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Scavenger Receptor BI Plays a Role in Facilitating Chylomicron Metabolism*

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The function of scavenger receptor class B type I (SR-BI) in mediating the selective uptake of high density lipoprotein (HDL) cholesterol esters is well established. However, the potential role of SR-BI in chylomicron and chylomicron remnant metabolism is largely unknown. In the present investigation, we report that the cell association of 160 nm-sized triglyceride-rich chylomicron-like emulsion particles to freshly isolated hepatocytes from SR-BI-deficient mice is greatly reduced (>70%), as compared with wild-type littermate mice. Competition experiments show that the association of emulsion particles with isolated hepatocytes is efficiently competed for (>70%) by the well established SR-BI ligands, HDL and oxidized low density lipoprotein (LDL), whereas LDL is ineffective. Upon injection into SR-BI-deficient mice the hepatic association of emulsion particles is markedly decreased (~80%) as compared with wild-type mice. The relevance of these findings for *in vivo* chylomicron (remnant) metabolism was further evaluated by studying the effect of SR-BI deficiency on the intragastric fat load-induced postprandial triglyceride response. The postprandial triglyceride response is 2-fold higher in SR-BI-deficient mice as compared with wild-type littermates (area-under-the-curve 39.6 ± 1.2 *versus* 21.1 ± 3.6; $p < 0.005$), with a 4-fold increased accumulation of chylomicron (remnant)-associated triglycerides in plasma at 6 h after intragastric fat load. We conclude that SR-BI is important in facilitating chylomicron (remnant) metabolism and might function as an initial recognition site for chylomicron remnants whereby the subsequent internalization can be exerted by additional receptor systems like the LDL receptor and LDL receptor-related protein.

Chylomicrons are triglyceride (TG)[1]-rich lipoproteins that transport dietary lipids from the intestine to the liver. Upon entering the circulation, chylomicrons are converted to remnants by the TG-hydrolyzing action of lipoprotein lipase (LPL) and the acquisition of apolipoproteins (apo) such as apoE. Chylomicron remnants are subsequently taken up by the liver by an apoE-mediated process (reviewed in Refs. 1–3). The essential role of apoE in remnant clearance was indicated by the accumulation of remnants in apoE-deficient mice (4). It has been suggested that several apoE-dependent recognition sites contribute to the removal of remnants, including the low-density lipoprotein (apoB and -E) receptor (LDLr) (4–9), and the LDLr-related protein/α_2-macroglobulin receptor (LRP) (8, 10, 11). It is generally accepted, however, that for the initial liver recognition of remnants, the so-called "capture step," additional systems are needed. The initial sequestration step was suggested to involve heparan sulfate proteoglycans (5, 12), the lipolysis-stimulated receptor (13–15), a TG-rich lipoprotein receptor (16, 17), the asialoglycoprotein receptor (18), LPL (19), and/or hepatic lipase (20), while we also provided evidence for a specific remnant receptor (21–23) to function as an initial recognition site for remnants. However, the removal pathways of chylomicrons and their remnants remain complex, and the precise mechanism of recognition is still unclear and under debate (1–3).

Here we introduce scavenger receptor class B type I (SR-BI) as a new player in postprandial lipid metabolism. SR-BI binds high density lipoproteins (HDL) and mediates the selective uptake of cholesteryl esters (CE) from HDL without concomitant uptake of HDL protein (24). The major apolipoproteins from HDL (apoAI, apoAII, and apoCIII) mediate the binding of HDL to SR-BI (25). Recently, it was shown that lipid-free apoE binds to SR-BI and enhances CE uptake from lipoproteins (26). In addition to HDL, SR-BI was found to bind a broad spectrum of ligands, including modified lipoproteins (acetylated LDL, oxidized LDL, and hypochlorite-modified LDL), native lipoproteins (HDL, LDL, and very low density lipoproteins (VLDL)), maleylated bovine serum albumin (BSA), and anionic phospholipids (27–30). In contrast, SR-BI does not bind polyanions (*e.g.* fucoidin and polyinosinic acid), which are well known ligands for scavenger class A receptors. In addition to CE, SR-BI selectively takes up a variety of other molecules, like lipoprotein-associated phospholipids (31, 32), HDL-associated CE hydroxides (33), and TG (32, 34).

The importance of SR-BI in cholesterol metabolism is readily observed in genetically altered mice. SR-BI knockout (KO) mice are characterized by an increase in serum cholesterol levels reflected in enlarged, cholesterol-rich HDL particles and impaired HDL cholesterol clearance (35). Conversely, adenoviral hepatic SR-BI overexpression results in decreased serum HDL

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[1] The abbreviations used are: TG, triglyceride; LPL, lipoprotein lipase; apo, apolipoprotein; LDLr, low-density lipoprotein receptor; LRP, LDLr-related protein; SR-BI, scavenger receptor class B type I; HDL, high density lipoprotein; CE, cholesteryl ester; VLDL, very low density lipoprotein; BSA, bovine serum albumin; KO, knockout; CO, cholesteryl oleate; TO, triolein; WT, wild-type; PBS, phosphate buffered saline; DMEM, Dulbecco's modified Eagle's medium.

cholesterol content, increased liver uptake, and subsequent delivery of HDL cholesterol to the bile (36). In transgenic mice with liver-specific overexpression of murine SR-BI, a decrease in serum HDL cholesterol, apoAI, and apoAII levels was observed (37, 38). Additionally, plasma TG, LDL cholesterol, VLDL, and LDL-apoB were also decreased. When these SR-BI transgenic mice were fed a high fat, high cholesterol diet, a lowering in plasma apoB levels and abolition of the dietary increase in VLDL-apoB and LDL-apoB was detected. Thus, studies in SR-BI transgenic mice suggest that, in addition to its major role in HDL metabolism, SR-BI can play an additional role in the metabolism of apoB-containing lipoproteins. However, despite the well known role of SR-BI in HDL metabolism, the role of SR-BI in TG-rich chylomicron (remnant) metabolism is largely unknown.

The aim of the present study was to evaluate the role of SR-BI in chylomicron (remnant) metabolism by assessing the effects of SR-BI deficiency on the association of chylomicron-like emulsion particles to hepatocytes *in vitro* and *in vivo*, as well as the effects on the intra-gastric fat load-induced postprandial TG response. Collectively, these *in vitro* and *in vivo* data indicate that SR-BI is crucially involved in chylomicron (remnant) metabolism. We conclude that SR-BI is important in facilitating chylomicron (remnant) metabolism and might function as an initial recognition site for chylomicron remnants, whereby the subsequent internalization can be exerted by additional receptor systems like the LDLr and LRP.

EXPERIMENTAL PROCEDURES

Materials—[1α,2α-^{3}H]Cholesteryl oleate ([^{3}H]CO), glycerol tri[9,10-^{3}H]oleate ([^{3}H]triolein); [^{3}H]TO), and [^{14}C]CO were purchased from Amersham Biosciences. Triolein (TO, ~99%), egg yolk phosphatidylcholine (99%), and cholesteryl oleate (CO) were obtained from Fluka (Buchs, Switzerland). L-α-lysophosphatidylcholine (99%), cholesterol (>99%), and BSA (fraction V) were obtained from Sigma. Emulsifier safe, Hionic Fluor, and Solvable were obtained from Packard Bioscience (Groningen, The Netherlands).

Animals—Heterozygous SR-BI mice on a 129(agouti)/C57Bl/6 background were kindly provided by Monty Krieger (Massachusetts Institute of Technology, Boston, MA). The offspring of the mice were analyzed for the presence of targeted or wild-type (WT) SR-BI alleles by PCR, as described by Rigotti *et al.* (35). All experiments were performed with 8- to 10-week-old male homozygous SR-BI-deficient mice and WT littermates as controls. Animal experiments were performed at the Gorlaeus Laboratories of the Leiden/Amsterdam Center for Drug Research in accordance with the national laws. All experimental protocols were approved by the Ethics Committee for Animal Experiments of Leiden University.

Preparation of Emulsion Particles—Large (160-nm sized) TG-rich emulsion particles were prepared according to the sonication and ultracentrifugation procedure of Redgrave and Maranhao (39) as modified by Rensen *et al.* (40). In short, 100 mg of total lipid at a weight ratio TO/egg yolk phosphatidylcholine/lysophosphatidylcholine/CO/cholesterol of 70/22.7/2.3/3/2 was dispersed in NaCl buffer of density 1.10 g/ml. For synthesis of radiolabeled emulsion particles, 20–100 μCi of [^{3}H]CO, or [^{3}H]TO and [^{14}C]CO was added to 100 mg of total lipid. The mixture was then sonicated for 30 min at 18 μm output using a Soniprep 150 (MSE Scientific Instruments, Crawley, UK) equipped with a water bath for temperature maintenance (54 °C). After sonication, a fraction containing large emulsion particles was obtained by flotation after density gradient ultracentrifugation using a Beckman SW 40 Ti rotor (22 min, 20,000 rpm, 27 °C) and was isolated from the top of the tube by aspiration. The emulsion was stored at room temperature under argon and used within 5 days after preparation.

Characterization of Emulsion Particles—Particle size and homogeneity of the emulsion particles was assayed by photon correlation spectroscopy using a Malvern Zetasizer (Malvern, Worcestershire, UK) at 25 °C and a 90° angle between laser and detector. Lipid composition of the emulsion particles was determined using enzymatic assays (Roche Diagnostics, Mannheim, Germany) for TG, phospholipids, and cholesterol. Precipath L was used as an internal standard. The emulsion contained 84.4 ± 2.4% (w/w) TG, 2.9 ± 0.2% cholesterol, 0.4 ± 0.1% CO, and 11.6 ± 0.3% phosphatidylcholine (mean ± S.E.; n = 3). The average particle size was 162.8 ± 3.2 nm (mean ± S.E.; n = 20).

Isolation and Lipoproteins—Human LDL and HDL were isolated from the blood of healthy volunteers as described by Redgrave *et al.* (41) and dialyzed at 4 °C against phosphate-buffered saline (PBS), 1 mM EDTA, pH 7.4. For oxidation of LDL, LDL was centrifuged twice according to Redgrave *et al.* (41) and dialyzed against PBS, 10 μM EDTA, pH 7.4. LDL was subsequently oxidized by exposure to $CuSO_4$ as described by Van Berkel *et al.* (42). Protein concentration was determined according to Lowry *et al.* (43), using BSA as a standard.

Association Studies of Emulsion Particles with Hepatocytes in Vitro—Hepatocytes were isolated from mice by linear perfusion at 37 °C according to the method of Seglen (44), as detailed earlier (45). In short, mice were anesthetized by subcutaneous injection of ketamine (60 mg/kg; Eurovet Animal Health), fentanyl citrate, and fluanisone (1.26 mg/kg and 2 mg/kg, respectively; Janssen Animal Health) and the heart was cannulated through the vena cava inferior. The portal vein was opened to allow the outflow of buffer, and a ligature, applied around the vena cava inferior (at the position of the bifurcation to the kidney), was closed. The liver was perfused with an oxygenated 6.7 mM KCl and 142 mM NaCl buffer containing 6.7 mM Hepes (pH 7.4) at a flow rate of 10 ml/min. After 10 min of perfusion, a 0.05% (w/v) collagenase type IV-containing buffer, containing 67 mM NaCl, 6.7 mM KCl, 4.8 mM $CaCl_2$, 67 mM Hepes, and 2% BSA, was flushed through the liver for 10 min at a flow rate of 14 ml/min. Subsequently, hepatocytes were isolated after mincing the liver in Hanks' buffer containing 0.3% BSA, filtered through nylon gauze, and centrifuged three times for 10 min at 50 × g. The cell pellets consisted of pure (>99%) hepatocytes as judged by light microscopy, and the viability of the cells was >90% as determined by trypan blue exclusion. Cells that were pelleted after the last centrifugation step were resuspended in oxygenated DMEM containing 2% BSA, pH 7.4. Hepatocytes (1–2 mg of cell protein) were incubated with increasing concentrations of [^{3}H]TO and [^{14}C]CO double-labeled emulsion particles (μg TG) for 3 h at 37 °C in 1 ml of DMEM containing 2% BSA. Cell incubations were performed in a circulating lab shaker (Adolf Kühner AG, Switzerland) at 150 rpm. Every 30 min, the incubations were briefly oxygenated. After the incubations, the cells were centrifuged for 2 min at 600 rpm in an Eppendorf centrifuge and washed two times with PBS containing 0.2% BSA, pH 7.4 and once with PBS without BSA. Cells were lysed in 0.1 N NaOH, and the protein and radioactivity contents were determined. For competition studies, hepatocytes (1–2 mg of cell protein) were incubated with [^{3}H]CO-labeled emulsion particles (100 μg TG) in the presence of increasing amounts of human HDL, LDL, or oxidized LDL (3 h at 37 °C) and subsequently processed as described above.

Distribution of Emulsion Particles in Mice in Vivo—The serum decay, liver uptake, and organ distribution of emulsion particles in mice were assessed as described by Rensen *et al.* (46) with some minor modifications. In short, fasted SR-BI KO and WT littermate mice were anesthetized as described above, and the abdomens were opened. [^{3}H]CO-labeled emulsion particles (150 μg of TG) were injected through the vena cava inferior. At the indicated times, 50 μl of blood were taken and allowed to clot for 30 min. ^{3}H radioactivity in 10 μl of serum, which was obtained after centrifugation for 3 min at 2500 × g, was counted in 4 ml of Emulsifier Safe. The total amount of serum radioactivity was calculated with the equation: serum volume (ml) = [0.0219 × body weight (g)] + 2.66. At the indicated times, liver lobules (not exceeding 15% of liver weight in total) were tied off, excised, and weighed. After dissolution overnight at 65 °C in 0.5 ml of Solvable, radioactivity was counted in 15 ml of Hionic Fluor. After 60 min, the mice were killed, and several organs (*i.e.* liver, heart, lungs, adrenals, spleen, muscles, and testes) were excised and weighed. The radioactivity in the heart, spleen, and adrenals was determined as described above for liver lobules. Radioactivity in other tissue samples was counted after combustion in a Tri-carb 306 Sample Oxidizer (Packard, Downers Grove, IL). The radioactivity in all tissue samples was corrected for the serum radioactivity assumed to be present at the time of sampling (40).

Intragastric Fat Load-induced Postprandial TG Response—Groups of 6–8 mice were fasted overnight. For basal TG and cholesterol levels, 50-μl blood samples were drawn just before 9.00 a.m. by tail bleeding into heparinized capillary tubes (t = 0). At 9.00 a.m., animals received an intragastric load of 400 μl of olive oil. After gavage, blood collection was continued every hour for 6 h. Plasma TG and cholesterol levels were measured at the various time points using enzymatic kits as described above. The distribution of TG over the different lipoproteins in plasma was analyzed by fractionation of 30 μl of pooled plasma using a Superose 6 column (3.2 × 30 mm, Smart-system, Amersham Bio-

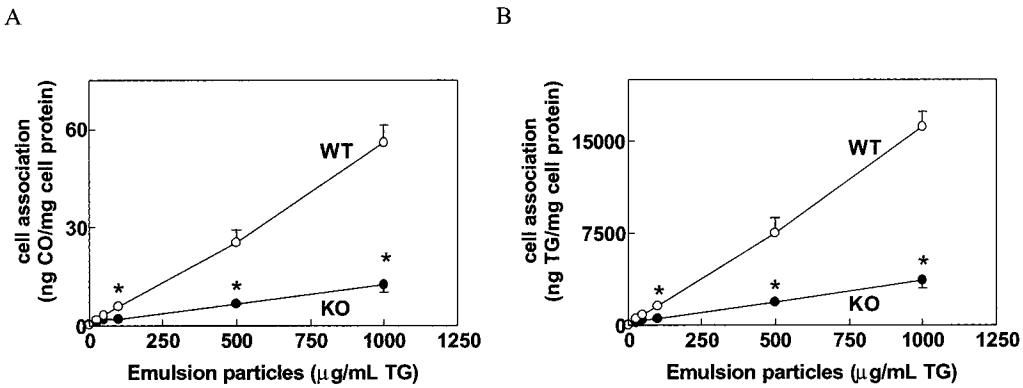

FIG. 1. **Effect of SR-BI deficiency on the association of TG-rich emulsion particles with isolated hepatocytes.** Hepatocytes of WT (○) and SR-BI KO (●) mice were incubated for 3 h at 37 °C with [^{14}C]CO and [^{3}H]TO-labeled emulsion particles in DMEM with 2% BSA. The cell associations of [^{14}C]CO (*A*) and [^{3}H]TO (*B*) were determined and expressed as ng lipid/mg of cell protein. Values are means ± S.E. of three separate cell isolations. *, $p < 0.05$.

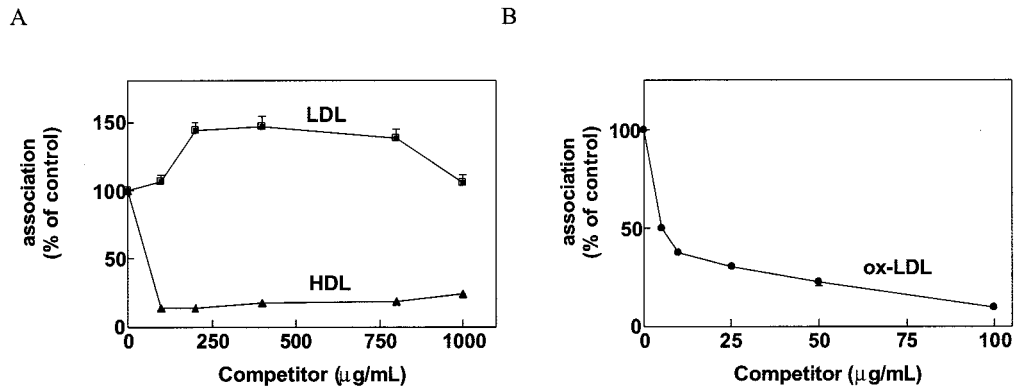

FIG. 2. **Effect of competitors for SR-BI on the association of TG-rich emulsion particles with hepatocytes.** Hepatocytes from WT mice were incubated for 3 h at 37 °C with [^{3}H]CO-labeled TG-rich emulsion particles (100 μg of TG) in the absence or presence of indicated amounts of unlabeled LDL (■) or HDL (▲) (*A*), or oxidized LDL (●) (*B*). The cell association of [^{3}H]CO was determined and expressed as the percentage of the association observed in the absence of competitor. Values represent the mean ± S.E. of triplicate determinations. For LDL, $p < 0.05$ at 200, 400, and 800 μg/ml *versus* 0 μg/ml. For all concentrations of HDL and oxidized LDL, $p < 0.0001$.

sciences), and determination of the TG content of the eluted fractions was made as described above.

RESULTS

Effect of SR-BI Deficiency on the Association of Chylomicron-like Emulsion Particles with Hepatocytes in Vitro—To study the role of SR-BI in the recognition of large chylomicron-like emulsion particles by hepatocytes *in vitro*, freshly isolated hepatocytes from SR-BI KO and WT littermate mice were incubated for 3 h at 37 °C with increasing concentrations of [^{3}H]TO and [^{14}C]CO double-labeled emulsion particle. SR-BI deficiency appeared to strongly decrease both the [^{3}H]TO and [^{14}C]CO association with hepatocytes (Fig. 1).

As SR-BI mediates the selective uptake of CE from HDL, whereby the uptake of CE is 30-fold higher than total particle uptake (24), we questioned whether CE in the large emulsion particle also could be taken up selectively. However, the ratio of [^{14}C]CO over [^{3}H]TO for the hepatocyte-associated values is identical to the ratio within the added emulsion, indicating that both components do associate to the cells as an unity. Because SR-BI can effectively interact with HDL and particularly with ox-LDL, we evaluated the effects of these particles on the association of emulsion-[^{3}H]CO with hepatocytes (Fig. 2). Similarly to HDL cholesteryl ester uptake, the cell association of emulsion particle cholesteryl esters was efficiently competed for by HDL and ox-LDL, whereas LDL was ineffective.

Effect of SR-BI Deficiency on the Kinetics of TG-rich Chylomicron-like Emulsion Particles in Vivo—To assess the relative contribution of SR-BI to the hepatic clearance of the large chylomicron-like emulsion particle *in vivo*, the kinetics of [^{3}H]CO-labeled emulsion particles were determined in SR-BI KO and WT littermate mice (Fig. 3). After intravenous injection of the emulsion particle (150 μg of TG), the serum decay (Fig. 3*A*) was similar for both groups of mice, with rapid clearance from the circulation ($t_{½} < 2$ min). In WT mice, the majority of the injected dose associated with the liver (50% of the dose at 10 min after injection) (Fig. 3*B*), which is in agreement with our previous observations (46). In contrast, the hepatic association of emulsion particles was strongly reduced in SR-BI KO mice (10% of the dose at 10 min after injection). Although SR-BI deficiency reduced the hepatic association for 80%, the serum decay was not affected (Fig. 3*A*). Examination of the organ distribution at 60 min after injection showed that a reduced association of emulsion particles with the liver in SR-BI KO mice was compensated for by a strongly increased association with the heart (2.5-fold, $p < 0.05$) and muscles (3.1-fold, $p < 0.05$) (Fig. 3*C*), thus explaining the similar serum kinetics for the emulsion in WT and SR-BI KO mice. Apparently, the reduced interaction of the chylomicron-like emulsion with the liver allows further interaction of the emulsion with LPL-expressing tissues.

Effect of SR-BI Deficiency on Chylomicron Metabolism in Vivo—Our combined data obtained with chylomicron-like emulsion particles suggest a prominent role for SR-BI in chylomicron metabolism. To confirm this hypothesis, we next investigated the effect of SR-BI deficiency on the postprandial TG response upon an intragastric fat load, which is an established

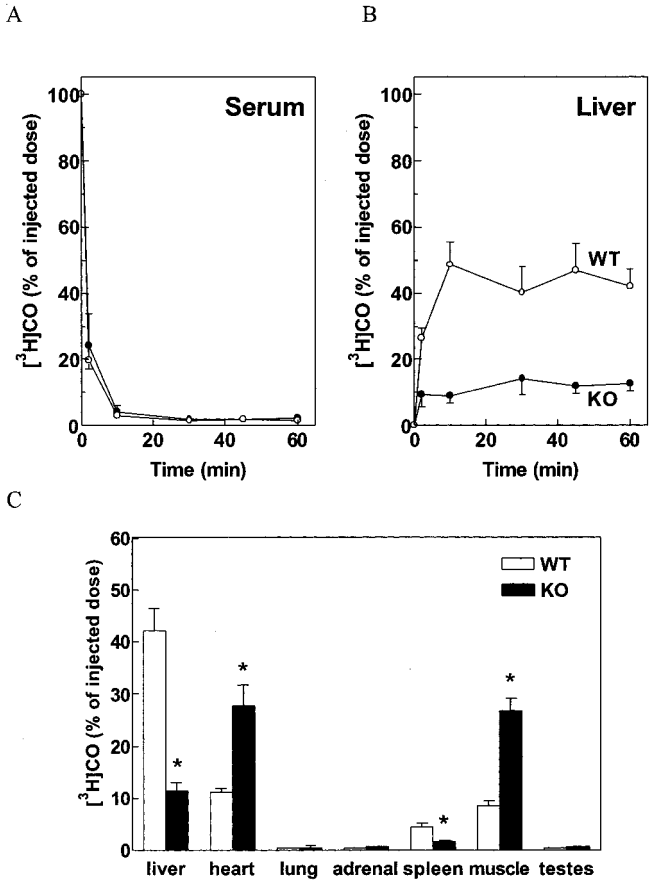

FIG. 3. **Effect of SR-BI deficiency on the serum decay, liver association, and organ distribution of TG-rich chylomicron-like emulsion particles.** [^{3}H]CO-labeled emulsion particles (150 μg of TG) were injected into fasted and anesthetized WT (○) and SR-BI KO (●) mice. At the indicated times, the serum decay (*A*) and liver association (*B*) of [^{3}H]CO were determined. At 60 min after injection, the mice were killed, and the uptake in the various organs was determined (*C*). Recoveries of ^{3}H radioactivity in SR-BI KO and WT mice were 81.3 ± 2.6% and 77.4 ± 1.5% of the injected dose, respectively. Values are means ± S.E. for three mice. For liver association, at 2, 10, 30, 45, and 60 min, $p < 0.005$ KO *versus* WT. *, $p < 0.05$.

procedure to study the kinetics of chylomicron metabolism. After an intragastric load with olive oil, plasma TG and cholesterol levels were determined over a period of 6 h in both WT and SR-BI KO mice (Fig. 4). The intragastric load of olive oil had no significant effect on cholesterol levels in SR-BI KO and WT mice (Fig. 4*A*); most of the cholesterol resides in the HDL fraction (data not shown). Before gavage, SR-BI KO mice had slightly but significantly higher basal levels of plasma TG compared with WT littermate mice (1.01 ± 0.08 mg/ml *versus* 0.79 ± 0.05 mg/ml, $p < 0.05$). At 4 h after olive oil administration, WT mice showed a postprandial increase in plasma TG (2.5-fold) (Fig. 4*B*), which returned to nearly basal TG levels at 6 h after administration. In contrast, SR-BI KO mice showed a 2-fold-increased TG response as compared with WT mice (area-under-the-curve 39.6 ± 1.2 *versus* 21.1 ± 3.6; $p < 0.005$). Specifically, the increase in plasma TG was significantly higher at 3, 4, 5, and 6 h after gavage. Analysis of lipoprotein profiles at 6 h after gavage (Fig. 4*C*) showed that chylomicron-TG was still 4-fold elevated in SR-BI KO mice, as compared with WT littermate mice, and resided mainly in the chylomicron/VLDL fractions (Fig. 4*C*).

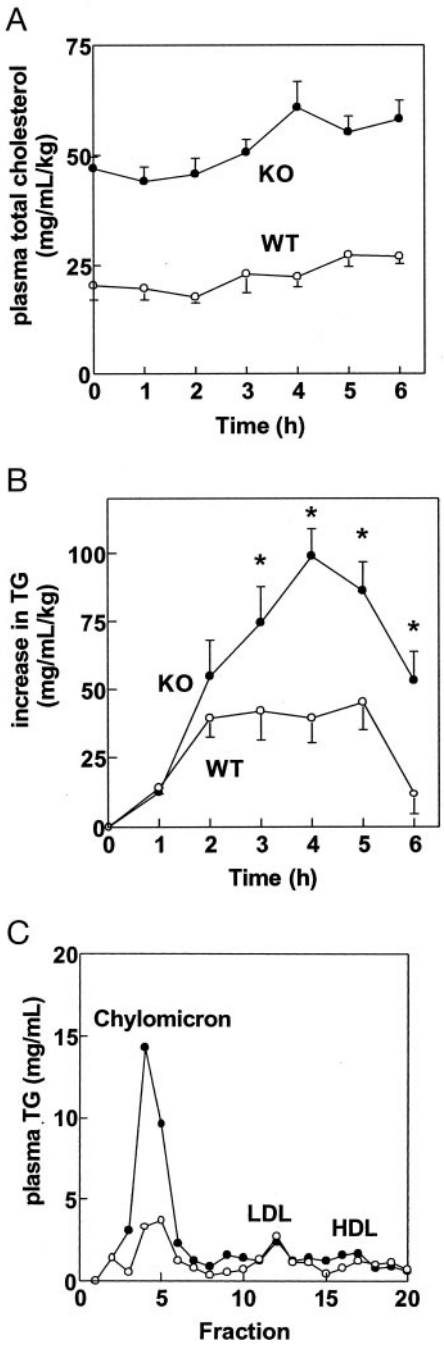

FIG. 4. **Effect of SR-BI deficiency on the postprandial TG response upon an intragastric fat load.** Overnight fasted WT (○) and SR-BI KO (●) mice received an intragastric load of 400 μl of olive oil at $t = 0$. Subsequently, plasma cholesterol (*A*) and TG (*B*) levels were determined at the indicated time points and expressed as total amount of cholesterol/kg of body weight and increase in TG levels relative to $t = 0$ (*B*), respectively. At 6 h after olive oil administration, TG levels in the lipoprotein profiles of pooled plasma of WT and SR-BI KO mice were determined (*C*). Values are means ± S.E. ($n = 6–8$ mice per group). *, $p < 0.05$.

DISCUSSION

SR-BI is a multi-ligand cell surface receptor and capable of binding HDL, LDL, VLDL, modified LDL, BSA, and liposomes containing anionic phospholipids (27–30). Although the function of SR-BI in the selective uptake of CE from HDL is indisputable (35), conflicting information on a potential role in VLDL and LDL turnover has been described (37, 38, 47). Ueda *et al.* (37) and Wang *et al.* (38) concluded that genetic overexpression of SR-BI does decrease levels of VLDL-apoB and LDL-apoB, while an accelerated clearance of non-HDL cholesterol was observed (37, 38). In contrast, Webb *et al.* (47) observed that overexpression of SR-BI by a recombinant adenoviral vec-

tor promotes the clearance of apoAI, but not apoB, in human apoB transgenic mice. The role of SR-BI in postprandial lipoprotein metabolism has not yet been addressed.

TG-rich emulsion particles have been widely used to mimic chylomicron metabolism (39, 40, 46, 48–50). In the present study, we utilized 160-nm chylomicron-like emulsion particles to study the effect of SR-BI deficiency on TG metabolism and the involvement of SR-BI in hepatic recognition of chylomicrons. Earlier studies from our group have indicated that small emulsion particles (*i.e.* 50 nm) are solely recognized by the LDLr *in vivo* (46), whereas the hepatic recognition of large emulsions (*i.e.* 150 nm) is exerted by a remnant receptor system of unidentified nature (22, 23, 51). The large emulsion follows a similar metabolic processing route as native chylomicrons, including efficient TG hydrolysis by LPL (50), acquisition of apoE (40), and apoE-mediated uptake by hepatocytes (46).

The *in vitro* studies with freshly isolated hepatocytes indicate that the association of the chylomicron-like emulsion particles to the cells is greatly diminished in the absence of SR-BI as well as in the presence of well established SR-BI ligands. Furthermore, upon injection of the large chylomicron-like emulsion particles into SR-BI KO mice, the hepatic association is greatly reduced (>80%) as compared with WT mice, establishing that for hepatic association the presence of SR-BI in the liver is apparently essential. In agreement with earlier data (46), we observed that in the absence of an efficient liver association of the chylomicron-like emulsion particles, an increased peripheral deposition may occur. Therefore, the strongly reduced hepatic association of the emulsion particles in SR-BI KO mice may have caused the increased binding to tissues such as heart and muscles, explaining the similar clearance kinetics of the emulsion in SR-BI KO and WT mice.

We then confirmed the role of SR-BI in endogenous chylomicron metabolism by giving an intragastric fat load to SR-BI KO and WT littermate mice. After administration of olive oil, the maximum level of TG reached in the blood circulation was increased 2-fold (area-under-the-curve 21.1 ± 3.6 *versus* 39.6 ± 1.2; $p < 0.005$) in the SR-BI KO mice as compared with control mice, and the clearance was greatly delayed. Our present experiments thus indicate that SR-BI can greatly facilitate the metabolism of postprandial TG-rich lipoproteins (*i.e.* chylomicrons) in mice.

In the past few years, despite the well defined role of LDLr and LRP in the endocytosis of chylomicron remnants, the mechanism responsible for initial liver capture has been under dispute (1–3). It is generally accepted, however, that for the initial liver recognition of remnants, the so-called "capture step," additional systems are needed. In mice without apoE-recognizing internalizing receptors (LRP/LDLr double-KO mice), the initial association of lipoprotein remnants (9) and large emulsion particles[2] with the liver is not affected, indicating that another molecular structure is responsible for the initial liver recognition. Our present experiments suggest that this initial remnant recognition site crucially involves SR-BI. Our data do not implicate that SR-BI is also the internalizing receptor as studies with LRP/LDLr double KO mice have clearly shown the decisive role of this combined system for the internalization and further metabolism of remnants (9, 52) and large emulsion particles[2] by the liver.

Our concept is supported by recent evidence from Fu *et al.* (53), who showed that the peroxisome-proliferator-activator receptor α agonist ciprofibrate leads to the increased accumulation of apoB-48-carrying lipoprotein remnants in the plasma of apoE KO mice as a result of down-regulation of hepatic SR-BI (53, 54). Restoration of SR-BI expression in ciprofibrate-treated apoE KO mice by recombinant adenoviral gene transfer of SR-BI abolished the accumulation of apoB48-carrying remnants. Fu *et al.* (53) concluded that SR-BI can function as a remnant receptor responsible for the clearance of remnants from the circulation in apoE KO mice. During the preparation of this manuscript, Webb *et al.* (55) showed that when SR-BI was over-expressed by recombinant adenoviral gene transfer in apoE KO mice, no detectable alterations in VLDL cholesterol were observed, suggesting that apoE is essential for the interaction of VLDL with SR-BI. Our present observations in SR-BI KO mice clearly indicate that SR-BI does function as a remnant-receptor in apoE-expressing mice, probably by utilizing its multi-ligand recognition properties as an initial capture system for chylomicron and their remnants. The function of SR-BI as initial remnant receptor might also explain the extreme phenotype of SR-BI deficiency on an apoE-deficient background (56, 57). In these double-KO mice, VLDL is strongly increased, as compared with single-apoE KO mice, and early coronary heart disease and death occur at a mean age of 6 weeks. We suggest that, in the absence of both functional LDLr and/or LRP by the absence of apoE and the initial recognition site SR-BI, no adequate escape mechanism for the uptake of postprandial atherogenic lipoproteins by the liver is available, leading to extremely rapid development of atherosclerotic lesions.

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