were suggested in the past by in vitro assays using CHO (42), COS (22), and Y1 2/3 adrenal cells (43). The first study revealed the importance of lipoprotein lipase, and the last showed a role for LDLr-related protein and apoE. It is possible that one or both of these pathways is (are) responsible for the non-SR-BI LDL-CE selective uptake pathway that we encountered. Given that the loss of SR-BI in females causes a more important catabolism of LDL-CE compared with homozygous SR-BI KO males and that the expression of CD36 is higher in female than in male human and rat livers (44), it is possible that CD36 is the SR-BI-independent pathway that we detected. Furthermore, considering the existence of SR-BI-dependent and -independent pathways for LDL-CE selective uptake, it is not surprising that the liver of male SR-BI KO mice accumulates as much LDL-CE as that of normal mice. Although SR-BI KO females showed higher SR-BI-independent activity, we noted a significant decrease of LDL-CE uptake by their livers. The reasons for this are obscure, but they may indicate that in female mice deficient in SR-BI a greater uptake occurs by a tissue/organ that was not investigated in this study. If this is true, it will highlight another difference between murine genders.

To further demonstrate that LDL-CE selective uptake occurs in vivo, injected LDLs in normal and SR-BI KO mice were recovered and analyzed. We found that SR-BI-

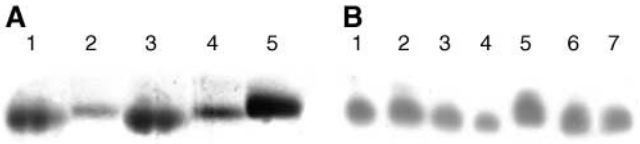

Fig. 5. Polyacrylamide gradient gel electrophoresis of noninjected or injected LDL into normal and SR-BI KO female or male mice. A: LDLs (1 mg of protein) were injected into normal and SR-BI KO female and male mice. Mice were bled 3 h after injection. Blood samples were pooled from five to six mice according to different genders and genotypes, and LDLs were reisolated by ultracentrifugation. LDLs were subjected to nondenaturing polyacrylamide gradient gel electrophoresis (NDGGE). Lanes 1, 2, injected LDL from normal or SR-BI KO males, respectively; lanes 3, 4, injected LDL from normal and SR-BI KO females, respectively; lane 5, noninjected LDL. B: ^{125}I-LDLs (480 μg of nonradiolabeled human LDL and 20 μg of LDL labeled with ^{125}I) were injected into normal or SR-BI KO male mice. Blood samples were taken at different times, and the plasma was subjected to NDGGE. The gel was dried and exposed to a film. Lane 1, noninjected ^{125}I-LDL; lanes 2–4, plasma from normal mice injected with ^{125}I-LDL and recovered at 2, 60, and 180 min, respectively; lanes 5–7, plasma from SR-BI KO mice injected with ^{125}I-LDL and recovered at 2, 60, and 180 min, respectively. These results are representative of at least two different experiments.

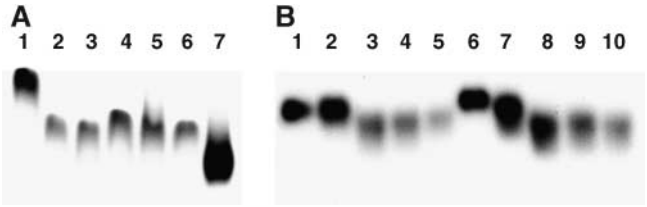

Fig. 6. Agarose-barbital gel electrophoresis of noninjected or injected LDL into normal and SR-BI KO female or male mice. A: LDLs (1 mg of protein) were injected into normal or SR-BI KO female and male mice. Mice were bled 3 h after injection. Blood samples were pooled from five to six mice according to different genders and genotypes, and LDLs were reisolated by ultracentrifugation. LDLs were thereafter loaded on a 0.5% agarose-barbital gel. Lane 1, noninjected LDL; lanes 2, 3, injected LDL from normal and SR-BI KO males, respectively; lanes 4, 5, injected LDL from normal and SR-BI KO females, respectively; lane 6, mouse LDL; lane 7, mildly oxidized LDL. B: ^{125}I-LDLs (480 μg of nonradiolabeled human LDL and 20 μg of LDL labeled with ^{125}I) were injected into normal or SR-BI KO male mice. Blood samples were taken at different times, and the plasma samples were subjected to electrophoresis on an agarose-barbital gel that was dried and exposed to a film. Lanes 1, 6, noninjected LDL; lanes 2–5, plasma from normal mice injected with ^{125}I-LDL and recovered at 2, 30, 60, and 180 min, respectively; lanes 7–10, plasma from SR-BI KO mice injected with ^{125}I-LDL and recovered at 2, 30, 60, and 180 min, respectively. These results are representative of at least two different preparations.

dependent and -independent pathways are able to capture CEs from LDLs (~30%) when those are harvested after a 3 h period in mice (Table 4). This experimental setup allowed the detection of a LDL size reduction only when recovered from normal mice (Fig. 5). However, when ^{125}I-LDL injected in normal or SR-BI KO mice were recovered and applied to NDGGE, the LDL size reduction was detectable with the two mouse genotypes. Furthermore, independent of the genotypes or genders, all LDLs migrate between noninjected LDLs and mildly OxLDLs on agarose-barbital gels (Fig. 6). This faster migration indicates that LDLs in the mouse blood suffered from a very mild oxidation by simple contact with blood constituents or vessels or by CE depletion. Alternatively, it is possible that the LDL charge-surface ratio increased because of the size reduction. Other experiments are required to discriminate between these possibilities.

Furthermore, as the LDL/HDL ratio is higher in humans than in rodents, it is tempting to speculate that under physiological conditions, CE selective uptake plays a greater role in human than in mouse LDL metabolism. In accordance with this notion, a very recent study by Schwartz, VandenBroek, and Cooper (45) conducted in vivo in humans showed that irreversible CE output was from VLDL, intermediate density lipoprotein, and LDL, and little was from HDL. Thus, our study in the mouse may give clues to the understanding of human LDL metabolism.

In summary, this in vivo study demonstrated that liver selective uptake from HDL and the catabolism rate of HDL are not influenced by mouse genders, despite significantly lower hepatic SR-BI expression in females. It also showed that SR-BI acts alone in HDL-CE selective uptake in both mouse genders. In contrast, LDL-CE selective uptake is accomplished by SR-BI-dependent and -independent pathways, and both have an impact on LDL composition and physiology.

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