The fate of HDL particles in vivo after SR-BI-mediated selective lipid uptake

Nancy R. Webb,[1,*] Lei Cai,[*] Kristine S. Ziemba,[*] Jin Yu,[†] Mark S. Kindy,[†] Deneys R. van der Westhuyzen,[*] and Frederick C. de Beer[*,§]

Departments of Internal Medicine* and Biochemistry,† University of Kentucky Medical Center, Lexington, Kentucky 40536; and Department of Veterans Affairs Medical Center,§ Lexington, Kentucky 40511

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Abstract Scavenger receptor class B type I (SR-BI) delivers cholesterol ester from HDL to cells via a selective uptake mechanism, whereby lipid is transferred from the core of the particle without concomitant degradation of the protein moiety. The precise metabolic fate of HDL particles after selective lipid uptake is not known. To characterize SR-BI-mediated HDL processing in vivo, we expressed high levels of this receptor in livers of apoA-I$^{-/-}$ mice by adenoviral vector gene transfer, and then injected the mice with a bolus of human HDL_2 traced with ^{125}I-dilactitol tyramine. HDL recovered from apoA-I$^{-/-}$ mice over-expressing SR-BI was significantly smaller than HDL recovered from control mice as measured by non-denaturing gel electrophoresis. When injected into C57BL/6 mice, these HDL "remnants" were rapidly converted to HDL_2-sized lipoprotein particles, and were cleared from the plasma at a rate similar to HDL_2. In assays in cultured cells, HDL remnants did not stimulate ATP-binding cassette transporter A1-dependent cholesterol efflux. When mixed with mouse plasma ex vivo, HDL remnants rapidly converted to larger HDL particles. These studies identify a previously ill-defined pathway in HDL metabolism, whereby SR-BI generates small, dense HDL particles that are rapidly remodeled in plasma. This remodeling pathway may represent a process that is important in determining the rate of apoA-I catabolism and HDL-mediated reverse cholesterol transport.—Webb, N. R., L. Cai, K. S. Ziemba, J. Yu, M. S. Kindy, D. R. van der Westhuyzen, and F. C. de Beer. **The fate of HDL particles in vivo after SR-BI-mediated selective lipid uptake. *J. Lipid Res.* 2002.** 43: **1890–1898.**

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Numerous epidemiological studies have indicated that HDL cholesterol (HDL-C) and apoA-I concentrations are inversely correlated to the risk for coronary heart disease. Consequently, much effort has been focused on the factors that regulate plasma HDL and apoA-I levels. Metabolic studies in humans have shown that variations in HDL-C and apoA-I concentrations are primarily associated with differences in the rate of apoA-I catabolism rather than apoA-I production (1–5). Many of the factors known to influence apoA-I catabolic rate have a major effect on the lipid composition of the HDL particle. For example, the accumulation of cholesterol ester (CE) in HDL (brought about in humans by a deficiency in CETP) is associated with delayed apoA-I catabolism (6). Conversely, LCAT deficiency, which results in a depletion of HDL CE, is associated with accelerated apoA-I catabolism (7). Accumulating evidence suggests that the kidney cortex is an important site for degrading lipid-poor apoA-I, perhaps through glomerular filtration followed by degradation in the proximal tubule. Cubilin, an endocytic receptor that is expressed on the apical surfaces of kidney proximal tubule cells, has been implicated in renal uptake of lipid-poor HDL (8, 9).

Scavenger receptor class B type I (SR-BI) is an HDL receptor that mediates selective lipid uptake from receptor-bound HDL. During this process, CE is transferred from the core of the HDL particle to cells without the concomitant degradation of HDL apolipoproteins. Despite the fact that SR-BI mediates only CE uptake, liver-specific SR-BI over-expression results in not only a depletion of plasma HDL-C, but also decreased concentrations of apoA-I (10–12). We and others have shown that increased SR-BI-mediated HDL selective uptake in the liver is associated with increased catabolism of apoA-I, and that at least some of this catabolism occurs in the kidney (11, 13). In this study, we investigated the metabolic fate of HDL and its constituent apoA-I following selective lipid removal by SR-BI.

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Abbreviations: ABCA1, ATP-binding cassette transporter A1; CE, cholesteryl oleoyl ester; CEt, cholesteryl oleoyl ether; CHO, Chinese hamster ovary; DLT, dilactitol tyramine; SR-BI, scavenger receptor class B type I.

[1] To whom correspondence should be addressed.
e-mail: nrwebb1@uky.edu

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EXPERIMENTAL PROCEDURES

HDL isolation and radiolabeling

Fresh human plasma was collected from consenting healthy volunteers and the HDL_2 (d = 1.11 to 1.13 g/ml) and HDL_3 (d = 1.13 to 1.18 g/ml) fractions were isolated by density gradient ultracentrifugation (14). HDLs were dialyzed against 150 mM NaCl/0.01% EDTA, sterile filtered, and stored under nitrogen gas at 4°C. For some experiments, HDL apolipoproteins and free apoA-I (Biodesign Intl, Saco, ME) were radioiodinated by the iodine monochloride method (15). For other experiments, HDL_2 was traced with the non-degradable, intracellularly trapped radiolabels ^{125}I-dilactitol tyramine (DLT) (16) or 1α,2α (n) [^{3}H]cholesteryl oleoyl ether (CEt) (17). Protein concentrations before and after radiolabeling were determined by the method of Lowry et al. (18).

HDL catabolism in apoA-I$^{-/-}$ mice

Ten-week old male apoA-I$^{-/-}$ mice (C57BL/6 background; Jackson Laboratories) were injected in the tail vein with 1×10^{11} particles of AdSR-BI, a replication-deficient adenovirus expressing mouse SR-BI, or a control virus, Adnull (19). Three days after adenovirus infusions, mice were injected via the jugular vein with a bolus of human HDL_2, which consisted of a mixture of 450 μg non-radiolabeled HDL_2 and 50 μg HDL_2 radiolabeled with either ^{125}I-DLT (85 cpm/ng) or [^{3}H]CEt (5.8 dpm/ng) in 100 μl PBS. The use of ^{125}I-DLT coupled by reductive amination was advantageous for tissue uptake experiments since this residualizing radiolabel is very efficiently trapped in tissues in vivo (20). At selected intervals after bolus injection, blood was collected from the retro-orbital sinus and radioassayed. At 4 h after tracer injection, animals were anesthetized, exsanguinated, and perfused with saline (30 ml per animal). Livers and kidneys were collected and radioassayed.

Production and characterization of SR-BI-modified HDL "remnants"

ApoA-I$^{-/-}$ mice weighing at least 25 g were injected in the tail vein with 1.5×10^{11} particles AdSR-BI or Adnull. Three days after adenovirus infusion, the mice were injected via the jugular vein with 750 μg of ^{125}I-HDL_2 (25–35 cpm/ng). Plasma was collected 1 h, 2 h, or 3 h after HDL injection. The size distribution of ^{125}I-HDL particles in apoA-I$^{-/-}$ mouse plasma was determined by non-denaturing polyacrylamide gradient (4–18% acrylamide) gel electrophoresis (21, 22) or by size exclusion chromatography on a Superose 6 column (Pharmacia LKB Biotechnology Inc.) as previously described (19). To re-isolate HDLs from apoA-I$^{-/-}$ mice after HDL bolus injection, plasma from 5–10 mice was pooled and the d = 1.09–1.25 g/ml fraction was separated by density gradient ultracentrifugation (14). The amount of HDL_2 apolipoprotein recovered in the d = 1.09–1.25 g/ml fraction was calculated from the yield of ^{125}I radioactivity in that fraction and the known specific activity of the injected HDL_2.

HDL remnant binding to SR-BI

A Chinese hamster ovary (CHO) line stably transfected with human SR-BI cDNA (CHO-SR-BI cells) has been described (23). Cell-association assays of CHO-SR-BI cells and non-transfected CHO cells incubated with varying concentrations of radiolabeled lipoprotein (HDL_2 or HDL remnants produced in vivo and re-isolated by density ultracentrifugation) were performed as described previously (19). Apparent K_d and maximum number of binding sites (Bmax) values for binding were determined by nonlinear regression analysis of the SR-BI-specific cell associated values (total cell-associated values minus corresponding values for untransfected control cells), using Prism software (GraphPad, San Diego, CA).

Efflux assays

Normal human skin fibroblasts and fibroblasts from a Tangier patient were provided by Dr. J. Oram (University of Washington). The intracellular cholesterol compartment of the fibroblast cell lines was radiolabeled as described by Francis et al., (24) with minor changes. Cells (∼70% confluent) in 12-well plates were incubated for 48 h with media containing 0.2 μCi/ml [^{3}H]cholesterol (40–60 Ci/mmol; Amersham). To induce ATP-binding cassette transporter A1 (ABCA1) expression, the radiolabeled cells were loaded with free cholesterol by incubating for 48 h with efflux media (serum-free DMEM supplemented with 0.2% BSA) containing 30 μg/ml cholesterol (Sigma) (25). After washing five times with PBS containing 0.1% BSA (PBS/BSA), the radiolabeled cells were incubated for 6 h in efflux media to allow for the equilibration of cellular cholesterol pools. Cells were then washed and incubated at 37°C for 16 h in efflux media with or without plasma collected from apoA-I$^{-/-}$ mice either 2 h or 3 h after ^{125}I-HDL bolus injection. These unpurified 2 h or 3 h remnants were dialyzed against efflux media and added to cells at a final concentration of 10 μg/ml ^{125}I-HDL (added mouse plasma constituted <5% of total culture volume). As controls, a corresponding volume of apoA-I$^{-/-}$ mouse plasma that was collected 3 days after AdSR-BI infusion with or without addition of apoA-I was dialyzed against efflux media and added to cells (final apoA-I concentration added to cells, 10 μg/ml). After incubation, culture media was collected at 4°C, centrifuged to remove cell debris, and radioassayed. Cells were rinsed three times with ice-cold PBS/BSA, twice with PBS, and lipids were extracted in hexane-isopropanol (3:2, v/v) for 1 h at room temperature and radioassayed. ABCA1-dependent efflux (defined as the difference in efflux from normal human fibroblasts and Tangier fibroblasts) was expressed as the ratio of [^{3}H]cholesterol counts in efflux media and total [^{3}H]cholesterol counts in media and lipid extracts of cells.

Catabolism of HDL_2 and HDL remnants in C57BL/6 mice

ApoA-I$^{-/-}$ mouse plasma containing ~15 μg of SR-BI-modified ^{125}I-DLT HDL "remnants" (∼4×10^5 cpm) was injected via the jugular vein into a C57BL/6 mouse. An equivalent amount of unmodified ^{125}I-DLT HDL_2 mixed with untreated apoA-I$^{-/-}$ mouse plasma was injected for comparison. At selected intervals after tracer injection, blood was collected from the retro-orbital sinus, radioassayed, and analyzed by non-denaturing gel electrophoresis. At 4 h and 24 h after tracer injection, the mice were humanely killed, perfused with 30 ml saline, and livers and kidneys were collected and radioassayed.

In vitro remodeling of SR-BI-modified HDL remnants

Plasma containing 2 h HDL remnants was collected from apoA-I$^{-/-}$ mice and then mixed 1:10 (v/v) with either mouse plasma, human HDL (1 mg/ml), or PBS. The samples were incubated at 37°C, transferred to ice, and then immediately applied to a non-denaturing acrylamide gradient gel (4–18% acrylamide) (21, 22), and visualized by autoradiography.

Statistical analysis

Unless stated otherwise, data was analyzed using a Student's *t*-test (Sigma Stat 2.03; SPSS, Inc). For this analysis, all data met the constraints of normality and equivalence of variance to permit parametric analysis.

RESULTS

SR-BI-mediated HDL processing in apoA-I$^{-/-}$ mice

To analyze SR-BI-mediated HDL particle processing in vivo, we over-expressed the receptor in livers of apoA-I$^{-/-}$

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mice using a recombinant adenoviral vector, AdSR-BI. Additional mice were administered a control adenovirus, Adnull. Three days after adenovirus infusion (at a time corresponding to maximal SR-BI expression after AdSR-BI-treatment) (19) mice were injected with a bolus containing 500 μg human HDL_2 traced with either ^{125}I-DLT or [^{3}H]CEt. Blood samples were collected at selected intervals after bolus injection and radioassayed to determine ^{125}I and ^{3}H content. A higher rate of removal from plasma of [^{3}H]CEt relative to ^{125}I-DLT indicated whole body selective uptake of HDL-CE in both AdSR-BI and Adnull-treated mice (**Fig. 1A**, **B**). Hepatic SR-BI over-expression resulted in a significantly increased rate of [^{3}H]CEt-HDL clearance compared to control (Fig. 1A). In addition to enhanced HDL-CE clearance, infusion of AdSR-BI resulted in more rapid clearance of ^{125}I-DLT-HDL (Fig. 1B). The analysis of livers 4 h after bolus injection showed more [^{3}H]CEt associated with the liver than can be accounted for by ^{125}I-DLT association, indicating selective lipid uptake in livers of both Adnull and AdSR-BI-treated mice (Fig. 1C). As expected, selective lipid uptake (defined as the difference between ^{3}H and ^{125}I uptake) was enhanced in livers as a result of AdSR-BI infusion. Interestingly, hepatic SR-BI over-expression also gave rise to a significant increase in hepatic and renal uptake of ^{125}I-DLT (Fig. 1C, D). [^{3}H]CEt was not detected in kidneys of either Adnull or AdSR-BI-treated mice 4 h after bolus injection, indicating that the ^{125}I-HDL taken up by the kidney was comprised of lipid-poor HDL.

At 2 h after bolus injection, 58.4% (±5.9%) of ^{125}I-DLT HDL and 17.2% (± 7.7%) of [^{3}H]CEt remained in the plasma of AdSR-BI-treated mice (Fig. 1A, B), indicating that the injected HDL_2 was markedly depleted of CE at this time point. To investigate the extent to which increased SR-BI activity in apoA-I$^{-/-}$ mice brings about alterations in HDL particle size, plasma was collected 2 h after bolus injection and the HDL fraction was re-isolated by density gradient ultracentrifugation. An aliquot of the re-isolated HDL (5 μg) was separated by non-denaturing gradient gel electrophoresis and visualized by Coomassie staining. Aliquots of unmodified human HDL_2 and HDL_3 were also analyzed for comparison. As shown in **Fig. 2A**, two distinct populations of HDL particles were detected in the HDL "remnant" preparation. Both of these were significantly smaller than the starting HDL_2 (mean radius ~5.2 nm). The larger HDL remnant migrated at a size corresponding to HDL_3 (mean radius ~4.1 nm).

To further characterize SR-BI-mediated HDL processing in vivo, plasma was collected from apoA-I$^{-/-}$ mice at various intervals after ^{125}I-HDL_2 bolus injection and ana-

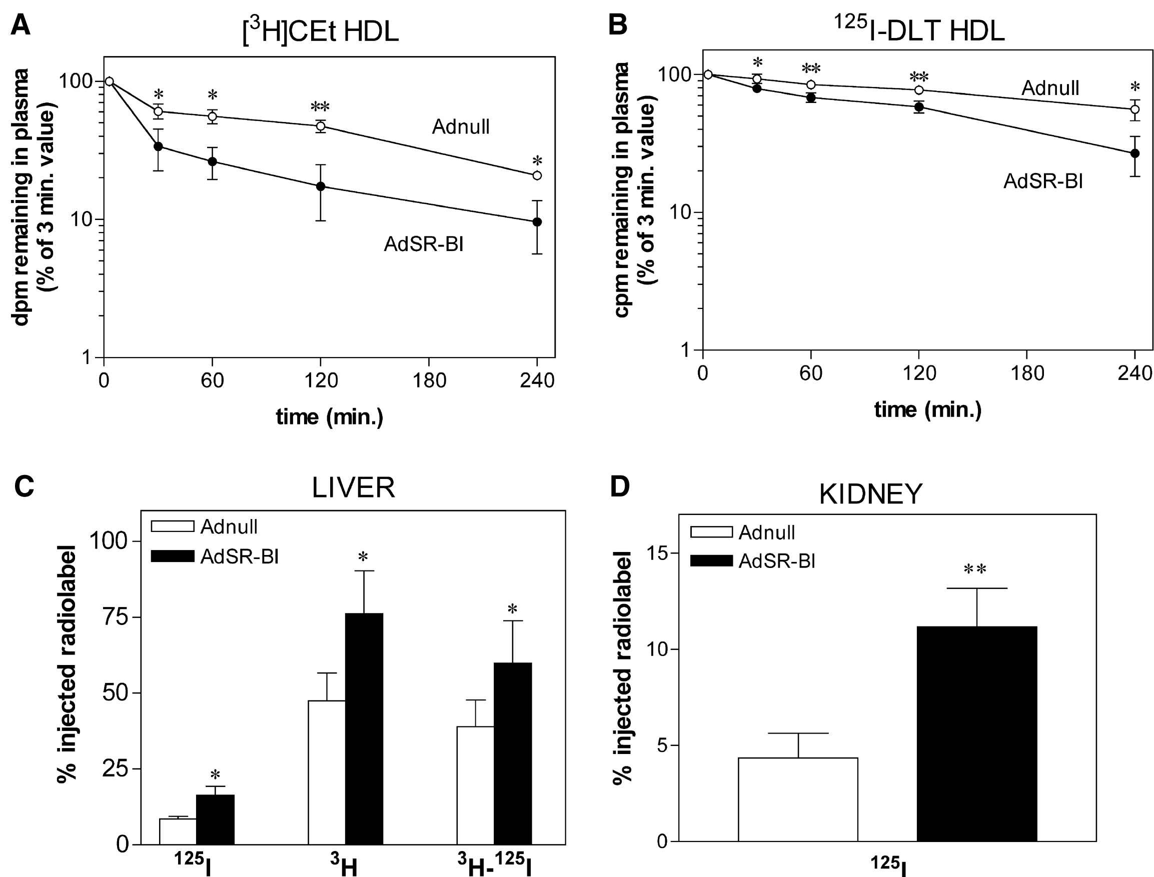

Fig. 1. Plasma clearance of [^{3}H]cholesteryl oleoyl ether (CEt) (A) or ^{125}I-dilactitol tyramine (DLT)-labeled (B) HDL injected into apoA-I$^{-/-}$ mice 3 days after infusion with 1×10^{11} particles Ad scavenger receptor class B type I (SR-BI) or Adnull. Liver (C) and kidney (D) uptake of ^{125}I-DLT and [^{3}H]CEt in apoA-I$^{-/-}$ mice 4 h after HDL bolus injection. Each data point represents the mean (±SD) of values from 3 individual mice. *$P < 0.05$; **$P < 0.01$.

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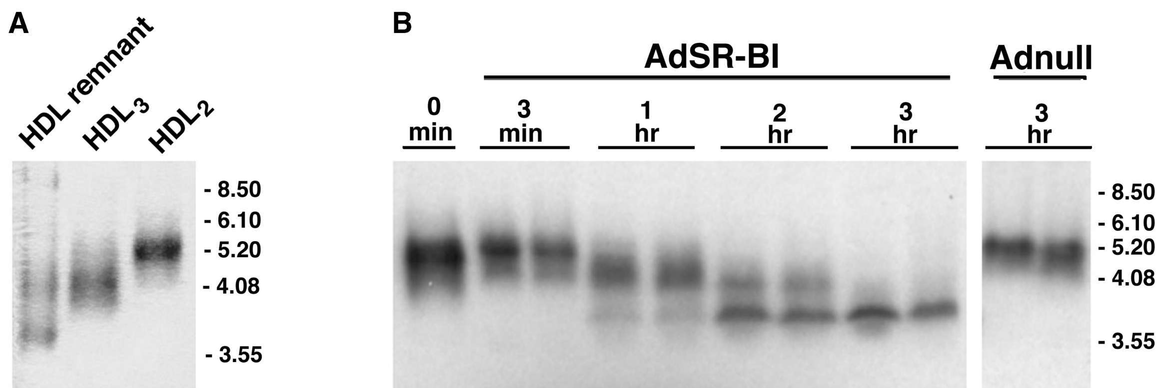

Fig. 2. Size distribution of HDL particles that accumulate in apoA-I$^{-/-}$ mice over-expressing SR-BI. Mice were injected with 1.5 × 10^{11} particles of adenoviral vector AdSR-BI or control adenovirus Adnull. Three days after adenovirus infusion, the mice were injected with 750 μg human HDL_2 traced with ^{125}I. A: Plasma was collected 2 h after ^{125}I-HDL bolus injection from 10 AdSR-BI-treated mice and pooled. The d = 1.09–1.25 g/ml fraction was isolated from the pooled plasma by density gradient ultracentrifugation. Aliquots (5 μg) of the HDL "remnant" preparation, human HDL_3, or human HDL_2 were separated by non-denaturing polyacrylamide gradient (4–18% acrylamide) gel electrophoresis and visualized by Coomassie blue. The mobility of standards with known radii is indicated. B: Aliquots of plasma from individual apoA-I$^{-/-}$ mice collected at the indicated times after ^{125}I-HDL bolus injection were separated on a non-denaturing 4–18% acrylamide gradient gel and visualized by autoradiography. An aliquot of the injected ^{125}I-HDL_2 was analyzed for comparison. The mobility of standards with known radii is indicated.

lyzed directly by non-denaturing gradient gel electrophoresis and autoradiography (Fig. 2B). Thus, for this analysis, HDLs were not subjected to density gradient ultracentrifugation. The generation of small HDL particles in apoA-I$^{-/-}$ mice appeared to be dependent on SR-BI over-expression, since ^{125}I-HDL from mice infused with Adnull co-migrated with the injected HDL_2. Interestingly, HDL particle remodeling appeared to occur in incremental steps in AdSR-BI-treated mice, such that discrete populations of increasingly smaller HDL particles accumulated 1 h, 2 h, and 3 h after bolus injection. Staining by Sudan black indicated that each of the HDL populations visualized by autoradiography contained lipids and thus did not represent lipid-free apoA-I (data not shown). Fractionation of plasma by size exclusion chromatography also showed that HDL particles that have accumulated in AdSR-BI-treated apoA-I$^{-/-}$ mice 2 h and 3 h after bolus injection (2 h and 3 h remnants, respectively) are distinctly larger than lipid-free apoA-I (**Fig. 3**).

To further analyze HDL particles after SR-BI-mediated processing, HDL remnants were isolated from apoA-I$^{-/-}$ mouse plasma by density flotation. The partitioning of HDL remnants during the ultracentrifugation steps was assessed by measuring the recovery of ^{125}I. In five separate experiments, plasma was pooled from 5–10 AdSR-BI-treated apoA-I$^{-/-}$ mice 2 h after ^{125}I-DLT HDL_2 injection. Plasma was initially spun at d = 1.09 g/ml, which removed the vast majority of apoB-containing lipoproteins without a substantial loss (<3%) of ^{125}I-HDL. For these 2 h remnant preparations, the mean recovery (±SD) of ^{125}I in the d = 1.09–1.25 g/ml fraction was 41.6% (± 15.8%). This compares to the recovery in two experiments (38.5% and 30%, respectively) where ^{125}I-DLT HDL particles were re-isolated from apoA-I$^{-/-}$ mice treated with the control virus, Adnull. In contrast, in the case of 3 h remnants, less than 19% of ^{125}I-HDL was recovered in the d = 1.09–1.25 g/ml fraction, suggesting that the bulk of these particles are too dense to float.

SR-BI binding of HDL remnants

We have shown that human SR-BI expressed in transfected CHO cells (CHO-SRBI cells) binds HDL_2 particles with higher affinity compared to HDL_3, suggesting that HDL particle size may influence receptor binding (26). Thus, it was of interest to determine whether 2 h ^{125}I-HDL remnants isolated by density flotation interacted with SR-BI differently than unmodified human HDL_2 (**Fig. 4**). In

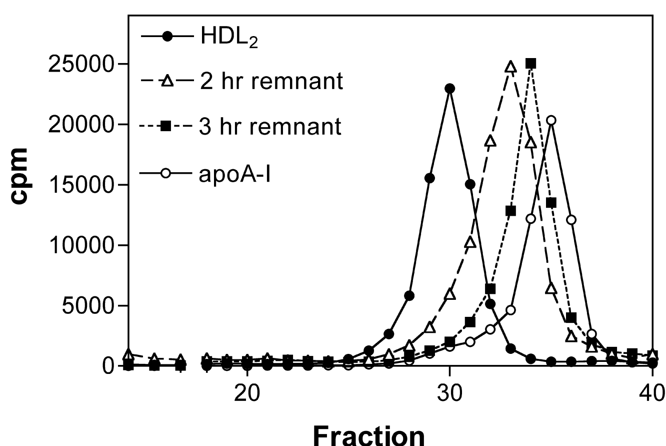

Fig. 3. Size distribution of HDL particles that accumulate in apoA-I$^{-/-}$ mice over-expressing SR-BI. Three days after infusion with 1.5 × 10^{11} particles AdSR-BI, mice were injected with 750 μg human HDL_2 traced with ^{125}I, and plasma was collected 2 h or 3 h after HDL injection. Plasma from individual mice containing ~5 μg HDL remnant was fractionated by size exclusion chromatography, and the ^{125}I content of 0.5 ml fractions was determined. The elution profiles of ^{125}I-HDL_2 (5 μg) and ^{125}I-apoA-I (5 μg) are also shown. For ^{125}I-apoA-I and ^{125}I-HDL_2 the cpms shown are one-tenth the actual values.

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three separate experiments utilizing different HDL preparations, both HDL_2 and 2 h remnants showed high affinity SR-BI binding (mean apparent K_d ± SD for HDL_2, 21 ± 3.1 μg HDL protein/ml; for remnant, 13 ± 3.0 μg HDL protein/ml). Maximum binding values for unmodified HDL_2 (1,020 ± 460 ng HDL protein/mg cell protein) were approximately 3-fold greater than for 2 h remnant particles (365 ± 52 ng HDL protein/mg cell protein). This could be explained at least in part by the possibility that smaller remnant particles contain less apolipoprotein per particle than HDL_2 (see Discussion).

Metabolic fate of SR-BI-generated HDL remnants in normal mice

We investigated whether SR-BI modification alters the HDL particle to promote its catabolism in vivo, perhaps through an increased rate of hepatic and/or renal clearance. For these experiments, apoA-$I^{-/-}$ mouse plasma (~150 μl) containing unmodified ^{125}I-DLT-HDL_2 or ^{125}I-DLT-HDL remnants was injected into normal C57BL/6 mice. The respective plasma clearance rates and hepatic and renal uptake of the different HDLs were compared. There was no measurable difference in the plasma clearance of unmodified HDL_2 and 1 h, 2 h, and 3 h HDL remnants (**Fig. 5A**). There was also no difference in liver or kidney uptake of HDL remnants compared to HDL_2 4 h after tracer injection (data not shown) or 24 h after tracer injection (Fig. 5B). After injection into C57BL/6 mice, only 10% of the ^{125}I-DLT-HDL remnants had been taken up by the liver or kidney in 24 h. This is in contrast to what occurred in apoA-$I^{-/-}$ mice over-expressing SR-BI by adenoviral vector, where more than 27% of the injected ^{125}I-DLT HDL_2 had accumulated in the liver and kidney in only 4 h (Fig. 1). We conclude from these results that small HDL remnants that accumulate in mice over-expressing SR-BI are not irreversibly modified to promote rapid clearance.

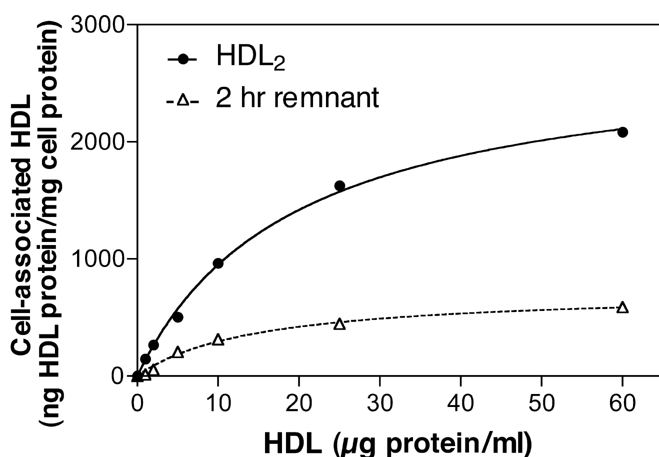

Fig. 4. Concentration-dependent association of human HDL_2 and 2 h HDL remnants with Chinese hamster ovary (CHO)-SR-BI cells. ^{125}I-HDL remnants were isolated from pooled apoA-$I^{-/-}$ mouse plasma (d = 1.09–1.25 g/ml). Cells were incubated for 2 h with the indicated concentrations of ^{125}I-HDL_2 or 2 h remnants and the cell-associated radiolabel was quantified as described in Experimental Procedures. Shown are SR-BI specific values, which were calculated as the difference between the binding to CHO-SR-BI cells and untransfected CHO cells. Values represent the mean of duplicate determinations. Similar results to those depicted here were obtained from two additional experiments utilizing different preparations of ligands.

HDL remnants and ABCA1-mediated cholesterol efflux

We investigated whether small, dense HDL particles that are produced as a result of SR-BI-mediated selective lipid uptake have the capacity to acquire lipid from cells through a pathway involving ABCA1. ABCA1-dependent efflux was defined as the difference in cholesterol efflux from normal human fibroblasts and Tangier fibroblasts that lack functional ABCA1. As shown in **Fig. 6**, control apoA-$I^{-/-}$ mouse plasma that contained no exogenous HDL promoted a small, but measurable amount of ABCA1-dependent [^{3}H]cholesterol efflux. ApoA-$I^{-/-}$ mouse plasma containing either 2 h or 3 h remnants had no additional effect on the amount of ABCA1-dependent efflux compared to apoA-$I^{-/-}$ mouse plasma alone. In contrast, the addition of purified apoA-I to apoA-$I^{-/-}$ mouse plasma significantly enhanced (3.5-fold) ABCA1-dependent cholesterol efflux. We conclude from this analysis that, compared to apoA-I, HDL remnants generated by SR-BI selective lipid uptake are relatively inefficient in stimulating ABCA-1-mediated cholesterol efflux.

HDL remnant remodeling in mouse plasma

The results depicted in Fig. 5A demonstrate that HDL remnants are not more rapidly cleared from C57BL/6 mouse plasma compared to unmodified HDL_2. Given the fact that these particles are not efficient acceptors for ABCA-1 mediated efflux (Fig. 6), it was of interest to determine whether they remain in the circulation as small HDLs. Accordingly, we analyzed the size distribution of ^{125}I-DLT HDL_2 remnants at various intervals after injection into C57BL/6 mice. Small 3 h remnants rapidly converted (within 3 min) to larger HDLs when injected into C57BL/6 mice (**Fig. 7A**). By 1 h after injection, the majority of the injected ^{125}I-radiolabeled apolipoproteins were in a larger HDL fraction that co-migrated with unmodified HDL_2 particles.

To assess the role of plasma components in the conversion of HDL remnants to larger particles, such remnants were incubated at 37°C in normal mouse plasma ex vivo and then analyzed by non-denaturing gradient gel electrophoresis. HDL remnants rapidly converted (within 10 min.) to normal-sized HDL_2 particles when incubated with mouse plasma but not with PBS (Fig. 7B). Significant but less remodeling occurred when remnants were incubated with 1 mg/ml human HDL. These results indicate that SR-BI-generated HDL remnants are efficiently converted to larger HDL particles through interactions with components in plasma. It is notable that plasma isolated from apoA-$I^{-/-}$ mice is just as efficient in promoting remnant remodeling as C57BL/6 mouse plasma (**Fig. 8A**), indicating that exogenous apoA-I is not required for the conversion to larger particles. HDL remnants underwent similar remodeling when incubated with apoA-$I^{-/-}$ mouse

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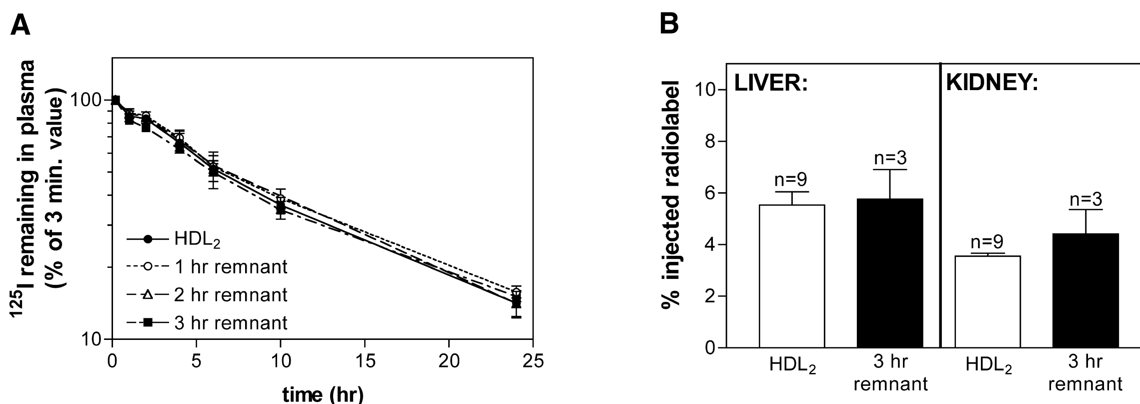

Fig. 5. Plasma clearance and liver and kidney uptake of HDL_2 and SR-BI-modified HDL remnants in C57BL/6 mice. HDL remnants traced with the residualizing radiolabel ^{125}I-DLT were generated in apoA-I$^{-/-}$ mice as described in the legend to Fig. 2. An aliquot of apoA-I$^{-/-}$ mouse plasma containing ~15 μg of SR-BI-modified ^{125}I-DLT HDL (5×10^5 cpm) was injected via the jugular vein into a C57BL/6 mouse. An equivalent amount of ^{125}I-DLT HDL_2 mixed with untreated apoA-I$^{-/-}$ mouse plasma was similarly injected for comparison. A: At the indicated times, blood was collected and radioassayed. Data points represent the mean (±SD) of values from three (3 h remnant), four (1 h and 2 h remnant), or nine (HDL_2) mice. Each mouse received an aliquot from a distinct HDL remnant preparation. B: Liver and kidney uptake of ^{125}I-DLT in C57BL/6 mice 24 h after tracer injection. Values represent the mean (±SD) from the indicated number of mice. In the case of kidney uptake, the data did not meet the constraints of equivalence of variance to permit parametric analysis; a Mann-Whitney rank sum test showed no significant difference in renal uptake of ^{125}I-HDL_2 and 3 h remnant.

plasma collected from mice 3 days after infusion of AdSR-BI. Taken together, these data suggest that the accumulation of small and dense HDL particles in apoA-I$^{-/-}$ mice is due to the highly increased selective uptake activity in these mice, rather than a deficiency in the remnant remodeling mechanism.

DISCUSSION

The ability of HDL to protect against atherosclerosis is widely attributed to its role in reverse cholesterol transport, whereby HDL promotes the flux of excess cholesterol from peripheral cells to the liver for excretion. In the first step of this pathway, lipid poor apoA-I (nascent HDL) accepts cholesterol from cells in a process involving the ATP-binding cassette transporter ABCA1. Subsequent to cholesterol removal from cells, HDL mediates cholesterol delivery to hepatocytes, either via apoB-containing lipoproteins or by direct transfer in a process involving SR-BI. The direct delivery of CE from HDL to cells is via a "selective uptake" mechanism whereby CE is selectively transferred from the core of the particle to cells. Thus, the centripetal transport of cholesterol from the periphery to the liver is envisioned to involve the lipidation (mediated by ABCA1) and de-lipidation (mediated by SR-BI) of HDL particles. Although the metabolic fate of HDL after SR-BI selective lipid uptake has not been precisely established, studies in mice have shown that increased SR-BI activity in the liver leads to increased apoA-I catabolism, and at least some of this catabolism occurs in the kidney (11, 13). To investigate this process further, we set out to assess the effect of SR-BI selective lipid uptake on HDL metabolism both in vivo and in vitro.

Our approach was to inject a bolus of radiolabeled human HDL into apoA-I$^{-/-}$ mice over-expressing SR-BI by adenoviral vector, and then re-isolating these particles at selected intervals for analysis. The dose of the injected bolus was estimated to result in a plasma apoA-I concentration of ~85 mg/dl, a concentration approximating the plasma apoA-I concentration in normal C57BL/6 mice (~110 mg/dl). We have shown (unpublished data) that increased SR-BI expression by adenoviral vector results in an almost complete depletion of HDL-C, apoE, and apoA-II in apoA-I$^{-/-}$ mice. Thus, the remodeling of exogenous HDL through the exchange with endogenous lipoprotein components would be minimal in our experimental system, and HDL fractions recovered from these mice would not contain substantial amounts of endogenous lipoproteins. Given the significant induction of SR-BI in AdSR-BI-treated mice (~20-fold increase), we expected that any alterations in HDL produced could be largely attributed to the activity of SR-BI. Our results show that hepatic SR-BI over-expression leads to an increased rate of HDL-CE and apolipoprotein clearance from the plasma, an increase in selective lipid uptake in the liver, and an increase in HDL apolipoprotein uptake in the liver and kidney. These effects on HDL clearance were associated with an accumulation of small HDL remnants in the plasma of apoA-I$^{-/-}$ mice. Thus, the effect of adenoviral vector-mediated SR-BI expression on exogenous HDL in apoA-I$^{-/-}$ mice is similar to what occurs with endogenous HDL in SR-BI transgenic mice (11, 12).

An interesting feature of SR-BI activity in apoA-I$^{-/-}$ mice is the fact that the formation of small HDL particles occurred in incremental steps during the experimental time course. One potential reason for the accumulation of particles of discrete size is the possibility that as HDL particles become smaller, they progressively lose their ability to interact with SR-BI. Thus, HDL remnants that accu-

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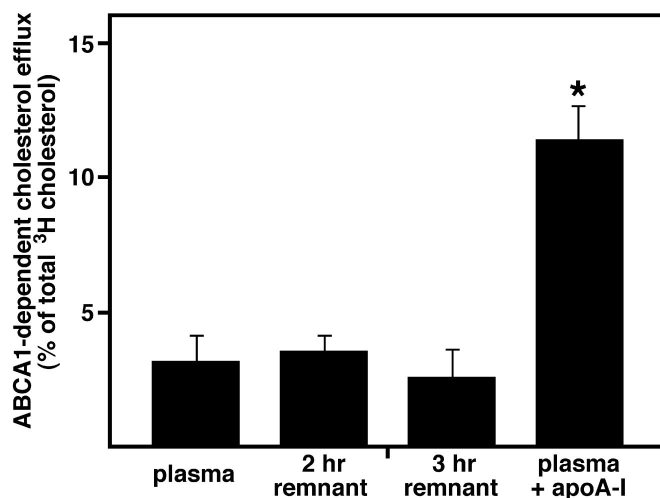

Fig. 6. ATP-binding cassette transporter A1 (ABCA1)-dependent cellular cholesterol efflux stimulated by apoA-I and HDL remnants. Normal human skin fibroblasts and Tangier fibroblasts were radiolabeled with [^{3}H]cholesterol, and then loaded with free cholesterol to induce ABCA1 expression as described in Experimental Procedures. 2 h remnant and 3 h remnant is plasma collected from apoA-I$^{-/-}$ mice 2 h and 3 h after ^{125}I-HDL bolus injection, respectively. "Plasma" and "plasma + apoA-I" is plasma collected from apoA-I$^{-/-}$ mice 3 days after AdSR-BI infusion with and without addition of apoA-I, respectively. In all cases, plasmas were dialyzed against efflux media and added to cells at a final concentration of 10 μg/ml HDL remnant or apoA-I (added mouse plasma constituted <5% of total culture volume). After 16 h incubations at 37°C, culture media was collected at 4°C, centrifuged to remove cell debris, and radioassayed. ABCA-1-dependent efflux (defined as the difference in efflux from normal human fibroblasts and Tangier fibroblasts) was expressed as the ratio of [^{3}H]cholesterol counts in efflux media and total [^{3}H]cholesterol counts in media and lipid extracts of cells. The results shown are the mean of triplicate determinations (±SE) and are representative of three assays using different preparations of remnants. *Efflux from cells incubated with plasma + apoA-I was significantly greater than from other cells, as determined by analysis of variance and Tukey test ($P < 0.005$).

mulate 1 h, 2 h, and 3 h after HDL injection may represent subpopulations of HDL that differ markedly in their affinity for SR-BI. We reported previously that HDL_2 binds SR-BI with greater affinity than HDL_3, suggesting that particle size may influence receptor binding (26). In studies using reconstituted HDL particles, we showed that apoA-I in 7.8 nm diameter discs bound poorly to SR-BI compared to apoA-I in larger 9.6 nm discs, and we concluded that differences in apoA-I conformation in different-sized particles markedly influence apoA-I recognition by SR-BI (26). In the current study, we investigated the possibility that small HDL remnants are deficient in SR-BI binding compared to HDL_2. Cell-association assays using transfected CHO cells showed that the apparent affinity of HDL remnants for SR-BI ($K_d = 13 \pm 3$ μg HDL protein/ml) was actually higher compared to unmodified HDL_2 ($K_d = 21 \pm 3.1$ μg HDL protein/ml). It was not possible to determine the K_d or Bmax of association of the remnant particles on a molar basis since their exact density and molar composition has not been determined. It is likely that HDL remnants contain fewer apoA-I molecules per particle than HDL_2, as discussed below. If this is the case, differences in apolipoprotein content would largely account for the observed differences in K_d and Bmax values when expressed in μg HDL protein rather than particle molarity. The fact that SR-BI-generated remnant particles, in contrast to either HDL_3 or small 7.8 nm reconstituted discs, bound with similar high affinity to SR-BI as did HDL_2 indicates that reduced particle diameter is not necessarily associated with decreased ability to bind SR-BI. In a recent report, Temel et al. showed that large (13–17 nm diameter) HDL particles isolated from apoA-I$^{-/-}$ mice bind to SR-BI with the same or higher affinity as smaller (11 nm diameter) HDL from normal mice (27). Clearly, properties of HDL particles in addition to apoA-I conformation or particle size can influence SR-BI binding.

Our data indicates that the accumulation of HDL particles of discrete sizes in apoA-I$^{-/-}$ mice is not due to a relative inability of HDL remnants to bind SR-BI. An alternative explanation for the incremental changes in HDL particle size may be constraints in HDL particle structure. It is possible that the discrete populations of HDL rem-

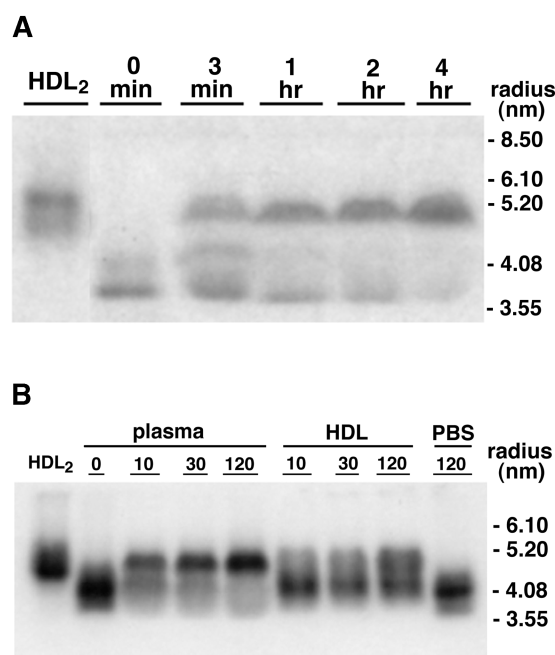

Fig. 7. Remodeling of HDL remnants in vivo and in vitro. A: Nondenaturing gel electrophoresis of 3 h HDL remnants before and after injection into C57BL/6 mice. ^{125}I-DLT-labeled 3 h remnants were injected into C57BL/6 mice as described in the legend to Fig. 5. At the indicated intervals, plasma samples were collected and an aliquot (15 μl) was separated on a 4–18% gradient acrylamide gel and visualized by autoradiography. Unmodified HDL_2 was analyzed for comparison. B: Size distribution of 2 h HDL remnants before and after incubations with C57BL/6 mouse plasma or human HDL. Plasma containing 2 h HDL remnants was mixed 1:10 (v/v) with either C57BL/6 mouse plasma, human HDL (1 mg/ml), or PBS. After incubating at 37°C for the indicated minutes, the samples were separated on a non-denaturing acrylamide gradient gel (4–18%) and visualized by autoradiography. Unmodified HDL_2 and starting 2 h remnants were analyzed for comparison.

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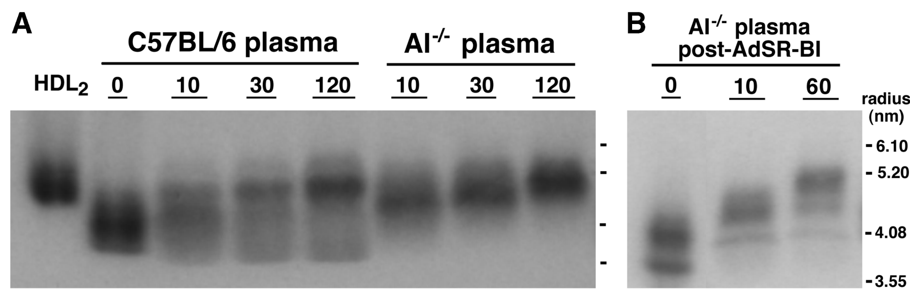

Fig. 8. Remodeling of HDL remnants in apoA-$I^{-/-}$ mouse plasma ex vivo. A: Plasma containing 2 h HDL remnants was incubated at 37°C with either C57BL/6 or apoA-$I^{-/-}$ mouse plasma for the indicated minutes and analyzed as described in legend to Fig. 7. Unmodified HDL_2 is shown for comparison. (B) Size distribution of 2 h HDL remnants after incubations with plasma collected from apoA-$I^{-/-}$ mice 3 days after AdSR-BI infusion.

nants in our experiments correspond to the small, medium, and large HDL particles described by Colvin et al. (28). These particles, which were isolated from African green monkeys, have been shown to contain two, three, or four apoA-I molecules, respectively (28). SR-BI selective uptake may lead to "shedding" of apolipoproteins from lipoprotein particles as they are progressively depleted of lipid to yield particles with decreased apoA-I content. The analysis of apoA-$I^{-/-}$ mouse plasma containing HDL remnants by both non-denaturing gel electrophoresis (Fig. 2) and by size exclusion chromatography (Fig. 3) showed no evidence that lipid-free ^{125}I-apoA-I is generated during SR-BI-mediated HDL processing. However, such lipid-free apoA-I may be either rapidly cleared by the kidney, or incorporated onto other HDL particles, and thus not be detected. Further studies are required to determine whether HDL remnant particles contain fewer apolipoprotein molecules compared to HDL_2.

We have shown that small, dense HDL particles that accumulate in apoA-$I^{-/-}$ mice over-expressing SR-BI are rapidly converted (within 10 min.) to HDL_2-sized particles when re-injected into normal mice. This rapid remodeling of HDL remnants appears to protect them from glomerular filtration since these particles were not more rapidly cleared in C57BL/6 mice compared to unmodified HDL_2. Given the propensity for SR-BI-generated HDL remnants to rapidly remodel to form larger particles, it seems unlikely that small remnants ever accumulate to detectable levels in normal HDL metabolism. In kinetic studies in nonhuman primates using HDL particles isolated by immunoaffinity and gel filtration, Colvin and Parks noted that apoA-I on large HDL particles cleared from the plasma without appearing on smaller HDL subfractions (29, 30). Although this suggests that apoA-I on large HDL particles is not recycled through small HDL intermediates, our data shows that apoA-I can indeed rapidly cycle between particles of different sizes. Studies in SR-BI transgenic mice show that small HDL particles accumulate in mice with normal apoA-I production as a result of greatly enhanced SR-BI expression. Ueda et al. assessed the size distribution of HDL particles in two lines of double transgenic mice (human apoB transgenic background) with either a 2-fold or more than 10-fold induction of SR-BI (31). Notably, the amount of large α-migrating HDL particles was not altered in low SR-BI/apoB transgenic mice, but was almost entirely depleted in high SR-BI/apoB mice. There also appeared in the high SR-BI/apoB mice a distinct population of small, α-migrating HDLs. Taken together, these studies suggest that the accumulation of detectable amounts of small HDL remnants requires substantially increased SR-BI expression, and that at physiological levels of SR-BI, equilibrium favors larger HDL particles.

The mechanism by which HDL remnants are converted to larger particles is not yet defined. Our data clearly shows that the ability of HDL remnants to promote ABCA1-mediated efflux is minor compared to apoA-I, and that the bulk of remnant remodeling can occur in plasma ex vivo. Previous studies have demonstrated that apoA-I, and not HDL_3, is an effective acceptor for cholesterol efflux by ABCA1 (32, 33). Thus, the lipidation state of apoA-I appears to markedly influence its ability to serve as a substrate for ABCA1. It is possible in our experiments that HDL remnants initially formed as a result of SR-BI selective lipid uptake interact rapidly with endogenous ABCA1, thereby producing particles that have lost the capacity to stimulate ABCA1-mediated efflux. Although the possible role of ABCA1 in early stages of remnant re-lipidation requires further study, our results show that significant remodeling of HDL remnants occurs in plasma independent of a direct involvement of ABCA1. Although the factor(s) that are involved in this process remain to be identified, the fact that efficient re-modeling occurred in mouse plasma establishes that CETP is not required. Interestingly, exogenous apoA-I is not essential for the remodeling process in vitro, since remnants rapidly converted to larger particles when incubated with plasma from apoA-$I^{-/-}$ mice. It is possible that the conversion of HDL remnants to larger particles involves a fusion of remnant particles. If fusion does occur, this process must require the contribution of plasma factor(s), since conversion to larger particles did not occur spontaneously when remnants were incubated in PBS.

In summary, we have shown in an in vivo model system that SR-BI has the capacity to generate, in a processive manner, small and dense HDL particles. Such particles are not rapidly cleared from the circulation, but rather remodel to form larger HDLs. Such remodeling appears to

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occur primarily in the plasma, independent of ABCA1 and exogenous apoA-I. This remodeling pathway may represent a significant determinant of the rate of apoA-I catabolism and HDL-mediated reverse cholesterol transport.

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REFERENCES

1. Fidge, N., P. Nestel, M. Ishikawa, T. Reardon, and T. Billington. 1980. Turnover of apoproteins A-I and A-II of high density lipoprotein and the relationship to other lipoproteins in normal and hyperlipidemic individuals. *Metabolism.* **29:** 643–653.
2. Schaefer, E. J., L. A. Zech, L. L. Jenkins, T. J. Bronzert, E. A. Rubalcaba, R. T. Lindgren, R. L. Aamodt, and H. B. Brewer, Jr. 1982. Human apolipoprotein A-I and A-II metabolism. *J. Lipid Res.* **23:** 850–862.
3. Brinton, E. A., S. Eisenberg, and J. L. Breslow. 1991. Increased apo A-I and apo A-II fractional catabolic rate in patients with low high density lipoprotein-cholesterol levels with or without hypertriglyceridemia. *J. Clin. Invest.* **87:** 536–544.
4. Brinton, E. A., A. Eisenberg, and J. L. Breslow. 1989. Elevated high density lipoprotein cholesterol levels correlate with decreased apolipoprotein A-I and A-II fractional catabolic rate in women. *J. Clin. Invest.* **84:** 262–269.
5. Rader, D. J., K. Ikewaki, N. Duverger, I. Feuerstein, L. A. Zech, W. Connor, and H. B. Brewer, Jr. 1993. Markedly decreased plasma high density lipoprotein levels without premature coronary atherosclerosis caused by rapid catabolism of apolipoproteins A-I and A-II. *Lancet.* **342:** 1455–1458.
6. Ikewaki, K., D. J. Rader, T. Sakamoto, M. Nishiwaki, N. Wakimoto, J. Schaefer, T. Ishikawa, T. Fairwell, L. Zech, H. Nakamura, M. Nagano, and H. B. Brewer, Jr. 1993. Delayed catabolism of high density lipoprotein apolipoproteins A-I and A-II in human cholesteryl ester transfer protein deficiency. *J. Clin. Invest.* **92:** 1650–1658.
7. Rader, D. J., K. Ikewaki, N. Duverger, H. Schmidt, H. Pritchard, J. Frohlich, M. Clerc, M. F. Dumon, T. Fairwell, L. Zech, S. Santamarino-Fojo, and H. B. Brewer, Jr. 1994. Rapid catabolism of apolipoprotein A-II and high density lipoproteins containing apoA-II in classic LCAT deficiency and fish-eye disease. *J. Clin. Invest.* **93:** 321–330.
8. Kozyraki, R., J. Fyfe, M. Kristiansen, C. Gerdes, C. Jacobsen, S. Cui, E. I. Christensen, M. Aminoff, A. de la Chapelle, R. Krahe, P. J. Verroust, and S. K. Moestrup. 1999. The intrinsic factor-vitamin B12 receptor, cubilin, is a high affinity apolipoprotein A-I receptor facilitating endocytosis of high-density lipoprotein. *Nat. Med.* **5:** 656–661.
9. Hammad, S. M., S. Sefansson, W. O. Twal, C. J. Drake, P. Fleming, A. Remaley, H. B. Brewer, Jr., and W. S. Argraves. 1999. Cubilin, the endocytic receptor for intrinsic factor-vitamin B12 complex, mediates high-density lipoprotein holoparticle endocytosis. *Proc. Natl. Acad. Sci. USA.* **96:** 10158–10163.
10. Kozarsky, K. F., M. H. Donahee, A. Rigotti, S. N. Iqbal, E. R. Edelman, and M. Krieger. 1997. Overexpression of the HDL receptor SR-BI alters plasma HDL and bile cholesterol levels. *Nature.* **387:** 414–417.
11. Wang, N., T. Arai, Y. Ji, F. Rinninger, and A. R. Tall. 1998. Liver-specific overexpression of scavenger receptor BI decreases levels of very low density lipoprotein apoB, low density lipoprotein apoB, and high density lipoprotein in transgenic mice. *J. Biol. Chem.* **273:** 32920–32926.
12. Ueda, Y., L. Royer, E. Gong, J. Zhang, P. N. Cooper, O. Francone, and E. M. Rubin. 1999. Lower plasma levels and accelerated clearance of high density lipoprotein (HDL) and non-HDL cholesterol in scavenger receptor class B Type I transgenic mice. *J. Biol. Chem.* **274:** 7165–7171.
13. de Beer, F. C., P. M. Connell, J. Yu, M. C. de Beer, N. R. Webb and D. R. van der Westhuyzen. 2000. HDL modification by secretory phospholipase A_2 promotes SR-BI interaction and accelerates HDL metabolism. *J. Lipid Res.* **41:** 1849–1857.
14. Strachan, A. F., F. C. de Beer, D. R. van der Westhuyzen, and G. A. Coetzee. 1988. Identification of three isoform patterns of human serum amyloid A protein. *Biochem. J.* **250:** 203–207.
15. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoproteins. *Biochim. Biophys. Acta.* **260:** 212–221.
16. Daugherty, A., S. R. Thorpe, L. G. Lange, B. E. Sobel, and G. Schonfeld. 1985. Loci of catabolism of β-very low density lipoprotein *in vivo.* Delineating with a residualizing label, ^{125}I-dilactitol tyramine. *J. Biol. Chem.* **260:** 14564–14570.
17. Rinninger, F., M. Brundert, S. Jackle, T. Kaiser, and H. Greten. 1995. Selective uptake of low-density lipoprotein-associated cholesteryl esters by human fibroblasts, human HepG2 hepatoma cells and J7743 macrophages in culture. *Biochim. Biophys. Acta.* **1255:** 141–153.
18. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and B. J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
19. Webb, N. R., P. M. Connell, G. A. Graf, E. J. Smart, W. J. S. de Villiers, F. C. de Beer, and D. R. van der Westhuyzen. 1998. SR-BII, an isoform of the scavenger receptor BI containing an alternate cytoplasmic tail, mediates lipid transfer between high density lipoprotein and cells. *J. Biol. Chem.* **273:** 15241–15248.
20. Strobel, J. L., J. W. Baynes, and S. R. Thorpe. 1985. 125I-glycoconjugate labels for identifying sites of protein catabolism in vivo: effect of structure and chemistry of coupling to protein on label entrapment in cells after protein degradation. *Arch. Biochem. Biophys.* **240:** 635–645.
21. Meyer, B. J., Y. C. Ha, and P. J. Barter. 1989. Effects of experimental hypothyroidism on the distribution of lipids and lipoproteins in the plasma of rats. *Biochim. Biophys. Acta.* **1004:** 73–79.
22. Silverman, D. I., G. S. Ginsberg, and R. C. Pasternak. 1993. High density lipoprotein subfractions. *Am. J. Med.* **94:** 636–645.
23. Graf, G. A., P. M. Connell, D. R. van der Westhuyzen, and E. J. Smart. 1999. The class B, type I scavenger receptor promotes the selective uptake of high density lipoprotein cholesterol esters into caveolae. *J. Bio. Chem.* **274:** 12043–12048.
24. Francis, G. A., R. H. Knoop, and J. F. Oram. 1995. Defective removal of cellular cholesterol and phospholipids by apolipoprotein A-I in Tangier disease. *J. Clin. Invest.* **96:** 78–87.
25. Lawn, R. M., D. P. Wade, M. R. Garvin, X. Wang, K. Schwartz, J. G. Porter, J. J. Seilhamer, A. M. Vaughan, and J. F. Oram. 1999. The Tangier disease product ABC1 controls the cellular apolipoprotein-mediated lipid removal pathway. *J. Clin. Invest.* **104:** R25–31.
26. de Beer, M. C., D. M. Durbin, L. Cai, A. Jonas, F. C. de Beer, and D. R. van der Westhuyzen. 2001. Apolipoprotein A-I conformation markedly influences HDL interaction with scavenger receptor BI. *J. Lipid Res.* **42:** 309–313.
27. Temel, R. E., R. L. Walzem, C. L. Banka, and D. L. Williams. 2002. Apolipoprotein A-I is necessary for the in vivo formation of high density lipoprotein competent for scavenger receptor BI-mediated cholesteryl ester-selective uptake. *J. Biol. Chem.* **277:** 26565–26572.
28. Colvin, P. L., E. Moriguchi, P. H. Barrett, J. S. Parks, and L. L. Rudel. 1999. Small HDL particles containing two apoA-I molecules are precursors in vivo to medium and large HDL particles containing three and four apoA-I molecules in nonhuman primates. *J. Lipid Res.* **40:** 1782–1792.
29. Colvin, P., E. Moriguchi, H. Barrett, J. Parks, and L. Rudel. 1998. Production rate determines plasma concentration of large high density lipoprotein in non-human primates. *J. Lipid Res.* **39:** 2076–2085.
30. Colvin, P. L., and J. S. Parks. 1999. Metabolism of high density lipoprotein subfractions. *Curr. Opin. Lipidol.* **10:** 309–314.
31. Ueda, Y., E. Gong, L. Royer, P. N. Cooper, O. L. Francone, and E. M. Rubin. 2000. Relationship between expression levels and atherogenesis in scavenger receptor class B, type I transgenics. *J. Biol. Chem.* **275:** 20368–20373.
32. Oram, J. F., and A. M. Vaughan. 2000. ABCA1-mediated transport of cellular cholesterol and phospholipids to HDL apolipoproteins. *Curr. Opin. Lipidol.* **11:** 253–260.
33. Wang, N., D. L. Silver, P. Costet, and A. R. Tall. 2000. Specific binding of ApoA-I, enhanced cholesterol efflux, and altered plasma membrane morphology in cells expressing ABC1. *J. Biol. Chem.* **275:** 33053–33058.

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